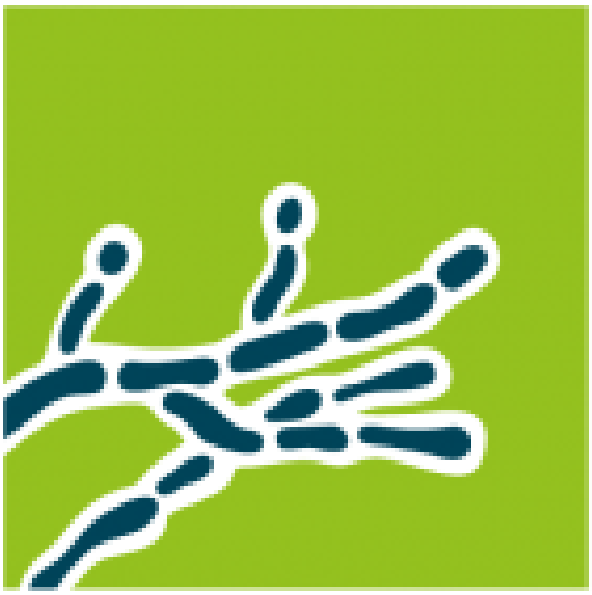
ECFG17

Book of Abstracts



ECFG17
DUBLIN · IRELAND
2025

Table of Contents

Opening Ceremony

OC1.1 - Killer toxins and evolution of the genetic code in yeasts

K. Wolfe[1], *P. Heneghan*[1], *L. Salzberg*[1]
[1]University College Dublin, Conway Institute, Dublin, Ireland

Zymocin (γ-toxin) is a killer toxin produced by a natural plasmid in *Kluveromyces lactis*. It is a secreted ribonuclease that kills its victims by cleaving tRNA-Glu. Zymocin-like toxins have not been found in eukaryotes other than yeasts, and even among yeasts they are rare. Only 4 zymocin-like toxins have previously been characterized: one targeting tRNA-Glu, two targeting tRNA-Gln, and one targeting rRNA. We hypothesized that a zymocin-like toxin might have been the evolutionary driving force behind the three parallel changes in the nuclear genetic code that occurred during budding yeast evolution, which resulted in clades that translate CUG codons as Ser or Ala instead of Leu. We therefore investigated the diversity and evolution of zymocin-like toxins. We found that they evolve extraordinarily quickly, and have a much broader phylogenetic distribution than previously realized. In budding yeasts (Saccharomycotina) we discovered 45 new toxins, from five different taxonomic orders including the two with CUG-Ser translation. Some toxins are encoded by cytosolic linear plasmids (Virus-Like Elements) but others are integrated into the nuclear genome. In filamentous fungi (Pezizomycotina), we found 55 nuclear gene clusters coding for a ribonuclease (toxin γ-subunit), a chitinase (toxin α/β-subunit), and in some cases an immunity protein. In the early-diverging fungi *Coemansia* (Zoopagomycota) and *Cunninghamella* (Mucoromycota) we found constellations of cytosolic linear plasmids containing homologs of yeast killer and helper plasmid genes, including some toxin candidates. Phylogenetic analysis suggests that the plasmids have mainly been inherited vertically, so zymocin-like killer toxins are as old as the kingdom Fungi.

PS1.1 - *Aspergillus fumigatus* conidial surface-associated proteome reveals factors for fungal evasion and host immunity modulation

G.H. Goldman[1]
[1]Universidade de São Paulo, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Ciências Farmacêuticas, Ribeirão Preto, Brazil

Most aspergillosis infections are caused by *Aspergillus fumigatus* and rely on asexual spores (conidia) to initiate host infection. There is scarce information about *A. fumigatus* proteins involved in fungal evasion and host immunity modulation. The section *Fumigati* is composed of *Aspergillus* fungal species presenting variable pathogenicity levels. Here, we used a phylogenetic approach analyzing the conidial surface proteome of *A. fumigatus*, two closely related non-pathogenic species, *Aspergillus fischeri* and *Aspergillus oerlinghausenensis*, as well as pathogenic *Aspergillus lentulus*, to identify such proteins. After identifying 62 proteins exclusively detected on the *A. fumigatus* conidial surface, we assessed null mutants for 42 genes encoding these proteins. The deletion of 33 of these genes altered susceptibility to macrophages, epithelial cells, and cytokine production. Notably, a gene that encodes a putative glycosylasparaginase modulates high levels of the host proinflammatory cytokines. We also identified a cysteine-rich protein, CyrA, that has reduced levels of proinflammatory cytokines but high levels of anti-inflammatory cytokine IL-10 and prostaglandins PGE2 and PDG2. Both mutants are virulent in the chemotherapeutic murine model but avirulent in an immunocompetent murine model of fungal disease. These results suggest that *A. fumigatus* conidial surface AspA and secreted CyrA proteins are important for evasion and modulation of the immune response at the onset of fungal infection.
Funding: NIAID-NIH, USA and FAPESP, Brazil

PS1.3 - Structure of fungal cell wall immune epitopes: the origins of immunity

N. Gow[1]
[1]University of Exeter, Biosciences, Exeter, United Kingdom

For a fungus, there may nothing as biologically variable and highly regulated as the glycans in its cell wall. This makes the wall challenging to study, but worth the effort because of the potential to reveal novel targets for antifungal drugs and mechanisms that are important for immune recognition. Differences and adaptations of cell wall composition can act to resist chemotherapy and create a moving target for efficient immune recognition.
We have used a variety of microscopic, forward and reverse genetic and immunological tools to generate a new spatially accurate model of the cell wall and to explore how dynamic changes in the wall influence drug efficacy and immune surveillance. Our molecular and cellular studies show that the cell has a mechanism to maintain wall robustness within physiological limits and has enabled the components of the wall to be defined with spatial precision. We have also

demonstrated that immune relevant epitopes can be diffuse or clustered, superficial or buried in the cell wall and they changed during batch culture and between yeast, hypha and other cellular morphologies. Unbiased screening of a haploid mutant library has revealed gene sets for both predicted and novel processes that are important for the assembly of the cell wall immune epitope. My presentation will describe a scaler and dynamic model of the cell wall that illuminates mechanisms of immune recognition and cell wall homeostasis.
Gow, N. & Lenardon, M. (2022). Architecture the dynamic fungal cell wall. *Nature Reviews Microbiology* PMID 36266346

PS1.5 - Epigenetic routes to antifungal resistance

R. Allshire[1]
[1]University of Edinburgh, Discovery Research Platform for Hidden Cell Biology, Institute for Cell Biology, Edinburgh, United Kingdom

Assembly of histone H3 lysine 9 methylation (H3K9me)-dependent heterochromatin over genes decreases their transcription. In fission yeast and several pathogenic fungi, heterochromatin is normally concentrated at telomeres and centromeres. However, changes in the cellular environment can result in the appearance of heterochromatin islands over euchromatic genes[1]. Such ectopic heterochromatin can be transmitted through cell division, provided the counteracting demethylase, KDM/Epe1, is absent[2]. Thus, cells may adapt by stochastically forming heterochromatin at various chromosomal locations, with resulting epimutations altering the phenotype of otherwise wild-type cells through reversible gene repression rather than changes in DNA. Heterochromatin-dependent epimutants that are resistant to caffeine and widely-used antifungals, form in fission yeast[1]. Isolates with reversible resistance exhibit distinct heterochromatin islands with reduced expression of underlying genes, including some whose mutation confers resistance. Both caffeine and antifungals down-regulate key counteracting activities (KDM/Epe1 and HAT/Mst2) that normally limit heterochromatin formation, thereby reprogramming heterochromatin [1,3]. Resulting epimutations allow wild-type cells to cope with unfavourable environments. We are currently investigating what upstream events lead to epimutation formation and the mechanisms underlying their resistance phenotypes. Key questions include: How do epimutants arise? Do they pre-exist in a cell population, or are they induced by external stressors? Do related epigenetic mechanisms contribute to antifungal tolerance/resistance in pathogenic fungi such as Cryptococcus neoformans, responsible for ~200,000 human deaths/annum, or Zymoseptoria tritici, which significantly reduces annual wheat yields globally?
1. Torres-Garcia et al. (2020) *Nature* 585:453.
2. Audergon et al. (2015) *Science* 348:132.
3. Yaseen, White, et al. (2022) *NSMB* 29:745.

PS2.1 - The genomic making of yeast metabolic and ecological diversity

C.T. Hittinger[1]
[1]University of Wisconsin-Madison, Madison, United States

Yeasts of the subphylum Saccharomycotina have evolved to occupy every continent and biome over the course of more than 400 million years. Much is known about the molecular genetics of model systems, such as *Saccharomyces cerevisiae* and *Candida albicans*, but little is known about how their diverse metabolisms and ecologies evoloved and are encoded in their genomes. Here, I will present research from the Y1000+ Project (http://y1000plus.org). We have sequenced, analyzed, and published genome sequences for nearly every known yeast species. The primary dataset of 1154 yeast genomes also includes quantitative growth rate data for 24 carbon and nitrogen and carbon, as well as a hierarchical ecological ontology (https://doi.org/10.1126/science.adj4503). Machine learning and phylogenetic analyses showed that differences in the breadths of carbon source utilization were due to intrinsic gene content differences in specific metabolic pathways, but we found little evidence for tradeoffs. I will introduce data to show how "keystone gene families" have influenced yeast genome evolution (https://doi.org/10.1101/2024.07.22.604484, https://doi.org/10.1093/molbev/msae228). I will also report the likely identification of an alternative *GAL*actose utilization pathway (https://doi.org/10.1073/pnas.2315314121). Finally, I will discuss ongoing challenges and progress toward predicting xylose metabolism in yeasts (https://doi.org/10.1093/molbev/msad111), which poses a substantial barrier to sustainable cellulosic biorefineries.

PS2.2 - Evolution of Mating type, population diversity, and genomic and phenotypic variation in *Rhodotorula* isolated from built and extreme environments.

X. Liu[1,2], *E. Ottum*[3], *A. Nguyen*[3], *C. Gostinčar*[4], *B. Turchetti*[5], *C. Coleine*[6], *L. Selbmann*[6], *I. Wheeldon*[3], *N. Gunde-Cimerman*[4], *J. Stajich*[2]
[1]Chinese Academy of Sciences, Institute of Microbiology, Beijing, China, [2]University of California-Riverside, Microbiology and Plant Pathology, Riverside, United States, [3]University of California-Riverside, Department of Chemical and Environmental Engineering, Riverside, United States, [4]University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia, [5]University of Perugia, Department of Agricultural, Food and Environmental Sciences, Perugia, Italy, [6]University of Tuscia, Department of Ecological and Biological Sciences, Viterbo, Italy

The basidiomycete yeasts in the *Rhodotorula* genus can be isolated from a broad range of environments from moist and temperate to extreme cold or hot and arid environments. Strains have been isolated from spoiled food, freshwater lakes, marine waters, and the human built environment. They inhabit extremes from glaciers to endoliths of rocks. *Rhodotorula mucilaginosa* can cause disease in mammals through skin and blood-borne infections. We

characterized solid media growth rates of 250+ strains from 18 species through at temperatures from 4-37 °C, on sole carbon sources glycerol and xylose, and high/low pH and salinity conditions. We developed PhenoScope to score colony growth rates from Petri plate images. The results establish a phenotyping dataset of growth rates, carotenoid production, and morphological traits. Using short-read sequencing, we assembled and annotated 300+ draft genomes of phenotyped isolates and additional samples from a diversity of environments. We compared these to examine the *Rhodotorula* pangenome, examine cryptic species and population structure within the most abundantly collected species *R. mucilaginosa*. These comparative analyses enable hypotheses generation of alleles for cold, hot, and saline environments. We also generated reference quality genomes from 9 named species of *Rhodotorula*, focusing on type strains, to support better study of the genus. We have used these reference and draft genomes to examine properties of gene synteny, mating type loci, and genome organization and content evolution in this ubiquitous and resilient yeast.

PS2.3 - Gene loss and horizontal gene transfer drive radical metabolic change in a floral yeast clade

P. Gonçalves[1]
[1]University of Lisbon, Lisbon, Portugal

Yeasts (Saccharomycotina) are excellent models for studying metabolic evolution due to their ease of cultivation, abundance of genomic data, and the suitability of many species for genetic modification.
Evolution of metabolism in yeasts seems to be dominated by processes associated with vertical gene inheritance, like gene duplication and loss (1). However, our work on metabolism remodelling in a yeast clade formed by the *Starmerella* and *Wickerhamiella* genera (W/S clade) showed that horizontal acquisition of genes from bacteria and from the Pezizomycotina played a pivotal role.
I will discuss the most exciting findings in this line of research, like the subversion of sugar preferences brought about by a transporter acquired from an Aspergillus related donor (2), the total remodelling of the alcoholic fermentation pathway resorting to bacterial genes (3,4) and the adaptation of a thiamine salvage pathway encoded by a bacterial pathway to the eukaryotic information flux environment (5). Finally, I will discuss factors in the molecular biology of W/S-clade species that may help explain why levels of horizontal gene transfer in this clade are an order of magnitude higher when compared with other yeast lineages.

1. Shen XX, et al. Cell.2018.175(6):1533-1545
2. Gonçalves C et al.MolBiolEvol.2016. 33:352-366.
3. Gonçalves C, et al. Elife.2018 .7:e33034.
4. Pontes A et al. BMCBiol.2024.22(1):128.
5. Gonçalves C and Gonçalves P. PNAS.2019. 116(44):22219-22228

PS2.4 - Genotype and phenotype diversity in Candida parapsilosis

G. Butler[1]
[1]University College Dublin, School of Biomolecular and Biomedical Science, Dublin, Ireland

Candida parapsilosis is the second most common cause of candidiasis in many countries, and epidemics associated with azole-resistant isolates have greatly increased since 2018. We collected and sequenced the genomes of >380 isolates of *C. parapsilosis* from Europe and from the US, and measured growth in >40 different conditions. In addition, we used homologous recombination and CRISPR-Cas9 editing to disrupt the function of >370 regulatory genes in the type strain, *C. parapsilosis* CLIB214. We identified several mechanisms associated with resistance to antifungal drugs, including amplification of the *ERG11* gene, of the *ERG11* promoter and of a drug transporter family. We also used variant and pangenome analysis to correlate genotype with specific phenotypes. We found that the most common variation resulted from expansion and contraction of gene families. In addition, we identified specific variants associated with nitrogen metabolism, which were confirmed by CRISPR-cas9 editing.

PS2.5 - Giant “Starship” transposons are a crucible of evolution

A. Vogan[1]
[1]University of Uppsala, Organismal Biology, Systematic Biology, Uppsala, Sweden

Horizontal gene transfer (HGT) disseminates genetic information between species and is a powerful mechanism of adaptation. Yet, we know little about its underlying drivers in eukaryotes. Giant Starship transposons have been implicated as agents of fungal HGT, providing an unprecedented opportunity to reveal the evolutionary parameters behind this process. These elements are unique in that they not only incorporate the genetic machinery for their own movement, but also mobilize a vast diversity of fungal genes. Through a combination of comparative genomic and molecular biology approaches, we have demonstrated that Starships are mobile within and between fungal genomes. We observe the recurrent transfer of Starships with adaptive cargo, such as genes for heavy metal resistance, between species including those in distinct taxonomic orders, showing how these elements frequently mediate rapid adaptation. Furthermore, we now have experimental validation that Starships can transfer between species under laboratory conditions. Our results demonstrate the key role Starships play in mediating HGT in fungi, elevating the importance of this process in eukaryotic biology.

PS3.1 - Evolution and ecological roles of isocyanides and their biosynthetic gene clusters

N. Keller[1]
[1]University of Wisconsin, Medical Microbiology and Immunology and Plant Pathology, Madison, United States

To the general public, fungal secondary metabolites (SMs) – if considered at all - are known due to accidental or purposeful ingestion (toxins and hallucinogens) or treatment of various infectious diseases (antibiotics). For practitioners in the science, we now understand that SMs are critical molecules steering the ecology and interactions of large numbers of fungi, particularly in specific Ascomycete and Basidiomycete taxa. A recently described new class of fungal natural products, the isocyanides encoded by isocyanide synthase (ICS) BGCs, present unique opportunities in SM research. Isocyanides are well known for their coordination chemistry with many transition metals, particularly copper and iron. Here we present unique contributions of fungal isocyanide natural products in mediating fungal success during copper starvation. Rather than involving import or export of copper, these isocyanides provide competitive fitness for the producing fungi against other microbes in low copper environments. The isocyanide xanthocillin chelates copper thus preventing other microbes from accessing copper ions and products of the isocyanide synthase CrmA – either only produced or increased in production during copper starvation – exhibit antimicrobial properties giving the fungus a survival advantage over other microorganisms. We present a bioinformatic view of the newly discovered isocyanide biosynthetic gene clusters in fungi, discuss a prospective of their role(s) in metal extreme environments and present our optimism of solving a fungal SM mystery affecting agriculture worldwide.

PS3.2 - Linking fungal RNA biology with development and metabolism

M. Feldbrugge[1]
[1]University of Dusseldorf, Dusseldorf, Germany

mRNA biology determines the precise timing and subcellular expression of encoded proteins. This spatiotemporal coordination is especially vital to coordinate development and metabolism in fungal microorganisms. Here, we study the developmental switch from yeast to hyphal growth in the plant pathogenic fungus *Ustilago maydis*. In this model organism, endosomal mRNA transport is essential to orchestrate cell growth, mitochondrial metabolism and polarity. Results on the underlying molecular mechanisms will be presented disclosing fundamental principles that are conserved from fungi to neurons.

PS3.3 - The role of transcription factors and chromatin modifiers in multicellular development in ascomycetes

M. Nowrousian[1]
[1]Ruhr-University Bochum, Department of Molecular and Cellular Botany, Bochum, Germany

The development of complex multicellular structures has evolved many times in eukaryotes, and at least twice in fungi. Fruiting bodies of filamentous ascomycetes are one example, and previous analysis of several ascomycetes has revealed drastic transcriptome changes during the transition

from early sexual stages to mature fruiting bodies. Our group focuses on the role of transcription factors and chromatin modifiers involved in this process. Molecular analyses of two genes, *asf1* and *pro44*, that are essential for fruiting body development in the model organism *Sordaria macrospora* will be presented in this talk. *asf1* encodes a histone chaperone, and its deletion leads to sterility, a reduction of DNA methylation, and upregulation of genes that are usually weakly expressed in the wild type. Mutations in *asf1* that result in loss of histone binding also prevent complementation of the sterile phenotype, but are still sufficient to overcome sensitivity to the DNA-damaging agent MMS indicating that histone binding of ASF1 is required for fruiting body formation, but not for genome stability. *pro44* encodes a transcription factor, and delta-pro44 strains are not only sterile, but produce fruiting bodies that are embedded in the agar, in contrast to the wild type that produces fruiting body at the agar-air interface. *pro44* is expressed throughout the mycelium, but most strongly in young fruiting bodies, where the protein is most abundant in the outer layers of the developing fruiting body. We are currently analyzing which regions of PRO44 are involved in maturation and the mislocalization of fruiting bodies.

PS3.4 - Panning for Gold in Mould Downunder: Can we increase the odds for fungal genome mining?

Y.H. Chooi[1]
[1]University of Western Australia, Perth, Australia

A paradigm that has emerged for microbial ecology and natural product discovery in the genomics era is that there are far more biosynthetic genes clusters (BGCs) that encode the production of these specialised metabolites than we previously assumed based on the chemical diversity we obtained from microbes growing in the laboratory. Heterologous biosynthetic pathway reconstruction has become a powerful approach to translate such cryptic BGCs to bioactive molecules. However, how can we increase the odds for fungal genome mining from the vast genomic information? Here, I will present several strategies that we used in our lab including discovery of cryptic virulence-related metabolites using host-pathogen interaction transcriptomics, taxonomic-guided discovery of novel metabolites and pathways from endemic Australian fungi, and our recent venture into resistance gene-guided genome mining. The latest which led to the discovery of the biosynthetic pathway for the first reported natural fatty acid synthase inhibitor, cerulenin, discovered over six decades ago. We discovered that the biosynthetic pathway to cerulenin starts with a C12 precursor synthesised by a polyketide synthase instead of via the fatty acid biosynthesis pathway. This precursor has both E and Z double bonds and undergoes amidation, followed by a series of epoxidations, double bond shifts, E/Z isomerization, and epoxide reduction. These findings highlight the potential of genome mining strategies to discover bioactive secondary metabolites from fungi, expanding our understanding of the relationships between BGC sequences, metabolite structures, and bioactivities.

PS3.5 - Microtubule-based organelle distribution inside a fungal hypha

X. Xiang[1]
[1]Uniformed Services University, Bethesda, United States

The fungal cytoskeleton is important for the distribution of organelles/vesicles, which support hyphal tip extension. Microtubules and the microtubule-motor proteins play critical roles in organelle distribution inside fungal hyphae. For example, the microtubule minus-end-directed cytoplasmic dynein motor is critical for the proper distribution of nuclei, early endosomes as well as other cargoes including those that hitchhike on early endosomes. Cytoplasmic dynein and its key regulator dynactin, which is a multi-protein complex, all accumulate at the dynamic microtubule plus ends near the hyphal tip, and this accumulation requires the plus-end-directed kinesin-1. In *Aspergillus nidulans*, defects in dynein or dynactin cause abnormal clustering of multiple nuclei inside the spore swelling and an abnormal accumulation of early endosomes at the hyphal tip near microtubule plus ends. This phenotype has facilitated mutant screens aimed at identifying cargo adapters as well as new players involved in dynein function. Recently, this genetic approach allowed us to reveal the functional importance of kinesin-1 autoinhibition in dynein-mediated organelle distribution, and it also led to the discovery of an important role of VezA/vezatin in assembly of the dynactin complex.

PS3.6 - Fungal diversity and adaptations highlight enzymatic systems for the retrieval of carbon from highly recalcitrant plant tissues

M.-N. Rosso[1]
[1]INRAE Institut national de recherche pour l'agriculture, l'alimentation et l'environnement, Paris, France

Among wood decomposers, Polyporales are basidiomycete fungi capable of degrading all lignocellulose polymers, via the secretion of oxidases, hydrolases and lyases that cleave polymers of lignin, cellulose, hemicellulose or pectin. Polyporales fungi thereby release the saccharides they use as nutrients, and increase the availability of organic carbon for uptake by other organisms. This single taxonomic order holds a variety of enzymatic arsenals to cope with the recalcitrance of wood to degradation.
Using phylogenomics, we have shown how gene family expansions or contractions have shaped the repertoires of lignocellulose degrading enzymes in the major taxonomic clades.
In a closer inspection of the Trametes clade, and using cross-species comparison of the early response of fungi to wood sawdust as a carbon source, we showed the conservation of a core set of enzymes and of yet overlooked genes that could contribute to the degradation of the lignocellulose polymers.
More recently, the analysis of the phenotype and genome polymorphism among strains from three Trametes species showed that strains of a same species have different capabilities to grow on recalcitrant lignocellulose, and that differences their repertoires of genes coding for lignocellulose degrading enzymes were not sufficient to explain this phenotype polymorphism.

These findings highlight that the abilities of Polyporales fungi to cope with recalcitrant carbon sources results from i) the adaptative evolution they went through during the last 183 million years, and ii) strain-specific gene regulation that calls for precaution when generalizing to a species the enzymatic capacities observed in individual strains.

CS 1.1.1 - *Candida albicans* strain diversity: Impact colonisation and host responses

K. Merga[1], *S. Chakraborti*[1], *I.D. Jacobsen*[1]
[1]Leibniz Institute for Natural Product Research and Infection Biology, Microbial Immunology, Jena, Germany

Systemic candidiasis in humans usually originates from strains that have long-term colonized mucosal surfaces – especially the gut – prior to the onset of infection. Likewise, mucosal candidiasis is caused by strains that colonize the oral or vaginal cavity, often for years without causing problems. While research has long focused on the pathogenesis of candidiasis, recent studies began to explore the consequences of colonization beyond infection risk.
It is becoming increasingly evident that *Candida albicans* colonization profoundly influences the host's immune system, including responses to subsequent candidiasis. It is also well established that *C. albicans* strains can differ significantly in their ability to colonize mucosal surfaces, virulence, and outcome of interaction with immune cells. However, if strain variability also impacts colonization-induced protection from systemic candidiasis is largely unknown.
We used strain 101 as a prototype "commensal" isolate characterized by low cytotoxicity and the ability to establish prolonged mucosal colonization, and compared it to the highly invasive thoroughly studied strain SC5314. *C. albicans* 101 colonized the murine gut at a higher level in the presence of intact microbiota, but was outcompeted by SC5314 in this setting. Despite higher intestinal fungal load, colonization with *C. albicans* 101 provided less protection against subsequent systemic challenge. We excluded a reduced Th17 response to 101 colonization as the underlying mechanism, but found differences in IgG induction and higher induction of tolerogenic cytokines as possible causes of reduced protection.
Thus, strain-to-strain variation affects colonization-induced immune responses, with consequences for the susceptibility to systemic candidiasis.

CS 1.1.2 - Beware the air?: Exploring indoor airborne fungal communities and urban chemical pollutants

S. Hemmings[1], *S. Fitzgerald*[1], *L. Barron*[1], *F. Kelly*[1], *M. Fisher*[1]
[1]Imperial College London, London, United Kingdom

In modern western societies, individuals spend up to 90% of their time indoors, yet while outdoor air quality has been extensively studied, the quality of indoor air—particularly the pollutants we inhale at home—has received less attention. Monitoring indoor air quality is essential, especially regarding exposure to mould, volatile organic compounds (VOCs), and particulate matter, all of which are linked to respiratory issues and infectious diseases. As part of the WellHomes project, this study analysed airborne fungal communities in 113 homes in North-West London through passive air sampling conducted between 2022 and 2024, with comparisons to outdoor environments. Amplicon sequencing revealed a significant prevalence of fungal genera such as

Penicillium and *Aspergillus* for indoor environments, alongside seasonal variations in fungal community profiles. Quantification of fungal burden using qPCR identified homes with elevated levels of specific fungal pathogens, which were linked to case studies of respiratory issues in occupants. Furthermore, significant correlations were observed between fungal community compositions and VOCs, suggesting potential interactions between biological and chemical pollutants. These findings highlight the need for comprehensive monitoring of indoor environments to better understand the combined effects of biological and chemical pollutants on air quality and public health.

CS 1.1.3 - Selective expression of Pneumocystis antigens during an outbreak

C. Meier[1], M. Pagni[2], S. Chevalley-Richard[1], K. Mühlethaler[3], P. Hauser[1]
[1]Institute of Microbiology, Lausanne, Switzerland, [2]Swiss Institute of Bioinformatics, Lausanne, Switzerland, [3]Institute of Infectious Diseases, Bern, Switzerland

The fungus *Pneumocystis* causes outbreaks of pneumonia among solid organ transplant recipients by interhuman transmission. We investigated the potential role of the surface antigenic variation system of *Pneumocystis* involving its major surface glycoproteins (*msg*). An outbreak including six renal transplant recipients was caused by a *Pneumocystis* genotype harbouring a specific repertoire of *msg* alleles. In contrast, the *msg* alleles of the repertoire that were expressed differed among the patients. This observation suggests that selective expression of surface antigens allowed evading each specific patient's immune response. This finding provides a rare indication of the function of the antigenic variation systems used by human pathogens. The outbreak was also driven by the previously described adaptation of *Pneumocystis* to transplant recipients through resistance to the immunosuppressant mycophenolate.

CS 1.1.4 - Collateral gains and fitness trade-offs of drug resistance in Nakaseomyces glabratus (Candida glabrata)

T. Gabaldón[1], J.C. Nunez-Rodriguez[1], M.-À. Schikora-Tamarit[1]
[1]Institute for Research in Biomedicine, Mechanisms of Disease, Barcelona, Spain

Invasive fungal diseases such as candidiasis, caused by *Candida* species, are a major public health problem. They are difficult to diagnose and have high mortality rates. Moreover therapeutic options are limited, and resistance to multiple antifungal drugs is increasingly reported, particularly in emerging species such as Nakaseomyces glabratus (*Candida glabrata)*. Over the last years we have used in vitro evolution, and comparative genomics and transcriptomics approaches to understand how N. glabratus can become resistant to the one or several drugs. We generated a comprehensive collection of drug and multidrug resistant strains representing a diverse set of mechanisms of resistance and conducted a comprehensive analysis of the stability of the resistance phenotypes and the possible trade-offs of drug adaptation. Our results show that the emergence of fitness trade-offs is a common feature of drug adaptation, but also indicates that it is highly heterogeneous, often comprising strain- and mechanism-specific

traits. Nevertheless, we identify some common trends and the underlying genetic drivers. Finally we provide a first proof of concept showing that trade-offs can be effectively actioned to limit the emergence of resistance in N. glabratus. Altogether, this study sheds light on the mechanisms underlying drug resistance-associated fitness trade-offs, outlines methods for their identification, and paves the way to develop innovative strategies to leverage these trade-offs in combating the growing challenge of antifungal drug resistance.

CS 1.1.5 - Experimental preclinical imaging-compatible animal models of mucormycosis

U. Binder[1], *A. Resendiz-Sharpe*[2], *J. Scheler*[1], *C. Kandlbauer*[1], *M. Navarro*[3], *F. Nicolas*[3], *I. Bauer*[1], *C. Lass-Flörl*[1], *V. Garre*[3], *G. Vande Felde*[2]
[1]Medical University Innsbruck, Innsbruck, Austria, [2]KU Leuven, Leuven, Belgium, [3]Universidad de Murcia, Murcia, Spain

Infections by mucormycetes are a serious threat in the clinical setting mainly due to their fast progression and limited treatment options.
We aimed to generate luciferase expressing Mucorales strains to be used for non-invasive monitoring of the infection in different animal models of mucormycosis – especially to establish multimodal, imaging-compatible preclinical mouse models - and as an alternative tool for *in vitro* drug testing.
Codon-optimized firefly luciferase without the peroxisomal target sequence, under the control of two different promoters was cloned into auxotrophic *M. lusitanicus* recipient strains. Positive transformants were checked for gene integration. Growth pattern and light emission under various conditions was determined by luminometer. Selected strains were used in Galleria infection assays and a neutropenic mouse model to determine infection by BLI imaging.
Firefly luciferase, with a single integration was successfully expressed in *M. lusitanicus.*, Light emission could be measured by luminometer and visualized in animal models. High light signal was obtained in infected *Galleria* larvae 48h after infection but decreased at 96h in those still alive. Similar results were obtained in mice and results correlated with the status of immunosuppression and weight loss. Overall, strains are usable for real-time, non-invasive infection monitoring and could also be used in the testing of antifungal efficacy by means other than survival.
The successful visualization of *M. lusitanicus* infection by a non-invasive method in insect and murine models, offers new ways to study mucormycosis and by extending this method to other species, will give valuable new insights in the pathogenesis of mucormycosis.

CS 1.1.6 - Fungi, flow, and resistance: what's lurking in Wales' waters?

P. Padmakumar[1], *R. Silvester*[1], *K. Farkas*[1], *D. L Jones*[1], *E. Jameson*[1]
[1]Bangor University, School of Environmental and Natural Sciences, Bangor, United Kingdom

Fungal infections are an emerging global health threat, exacerbated by increasing antifungal resistance (AFR). Wastewater systems and environmental waters may potentially serve as reservoirs for AFR fungi due to their prolonged exposure to human, industrial, and pharmaceutical waste, creating selection pressure for persistence and spread of AFR fungi. This study aimed to isolate and identify fungi from wastewater (hospital and municipal) and environmental reservoirs in North Wales, assessing their pathogenic potential and role in AFR dissemination. Fungal species were isolated using CHROMagar Candida Plus media, with species identification confirmed by Internal Transcribed Spacer (ITS) sequencing. Quantitative PCR (qPCR) and genotyping techniques are being utilized to detect AFR genes, while metagenomic sequencing aimed to identify novel strains. Preliminary results show distinct fungal distributions, with hospital wastewater samples exhibiting higher diversity and clinically relevant species such as *Candida albicans*, *C. parapsilosis*, *C. glabrata*, and *C. tropicalis*. Environmental waters exhibited lower diversity. Antifungal susceptibility testing is ongoing to confirm resistance patterns. Hospital wastewater is a critical reservoir of pathogenic fungi, with environmental waters potentially contributing to their spread. Effective monitoring is essential to prevent AFR dissemination, and rapid detection methods can enhance our response to fungal outbreaks, reducing associated public health risks. Wastewater-based epidemiology (WBE) offers a promising approach for tracking AFR and informing public health interventions by integrating environmental and public health strategies.

CS 1.2.1 - Beyond infection: exploring the ecology of human fungal pathogens

A. Pereira da Costa Filho[1], *H. Lin*[1], *G. Panagiotou*[2], *A.E Barber*[1]
[1]Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany, [2]Leibniz Institute for Natural Product Research and Infection Biology-Hans Knöll Institute, Department of Microbiome Dynamics, Jena, Germany

While a few human pathogenic fungi are commensal members of the human microflora, the majority of fungal species that infect humans live in the environment and have primary lifestyles independent of human habitats. Despite this, little is known about their ecology, including a comprehensive understanding of their geographic distribution, as well as the how their environmental interactions shape their virulence in humans. We are addressing this gap using both publicly available and *de novo* datasets. To define the global prevalence of involving human fungal pathogens in the environment, we assembled and analyzed amplicon microbial community data from >7,500 publicly available soil samples. We find that their abundance in the environment frequently often does not reflect their incidence in the clinic. For example, *Aspergillus fumigatus* has a high environmental and clinical prevalence, while *Fusarium* spp have an even higher environmental prevalence but cause relatively few infections. Modeling the environmental interactions of fungal pathogens with other bacteria and fungi reveals that human fungal pathogens each belong to unique interaction modules, suggesting that they each play distinct ecological roles. Because amplicon data cannot provide functional insight into microbial communities of human-pathogenic fungi, we are also establishing methods for their analysis using whole metagenome sequencing, including the development of a cell-based spike-in for absolute quantification of bacterial and fungal abundance in soil. This knowledge can be used to

better understand the link between environmental exposure and subsequent clinical infection and inform 'One Health' approaches to combat antifungal resistance.

CS 1.2.2 - A pangenome analysis of structural variants and transposable elements across *Candida albicans*

U. Oggenfuss[1], *R.T Todd*[2], *N. Soisangwan*[1], *B. Kemp*[1], *A. Guyer*[1], *A. Beach*[1], *A. Selmecki*[1]
[1]University of Minnesota, Department of Microbiology and Immunology, Minneapolis, United States, [2]Bard College, Biology, Annandale-on-Hudson, United States

The human fungal pathogen *Candida albicans* represents a major challenge on global health, largely due to its rapid evolution of antifungal drug resistance. Beyond point mutations, structural variants (SVs) are an important but understudied source of adaptation. To comprehensively investigate SVs across clinical isolates of *C. albicans*, we conducted long read sequencing and genome-wide SV analysis in distantly related clinical isolates. Our work included a new, comprehensive analysis of transposable element (TE) composition, location and diversity. Our findings reveal that SVs and TEs are distributed genome-wide, and are frequently located close to coding sequences. We found TE polymorphism between clinical isolates, including indication of recent TE activity. Most SVs are uniquely present in only one clinical isolate, and often are heterozygous. SVs and TEs thus likely impact gene and allele-specific expression, and represent a significant source of intra-species genetic variation. We identified multiple, distinct SVs and TEs at the centromeres of Chromosome 4 and Chromosome 5, including inversions and transposon polymorphisms. These two chromosomes are often aneuploid in drug resistant clinical isolates, and can form isochromosome structures with breakpoints near the centromere. Our work shows the importance of genome plasticity in *C. albicans* and identifies SVs and TEs as key contributors. The widespread heterozygosity of these variants further suggests that genomic rearrangements may drive phenotypic diversity, which in turn could facilitate adaptation to changing environments in the host and during antifungal therapy.

CS 1.2.3 - Genome-wide association studies reveal adaptation mechanisms in a major wheat pathogen

C. Lorrain[1], *S. Minana-Posada*[1], *A. Feurtey*[1], *J. Alassimone*[1], *B. McDonald*[1]
[1]ETH Zurich, Institute of Integrative Biology - Plant Pathology, Zurich, Switzerland

Human activity significantly influences the evolutionary dynamics of both crops and their pathogens, especially in agroecosystems. *Zymoseptoria tritici*, a major wheat pathogen, poses severe economic threats to wheat production worldwide. We utilize genome-wide association studies (GWAS) to uncover the genetic mechanisms behind *Z. tritici* adaptation to environmental and host factors. We identified candidate genes associated with thermal and host adaptation by integrating population sequencing, bioclimatic, and host genotype metadata. We conducted a high-throughput phenotyping approach, assessed the thermal response of over 400 *Z. tritici* strains at multiple growth temperatures, and used GWAS to identify candidate genes associated with climatic adaptation[1].

Additionally, we applied a genome-host association (GHA) approach, correlating 800 pathogen genotypes with natural infection phenotypes across various wheat cultivars[2]. These approaches identified multiple gene candidates, 20 of which were selected for functional validation, revealing that over half of the validated genes significantly affect the virulence of *Z. tritici* when deleted. Our study provides a novel application of GWAS in plant pathogens that transcends the limitations imposed by traditional phenotyping methods. By enhancing GWAS with environmental and host metadata, we gain insights into the evolutionary mechanisms driving pathogen adaptation.

1. Miñana-Posada, S., Lorrain, C., McDonald, B. A. & Feurtey, A. Thermal adaptation in worldwide collections of a major fungal pathogen. *bioRxiv* 2024.09.12.612681 (2024) doi:10.1101/2024.09.12.612681.
2. Lorrain, C., Feurtey, A., Alassimone, J. & Mcdonald, B. A novel genome-wide association approach reveals wheat pathogen genes involved in host specialization. (2024) doi:10.21203/RS.3.RS-4486034/V1.

CS 1.2.4 - Investigating the host specificity of Stemphylium vesicarium on pear through genome and transcriptome analyses

X. Chen[1], F. Negrini[1], M. Karas[1], M. Menghini[1], A. Menicucci[1], C. Fiorenzani[1], E.G. Kabeto[1], M. Collina[1], R. Baroncelli[1,2], E. Baraldi[1]
[1]University of Bologna, Department of Agricultural and Food Sciences (DISTAL), bologna, Italy, [2]University of Napoles Federico II, Center for Studies on Bioinspired Agro-Enviroental Technology, Portici, Italy

The brown spot of pears, caused by the pathogenic fungus *Stemphylium vesicarium*(*Sv*), poses a serious threat to European pear (*Pyrus communis*) production, leading to substantial yield reductions and severe economic losses. This disease has significantly impacted on Italy, traditionally a leader country for European pear production. From 2012 to 2018, Italy averaged 720k tons of pears annually, but production has sharply declined to 250k tons in 2023. A major cause underneath this dramatic decline is the spread of *Sv* in pear producing regions. This pathogen exhibits a strong host specificity at the strain level, with only specific *Sv* strains capable to infect pear, suggesting a high genetic adaptation at the strain level. To highlight key virulence genes responsible for *Sv* infection in pear we made an extensive comparative genomic analysis on 22 Sv from different hosts, including pear, onion, asparagus, hazelnut and apple. We identified 17 specific genetic elements within strains of pear pathogenic *Sv*, possibly enabling the pathogen to infect pears. The functions of these genes involve toxin transport, glycosylation, sugar isomerase, fungal-specific transcription factors, fatty acids, TPR, catalytic activity, oxidoreductase activity, and serine carboxypeptidase. In parallel we undertook RNA-seq analysis on pear leaves infected by *Sv* at early stage of interaction to highlight both host and pathogen genes expressed during interaction. Our result provides important information to develop new control strategies effective against this pathogen.

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

CS 1.2.5 - How safe is *Trichoderma*? AI-driven genotype-phenotype insights reveal environmental adaptations and propose a biosecurity framework for agricultural applications

A. Steindorff[1], *F.M. Cai*[2], *E. Shelest*[3], *J. TrichoCosm*[4], *R.P. de Vries*[5], *I.V. Grigoriev*[1], *I.S. Druzhinina*[6]
[1]Joined Genome Institute, DOE, Berkeley, United States, [2]Sun Yat-sen University, Ecology School, Shenzhen, China, [3]University of Portsmouth, Portsmouth, United Kingdom, [4]The Community Sequencing Project for Whole-Genus Trichoderma Genomics, Berkeley, United States, [5]Westerdijk Institute of Fungal Biodiversity, Utrecht, Netherlands, [6]Royal Botanic Gardens Kew, Accelerated Taxonomy, Richmond, United Kingdom

Mycoparasitic fungi from the genus *Trichoderma* (Hypocreales, Ascomycota) are widely recognized for their ability to support sustainable agriculture by suppressing soil-borne pathogens and promoting plant immunity, stress resilience, and growth. However, the rising occurrence of pathogenicity in plants, humans, and cultivated mushrooms calls for comprehensive risk assessment related to *Trichoderma*-based products. This study utilized machine learning to analyze genomic data from 37 *Trichoderma* strains representing diverse ecological backgrounds, mapping them against over 150 phenotypic traits. Results position *Trichoderma* as an ancient, genetically distinct genus putatively predominantly associated with microbial biofilms in the phyllosphere of tropical rainforests or other arboreal habitats. The genomic data highlighted the roles of specific regulatory proteins, enzymes, transport proteins, and small secreted proteins in facilitating environmental adaptability, resilience, and ecological fitness. Several strains, particularly those used as agricultural bioeffectors, such as *T. afroharzianum* T22, strains of *T. asperellum*, and *T. atroviride*, exhibited outstandingly high environmental opportunism, raising biosecurity concerns. Ecophysiological traits also indicated drastic phenotypic variation among closely related strains, suggesting ongoing evolutionary processes and the lack of cryptic species if physiology is addressed. Despite these risks, the study shows that the extensive genetic diversity within the genus permits the selection of bioeffector strains that are both effective and safe. These findings emphasize the need for science-based safety guidelines to support the responsible use of *Trichoderma* in agriculture, balancing the benefits of improved crop health and sustainability with the mitigation of potential risks.

CS 1.2.6 - Machine learning classifiers reveal the evolutionary drivers of virulence and drug resistance in the fungal pathogen Candida parapsilosis

M.À. Schikora Tamarit[1,2], *Ó. Zaragoza*[3,4], *T. Gabaldón*[1,2]
[1]Institute for Research in Biomedicine (IRB Barcelona), Barcelona, Spain, [2]Barcelona Supercomputing Centre (BSC-CNS), Barcelona, Spain, [3]Mycology Reference Laboratory, National Centre for Microbiology, Instituto de Salud Carlos III, Madrid, Spain, [4]Center for Biomedical Research in Network in Infectious Diseases (CIBERINFEC-CB21/13/00105), Madrid, Spain

Fungal pathogens pose a serious health threat, causing ~4 million yearly deaths, requiring improved therapeutic and diagnostic tools. Candida species are major contributors to hospital-acquired infections, particularly among immunocompromised patients. Given the high dynamism of Candida genomes, a promising strategy to improve current therapies and diagnostics is to elucidate the evolutionary mechanisms generating virulence and antifungal drug resistance. These remain obscure because previous studies had small sample sizes, lacked rigorous statistical analyses, focused only on expected genes and/or ignored the role of genetic interactions.
Here, we investigated such evolutionary processes in Candida parapsilosis, an emergent pathogen frequently causing nosocomial outbreaks. We sequenced the genomes and measured several phenotypes (e.g. drug susceptibility, invasiveness) for hundreds of clinical isolates from six Spanish cities. This large dataset enabled unprecedented statistical power to study drug resistance and virulence. For this, we first performed a convergence genome-wide association study to pinpoint variants (including structural variants) underlying these traits. Additionally, we used explainable machine learning classifiers (e.g. based on random forests) to predict each phenotype from genetic variants.
Our analyses revealed hundreds of potential genetic drivers of virulence and resistance, confirming expected evolutionary mechanisms, and suggesting novel ones. We were able to build highly accurate classifiers for some phenotypes. For instance, we can predict changes in fluconazole resistance, the mainly used drug for Candida parapsilosis, with high confidence. Our results provide insights on the emergence of clinically-relevant traits in a major fungal pathogen, and set the foundations for sequence-based characterization of antifungal susceptibility in the clinics.

CS 1.2.7 - Clade-wide exploration of small RNA loci and machinery shows distinct RNAi systems across fungi

N. Johnson[1], *L. Melet*[1], *E. Vidal*[1], *F. Gonzalez*[1]
[1]Universidad Mayor, Center of Genomics and Bioinformatics, Santiago, Chile

Small RNAs (sRNAs) are the functional element of this RNA interference and are important in wide-ranging processes, including gene-regulation, defense, and genomic integrity. This process is ancient and common throughout eukaryotic organisms, with many of the fundamental processes first identified in fungi. Much of the genomics of sRNAs in fungi are still undefined, compared to plants and animals where we have rigid definitions of several gene classes and where they are found in the genome. Here, we use public sRNA-seq data from over 200 projects in 86 species to build a comprehensive understanding of sRNA-expressing loci in fungi. Using our high-precision annotation pipeline for fungal sRNAs, we can discretely define loci in these organisms. We find a uniquely small number compared to other eukaryotes, even when accounting for genome size and that fungi produce sRNAs in a unique spectrum of sizes, dependent on the organism. A comprehensive search of fungal Dicers shows that these proteins vary greatly from other eukaryotes particularly in the PAZ domain, a possible explanation for this variation. Considering the evolutionary retention of this process, this points important roles for these few loci. We identify some miRNAs similar to other eukaryotes, while many differ hinting to more complexity in fungi. These also show some signs of inter-species conservation of loci.

Overall, this process is building a comprehensive understanding of what this ancient and important process looks like in fungi, enhancing our ability to explore its biological functions.

CS 1.2.8 - A comparative analysis of transposable elements in the subphylum Pucciniomycotina reveals a massive retrotransposon-driven expansion in the genomes of rust fungi

E. CORRE[1], *E. Morin*[1], *S. Duplessis*[1], *C. Lorrain*[2]
[1]INRAE, UMR 1136, Champenoux, France, [2]ETH-Institute of Integrative Biology, Plant Pathology Group, Zurich, Switzerland

Transposable elements (TEs) play a crucial role in genome evolution, influencing gene regulation, diversity, and genome architecture. Rust fungi (Pucciniales) are the largest group of obligate biotrophic plant pathogens and harbor some of the largest and most TE-rich genomes compared to other fungi. This genomic expansion contrasts with the smaller genomes and minimal mobilome found in other Pucciniomycotina species. Despite the availability of high-quality genome assemblies, our understanding of TE dynamics in Pucciniales remains limited due to inconsistent and incomplete TE annotations.
This study investigates three evolutionary models to explain TE proliferation in Pucciniales: (i) an ancestral TE burst in Pucciniomycotina, lost in other species but retained in Pucciniales; (ii) recent, species-specific TE bursts; or (iii) a combination of ancestral and species-specific expansions. We analyzed the mobilomes of 13 Pucciniomycotina species, exploring TE diversity, evolutionary history, and impact on genome architecture. We found no Pucciniales-specific deficiency in known TE control mechanisms that could explain the TE proliferation in Pucciniales compared to the other Pucciniomycotina. Molecular dating of the most abundant TEs, the Gypsy retrotransposons, revealed that ancient elements have been retained since the early evolution of Pucciniales. The significant number of TE families that are shared at the genus level also support the third hypothesis of TE proliferation in Pucciniales as an ancestral genomic trait, from which species-specific waves of TE expansion have independently occurred over time. This study highlights the profound influence of TE activity on rust genome architecture and provides valuable insights into the evolutionary history of Pucciniales.

CS 1.2.9 - Exploring mucoromycota and endosymbiont diversity and biology using whole genome based life identification numbers

H. Johnson[1], *K. Belay*[2], *R. Mazloom*[2], *L. Heath*[2], *B. Vinatzer*[2], *D. Haak*[2], *N. Grunwald*[1], *J. Uehling*[1]
[1]Oregon State University, Corvallis, United States, [2]Virginia Tech, Blacksburg, United States

Advancements in high-throughput sequencing technology have increased the availability of fungal whole genome sequences (WGSs); however, the ever-increasing number and length of sequences in DNA databases represents a computational challenge. Additionally, only a fraction of kingdom fungi has been described and new taxa often disrupt current naming conventions. To meet these challenges, we propose adapting the life identification number (LIN) concept, a

concept designed for prokaryotes that assigns labels known as LINs to individual genomes reflecting genomic similarity, for the analysis of fungi. The LIN workflow combines efficient k-mer and hashing algorithms for initial database-wide pairwise comparisons followed by a single average nucleotide identity computation which, in conjunction with the LIN concept, permits rapid, sensitive, and scalable WGS-based identification. This research focuses on the use of LINs to understand taxonomic relationships and elucidate biology of the Mucoromyota as well as the diversity and evolution of their endohyphal bacteria, and taxonomy determined via the LIN system will be compared to orthologous gene-based phylogeny. We expect LIN-based classifications will resolve Mucoromycota from the genus to intraspecific variants including variation in accessory or repetitive chromosomes. Further, we expect LIN-based endosymbiont phylogeny and its comparison to free-living relative and host Mucoromycota phylogenies will reveal insights into the evolutionary history of this symbiosis. We anticipate this approach of LIN-based Mucoromycota identification will be extensible to other fungal phyla, permitting strain level identification of isolates with no need for conventional Linnean classification and allowing rapid inference of phenotypes like fungicide resistance and virulence of pathogenic fungi.

CS 1.2.10 - Transposable elements on the move: horizontal transfer of transposable elements in fungi

J. Romeijn[1], *I. Bañales Belaunde*[1], *M.F. Seidl*[1]
[1]Utrecht University, Biology, Utrecht, Netherlands

Transposable elements (TEs) are mobile DNA sequences that are found in almost all living organisms. TEs can transpose within their host, shaping host genomes and functions. Recent findings suggest that TEs are frequently horizontally transferred between species, including fungi. Our preliminary data in a set of over 1,000 publicly available fungal genomes from diverse taxa revealed significant variation in TE composition and genome size (between 2.2Mb and 1.2Gb), which is strongly correlated with TE content. Since we did not observe a strong phylogenetic signal underlying these patterns, we interpret these as signals of frequent horizontal transfer of TEs, suggesting that transferred TEs shape fungal genomes and their evolution.

Here, we aim to systematically detect horizontal transfers of TEs using a comprehensive bioinformatics approach which yielded 16 million TEs in total, with 2 million encoding TE-related protein domains. Among these, approximately 680,000 TE pairs between species exhibit higher sequence similarity than expected for vertical inheritance and are therefore likely horizontally transferred. From these TE pairs, we are currently in the process to detect unique and independent horizontal transfer events and to uncover how these contribute to TE expansions. Moreover, we will elucidate the prominent TE types or fungal species involved in horizontal transfer events.

We anticipate that our efforts will contribute to a better understanding of the mechanisms driving horizontal transfer of TEs and TE expansions, and the role of horizontal transfer of TEs in fungal genome evolution.

CS 1.2.11 - Integration of dual multi-omics data reveals important pathways involved in the host-pathogen interactions of *Candida albicans* in context of the vaginal microenvironment

K. Rahate[1], *T. Madan*[2], *S. Thomas*[1]
[1]ICMR-National Institute for Research in Reproductive and Child Health, Biomedical Informatics Centre, Mumbai, India, [2]Indian Council of Medical Research, Development Research, New Delhi, India

Candida spp. are common commensals that colonize the mucosal surfaces of humans and possess the ability to transform into aggressive pathogens in conditions that disturb the host functions. Infections by *Candida* species such as *C. albicans, C. glabrata, C. krusei, and C. auris* are widespread, yet have limited treatment options in the form of safe and effective antifungal agents. The increase in resistance of these species to standard antifungal drugs makes *Candida* infections more challenging to manage. This necessitates the discovery and development of novel antifungal targets and drugs, for which an improved understanding of the intricate biology of *Candida* spp. in the context of host-pathogen interactions is crucial.
This study uses an *in vitro* system wherein the reciprocal effect of the human vaginal epithelial cell line A-431 and *C. albicans* growing in co-culture was studied using a multi-omics approach. Untargeted RNA-sequencing and LC-MS/MS techniques were used to generate the transcriptomic, proteomic and metabolomic profiles of *C. albicans* and the host cell line A-431, post 3 hours and 6 hours of co-culture, in conditions mimicking the physicochemical properties of the human vaginal niche. This provides a global overview of their interactions and helps connect the host and pathogen at different biological levels. Several metabolic and signalling pathways of both, the host as well as the pathogen, were identified to be enriched during co-culture compared to their corresponding monocultures, indicating their importance in host-pathogen interactions.

CS 1.2.12 - Coordinated adaptation of gene families has shaped the genome of dimorphic fungi

G. Tahiri Zainane[1], *C. Lax*[1], *T. Gabaldón*[2], *V. Garre*[1], *F.E. Nicolás Molina*[1]
[1]University of Murcia, Genetics and Microbiology, Murcia, Spain, [2]Barcelona Supercomputing Center, Life Sciences - Comparative Genomics, Barcelona, Spain

Dimorphism is a critical virulence factor in fungi, enabling pathogens to transition between yeast and the invasive mycelium form, which helps them evade the host immune response. Through comprehensive transcriptomic analysis of the yeast and mycelial forms of the dimorphic fungus *Mucor lusitanicus*, we identified that approximately 30% of its protein families are dimorphic, characterized by paralog genes that are expressed in yeast and those specifically expressed in mycelium. These dimorphic families are randomly distributed along genome and are involved in multiple biological processes.
Notably, a thorough genomic analysis revealed that *M. lusitanicus* utilizes head-to-head (H2H) genes to coordinately regulate genes from dimorphic families associated with related functions.

These conserved genetic structures across the Mucorales control around 78% of the H2H genes co-expressed within the same morphology. This indicates that these genetic structures could serve as predictors of the morphology in which a particular paralog is expressed, as orthologs expressed in mycelium cluster together in phylogenetic analyses.
Moreover, comparative genomic analysis between dimorphic and non-dimorphic fungi showed that dimorphic fungi have an expanded set of genes within dimorphic families, whereas non-dimorphic fungi possess an expanded array of genes predominantly expressed in the mycelial form.
Altogether, the integration of transcriptomic, genomic, and phylogenetic analyses enables the development of a predictive model to distinguish between dimorphic and non-dimorphic fungi. This model enhances our understanding of their evolutionary mechanisms and potential strategies for evading the immune response.

CS 1.3.1 - Delineating the evolution of antifungal resistance evolvability in Candida auris

D. Santana[1], *T. Peters*[1], *P. Ma*[1], *P.D. Rogers*[1]
[1]St. Jude Children's Research Hospital, Pharmacy and Pharmaceutical Sciences, Memphis, United States

The evolution of antimicrobial resistance is a critical global health threat. *Candida auris* exhibits markedly high rates of acquired resistance compared to related species, contributing to its public health burden. Here, we exploit *C. auris* as a model to understand the evolution of evolvability by exploring the biology that has allowed it to establish such high rates of resistance. First, we observed widespread variability in the rates at which different lineages of *C. auris* acquire resistance mutations. Our findings suggest resistant lineages were more predisposed to develop resistance mutations than susceptible lineages. To explore the mechanisms driving this variation, we have developed single-cell genetic approaches to allow tracking resistance emergence in subpopulations under selection. Second, we asked how resistance mutations are stably maintained. A common resistance mechanism - overexpression of the efflux pump Cdr1 – carries no discernable fitness defect. However, its overexpression stimulates a substantial transcriptional response, suggesting an essential physiological compensation. We have also identified small molecules that selectively inhibit only cells overexpressing Cdr1, further highlighting a hidden cost of this resistance mechanism. Together, these data provide key insights into the mechanisms by which *C. auris* establishes and maintains resistance.

CS 1.3.2 - Battling drug resistance in *Cryptococcus:* using microevolution to understand drug resistance development in a susceptible strain

D. Tamayo[1], *H. Gifford*[1], *R. Anand*[1], *Q. Ma*[1], *N. Helmstetter*[1], *D.M Engelthaler*[2], *M.C Fisher*[3], *R.C May*[4], *R.A Farrer*[1]
[1]University of Exeter, Medical Research Council Centre for Medical Mycology, Exeter, United Kingdom, [2]Translational Genomics Research Institute, Flagstaff, Arizona, United States, [3]Imperial College, Department of Infectious Disease Epidemiology, London, United Kingdom, [4]University of Birmingham, Institute of Microbiology and Infection, Birmingham, United Kingdom

Cryptococcosis causes approximately 181,000 deaths annually, with the majority occurring in LMIC, mainly in sub-Saharan Africa, where a lack of access to standard antifungals (amphotericin and flucytosine) limits their use. Even with the appropriate and recommended treatments, *Cryptococcus* can readily evolve resistance (*e.g.*, fluconazole) and has inherent resistance to some antifungals (*e.g.*, echinocandins), contributing to mortality. It is largely unknown how *Cryptococcus* acquires or develops antifungal resistance. To understand this, we micro-evolved a susceptible strain of *C. gattii* (CgWM1243) in the presence of sub-MIC concentrations of amphotericin and fluconazole. Our microevolution experiment was coupled with whole-genome sequencing, together with phenotypic analyses (broth microdilution, disc-diffusion, spot assays, lipid, DNA and chitin content by flow cytometry) to obtain a global view of the adaptive mutations and phenotypic changes occurring in the cells to adapt to the antifungals. We discovered that the cells micro-evolved in fluconazole and amphotericin grew at higher MIC than the established breakpoint for *Cryptococcus* (>320mg/L for fluconazole micro-evolved strain and >3.2mg/L for amphotericin micro-evolved strain). Amphotericin micro-evolved cells had increased DNA (>4C) and chitin content (MFI 4088 (parental) vs 22003 (micro-evolved)), together with an increase in the population with low order lipids (0.23% (parental) vs 9.81% (micro-evolved)). Collateral-sensitivity to azoles was also observed in the cells micro-evolved in amphotericin, which represents a potential mechanism by which combination therapy is able to more successfully treat resistant strains. Our experiments reveal new genetic and cellular traits involved in drug resistance that may help guide new therapeutic strategies to control cryptococcosis.

CS 1.3.3 - Population genomics of the pathogenic fungus *Aspergillus fumigatus* in Japan

H. Takahashi[1], *X. He*[2], *Y. Kusuya*[3], *D. Hagiwara*[4], *T. Toyotome*[5], *T. Arai*[1], *C. Bian*[6], *M. Nagayama*[1], *S. Shibata*[1], *A. Watanabe*[1]
[1]Chiba University, Medical Mycology Research Center, Chiba, Japan, [2]Chiba University, Graduate School of Medical and Pharmaceutical Sciences, Chiba, Japan, [3]National Institute of Technology and Evaluation, Kisarazu, Japan, [4]University of Tsukuba, Tsukuba, Japan, [5]Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan, [6]BGI-Shenzhen, Shenzhen, China

Aspergillus fumigatus is a pathogenic fungus with a global distribution. Although azole-resistant TR-mutants (TR34 and TR46) are widely distributed, a few TR-mutants have been isolated in Japan. The emergence of azole-resistant *A. fumigatus* (AR*Af*) other than the TR-mutants is a problem in Japan. Additionally, the genetic diversity of *A. fumigatus*strains in Japan remains relatively unknown. In this study, we analyzed the genome sequences of 171 strains from Japan as well as the antifungal susceptibility of these strains. We found that 22 strains were highly tolerant to itraconazole. Next, we conducted a population analysis of 876 strains by combining the available genomic data for strains isolated worldwide, which were grouped in six clusters. We observed the geographic characteristics of clusters such as Cluster 2 where the strains from Japan were over-represented. Finally, a genome-wide association study identified the genomic loci associated with AR*Af* strains, but not the TR-mutants. Furthermore, we revealed pan-genome in all clusters. These results highlight the complexity of the genomic mechanism underlying the emergence of AR*Af* strains other than the TR-mutants.

CS 1.3.4 - Reversing antifungal resistance through protein disruption

M. Woods[1], K. Woolnough[1], S. Sieber[2], A. Hamilton-Wright[1], J. Geddes-McAlister[1]
[1]University of Guelph, Guelph, Canada, [2]Technical University of Munich, Munich, Germany

Fungal disease impacts the lives of almost a billion people across the globe. The opportunistic human fungal pathogen, *Cryptococcus neoformans*, causes cryptococcal meningitis in immunocompromised individuals with high fatality rates in response to limited treatment options. Moreover, the emergence of azole-resistant isolates in the clinic following prolonged treatment regimes, environmental fungicide exposure, and fungal evolution, threatens the outcome of current therapeutic options, endangering the survival of infected individuals. By quantitatively characterizing the proteomes of fluconazole-susceptible and -resistant *C. neoformans* strains using state-of-the-art tandem mass spectrometry, we defined ClpX, an ATP-dependent unfoldase, as a target to overcome resistance. We discovered that disruption of ClpX through deletion or inhibition re-introduces fluconazole susceptibility into the resistant strains, rendering treatment effective once again. We further explored the mechanism of resistance and determined interruption to heme biosynthesis and ergosterol production associated with ClpX. Finally, we leverage computational strategies to optimize inhibitor design towards ClpX for specific and effective therapeutic design. Our results contribute to the understanding of novel mechanisms driving fluconazole resistance and provide support for targeting proteins as a therapeutic strategy to combat resistance.

CS 1.3.5 - Nation-wide air sampling reveals land use is linked to voriconazole resistance and TR_{46} prevalence in *Aspergillus fumigatus* in the Netherlands

B. Briggeman[1,2], H. Kortenbosch[1], F. Reyes-Marquez[1], B. Zwaan[1], E. Snelders[1]
[1]Wageningen University, Laboratory of Genetics, Wageningen, Netherlands, [2]National Institute for Public Health and the Environment, Centre for Infectious Disease Research, Diagnostics and Laboratory Surveillance, Bilthoven, Netherlands

Cross-resistance between agricultural and medical triazoles burdens treatment of opportunistic fungal pathogen *Aspergillus fumigatus*. Medical triazoles are the first-line treatment for *A. fumigatus* infections; however, mutations in the *cyp51A* gene confer cross-resistance. Environmentally selected triazole resistance is characterized by a tandem repeat (TR) in the promotor region of this gene with non-synonymous point mutations in the coding region.
In the fall of 2023, Citizen Science project Fungal Radar (Schimmelradar) sent out 500 air sampling kits divided over 100 equal-sized regions in The Netherlands. The seals were selectively cultured, and per location a control, voriconazole (VOR) and itraconazole (ITR) growth treatment was applied. VOR and ITR resistance fractions and the TR haplotypes were determined and linked to publicly available land-use data via optimized generalized linear models.
The median resistance fraction for ITR (3.9%, n= 1,346) and VOR (2.2%, n= 914) was calculated (total n = 98,064). From colonies resistant to VOR or ITR, the TR_{34} (46.5%) and TR_{46} (43.0%) were most prevalent. Yet, VOR resistance has a more pronounced link to flower bulb cultivation, potato cultivation, and greenhouse horticulture than ITR resistance. Also, the TR_{46} haplotype is spatially heterogeneous, and increased TR_{46} haplotype frequency is associated with local land use, most notably flower bulb cultivation and horticulture. The spread of the TR_{34} haplotype had no land-use association and was more spatially homogeneous. This study demonstrates the differences in relative exposure to triazole-resistant *A. fumigatus* (ranging between 0-20% resistance) and notable differences in exposure to the two most common TR haplotypes.

CS 1.3.6 - Investigation of a novel azole-resistance mechanism in *Aspergillus fumigatus*

M. Uchida[1], *S. Shibata*[1], *T. Arazoe*[2], *T. Kamakura*[2], *H. Takahashi*[1,3]
[1]Chiba University, Medical Mycology Research Center, Chiba, Japan, [2]Tokyo University of Science, Department of Applied Biological Science, Noda, Japan, [3]Chiba University, Molecular Chirality Research Center, Chiba, Japan

Aspergillus fumigatus is a pathogenic filamentous fungus that causes aspergillosis, with an estimated 600,000 deaths annually. Azoles are the most commonly used antifungals for treating this fungus, but azole resistance has been recognised in many clinical isolates, making treatment more difficult. A typical resistance mechanism is mutations on the coding sequence or promoter region of *Cyp51*, which codes the targeted enzyme involved in ergosterol biosynthesis. In addition, it has also been reported that mutations in the HMG-CoA reductase *Hmg1* could lead to azole resistance. On the other hand, many azole-resistant isolates have been isolated in which there is no mutation in these genes, or the resistance is so high that it cannot be explained by these genes alone, suggesting other unknown mechanisms.
This study aims to identify a novel mechanism of azole resistance using genomic recombination by mating. Crossings between azole-susceptible and -resistant isolates (without known mutation) with all combinations resulted in a successful acquisition of progenies between one susceptible (S) and two resistant (R-1 and R-2) isolates, with over 160 F_1 progeny each. The comparative genomic analysis of five susceptible and five resistant progenies from the S×R-1 identified a single genomic region of 24 kb, predicted to be responsible for the resistance. This region contained 17 genes, of which 6 had single amino acid substitutions, and 2 had frameshift

mutations. Disruptants and point mutants of these genes are being generated to verify their association with azole resistance.

CS 1.3.7 - Multi-drug resistance in the fungal wheat pathogen *Zymoseptoria tritici*: a hint to complex mechanisms

S. Patry-Leclaire[1], *E. Neau*[2,1], *A. Laleve*[1], *A.-S. Walker*[1], *S. Fillinger*[1]
[1]INRAE, BIOGER, Palaiseau, France, [2]University Paris-Saclay, Gif-sur-Yvette, France

The agricultural sector faces increasing development of fungicide resistance in *Zymoseptoria tritici*, the agent of septoria leaf blotch in wheat. Since the early 2010's, strains with 'Multi-Drug Resistance' (MDR) phenotype have been detected in natural populations. This phenotype is associated with the overexpression of the membrane efflux pump gene *MFS1* due to three distinct promoter inserts, resulting in increased efflux of fungicides. A *Z. tritici* population survey for MDR strains in 2020/21 led to the isolation of new MDR strains whose resistance profiles differ from previous field isolates and transgenic constructs indicating that additional mechanisms to *MFS1* promoter inserts may be at play.
Experimental evolution studies have also led to the selection of MDR strains unlinked to *MFS1* overexpression. To elucidate whether increased efflux is involved in these phenotypes, efflux tests were carried out on a selection of MDR isolates. The majority of the studied isolates exhibited an increase in comparison to the ancestor strain suggesting that their MDR phenotype may be attributed to increased fungicide efflux. Other isolates showed different phenotypes, indicating the involvement of alternative MDR mechanisms. Whole-genome sequencing revealed unknown genes potentially involved MDR. The involvement of a previously uncharacterized transcription factor in MDR is currently under investigation.

CS 1.3.8 - Effects of synonymous and nonsynonymous Cyp51 mutations on DMI resistance in *Cercospora beticola*

M. Bolton[1], *I. Courneya*[1], *N. Wyatt*[1], *G. Secor*[2]
[1]U.S. Dept. Agriculture, Fargo, United States, [2]North Dakota State University, Plant Pathology, Fargo, United States

Cercospora leaf spot (CLS), caused by the fungal pathogen *Cercospora beticola*, is the most economically important disease of sugarbeet worldwide. Demethylation inhibitors (DMIs) are a class of fungicide that target Cyp51, a key enzyme in the synthesis of the essential fungal cell membrane component ergosterol. While DMIs are important for managing CLS, resistance to these fungicides has been observed. Previous work in our lab has shown that resistance to DMIs is highly correlated with synonymous and non-synonymous mutations in the Cyp51 gene. One such mutation, L144F, is found in two codon variants TTC and TTT, where TTC is associated with resistance and TTT is associated with sensitivity even though both codons encode phenylalanine. Notably, resistance is also strongly associated with the synonymous mutation E170. We have identified five Cyp51 haplotypes exhibiting different combinations of these mutations. To improve our understanding of the effects of silent mutations on DMI resistance, we

have undertaken a variety of studies to characterize the role of this gene in mediating DMI resistance. The results and potential implications of these studies will be presented.

CS 1.3.9 - Characterisation of the antifungal effects of manogepix on the human-pathogenic mould *Aspergillus fumigatus*

S. Brazil[1], *I. Fouquet*[1], *J. Wagener*[1]
[1]Trinity College Dublin, Clinical Microbiology, Dublin, Ireland

Manogepix is a new antifungal and has been reported to have a fungistatic activity against the opportunistic pathogenic mould *Aspergillus fumigatus*. Manogepix targets Gwt1, an enzyme required to form glycosylphosphatidylinositol (GPI)-anchored proteins in the endoplasmic reticulum. GPI-anchored proteins are localized to the cell wall and play roles in maintaining cell wall strength. We show that manogepix triggers activation of the cell wall integrity (CWI) signalling pathway of *A. fumigatus*, which results in upregulation of cell wall chitin biosynthesis. Deletion of genes that encode key components of CWI pathway typically results in an increased susceptibility to antifungal agents that target the cell wall (e.g., echinocandin antifungals, calcofluor white). Surprisingly, some of these CWI deletion mutants grow better in the presence manogepix when compared to wild type. In agreement with this, overexpression of a key player of *A. fumigatus'* CWI pathway results in reduced growth in the presence of manogepix. Our results support a model where manogepix triggers activation of the CWI signaling pathway, which contributes to the fungistatic effect of manogepix. Furthermore, our data reveal an alternate stress signaling pathway triggered by manogepix which alters the susceptibility of *A. fumigatus* to other antifungal agents.

CS 1.3.10 - Understanding of primary resistance to echinocandin in *Mucor*

A. Rendon[1], *A. Garcia*[1], *E.Y. Huh*[2], *A. Ko*[1], *S.C. Lee*[2]
[1]University of Texas at San Antonio, San Antonio, United States, [2]Texas Tech University Health Science Center, Immunology and Molecular Microbiology, LUbbock, United States

Mucormycosis, which is caused by Mucorales fungi, is poses a challenge to immunocompromised patients. As the susceptible cohort increases, the incidence of mucormycosis is rising. The mortality rate for mucormycosis reaches 90% in cases of disseminated infection. However, there are limited options in the current armamentarium for mucormycosis due to the high intrinsic resistance of Mucorales to available antifungal drugs. Echinocandins are the newest antifungal drugs that are highly efficacious in the treatment of many fungal infections. Mucorales, however, exhibit primary resistance to these drugs. Echinocandins inhibit the *fks* genes that encode beta 1,3-glucan synthases, and the model Mucorales *Mucor* genome harbors three copies of *fks* genes. To understand the primary resistance mechanism, we looked at the amino acid sequence of the gene products and found that the three *fks* genes in *Mucor* encode intrinsically altered amino acids in the Hotspot 1 region. Nonsynonymous mutations in Hotspot 1 in *Candida* species have been known for major

resistance mechanisms to echinocandin. To elucidate if these inherited changes in Hotspot 1 of the *Mucor* Fks's are involved in echinocandin resistance, we expressed *Mucor fks* genes and measure drug resistance and susceptibility in *Candida albicans*. The expression and function of *Mucor* Fks were confirmed. Interestingly, the engineered *C. albicans* strains expressing *Mucor fks ge*nes are similarly susceptible to echinocandin. These results demonstrate that the *Mucor* beta 1,3-glucan synthases themselves, despites the inherited changes in Hotspot1, are also susceptible for echinocandin and other unknown mechanism(s) is involved in the intrinsic resistance.

CS 1.3.11 - High-throughput identification of genetic factors driving tissue invasion in *Aspergillus fumigatus* through an integrated *in vitro* and *ex vivo* screening platform

S.Y. Tang[1], *C. Valero*[2], *N. Van Rhijin*[2], *M. Bromley*[2], *C. Zhao*[1,2]
[1]Manchester Metropolitan University, Life Sciences, Manchester, United Kingdom, [2]University of Manchester, Manchester Fungal Infection Group, Manchester, United Kingdom

Over 10 million people globally suffer from lung diseases caused by *Aspergillus fumigatus*, with limited treatment options and increasing antifungal resistance. Understanding the genetic factors that enable *A. fumigatus* to penetrate host tissues is crucial for developing new therapies, yet these genomic determinants remain largely unexplored.
As part of the *A. fumigatus* genome-wide knockout program (COFUN), we have completed the generation of a library that consists of over 6000 genetically dual-barcoded null mutants. Recently, we developed a novel high-throughput screening platform, using competitive fitness profiling in an *in vitro* barrier model to identify key genetic factors involved in invasive capacity. To expand this screening approach, we also incorporated a second-stage, high-throughput *ex vivo* screening employing a porcine corneal infection model of fungal keratitis (FK), which mimics tissue invasion more closely. This is designed to screen mutants identified from the *in vitro* stage, providing a more clinically relevant assessment of tissue invasion.
As a proof of concept, a subset of the COFUN collection, containing knockout mutants of protein kinases, was screened through this integrated pipeline. Key kinases critical to barrier penetration and tissue invasion were identified, including YakA, a known stress-activated kinase that plays a crucial role in septal plugging and contributes to the pathogenicity of *A. fumigatus*.
This study showed that the our methodology can provide a powerful tool for uncovering fungal invasion mechanisms and identifying potential therapeutic targets for FK and other invasive fungal infections.

CS 1.3.12 - Dectisomes increase efficacy of antifungal drugs by targeting fungal polysaccharides

Q. Choudhury[1], *S. Ambati*[1], *T. Pham*[1], *X. Lin*[1], *R. meagher*[1], *Z. Lewis*[1]
[1]University of Georgia, Microbiology, Athens, United States

Fungal infections account for approximately 2.5 million deaths globally each year and tens of billions of dollars in healthcare costs each year in just the United States. However, only three

primary antifungal classes exist to treat these infections. Issues with these classes include limited options to choose from, organ toxicity, and rising levels of drug resistance. The polyene amphotericin B (AmB), which targets ergosterol in the fungal cell membrane, has been described as the "gold standard" of antifungal treatments due to its broad spectrum of activity. However, it also has an affinity for cholesterol, thus leading to severe side effects that limit its use. The liposomal formulation of AmB was designed to address these issues; although this was an improvement over the original formulation, the toxicity concerns are still a prevalent limiting factor. In order to address these issues, our research group created a targeted antifungal liposome (DectiSome), in which the liposome's surface is covered with Dectin immune receptors that specifically recognize fungal cells. Dectisomes improve efficacy of AmB against *Cryptococcus neoformans, Candida albicans,* and *Aspergillus fumigatus* in cell culture and mouse models of fungal disease. Dectin-1 and 3-targeted Dectisomes are more effective than untargeted AmB-liposomes in *Rhizopus delemar.*

CS 1.4.1 - Gene expression dynamics associated with the developmental processes of conidial germination and polar growth in filamentous fungi

V. Halder[1], *W. Zheng*[1], *C. Dong*[2], *F. Trail*[3], *O. Yarden*[4], *J.P. Townsend*[1]
[1]Yale University, New Haven, United States, [2]Chinese Academy of Sciences., Beijing, China, [3]Michigan State University, East Lansing, United States, [4]Hebrew University of Jerusalem, Rehovot, Israel

Polar growth, the dominant form of growth in filamentous fungi, plays a crucial role in their exploring the environment, interacting with each other, and establishing infection in hosts. Unraveling the molecular mechanisms underlying polar growth is critical in advancing our knowledge of fungal biology, from their initial response to the environment to the development of pathogenicity. To better understand the genetic basis of asexual spore germination, we aimed to identify the genes associated with this developmental process and their divergences of function in different fungal species.
We performed a comparative genomic analysis to identify conserved and species-specific mechanisms regulating polar growth in filamentous fungi. This analysis examined gene expression during conidial germination extending from isotropic to polarized growth to the first hyphal branch, in several fungal species grown in a common medium representing saprobic and pathogenic lifestyles. Additionally, we examined gene expression patterns in pathogenic fungi grown in media representative of host environments to investigate how polar growth is modulated during the initiation of fungal infection.
The model fungus *Neurospora crassa*, with its intricate branching and hyphae fusion, represents an ideal non-pathogenic reference of polar growth. By leveraging the *Neurospora crassa* knockout library, we delved into the cognate functional roles of the genes highlighted in our comparative analyses. This approach validates the significance of specific genes in polar growth dynamics and offers insights into both fungal development and pathogenicity, enriching our understanding of these complex biological processes.

CS 1.4.2 - Mechanistic aspects of the combinatorial growth inhibition of *Aspergillus fumigatus*

A. Traynor[1], *M. Dwyer*[2], *G. Jones*[3], *D. Fitzpatrick*[2], *S. Doyle*[2]
[1]Maynooth University, Maynooth, Ireland, [2]Maynooth University, Biology, Maynooth, Ireland, [3]Leeds Beckett University, Leeds, United Kingdom

Aspergillus fumigatus causes over 400,000 cases of invasive aspergillosis per annum. Here, we show the antifungal properties of celastrol, a natural product of plant origin, against *A. fumigatus* under both metal-deplete and replete conditions. *A. fumigatus* growth inhibition by celastrol is augmented by co-addition of compound ADL2022, which decreases the inhibitory concentration of celastrol from > 100 µM to ~ 10 µM in iron-replete conditions. The combination of ADL2022 and celastrol in both iron and zinc-deplete, and replete, liquid cultures increases the observed inhibitory effect compared to celastrol alone. Celastrol increases the level of siderophore fusarinine C and decreases the amount of triacetylfusarinine C produced by *A. fumigatus*, in a concentration dependent manner. LFQ proteomics reveal molecular insights into the effect of celastrol on *A. fumigatus* whereby celastrol, and not ADL2022, is the active agent causing alterations to protein abundances and influencing growth. Several enzymes from the ergosterol biosynthetic pathway are uniquely abundant in the combined treatment compared to ADL2022 alone. Generally, DNA/RNA processing proteins and membrane-domain proteins are most influenced by treatment with celastrol and ADL2022 compared to either component alone. The antifungal properties of celastrol and ADL2022 may offer a potential alternative modality for treatment of *A. fumigatus* infections.

CS 1.4.3 - Dissecting the genetic machinery of aryl hydrocarbon receptor (AhR) agonist production in the skin commensal yeast Malassezia

G. Ianiri[1], *G. Barone*[1], *E. Patriarca*[1], *E. Gushiken-Ibañez*[2], *A. Staropoli*[3], *F. Vinale*[3], *S. Leibundgut-Landmann*[2]
[1]University of Molise, Department of Agricultural, Environmental and Food Sciences, Campobasso, Italy, [2]University of Zurich, Section of Immunology at Vetsuisse Faculty and Institute of Experimental Immunology, Zurich, Switzerland, [3]University of Naples Federico II, Department of Agricultural Sciences, Naples, Italy

The lipophilic yeast *Malassezia* is by far the most abundant member of the skin mycobiome, representing over 90% of all commensal skin fungi. While being a commensal fungus, *Malassezia* has also been associated with some skin disorders like seborrheic dermatitis (SD), atopic dermatitis (AD) and pityriasis versicolor (PV). Recent studies also report an involvement of *Malassezia* species in extracutaneous diseases such as Crohn's disease. Our understanding of the interaction between *Malassezia* and the host remains incomplete, both in the healthy and in the diseased skin. Previous studies found that *Malassezia furfur* converts tryptophan into brown-pigmented indoles that activate aryl hydrocarbon receptor (AhR) signaling in human keratinocytes. To elucidate the biochemical pathway responsible for AhR ligand production in *M.*

furfur, we used a combination of random and targeted mutagenesis coupled with transcriptomics and metabolomics. We developed a genetic screen that allowed the isolation of mutants impaired in the production of the characteristic brown indoles in media supplemented with tryptophan as the sole nitrogen source, and defective AhR activation in human keratinocytes. The availability of *M. furfur* mutants and ex vivo and in vivo models allows to establish the mechanism by which *Malassezia* AhR agonists affect the antifungal response, with implications for therapeutical approaches targeting the tryptophan metabolic pathway in the fungus or AhR signaling in the host.

CS 1.4.4 - A rewired transcription factor in Candida albicans and its role in regulating sulfur metabolism and tolerance to reactive sulfur species

A.M. Cheppanakozhummal Thazhathidam[1,2], *F. Tebbji*[2], *A. Vincent*[3], *A. Sellam*[1,2]
[1]Université de Montréal, Department of Microbiology, Infectiology and Immunology, Montréal, Canada, [2]Montréal Heart Institute, Montréal, Canada, [3]Université Laval, Department of Animal Sciences, Laval, Canada

Candida albicans is an opportunistic human fungal pathogen and a leading cause of nosocomial infections in immunocompromised patients. Metabolic flexibility is an important virulence trait of *C. albicans* that allows it to colonize contrasting host niches with varying nutritional compositions. While the majority of works investigating the contribution of metabolic flexibility to fungal virulence have focused mainly on carbon and nitrogen sources, sulfur have received only little attention. To explore the genetic circuit through which *C. albicans* coordinate the utilization of alternative sulfur sources, we undertook a systematic analysis using both genetic screen and transcriptional profiling. We identified a core transcriptional module of three transcription factors: Met32, Cbf1 and Met4, that governs the utilization of different sulfur sources found in different niches of the human host (free and bile acid-conjugated taurine, glutathione, diverse sulfonates). Genome-wide occupancy of Met32 using ChEC-seq uncovered that, in addition to the control of sulfur utilization genes (desulfonating enzymes), Met32 modulate genes related to sulfur detoxification such as the sulfite exporter *SSU1*. Accordingly, *met32* mutant was highly sensitive to excess of sulfite. This suggests that Met32 play a critical role by tuning intracellular sulfur contents through the modulation of both uptake and efflux of sulfur metabolites. Using several infection models, we found that Met32 and other desulfonating enzymes were required for fungal virulence. Our study delineates, to our knowledge, the first transcriptional circuit that mediates sulfur utilization in *C. albicans* and its contribution to fungal virulence.

CS 1.4.5 - RNA-binding protein complex regulated specialized secondary metabolites contribute to fungal virulence

W.-B. Yin[1,2], *Z. Song*[1,2], *H. Zhang*[1,2]
[1]State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China, [2]Medical School, University of Chinese Academy of Sciences, Beijing, China

The intricate interplay between secondary metabolism and fungal pathogenesis remains incompletely understood. We have discovered an RNA-binding protein CsdA, which coordinates fungal virulence and secondary metabolism by regulates splicing in *Aspergillus fumigatus*. Mechanistically, CsdA specifically binds to the 5' splice site “AUGUCAGC” of the *laeB* intron 3, controlling its recruitment to the spliceosome components Usp102 and Usp106, thereby affecting mRNA splicing and ultimately regulating the production of multiple specialized metabolites, mainly fumiquinazoline C, to mediate the infection process of *A. fumigatus*. Surprisingly, CsdA can also directly interact with LaeB to form a complex that regulates secondary metabolism at both the transcriptional and post-transcriptional levels. This work provides insights into a novel paradigm for regulating fungal infection and global secondary metabolism at both transcriptional and post-transcriptional levels, illuminating the molecular intricacies of fungal pathogenesis and secondary metabolism.

CS 1.4.6 - Novel insights into endogenous biotinylation in fungi

Z. Dong[1], *H.P. Leng*[1], *Q. Lan*[1], *K.H. Wong*[1]
[1]University of Macau, Macau, China

Biotin, an essential vitamin, acts as a coenzyme for holocarboxylases that catalyze key steps in gluconeogenesis, amino acid catabolism, and fatty acid synthesis. Biotin is covalently attached to holocarboxylases at the lysine residue in the conserved Met-Lys-Met sequence motif by holocarboxylase synthetase (HCS) through a process called biotinylation. Carboxylase family proteins are generally believed to be the only targets of HCS. However, we and others have observed from Western blot analysis many more biotinylated proteins than expected for carboxylases in different fungal species. Mass spectrometry analysis of streptavidin pull-down showed that many of the endogenous biotinylated proteins in *Aspergillus nidulans* are unrelated to carboxylases and are highly abundant in the cell; e.g. histones and tubulin. Interestingly, unlike carboxylases, these other endogenous biotinylated proteins lack the canonical Met-Lys-Met biotinylation motif. This raises the question of how biotin is introduced to these proteins and suggests the existence of a non-canonical biotinylation pathway. We are currently testing different potential mechanisms of non-canonical biotinylation and studying the role of these biotinylation events. Endogenous biotinylation is commonly observed in different organisms, including bacteria, various fungal species, and mammalian cells, indicating an evolutionarily conserved mechanism for the non-canonical biotinylation pathway.

CS 1.4.8 - Trichoderma reesei QM6a harbors an endohyphal Methylobacterium, which impacts carbohydrate degradation and growth in light

M. Schalamun[1], *S. Beier*[1], *I. Scalmani*[1], *S. Compant*[1], *W. Hinterdobler*[1], *M. Schmoll*[1,2]
[1]AIT Austrian Institute of Technology GmbH, Center for Health and Bioresources, Tulln, Austria, [2]University of Vienna, Centre for Microbiology and Environmental Systems Science (CeMESS), Vienna, Austria

In nature, complex organismic communities have evolved for optimal colonization of habitats. Interkingdom interactions between fungi and bacteria can be mutualistic, but also parasitic. We found evidence for the presence of endohyphal bacteria of different species in the majority of *Trichoderma* strains tested. In the saprophyte *Trichoderma reesei* QM6a, we detected endohyphal bacteria by confocal microscopy and specific staining. We confirmed the presence of a *Methylobacterium* species in the hyphae by sequencing 16S rRNA and genome sequencing after isolation from the fungal host and that this bacterium is not an obligate biotroph.
To evaluate the interrelationship between *Methylobacterium* and *T. reesei*, we cured QM6a from the bacterium and applied BIOLOG phenotype microarrays. This clearly showed a light dependent alteration of growth in the cured strain especially on xylitol, an intermediate of hemicellulose degradation, on D-mannitol and several other carbon sources. Antagonism against pathogenic fungi on plates was not altered in the cured strain and no general growth defect was obvious. Transcriptome analysis between wild-type and cured QM6a indicates a substantial influence of the endohyphal *Methylobacterium* on mannan and glucan metabolic genes of *T. reesei* QM6a, on a hydrophobin gene as well as on several sugar transporters, predominantly in light. Accordingly, the genome of the *Methylobacterium* comprises multiple genes associated with light response and CAZyme genes to explain its contribution.
Consequently, the endohyphal bacterium of *T. reesei* QM6a supports the metabolic adaptation of the fungus to growth in light and may be beneficial to broaden the nutrient versatility of the fungus.

CS 1.4.9 - Multi-omics analyses reveal divergent molecular mechanisms underlying plant biomass conversion of five fungi

M. Peng[1], *J. Li*[1], *L. Xu*[1], *N. Tolic*[2], *C. Clendinen*[2], *R.P. De Vries*[1]
[1]Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands, [2]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, United States

Fungal plant biomass conversion is of great importance in the Earth's carbon cycle, as well as in many industrial applications, such as the production of biofuel and biochemicals from plant lignocellulose materials. To further investigate the diversity of fungi with respect to their approach for converting crude plant biomass, we comparatively analyzed the transcriptome, proteome and metabolome profile of five diverse fungi during their growth on two common feedstocks, soybean hulls and corn stover, at three time points. We selected the ascomycetes *Aspergillus niger*, *Aspergillus nidulans*, *Penicillium subrubescens*, *Trichoderma reesei* and the white-rot basidiomycete *Phanerochaete chrysosporium* for this study. The gene expression profile of lignocellulose degrading enzymes, sugar transporters and sugar metabolic enzymes showed strong time, substrate and species specific differences. The proteome and metabolome profiles also shown significant difference among the response of different fungi to crude plant biomass. These results improve our understanding of the diversity of molecular mechanisms driving plant biomass conversion by fungi, which will facilitate future studies of fungal ecological roles and further development of fungal biotechnology.

CS 1.4.10 - Understanding and harnessing the carbon catabolic potential of *Fomes fomentarius* for industrial applications

A.S. Birke[1], *T. Cairns*[1], *C. Freidank-Pohl*[1], *C. Regner*[1], *S. Jung*[1], *V. Meyer*[1]
[1]Technical University Berlin, Applied and Molecular Microbiology, Berlin, Germany

Fomes fomentarius is a promising candidate for fungal mycelium-based composite material production. However, its application in industry is hampered by a limited knowledge of its carbon catabolism. To accelerate the development of an industrially feasible composite production process based on *F. fomentarius*, we have grown it under 24 cultivation conditions, analysed gene expression patterns during substrate degradation, and constructed gene co-expression networks. Our analyses suggest that genes encoding putative lignocellulolytic CAZymes have been the subject of gene family expansion followed by transcriptional divergence, which will have contributed to niche specificity and metabolic robustness. Transcriptional regulation might be directed by two putative regulators, CalA and CalB, that we have identified using gene co-expression networks. A subset of CAZyme-encoding genes were found to be arranged in a novel type of metabolic gene cluster, termed contiguous co-expression clusters, that have not been described previously in fungi. This discovery has given us further insight into the transcriptional organisation and regulation of *F. fomentarius* genes. Lastly, we identified 35 sugar transporters *in silico*. Applying SPOT, a state-of-the-art model, we predicted potential substrates and analysed their respective gene expression patterns, the results of which indicate that sugar transporters in *F. fomentarius* might be promiscuous towards more than one substrate. By carrying out gene expression analysis and constructing co-expression networks focused on *F. fomentarius* carbon catabolism, we have been able to single out target genes for future studies aiming to optimise substrate utilisation. Ultimately, this insight will facilitate strain improvement approaches of *F. fomentarius* for industrial applications.

CS 1.4.11 - Bioconversion of wood and lignocelluloses by fungi of *Basidiomycota* and consortia: sustainable solutions for production of metabolites, bioethanol and bioactive natural compounds

T. Lundell[1], *E. Kiviniemi*[1], *M. Qurashi*[1], *M. Wahlsten*[1], *J. Österman-Udd*[1]
[1]University of Helsinki, Department of Microbiology, Helsinki, Finland

In forest ecosystems, wood decay fungi of *Basidiomycota* are key players for degradation of dead plant biomass and maintenance of carbon cycling. Hyphal contacts and interspecies interactions of fungi are on-going processes in deadwood, together with associations with diverse bacteria adapted to the chemically and physically changing environment. We explored these interactions on wood substrates to follow changes in enzyme expression and metabolite profiles together with transcriptomic analyses to assess potential of fungal combinations for bioconversions. Fermentative metabolism, its regulation under hypoxia or oxidative stress, production of bioethanol, organic acids and sugars from lignocellulosic materials was further explored with the lignin-degrading white-rot fungus *Phlebia radiata*. We also isolated novel nitroaryl secondary metabolites from this species produced *de novo*.

Combination of white rot – brown rot fungi lead to mitigation of brown rot activity and domination of white rot degradation in a one-year experiment on spruce wood. On birch wood, fungal co-cultures indicated potential for xylanase production, while single- and two-species combinations were the most effective in conversion of the substrate into bioactive compounds. A few culture fluid extracts showed antimicrobial and antioxidant potential, and respective compounds were identified. On-going studies focus on fungi-bacteria combinations with a similar approach.
Our results imply that fungal co-cultures exhibit potential for production of carbohydrate-active and oxidoreductase enzymes as well as conversion of plant biomass wastes into added-value natural compounds, many of them showing medicinal and health-promoting abilities. Next step is to open the biosynthetic and degradative metabolic pathways of deadwood microbiota by multi-omics approach.

CS 1.4.12 - *Colletotrichum orbiculare* as a fungal model for Niemann-Pick Type C autosomal recessive lysosomal disorder

Y. Kubo[1], *H. Sano*[1], *S. Takezawa*[1], *H. Horiguchi*[1], *N. Saomoto*[1], *A. Chiba*[1], *C. Tanaka*[1], *N. Kato*[1], *T. Nishiuchi*[2], *H. Nishisaka*[3], *K. Kitatani*[3], *S. Kodama*[1]
[1]Setsunan University, Faculty of Agriculture, Hirakata, Japan, [2]Kanazawa University, Bioscience Core Facility, Kanazawa, Japan, [3]Setsunan University, Faculty of Pharmacy, Hirakata, Japan

Molecular genetic studies on the anthracnose fungus *Colletotrichum orbiculare*, a plant pathogenic filamentous fungus, have revealed that the formation of appressoria during host invasion requires precise environmental responses and advanced functional differentiation. Our research further highlights *C. orbiculare* as a powerful model for studying lysosomal dysfunction and its implications for both plant and human diseases. Niemann-Pick disease type C (NPC), a refractory human lysosomal disease, leads to abnormal cholesterol accumulation and sphingolipid metabolism disorders in lysosomes, resulting in severe neurological disorders. In our groundbreaking study, we discovered that miglustat, the only clinically approved drug for NPC, can restore the impaired invasion capability of *C. orbiculare npc* mutant strains. This fungal *npc* mutation causes lysosomal dysfunction, which disrupts appressoria formation, a key factor in host invasion. Miglustat not only restores the invasive function in these mutants but also affects sphingolipid metabolism within the fungus, mirroring its effects in human NPC disease. This novel finding highlights the conservation of lysosomal processes across species and highlights the utility of *C. orbiculare* as a model for investigating human lysosomal disorders, particularly those involving sphingolipid metabolism. Unlike yeast, where NPC gene deficiency does not lead to significant functional consequences, *C. orbiculare* offers a unique microbial system to study the pathogenic mechanisms of lysosomal dysfunction. This approach has the potential to contribute to the development of new therapeutic strategies for lysosomal diseases, advancing drug discovery efforts and providing societal benefits through the treatment of these intractable disorders.

CS 1.5.1 - Developing RNAi-based fungicides to control fusarium head blight

M. Djavaheri[1], T. Bender[1], K. Horner[1], M.-A. Henriquez[2], O. Molina[2], S. Berraies[3], S. Rosa[4], R. Kutcher[5], B. Blackwell[6], S. Sharpe[1], S. Clarke[7], G. Subramaniam[6], H. Borhan[1], S. Robinson[1]
[1]Agriculture Canada, Saskatoon, Canada, [2]Agriculture Canada, Morden, Canada, [3]Samia Berraies, Swift Current, Canada, [4]Cerom, Saint-Mathieu-de-Beloeil, Canada, [5]University of Saskatchewan, Plant Science, Sask, Canada, [6]Agriculture Canada, Ottawa, Canada, [7]National Research Council, Saskatoon, Canada

Fusarium head blight (FHB) is a serious fungal disease of cereal crops including wheat, durum, barley, oats and corn. FHB is caused by several species from the genus Fusarium with *F. graminearum* causing the most concern. FHB affects kernel development, reducing both yield and grade. Furthermore, the disease contaminates grain with mycotoxins which can render it worthless should toxins exceed strict threshold limits.
Genetic resistance to FHB is limited and where identified, it exhibits polygenic inheritance complicating breeding strategies. FHB management relies on timely fungicide application to suppress disease outbreaks. Chemical pesticides are subject to increasing scrutiny by regulatory bodies over health and environmental concerns, accelerating the need to develop alternative solutions for pest management. RNAi is a promising approach for crop disease management where dsRNA designed to key pathogen gene targets can trigger silencing, leading to fungal cell death. The sequence specificity encoded in RNA restricts fungicide control to the pathogen of concern, leaving beneficial species unharmed. Since dsRNA can be applied as a foliar spray, the application of these Next-Generation Fungicides avoids the need for transgenic crop production, maintaining access to lucrative export markets.
Here, we present the potential of RNAi-based gene silencing to control FHB in wheat. The strategy used to rapidly identify effective dsRNA trigger sequences from greenhouse-based disease assays will be presented. We will discuss the challenges of evaluating efficacious dsRNA trigger sequences in replicated field trials which include meeting regulatory compliance, scaling dsRNA production and dsRNA application.

CS 1.5.2 - Small RNAs-mediated fungal interactions relevant to biocontrol

E. Piombo[1], N.C. Konakalla[2], P. Kalyandurg[2], P. Sundararajan[2], D. Funck Jensen[1], M. Karlsson[1], R. Raju Vetukuri[2], M. Dubey[1]
[1]Swedish University of Agricultural Sciences, Uppsala, Sweden, [2]Swedish University of Agricultural Sciences, Alnarp, Sweden

Small RNA (sRNAs)- mediated RNA silencing is emerging as a critical player in host-microbe interactions. However, its role in fungal interactions relevant to biocontrol is yet to be fully explored. Our recent research explored small RNA (sRNA)-mediated interactions between the biocontrol fungus *C. rosea* and its fungal and plant hosts. We showed that deletion of the DCL2 *gene*, which acts upstream in RNA silencing pathways, in *C. rosea* resulted in mutants with

reduced specialized metabolite production, mycoparasitism and biocontrol ability. However, its root colonization ability was increased. Dual-RNA seq analysis revealed the downregulation of defense response-related genes in wheat during interactions with Δ*dcl2* strains compared to the WT, which aligns with the increased root colonization ability of DCL2 deletion strains. Furthermore, our sRNA sequencing identified 18 wheat miRNAs responsive to *C. rosea*, with three predicted to target the *C. rosea* polyketide synthase gene *pks29*, known for its role in fungal antagonism and biocontrol. Two of these miRNAs were shown to enter *C. rosea* from wheat roots with fluorescence analyses and silenced the expression of a polyketide synthase gene *pks29*, previously shown for its role in fungal antagonism and biocontrol. This provides compelling evidence for cross-kingdom RNA silencing of the *C. rosea* gene by wheat miRNAs. Our work provides insights into the mechanisms underlying biocontrol fungi-plant interactions and holds promise for future studies on sRNA-mediated RNA silencing in fungal biocontrol.

CS 1.5.3 - Integrating *Trichoderma gamsii* T6085 and *Clonostachys rosea* IK726 to enhance Fusarium Head Blight control in wheat

A. Petrucci[1], *M. Cesarini*[1], *I. Vicente*[2], *L. Merani*[3], *B. Jensen*[4], *D.B. Collinge*[4], *S. Sarrocco*[1]
[1]University of Pisa, Department of Agriculture, Food and Environment (DAFE), Pisa, Italy, [2]University of Salamanca, Department of Microbiology and Genetics, Institute for Agribiotechnology Research (CIALE), Salamanca, Spain, [3]University of Bologna, Department of Agricultural and Food Sciences (DISTAL), Bologna, Italy, [4]University of Copenhagen, Department of Plant and Environmental Science (PLEN), Copenhagen, Denmark

Wheat is the third staple food globally, and Fusarium Head Blight (FHB) is an important disease caused by several *Fusarium* spp., with *F. graminearum* (*Fg*) as the most relevant in Europe. The major concern of FHB is the production of mycotoxins, thereby food safety.
Biocontrol is a sustainable approach, with the biocontrol agents (BCAs) *Trichoderma gamsii* T6085 (*Tg*) and *Clonostachys rosea* IK726 (*Cr*) proving effective individually. This study aims to improve efficiency and stability in field by combining *Tg* and *Cr*.
Compatibility was assessed *in vitro* and *in planta*. On solid agar, growth inhibition due to diffusible compounds was absent but *Tg* volatile compounds slightly inhibited *Cr* growth. In liquid cultures, *Cr* cultural filtrates inhibited *Tg* spore germination, and *Cr* inhibited *Tg* growth. Mutual growth inhibition did not occur on wheat spikes or on straw.
Following root application, no evidence of systemic modulation of defence-related (DR) genes occurred in leaves but in spikes inoculated with *Cr* (alone and combined) *Pal1*, *PR1* and *Lox1* were up-regulated 96hpi (hours post-inoculation). In spikes subsequently inoculated with *Fg*, *PR1* was slightly up-regulated by *Cr* at 24hpi. *Tg* highly up-regulated DR genes at 72hpi. BCAs co-inoculation up-regulated *Lox1* and *Pgip2* to the highest level at 96hpi without *Fg* and at 24hpi with *Fg*, respectively. Disease incidence and pathogen inoculum for the next cropping season were reduced by >90% and 99%, respectively, across treatments.
Compatibility on spikes, upregulation of DR genes and reduction of FHB symptoms suggest that combining *Tg* and *Cr* potentially enhance FHB management.

CS 1.5.4 - A secondary metabolite from *Beauveria bassiana* reprograms root exudate recognition and virulence in *Fusarium oxysporum*

M. Ranesi[1], *S. Vitale*[1], *A. Staropoli*[1], *L. Di Costanzo*[1], *M. Lorito*[1], *S.L. Woo*[2], *F. Vinale*[3], *D. Turrà*[1]
[1]Università degli Studi di Napoli Federico II (Napoli), Department of Agricultural Sciences, Portici, Italy, [2]Università degli Studi di Napoli Federico II (Napoli), Department of Pharmacy, Naples, Italy, [3]Università degli Studi di Napoli Federico II (Napoli), Department of Veterinary Medicine and Animal Production, Naples, Italy

Several soil-borne microorganisms secrete organic acids (OAs) that serve multiple ecological functions including pH modulation, ion availability regulation, mineral acquisition facilitation, and inter-species communication. However, how such molecules regulate tritrophic interactions among pathogens, plants, and biocontrol agents remains largely unknown. Here, we characterized OAs production among different isolates of the endophytic biocontrol fungus *Beauveria bassiana* (*Bb*) and identified a novel metabolite, Bb1993, with dual functionality: reducing environmental pH at high concentrations and inhibiting germ tube formation and growth of *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) at low concentrations. Root treatment with either *Bb* spores or Bb1993 altered Root Exudates (REs), making them repellent to *Fol* germ tubes despite increased peroxidase accumulation, a major fungal chemoattractant. Dual confrontation assays between *Fol* and *Bb* colonies, and direct Bb1993 application, enhanced *Fol*'s invasive growth on cellophane membranes. Our results demonstrate that Bb1993 exerts both direct and plant-mediated effects on *Fol* by modulating spore germination, plant recognition, and penetration. Ongoing experiments aim to identify the biologically active molecules from REs of Bb1993-treated tomato plants that confer repellent activity against *Fol*. These findings provide new insights into the chemical dialogue mediating plant-microbe interactions and suggest novel strategies for metabolite-based biological control.

CS 1.5.5 - The decision for or against mycoparasitic attack by *Trichoderma* spp. is taken already at a distance in a prey-specific manner and benefits plant-beneficial interactions

P. Stange[1], *P. Balaji Sivaprakasam Padmanaban*[2], *J.-P. Schnitzler*[2], *M. Rosenkranz*[3], *J.P. Benz*[1]
[1]Technical University of Munich, Holzforschung München, TUM School of Life Sciences, Freising, Germany, [2]Helmholtz Munich, Research Unit Environmental Simulation, Neuherberg, Germany, [3]University of Regensburg, Institute of Plant Sciences, Ecology and Conservation Biology, Regensburg, Germany

The application of biocontrol agents is becoming more important as both agriculture and forestry are facing the challenges of climate change. *Trichoderma* spp. are gaining popularity due to their multifaceted roles in promoting plant health, including direct antagonism of plant-pathogenic fungi in the rhizosphere. However, plants also associate with a range of plant-beneficial fungi, such as mycorrhiza. The mycotrophic nature of *Trichoderma* therefore bears the risk that also

these might be antagonized, which could result in negative consequences for the plant hosts. So far, it remained unclear whether *Trichoderma* spp. are able to differentiate between plant-beneficial and -pathogenic fungi they might encounter in the rhizosphere.
We therefore developed a novel system to evaluate the decision-making process. With this "olfactometer", we observed a positive chemotropism of *T. harzianum* and *T. atrobrunneum* towards two plant pathogens, *Alternaria alternata* and *Fusarium graminearum*, already at a distance of 10-15 cm. In contrast, the presence of ectomycorrhiza (*Laccaria bicolor* and *Hebeloma cylindrosporum*), led to an avoidance behavior of both *Trichoderma* spp..
Transcriptomics confirmed that mycotrophic programs were induced only in the presence of the pathogens.
The findings shed light on the entangled interactions of fungi in the rhizosphere and suggest that *Trichoderma* spp. can distinguish between fungi of different trophic modes already from afar and in the interest of a potential host plant. It adds a mechanistic understanding and a promising blueprint for risk assessments of fungal biological control agents such as *Trichoderma* to consider application in the rhizosphere as safe.

CS 1.5.6 - Insights into 6-Pentyl-α-Pyrone biosynthesis in Trichoderma atroviride

A. Eschlböck[1], *D. Flatschacher*[1], *S. Pierson*[1], *U. Schreiner*[1], *V. Stock*[2], *A. Schiller*[2], *D. Ruso*[3], *M. Doppler*[3], *V. Ruzsanyi*[2], *M. Gründlinger*[1], *R. Schuhmacher*[3], *S. Zeilinger-Migsich*[1]
[1]University of Innsbruck, Department of Microbiology, Innsbruck, Austria, [2]University of Innsbruck, Institute for Breath Research, Innsbruck, Austria, [3]University of Natural Resources and Life Sciences Vienna, Department of Agrobiotechnology, IFA-Tulln, Vienna, Austria

The filamentous fungus *Trichoderma atroviride* is a widely recognized biocontrol agent utilized to combat crop diseases caused by other fungi. This is due to the production of a wide variety of specialized metabolites (SMs) with a broad spectrum of bioactivities and its ability to parasitize phytopathogenic fungi. One of the most prominent SMs of *T. atroviride* is 6-pentyl-alpha-pyrone (6-PP), known for its role in inhibiting bacterial and fungal growth as well as promoting plant development. 6-PP production has been shown to be inhibited by blue and white light, reaching peak production under cultivation in complete darkness.
In this study, we identified the core gene (*pks1*) responsible for 6-PP production in *T. atroviride* and confirmed the polyketide nature of this pyrone. Transcriptomic analysis revealed that *pks1* is significantly upregulated under dark cultivation compared to light, which aligns with the observed increase in 6-PP production in the absence of light. Using CRISPR/Cas9, deletion mutants lacking the *pks1* polyketide synthase gene were generated and functionally characterized. HPTLC and LC-MS analysis confirmed the inability of the Δ*pks1* mutants to produce 6-PP and related compounds. Although *pks1* deletion strains showed a similar morphology and growth behavior as the WT, they exhibited a significant reduction in their mycoparasitic activity against host fungi such as *Botrytis cinerea* and *Rhizoctonia solani*.

CS 1.5.7 - The new frontier of biocontrol: breeding for biologicals and microbiome resilience

B. Dotson[1,2], C.B. Andersen[1], Å. Lankinen[1], A. Rasmusson[2], L. Grenville-Briggs Didymus[1]
[1]Swedish University of Agricultural Sciences, Department of Plant Protection Biology, Alnarp, Sweden, [2]University of Lund, Department of Biology, Lund, Sweden

In a one health context where we consider the plant as a holobiont, traits encoded for by symbiotic microbes and the microbiome are important contributors to overall plant health. We are studying the impact of fungal (*Trichoderma afroharzianum* T22) and oomycete (*Pythium oligandrum*) biocontrol agents on sugar beet and potato health. *Trichoderma*- and *P. oligandrum* induced growth and disease protection have been shown in many cultivated plants. However, variable efficiencies have been seen, and a lack of genetic understanding of the interaction likely limits the present agricultural benefits. Studies on crop plant rhizosphere microbiomes have focused mainly on spatio-and-temporal dynamics between plant growth stages, genotypes and cropping systems. Few studies have investigated manipulation of the plant rhizosphere microbiome with the amendment of a Biological Control Agent (BCA), which is of importance for our understanding of the function of BCAs in the environment and their impact on soil/plant health. We show that *P. oligandrum* has a biostimulatory effect in a cultivar-dependent manner in potato and that it induces changes in the rhizosphere microbiome. In sugar beet we observed significant variation in the biocontrol of damping off and in growth promotion by T22 within sugar beet elite breeding lines. Results indicate that plant genotype and development are the major factors explaining the observed variation. yet different Trichoderma species/strains will differentially affect the growth outcome of each sugar beet line. This implies that efficient technological use of these BCAs will demand a tight interaction between microbiological optimisation and plant breeding.

CS 1.5.8 - Exploring the anticancer potential of recombinant fungal defensin-like peptides from *Aspergillus udagawae* and *Hyaloscypha hepaticicola*: production, characterization, and mechanistic insights into oligomerization

B. Gundogdu[1], B. Ozcan[1], G. Bayram Akcapinar[1]
[1]Acıbadem University, Institute of Health Sciences, Department of Medical Biotechnology, Istanbul, Turkey

Defensins are small, cationic peptides with conserved cysteine framework which confer stability and functional versatility. They are primarily recognized for their role in innate immune response and essentially all living organisms produce defensin-like peptides to protect themselves against pathogens and external threats. These peptides have a broad range of biological functions such as antimicrobial, antiviral, and antifungal activities. Notably, defensin-like peptides (DLPs) can also target cancer cells.
Similar to their antimicrobial function, these peptides may disrupt cancer cell membranes, may induce apoptosis or modulate immune response by acting like chemoattractants. Fungal defensins

that show anticancer properties often rely on oligomerization to enhance their efficacy. By oligomerizing, these defensins can increase their ability to create pores or disrupt key membrane components, leading to cell death and offering a potential therapeutic route for cancer treatment. Fungal defensins are promising candidates for anti-cancer therapeutics. Uncharacterized fungal defensin-like peptides with sequence homology mined from NCBI BLASTp database.
In this study, two candidates from *Aspergillus udagawae* and *Hyaloscypha hepaticicola* were selected according to their structural and physicochemical properties and recombinantly produced in the methylotrophic yeast *Pichia pastoris (aka Komagataella phaffi)*, expression system. Codon-optimized defensin-like peptides produced in *P. pastoris* KM71H (Muts) strain. Candidate proteins are secreted into the medium collected and purified and characterized. Cytotoxic properties of peptides tested on cancer cell lines to evaluate the anti-cancer properties of the peptides. Peptides' oligomerization tendency in different conditions measured via DLS and their oligomerized structures were modeled.

CS 1.5.10 - When the pathogen becomes the prey: responses of *Botrytis cinerea* to mycoparasitism

F. Rocher[1], *R.R. Vetukuri*[2], *L. Grenville-Briggs Didymus*[1]
[1]Swedish University of Agricultural Sciences, Plant Protection Biology, Alnarp, Sweden,
[2]Swedish University of Agricultural Sciences, Plant Breeding, Alnarp, Sweden

Botrytis cinerea is a plant necrotrophic fungal pathogen characterized by its broad host range encompassing more than 200 species. *B. cinerea* infection mechanisms have been thoroughly characterized and involve the secretion of an arsenal of toxins, cell wall- degrading enzymes and effectors that induce plant cell death. However, plant pathogens share their ecological niche with a multitude of other microorganisms that compose the plant holobiont including mycoparasite species that represent an important resource to control plant diseases. The mechanisms involved in interactions between plant pathogens and other microorganisms in the plant holobiont have been overlooked and must be addressed to improve plant protection strategies. In this context, the molecular components underlying the responses of *B. cinerea* facing the oomycete mycoparasite *Pythium periplocum*, have been characterized *in vitro* using an RNA-seq approach. In response to mycoparasitism, *B. cinerea* exhibits extensive gene expression changes with around 61% of its genes being differentially expressed. Using a biologically oriented gene mining approach, we depicted a near-complete map of the processes involved in *B. cinerea* defence strategy to mycoparasitism covering sensing, signal transduction, gene regulation, detoxification and secreted defence compounds. Subsequent identification of genes within the Pathogen-Host Interactions database allowed us to provide a list of candidates for further validation studies. Taken together, our results shed light on a poorly understood part of *Botrytis cinerea* biology and represent a valuable resource to understand its responses in adverse environments.

CS 1.5.11 - *Bacillus velezensis*: a potential biocontrol agent against *Rhizopus microsporus*, a fungus causing tomato fruit infections

A. Kock[1], *M. Napo*[1], *K. Alayande*[1], *J. Uehling*[2], *T. Pawlowska*[3], *R. Adeleke*[1]
[1]North-West University, Microbiology, Potchefstroom, South Africa, [2]Oregon State University, Department of Botany and Plant Pathology, Corvallis, United States, [3]Cornell University, Plant Pathology and Plant-Microbe Biology, Ithaca, United States

Rhizopus microsporus is a necrotrophic opportunistic pathogen that causes significant economic losses in the agricultural sector. The use of chemical fungicides is harmful to the environment and can contribute to the development of fungicide-resistant strains. To explore alternative strategies for the mitigation of post-harvest infections, we investigated the potential biological control efficacy of an endophytic bacteria, *Bacillus velezensis*, against *R. microsporus*, using tomatoes as a model species. Two *B. velezensis* strains (KV10 and KV15) were evaluated against three *R. microsporus* strains (W2-50, W2-51, and W2-58) both *in vitro* and *in vivo*. Culture-based assays assessed the inhibitory effects of *B. velezensis* against *R. microsporus*. Results demonstrated strain-specific antifungal activity with reduction in fungal growth across treatments. Further analysis investigated the production of volatile organic compounds (VOC) by *B. velezensis*, and the antifungal potential of these VOCs. Results further verified the mycelial inhibition of *R. microspores* strains across treatments with *B. velezensis* strains. The *in vivo* biocontrol efficacy was evaluated by inoculating tomato fruits with *R. microsporus* and subsequently treated with *B. velezensis*. This supported the strain specific reduction in tomato spoilage, resulting in various spoilage incidences observed across treatments. Our findings highlight the potential of *Bacillus velezensis* as a promising biocontrol agent for the management of *Rhizopus microsporus* post-harvest infections in tomatoes. Further research is warranted to optimize the application *of B. velezensis* as a sustainable and environmentally friendly approach for controlling post-harvest diseases in tomatoes.

CS 1.5.12 - The sugarbeet phyllosphere harbors bacteria capable of inhibiting *Cercospora beticola*, the causal agent of Cercospora leaf spot

M. Christenson[1], *D. Back*[2], *M. Natwick*[1], *L. Rangel*[3], *N. Wyatt*[2], *M. Bolton*[2]
[1]North Dakota State University, Plant Pathology, Fargo, United States, [2]U.S. Dept. Agriculture, Fargo, United States, [3]The James Hutton Institute, Dundee, United Kingdom

Cercospora beticola is a fungal pathogen of sugarbeet (*Beta vulgaris* L. ssp. *vulgaris*) that causes Cercospora leaf spot (CLS). Unfortunately, *C. beticola* has proven adaptable to current management practices due in part to high levels of genetic diversity found in most field populations. We have shown previously that *C. beticola* alters the microbial community structure of the phyllosphere. We hypothesize that the sugarbeet phyllosphere harbors bacterial communities that may have properties to ward off plant pathogens like *C. beticola*. To that end, CLS-infected sugarbeet leaves were utilized to create a collection of culturable epiphytic bacteria. A total of 447 bacterial isolates were sequenced from CLS-resistant and -susceptible cultivars

across five sampling timepoints. *Cercospora beticola* isolates were challenged with bacteria from the collection to ascertain antagonistic phenotypes. The assays revealed that *Burkholderia contaminans* reduces fungal growth without direct mycelial contact or via volatile compounds. Bacterial and fungal RNA-seq was conducted to provide insight into the interactions between *B. contaminans* and *C. beticola.* Characterization of secondary metabolite candidates involved with *C. beticola* growth inhibition will be discussed. Identification of the compound(s) responsible for inhibition could provide us with a new management tool for CLS and will shed light on the biology of this pathogen.

CS 1.6.1 - Caught in the air: rethinking fungal virulence through the lens of exposure

E. Snelders[1], *B. Auxier*[1], *A. Barber*[2]
[1]Wageningen University & Research, Laboratory of Genetics, Wageningen, Netherlands, [2]Friedrich Schiller University, Research Group Fungal Informatics, Institute of Microbiology, Jena, Germany

Infection rates vary between fungal species, with certain fungi showing a higher incidence of infection than others. Research into the causes of these differences, virulence, typically focuses on identifying genes by comparing pathogenic fungi to nonpathogenic relatives, linking specific genes to virulence. However, the relative contribution of a species to the total exposure of fungal spores is rarely considered. Air is a primary transmission medium for many human and animal pathogenic fungi. Although some fungi are endemic, others have a wide geographic range. The ecology of an environment, including urbanization, land use, and climate, significantly impacts airborne fungal concentrations, but the occurrence of spores remains understudied due to technical sampling limitations.
A new method allows cost-effective air sampling at a multitude of sample sites. Our results show that in the Netherlands, *Aspergillus fumigatus* spores are most prevalent, while interestingly spore concentrations of *Aspergillus niger* and *Mucorales species* are orders of magnitude lower. This variation in spore prevalence may explain differences in disease burden. I propose shifting fungal virulence studies from a strong genetic focus, to investigating the environment as an exposure source. Recent pangenome studies in *Aspergillus fumigatus* and *Aspergillus flavus* also show large population-level variation that may represent local habitat adaptations. Using air sampling across various types of land use, we can understand fungal exposure, concentrations, antifungal resistance levels, and uncover hidden transmission patterns. This broader perspective integrates epidemiology into fungal virulence, guiding prevention strategies.

CS 1.6.2 - Dysbalance of the murine lung microbiome by the human pathogenic fungus *Aspergillus fumigatus*

L. Nikitashina[1,2], *X. Chen*[3,2], *L. Radosa*[1], *K. Li*[3,2], *M. Straßburger*[4], *B. Seelbinder*[3], *W. Böhnke*[5,2], *S. Vielreicher*[5,2], *S. Nietzsche*[6], *T. Heinekamp*[1], *I.D. Jacobsen*[5,2], *G. Panagiotou*[3,2], *A.A. Brakhage*[1,2]
[1]Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Department of Molecular and Applied Microbiology, Jena, Germany, [2]Friedrich Schiller

University, Institute of Microbiology, Jena, Germany, [3]Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Department of Microbiome Dynamics, Jena, Germany, [4]Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Transfer Group Anti-infectives, Jena, Germany, [5]Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Research Group Microbial Immunology, Jena, Germany, [6]University Hospital Jena, Electron Microscopy Center, Jena, Germany

The role of the lung microbiome in health and disease remains debated. Here, we demonstrate significant shifts in the lung microbiome and metabolome of mice under immune suppression, following infection with virulent and avirulent strains of *Aspergillus fumigatus*, a major air-borne fungal human pathogen, and after treatment with the antifungal drug voriconazole. Our analysis of the gut-lung axis indicates the crucial role of the gut microbiome for the lung homeostasis mediated through the plasma metabolome. Notably, we observed that invasive aspergillosis induced gut dysbiosis in the mouse model.
In the lung, *A. fumigatus* infection significantly increased abundance of *Ligilactobacillus murinus*, the dominant bacterium in murine lungs, confirmed by isolating live bacteria from the lower respiratory tract. *In vitro*, *L. murinus* is tolerated and even internalized by alveolar epithelial cells. Co-cultivation with *A. fumigatus* enhanced *L. murinus* growth while reducing oxygen levels. This implies that the fungus creates a microaerophilic niche along its hyphae, fostering anaerobic bacterial growth. The fungal-induced promotion of *L. murinus* both *in vivo* and *in vitro* suggests a possible direct impact of *A. fumigatus* on the resident lung bacteria. Further investigation of the lung microbiome during invasive aspergillosis could provide valuable insights into the interdependence of lung microbiota, infection, and the host immune response.

CS 1.6.3 - Role of secretory proteins and pathways in the pathogenicity of Aspergillus fumigatus

J. Gosciniak[1], V. Francis[1], B. McCann[1], C. Duarte-Oliveira[1], R. Fortune-Grant[2], Z. Carter[2], D. Macedo[2], D. Weaver[2], M. Bertuzzi[2], E. Bignell[1]
[1]University of Exeter, Department of Biosciences, Exeter, United Kingdom, [2]University of Manchester, Manchester Fungal Infection Group, Manchester, United Kingdom

Aspergillus fumigatus is the major causative agent of multiple types of human lung diseases named aspergilloses of which invasive pulmonary aspergillosis is associated with alarming mortality rates (40-80%). *A. fumigatus* must establish infection under stressful conditions before residual host immunity can clear the fungus. Secreted and cell surface-associated fungal proteins, as well as the proteins that process them and facilitate their secretion, are vital for host-pathogen interactions that drive adverse pathology in host tissue, however few of the many hundreds of secreted proteins encoded by the *A. fumigatus* genome have been the subject of focussed study. Previously, a transcriptomics approach identified over 300 *A. fumigatus* signal peptide-encoding genes that are upregulated during murine lung infection relative to ungerminated spores grown under laboratory conditions. Subsequent analyses of corresponding null mutants revealed 40 and 36 null mutants exhibiting significantly reduced fitness in corticosteroid and leukopenic mice,

respectively. Here, nine such mutants have been selected for further investigation. Culture filtrates of eight of the mutants exhibited decreased cytolytic killing of epithelial cells, assessed by LDH activity, relative to wild type. Intriguingly one of the encoded proteins is homologous to Lhs1 in *Magnaporthe oryzae* involved in the secretion of virulence factors important to establishing infection of plants. Despite reduced capability to kill epithelial cells, this mutant germinates faster than the wild type but displays reduced fitness relative to wild type during metal ion depletion. Investigations are being made into whether this mutant is involved in the secretion of effectors important for survival and virulence.

CS 1.6.4 - Study of the value of NL1 as an anti-persulfidation and anti-virulence compound in *Aspergillus fumigatus*

Á. González-Camuesco[1], C. Zhao[2], J. Amich[1]
[1]Instituto de Salud Carlos III, Mycology Reference Centre / National Centre for Micorbiology, Majadahonda, Spain, [2]Metropolitan University Manchester, Manchester, United Kingdom

We previously showed that deleting the cystathionine-γ-lyase (CSE) encoding gene in *Aspergillus fumigatus* caused a decrease in the levels of the post-translational modification persulfidation, which altered the functionality of proteins relevant for fungal pathogenicity, and translated into a reduction in its virulence potential [1]. With the aim of targeting persulfidation during infection, and therefore reduce fungal virulence, we are now investigating the capacity of the compound NL1, designed against bacterial pathogens [2], to inhibit *A. fumigatus* cystathionine-γ-lyase.
We firstly performed an *in-silico* analysis and found that NL1 docks well into the fungal enzyme. Similarly to the bacterial proteins there is an aromatic amino acid (H120) close to the ligand binding pocket (which is absent in the human counterpart) which function seems to be blocked by NL1 [2]. Accordingly, NL1 efficiently inhibited recombinantly expressed *A. fumigatus* CSE, but not human CSE, *in-vitro*. As expected, this translated into the capacity to reduce persulfidation levels in *A. fumigatus*, as tested on five different strains. In agreement with previous results with the null mutant, we found that NL1 exposure increased *A. fumigatus* susceptibility to peroxide. In contrast to the reports in bacteria, NL1 did not potentiate the action of antifungal drugs (azoles or amphotericin-B) against wild-type or resistant (TR34-L98H) isolates. At the time of submission of this abstract we are investigating the NL1 capacity to reduce fungal virulence using *in-vitro* and *in-vivo* models of infection.
[1] Sueiro-Olivares et al. PLoS Biol. 2021. Doi: 10.1371/journal.pbio.3001247
[2] Shatalin *et al*. Science 2021. Doi: 10.1126/science.abd8377

CS 1.6.5 - Evolutionary significance of fungal hypermutation

J. Rhodes[1,2], N. van Rhijn[3], Y. Song[4], M. Bottery[3], X. Wang[5], H. Gifford[6], D. Wilson[6], R. Farrer[6], R. Leitao[5], A. Brackin[5], A. Abdolrasouli[7], M. Fisher[5], P. Verweij[2], D. Armstrong-James[5]
[1]University of Birmingham, Birmingham, United Kingdom, [2]Radboudumc, Nijmegen, Netherlands, [3]University of Manchester, Manchester, United Kingdom, [4]Peking University, Beijing, China, [5]Imperial College London, London, United Kingdom, [6]University of Exeter, Exeter, United Kingdom, [7]King's College London Hospital, London, United Kingdom

Elevated spontaneous mutation rates in pathogenic fungi, similar to their bacterial cousins, result in 'hypermutators'. Whilst deleterious mutations may result in a fitness defect, the accumulation of beneficial mutations can confer antifungal drug resistance and host adaptation. This could potentially lead to a serious public health threat when hypermutator infections occur in the clinic. Defects in DNA mismatch repair (MMR) genes result in hypermutator states. We have previously shown that nonsense mutations in MMR genes result in truncated MSH2, MSH5 and RAD5 proteins in *Cryptococcus neoformans*. These nonsense mutations caused an elevated mutation rate, an antifungal drug resistant phenotype, and likely contributed to a relapse infection.
We are now observing (hyper)mutator states in more human pathogenic fungi. Non-synonymous mutations conferring amino acid substitutions in *mre11* in a drug-susceptible background of *Aspergillus fumigatus* also result in a mutator state; over time this could result in spontaneous mutations conferring antifungal drug resistance. Similarly, we have observed non-synonymous and nonsense mutations in MMR genes in major lineages of *C. auris*, resulting in an elevated mutation rate. In some isolates this mutation switches to encode a stop codon, resulting in hypermutation. It is possible that this (hyper)mutator state in *C. auris* is responsible for the rapid evolution of its pan-drug resistance profile, and swift host adaptation and worldwide dispersal.

CS 1.6.6 - The Kynurenine Pathway is required for virulence in *Cryptococcus neoformans* through mitochondrial contributions

C.W. Lee[1], *A. Brisland*[2], *M. Ho*[3], *X. Qu*[3], *L. B. R. Da Silva*[3], *G. Hu*[3], *J. Kronstad*[3]
[1]University of British Columbia, Microbiology, Vancouver, Canada, [2]University of British Columbia, Biology, Vancouver, Canada, [3]University of British Columbia, Vancouver, Canada

The basidiomycete fungus *Cryptococcus neoformans* serves as a useful model for investigating mechanisms of fungal pathogenesis. This pathogen is the causative agent of cryptococcal meningitis in immunocompromised patients and is listed in the critical priority group of the World Health Organization fungal priority pathogens list. Here, we investigate the role of the kynurenine pathway and characterize its role in cryptococcal virulence. The kynurenine pathway (KP) plays a major role in the biosynthesis of nicotinamide adenine dinucleotide (NAD+) and therefore mitochondrial regulation. By generating a collection of kynurenine deletion mutants consisting of bna2Δ (tryptophan 2,3-dioxygenase), bna3Δ (kynurenine aminotransferase), bna5Δ (kynureninase), and bna1Δ (3- hydroxyanthranilate 3,4-dioxygenase), we show that mutants defective in the KP have impaired mitochondrial function and impaired response to reactive oxygen species. Moreover, we show that this pathway contributes to downstream processes regulated by the mitochondria such as iron uptake, glutathione biosynthesis, capsule formation, and cell wall biogenesis. We further characterized impacts on iron uptake mechanisms by analysing the transcriptome of the *bna5*Δ mutant in low and high iron conditions and found significant upregulation of mitochondrial and iron uptake related pathways. All mutants except bna3Δ also displayed significantly reduced capsules compared to the wildtype implying a role in capsule formation. Moreover, the bna5Δ was found to be avirulent in a mice model of cryptococcosis and was significant reduced in fungal burden compared to the wildtype. These

findings highlight key contributions of the KP in mitochondrial function and support a major role for the KP in during cryptococcal infections.

CS 1.6.7 - Coccidioidomycosis, climate change, and risk for future outbreaks

B. Barker[1], *D. Kollath*[1], *D. Wagner*[1], *P. Keim*[1], *J. Sahl*[1], *D. Birdsell*[1], *J. Galgiani*[2], *M. Teixeira*[3], *J. Stajich*[4]
[1]Northern Arizona University, Biological Sciences, Flagstaff, United States, [2]University of Arizona, Medicine, Tucson, United States, [3]University of Brasilia, Brasilia, Brazil, [4]University of California Riverside, Riverside, United States

Coccidioides is a pathogenic genus that causes coccidioidomycosis, aka Valley fever, in arid regions of the Americas. *Coccidioides immitis* causes disease along the Pacific range of North America. *C. posadasii* is found in southwester US and into Mexico and South America. The exact range of *C. posadasii* and its role on human and animal infections in Central and South America is not well defined. The Caribbean region is bordered by the Caribbean Sea, and its surrounding continental landscapes and islands may play an important role in the dispersion of *C. posadasii* across South America through Southeastern Mexico, Honduras, Guatemala, and Venezuela. To better define the distribution and population dynamics of *C. posadasii*, we have prospectively collected clinical isolates from patients in southern Arizona. We performed phylogenomic and population genomics analysis to determine if outbreaks among patients could be detected. Phylogenomic analyses of the *C. posadasii* complex reveal that clinical strains do not appear to have evidence of frequent clonal outbreaks. Population genomics data indicates that limited gene flow exists between neighboring county-level populations in Arizona. We are now assessing the environmental reservoir, to determine if there are certain times of the year or specific conditions that result in higher numbers of reported cases in southern Arizona. Data show that the pathogen is detected in soil and air all year, thus exposure risk appears constant. In summary, southern Arizona is a hotspot for this endemic disease and specific factors affecting growth and outbreaks are being elucidated.

CS 1.6.8 - Host brain environment triggers RNAi epimutation in the human pathogen *Mucor circinelloides*

M. Navarro-Mendoza[1], *C. Pérez-Arques*[1], *E. Cao*[1], *Z. Xu*[1], *Y. Choi*[1], *Z. Chang*[1], *Y.-S. Bahn*[2], *A. Moseman*[3], *J. Heitman*[1]
[1]Duke University, Department of Molecular Genetics and Microbiology, Durham, United States, [2]Yonsei University, Department of Biotechnology, Seoul, Korea, Republic of, [3]Duke University, Department of Integrative Immunobiology, Durham, United States

Mucorales are basal fungi causing severe human infections known as mucormycoses, particularly in immunocompromised and COVID-19 patients. Despite aggressive treatments, mortality can reach 90% in disseminated infections, mainly due to poor diagnosis and antimicrobial drug resistance. *Mucor* species can develop transient antimicrobial drug resistance through RNAi-

based epimutations, adapting to different stresses through a reversible epigenetic mechanism. These epimutations exhibit organ-specific induction, being more prone to arise after central nervous system (CNS) infections. Immunofluorescence cryosections of brain tissue infected with *Mucor* fluorescent strains revealed a specific strong inflammation and granuloma formation compared to other organs. We identified seven loci targeted by RNAi epimutations during brain invasion in mice, named *bep* for brain epimutations (*bepA* to *bepG*). *bepA* encodes a serine-threonine kinase related to Mpk1, involved in cell wall stress responses. *M. circinelloides bepA* mutants were resistant to calcineurin inhibitors FK506 and cyclosporine A, showing mycelial growth in the presence of these inhibitors. On the other hand, *bepA* overexpression reversed this phenotype. This suggests BepA is an antagonist to the phosphatase calcineurin that promotes the yeast-to-hyphal transition. To understand BepA roles in host adaptation, we studied *Mucor* interactions with brain endothelial cells and crossing the blood-brain barrier (BBB). We also monitored infections in immunosuppressed mice, analyzing cryosections of infected organs with *bepA* mutant fluorescent strains. Preliminary data indicate that *bepA* mutants cause more severe BBB damage compared to wild type. Ongoing research will clarify how *bepA* silencing is triggered in the CNS.

CS 1.6.9 - Xenosiderophore transporter gene expression and clade-specific filamentation in *Candida auris* killifish infection

H. Gifford[1], *T. Bedekovic*[1], *N. Helmstetter*[1], *J. Gregory*[1], *Q. Ma*[1], *A.C. Brand*[1], *D. Wilson*[1], *J. Rhodes*[2], *M. Ramsdale*[1], *T. Kudoh*[1], *R. Farrer*[1]
[1]University of Exeter, Exeter, United Kingdom, [2]Imperial College London, London, United Kingdom

Candida auris is an emerging infection and World Health Organisation (WHO) critical priority fungal pathogen. Rising drug resistance, nosocomial outbreaks and diagnostic challenges complicate clinical infections with a patient mortality rate of ~45%. Before now, gene expression profiles of *C. auris* have not yet been described during infection *in vivo*. Wee developed a thermo-relevant fish embryo yolk-sac microinjection model (*Aphanius dispar*; Arabian killifish; AK) that mimics human body temperature, allowing us to interrogate infection dynamics through dual host-pathogen RNA-seq at 24 and 48 h post injection (HPI) at 37 °C across the five major clades (I-V) of *C. auris*. Host gene expression following infection featured heat shock, complement activation, and nutritional immunity, including haem oxygenase (*HMOX*) expression in response to clade IV infection. We identified an *in vivo* transcriptional signature across five clades of *C. auris* that was highly enriched for putative xenosiderophore transmembrane transporters. We describe this newly-discovered seventeen-member xenosiderophore transporter candidate (*XTC*) family in terms of individual gene expression patterns, and a sub-clade of five putative haem transport-related (*HTR*) genes, also up-regulated during infection. Only the basal clade V isolate formed filaments during infection, coinciding with typical and atypical regulators of morphogenesis, including *UME6*, *HGC1*, and the novel adhesin *SCF1*. Clades I and IV demonstrated increased virulence, coinciding with up-regulation of three *HTR* genes in clade IV, and the mating-type locus (*MTL*) non-mating gene *PIKA* in both. *XTC* and *HTR* genes may play critical roles in *C. auris* virulence, making excellent targets for further investigation and potential therapy.

CS 1.6.10 - Mechanisms of evolution of transcription regulation in the fungal lineage

M. Garber[1], *H. Gause*[1], *M. Lohse*[1], *J. Zhang*[1], *A. Johnson*[1]
[1]UCSF, Microbiology and Immunology, San Francisco, United States

For nearly 50 years, it has been theorized that changes in gene expression patterns, rather than the invention of new genes, are a major driver of evolutionary change. Yet, our understanding of transcription circuit evolution remains limited beyond a few well-studied cases. Transcription circuits—composed of transcription regulators, DNA binding sites, and their target genes—evolve rapidly over relatively short evolutionary timescales. In fungi, entire regulatory networks have shifted within 100-200 million years, a timeframe comparable to the divergence of the mammalian lineage. However, we still need a deeper understanding of the diverse mechanisms by which transcription regulators evolve to control new sets of genes while avoiding fitness barrier caused by breaking pre-existing pathways.
In this study, we computationally predict and experimentally test examples of transcription circuit evolution in fungi to explore how transcription regulators acquire regulation of new gene sets. Given that structured DNA binding domains of transcription regulators are preserved by strong selection pressures, we hypothesized that unstructured regions provide a source of plasticity where function-changing mutations can arise neutrally. We found that Gcn4, a key regulator of amino acid starvation, has acquired two new peptide-motifs—short sequences conserved exclusively in the *Candida* clade—that broaden its regulatory function to include new genes. To test these hypotheses, we compared *Candida* Gcn4 to peptide-motif variants in *Candida albicans* using RNA-seq and ChIP-seq experiments, revealing that Gcn4 evolved to regulate new genetic targets involved in pathogenesis. These findings provide critical insights into the mechanisms underlying transcription circuit evolution in fungi.

CS 1.6.11 - Fungal glycogen contributes to *Candida albicans* β-(1→3)-glucan masking, immune activation, and oral commensalism

J. Miao[1,2], *J. Sekar*[2], *A. Hopke*[3], *M. Kruppa*[3], *M. Swidergall*[2], *D. Williams*[3], *B. Peters*[1]
[1]University of Tennessee Health Science Center, Memphis, United States, [2]Lundquist Institute, Los Angeles, United States, [3]East Tennessee State University, Johnson City, United States

Complex carbohydrates are major components of the fungal cell wall and serve as pathogen associated molecular patterns (PAMPs) to potentiate innate immunity. We recently reported that glycogen and β-(1→3)-glucan form a covalently linked macromolecular complex at the *Candida albicans* cell wall. Using a combination of biochemical and genetic approaches, we confirmed that loss of *GSY1* (*i.e.*, glycogen synthase) ablated cell wall glycogen content. Challenge of macrophages with fixed or live *gsy1*Δ/Δ led to exacerbated cytokine secretion (*e.g.*, IL-1β) or neutrophil swarming. Analysis of cell wall components by fluorescence staining revealed that levels of total glucan, total mannan and total chitin remained similar, while reduced glycogen content significantly correlated with increased β-(1→3)-glucan exposure. Antibody-mediated blockade confirmed that exacerbated cytokine release observed in *gsy1*Δ/Δ (and isolated glucan-

glycogen particles) was partially dependent on Dectin-1 signaling. To establish translational impact of our findings, a collection of *C. albicans* clinical isolates was screened for glycogen content, which revealed significant intra-species heterogeneity. Remarkably, overexpression of *GSY1* in a subset of reduced glycogen accumulation isolates reversed their hyperinflammatory phenotype and deletion of *GSY1* in a subset of WT-like glycogen accumulation isolates induced a hyperinflammatory phenotype during human macrophage challenge. Moreover, using an immunocompetent model of oropharyngeal candidiasis, mice inoculated with *gsy1*Δ/Δ in multiple strain backgrounds showed remarkably decreased early fungal burdens. Collectively, our data demonstrate that the glucan-glycogen macromolecular complex may be a novel cell wall determinant important for governing the host-*Candida* interaction and that fungal glycogen content contributes to commensalism in the oral cavity.

CS 1.6.12 - Clade-specific deletion of *TLO* genes in *Candida albicans in AGO1⁺ and Ago1⁻ backgrounds*

A.A. Abu Alhaija[1], *G. Moran*[1], *D. Sullivan*[1]
[1]Dublin Dental University Hospital, Oral Bioscience, Dublin, Ireland

Background: *Candida albicans* is a key fungal pathogen responsible for opportunistic infections. The *TLO* gene family, comprising 14 highly similar genes divided into three clades—alpha, beta, and gamma—plays essential roles in transcriptional regulation, virulence, and antifungal drug tolerance. Recent studies have shown that *TLO* gene expression is regulated by RNA interference (RNAi). Understanding the function of *TLO* genes is critical for elucidating *C. albicans* pathogenesis and developing new therapeutic strategies.
Objective: This study aims to create clade-specific *TLO* knockout strains using CRISPR-Cas9 in strains with functional and non-functional RNAi systems, focusing on their impact on growth, virulence, and antifungal responses.
Methods: A missense mutation in *AGO1* (RNAi pathway) of *C. albicans* SC5314 was corrected using CRISPR-Cas9. Clade-specific gRNAs were designed and used for targeted *TLO* deletions, confirmed via PCR. Knockouts were assessed through growth assays, RNA and long-read sequencing, biofilm assays, drug susceptibility, and metabolic studies.
Results: Correcting the Argonaute gene (wild-type *AGO1+*) had no significant effect on planktonic growth compared to the mutant (*ago1-*) strain, however significant reduction in biofilm formation was observed. *TLO* expression, measured by qRT-PCR, was reduced in the *AGO1+* strain. Deletion of *TLO2* (beta clade) showed no effect on growth under various conditions, but biofilm formation was significantly reduced. Phenotypic analysis of alpha and gamma clade mutants will be presented.
Significance: These data show that the RNAi system and members of the *TLO* family play role in biofilm formation. Ongoing studies focus on creating alpha and gamma clade knockouts to determine clade-specific phenotypes.

CS 1.7.1 - Virulence of banana wilt-causing fungal pathogen *Fusarium oxysporum* tropical race 4 is mediated by nitric oxide biosynthesis and accessory genes

Y. Zhang[1], *C. Li*[2], *D. Mostert*[3], *S. Haridas*[4], *I. Grigoriev*[4], *A. Viljoen*[3], *L.-J. Ma*[5]
[1]Georgia Southern University, Statesboro, United States, [2]Institution of Fruit Tree Research, Guangdong Academy of Agricultural Sciences, Guang Zhou, China, [3]Stellenbosch University, Stellenbosch, South Africa, [4]Joint Genome Institute, Berkeley, United States, [5]University of Massachusetts Amherst, Amherst, United States

Fusarium wilt of banana, caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), is considered one of the most damaging plant diseases in agricultural history. *Foc* race 1 decimated the Gros Michel-based banana trade, and now *Foc* tropical race 4 (TR4) threatens global production of its replacement, the Cavendish banana. Since 2013, *Foc* TR4 has been spreading rapidly from Southeast Asia to the Indian subcontinent, the Middle East, Africa, and South America. To understand the evolutionary history behind its spread and the molecular mechanisms underpinning the resurgence of the Fusarium wilt epidemic on Cavendish bananas, we conducted a comprehensive population genomic analysis using 36 geologically and genetically diverse *Foc* strains. These strains formed three distinct populations, with the race 4-only population including *Foc* TR4 strains and other race 4 strains. Population structures as well as genomics data suggest that *Foc* TR4 was derived from a sexually reproducing population. Analysis of the *Foc* TR4 genome revealed accessory sequences enriched for pathogenesis- and mitochondrial-related functions. Meta-transcriptomics revealed the unique induction of the entire mitochondrion-localized nitric oxide (NO) biosynthesis pathway upon TR4 infection. Knocking out genes involved in the fungal NO biosynthesis pathway significantly reduced NO production in mutants and fungal virulence, indicating that the induction of a NO burst contributes to TR4 infectivity. Collectively, our study points to a unique evolutionary origin for *Foc* TR4, with its virulence mediated by NO biosynthesis and accessory genes.

CS 1.7.2 - Identification and characterization of a large multigene family of cooperating effector genes facilitating cell-to-cell mobility conserved in Dothideomycetes and Sordariomycetes

N. Talbi[1,2], *M. Blekemolen*[3], *L. Fokkens*[3], *C. Audran*[4], *Y. Petit-Houdenot*[2], *C. Pouzet*[5], *T. Rouxel*[2], *M.-H. Balesdent*[2], *M. Rep*[3], *F.L.W. Takken*[3], *I. Fudal*[2]
[1]Paris-Saclay University, Palaiseau, France, [2]INRAE, BIOGER, Palaiseau, France, [3]University of Amsterdam, Molecular Plant Pathology, Amsterdam, Netherlands, [4]INRAE, LIPME, Castanet-Tolosan, France, [5]CNRS, FRAIB-TRI, Castanet-Tolosan, France

With the increasing availability of high-quality fungal genomes, effectors conserved among species and genera have been uncovered. Two avirulence effectors, *AvrLm10A* and *AvrLm10B*, of *Leptosphaeria maculans*, responsible for stem canker of oilseed rape, are members of such a large family of conserved effectors. *AvrLm10A* and *AvrLm10B* are neighboring genes in divergent transcriptional orientation. Sequence searches within the *L. maculans* genome showed

that *AvrLm10A*/*AvrLm10B* belong to a multigene family comprising five pairs of genes with similar tail-to-tail organization, specifically expressed during biotrophic stages of infection. Two of the corresponding protein pairs, including AvrLm10A and AvrLm10B, have the ability to physically interact. AvrLm10A homologues were identified in more than 30 Dothideomycete and Sordariomycete plant-pathogenic fungi. One of them, SIX5, is an effector from *Fusarium oxysporum* f.sp. *lycopersici* (*Fol*) physically interacting with the avirulence effector Avr2 and required for the movement of Avr2 from cell-to-cell through plasmodesmata. We demonstrated that members of the AvrLm10A family in *L. maculans* can complement SIX5 function in plant cell-to-cell mobility assays and *Fol* virulence. We found that AvrLm10A/SIX5 homologues were associated with at least eight distinct effector families, suggesting an ability to cooperate with different effectors. These results point to a general role of the AvrLm10A/SIX5 proteins as "cooperator proteins".

CS 1.7.3 - Elucidating population structure, genetic diversity and host specificity of *Alternaria alternata* from diverse regions and wild and domestic host plants

G. Clasen[1], *R. Stam*[1], *Ž. Ivanović*[2]
[1]Christian-Albrechts-University Kiel, Chair of Phytopathology, Kiel, Germany, [2]Department of Plant Disease, Institute for Plant Protection and Environment, Belgrade, Serbia

Alternaria spp. are cosmopolitan, ubiquitous necrotrophic fungal pathogens that infect a broad range of hosts. They cause early blight and leaf spot disease, which induce significant harvest losses.
We collected suspected *Alternaria*-infected leaves from potatoes, tomatoes, and wild hosts in three different climate zones and from different farm types at sites equal in distance in Serbia and Germany.
Using molecular markers, we identified the pathogens. The infections were caused predominantly by small-spored species of *Alternaria* of the section *Alternaria*, like *Alternaria alternata*. Morphological observations confirmed the molecular identification.
The sequencing of the three molecular markers Alt A1, RPB2 and GAPDH reveals an equal distribution of five haplotypes of section *Alternaria* for all host plants, farm types, and climate zones.
Comparative genetic diversity analyses such as Pi and Tajima´s D also show a similar genetic diversity for isolates from different host plants.
Using only these three markers, *Alternaria* populations seem to be genetically heterogeneous at a continental scale and across hosts and climates, indicating the pathogen's ability to thrive in diverse environments.
Nevertheless, phenotyping by detached leaf assays revealed a higher virulence in isolates originating from wild host plants.
Whole-genome sequencing of 220 *A. Alternata* isolates from our sample set further elucidates their populations admixture, the influence of the host and potential for gene transfer or recombination.
The widespread occurrence of distinct genotypes, combined with evidence for admixture and

recombination, suggests a higher adaptive capacity in this pathogen, which poses challenges for disease management.

CS 1.7.4 - DNA repair mechanisms generate cryptic diversity in the invasive oomycete pathogen *Phytopthora ramorum*

J. LeBoldus[1], *S. Chase*[2], *M. Sudermann*[2], *A. Carson*[3], *N. Cauldron*[4], *J. Chang*[2], *N. Grunwald*[5]
[1]Oregon State University, Botany and Plant Pathology & Forest Engineering, Resources and Management Department, Corvallis, United States, [2]Oregon State University, Botany and Plant Pathology, Corvallis, United States, [3]Oregon State University, Forest Engineering, Resources and Management Department, Corvallis, United States, [4]Brown University, Deaprtment of Molecular Micorbiology and Immunology, Providence, United States, [5]USDA, Horticultural Crop Disease and Pest Management Unit, Corvallis, United States

Phytophthora pathogens continue to emerge on diverse hosts causing major diseases. The invasive oomycete, *Phytophthora ramorum*, is responsible for sudden oak death, causing disease of more than 170 plant species across the United States and Europe. The pathogen exists as 4 distinct clonal lineages which reproduce asexually. The emergence of this pathogen globally in both nursery and forest ecosystems is poorly understood. We will focus on isolates collected from Oregon, where the NA1 clonal lineage of *P. ramorum* was first discovered in 2001. Two more lineages, EU1 and NA2, were discovered in Oregon more recently. We characterized invading populations sampled during the five years following first introduction, between 2001-2005 for NA1 and 2015-2019 for EU1, using whole-genome sequencing. In addition, we downloaded *P. ramorum* genomes from the NCBI SRA database. A total of 725 genomes were analyzed for diversity and adaptive potential revealing that the NA1 lineage has accumulated runs of homozygosity (ROH) at greater frequency compared to either EU1 or NA2. These differences in ROH correlate to phenotypic changes in sporulation and disease severity. We hypothesize that the mechanisms of DNA repair have been altered in the NA1 lineage, and correlate to expression of transcripts involved in double stranded break repair (e.g. XRCC4). Recently, we have developed an approach to experimentally induce ROH to better study this phenomenon under controlled conditions.

CS 1.7.5 - Lem2-mediated tethering of telomeres to the nuclear periphery is essential for *Ustilago maydis* pathogenesis

E. Sanz Marti[1], *J.I. Ibeas Corcelles*[1], *R. Ramos Barrales*[1]
[1]Universidad Pablo de Olavide/ Centro Andaluz de Biología del Desarrollo, Departamento de Biología Molecular e Ingeniería Bioquímica, Sevilla, Spain

The transcriptionally silent heterochromatin is predominantly located at the nuclear periphery, anchored mainly by lamina-associated proteins (LAPs). This peripheral localization of heterochromatin is essential for its complete silencing and has important implications in cell fate and development. However, its impact on fungal pathogenesis remains unexplored. In this study, we investigate the role of the LAP homolog Lem2, involved in the perinuclear tethering of

centromeres and telomeres and their silencing in yeast, in the pathogenesis of *Ustilago maydis*. Our results demonstrate that Lem2 is essential for virulence, specifically for plant penetration, as most of the Δ*lem2* mutant filaments become arrested at the appressorium stage, a specialized structure involved in penetration. We found that the Δ*lem2* mutant exhibited impaired nuclear migration and telomere detachment from the nuclear periphery. Interestingly, nuclear migration issues are partially restored when we artificially tether telomeres to the nuclear periphery in a Δ*lem2* mutant, suggesting that telomere attachment to the nuclear envelope is essential for nuclear migration from the appressorium to the penetrating filament. To investigate why telomere detachment prevents nuclear migration, we performed a transcriptomic analysis during filamentation and found that the Δ*lem2* mutant shows upregulation of G1-S specific genes and several kinases involved in cell cycle checkpoints that could explain the nuclear migration block. Overall, our data not only enhances our understanding of fungal virulence but also opens new avenues for exploring how chromatin location can shape pathogen behavior.

CS 1.7.6 - Dihydroxyhexanoic acid biosynthesis controls turgor in pathogenic fungi

N. Kumakura[1], *T. Motoyama*[2], *K. Miyazawa*[3], *T. Nogawa*[2], *Y. Katsuma*[1,4], *K. Sakai*[5], *N. Ishihama*[1], *K. Matsumori*[3], *P. Gan*[1], *H. Koshino*[2], *T. Fukuma*[3], *R.J. O'Connell*[5], *K. Shirasu*[1,4]
[1]RIKEN, Center for Sustainable Resource Science, Yokohama, Japan, [2]RIKEN, Center for Sustainable Resource Science, Wako, Japan, [3]Kanazawa University, Faculty of Frontier Engineering, Kanazawa, Japan, [4]The University of Tokyo, Bunkyo, Japan, [5]Université Paris-Saclay, UR BIOGER, Palaiseau, France

Many plant pathogenic fungi penetrate the host surface mechanically using turgor pressure, especially through specialized cells called appressoria. These cells accumulate osmolytes in their cytoplasm to create turgor by osmosis, and develop a semipermeable cell wall, which excludes molecules larger than water. While melanin provides the wall with physical rigidity to withstand high turgor pressures, the mechanisms conferring wall semipermeability to restrict osmolyte leakage remain unclear. Here, we identify a pair of secondary metabolite biosynthetic enzymes, PKS2 and PBG13, as essential components in the formation of the appressorial semipermeable barrier in both anthracnose fungi (*Colletotrichum* species) and the rice blast fungus (*Magnaporthe oryzae*). We demonstrate that these enzymes are involved in synthesizing 3,5-dihydroxyhexanoic acid polymers that are critical for the semipermeable properties of the cell wall. Deletion of these enzymes reduces both pathogenicity and appressorial turgor by impairing the reduction of cell wall porosity, with no detectable effect on appressorial melanization. This links enzyme function directly to pathogen penetration ability and disease potential. Our findings reveal a hitherto undescribed mechanism of turgor generation in these major crop pathogens and identify potential new targets for disease control.

CS 2.1.1 - Armed with the ‘Red Button’ - How does a fungal pathogen activate its necro ('nuclear') trophic effector arsenal?

K.-C. Tan[1], *E. John*[1,2], *C. Verdonk*[1], *K. Singh*[1,3], *R. Oliver*[4], *L. Lenzo*[1], *S. Morikawa*[1], *J. Soyer*[5], *J. Muria-Gonzalez*[1], *D. Soo*[1], *C. Mousley*[6], *S. Jacques*[1]
[1]Curtin University, School of Molecular and Life Sciences, Perth, Australia, [2]University of Bonn, Bonn, Germany, [3]Commonwealth Scientific and Industrial Research Organisation, Perth, Australia, [4]University of Nottingham, Nottingham, United Kingdom, [5]Université Paris-Saclay, Paris, France, [6]Curtin University, Curtin Health Innovation Research Institute, Perth, Australia

Fungal pathogens cause large crop losses worldwide. These pathogens use effectors, which require coordinated expression at specific stages of the pathogenic lifecycle, to manipulate the host plant metabolism in favour of infection. The Zn_2Cys_6 class Pf2 transcription factor (TF)-orthologue family underpins virulence and necrotrophic effector gene expression in several major fungal pathogens including *Parastagonospora nodorum*, the causal agent of septoria nodorum blotch of wheat. We used a combination of reverse genetics, transcriptomics and chromatin-immunoprecipitation sequencing (ChIP-Seq) to define the role of Pf2 in the pathogenicity of *P. nodorum* on wheat. We observed that Pf2 functions as a positive regulator of several necrotrophic effectors and is required for host genotype-specific virulence. In addition, Pf2 regulates a suite of genes that are associated with the necrotrophic lifestyle on the host such as nutrient assimilation and plant cell wall degradation. ChIP-Seq analyses revealed a functional DNA motif sequence at the promoter region of major effector genes and other putative virulence genes where Pf2 binds to control gene expression. Alluding to the title, Pf2 is analogous to a doomsday device where it plays a major role in coordinating the production of fungal effectors and other virulence factors that cause tissue necrosis as a vanguard during wheat infection.

CS 2.1.2 - Investigating the role of secreted phospholipases in fungal virulence; the case of Verticillium longisporum

V. Rafiei[1], *E. Piombo*[1], *H. Velez*[1], *G. Tzelepis*[1]
[1]Swedish University of Agricultural Sciences, Forest Mycology and Plant Pathology, Uppsala, Sweden

Phospholipases are ubiquitous enzymes capable of hydrolyzing phospholipids. They play a crucial role in several biological processes, but their involvement in fungal-plant interactions remains understudied. In this study, the role of a secreted phospholipase A2 ($VIPLA_2$) was investigated in the soil-borne plant pathogenic fungus *Verticillium longisporum*, a global threat to rapeseed crops worldwide. The *$VlPLA_2$* gene encodes an active phospholipase A2, and transient expression of this gene in *Nicotiana benthamiana* plants resulted in increased production of certain phospholipids. In addition, $VlPLA_2$ was able to suppress the chitin-induced ROS burst and hypersensitive response (HR) triggered by a PRR complex. Furthermore, fungal *$VlsPLA_2$* overexpression strains showed increased virulence and induction of fungal genes with a confirmed role in pathogenicity. Confocal microscopy showed that VlPLA2 was initially localized in the plant nucleus, whereas it was translocated to the chloroplasts at later time points.

In addition, our results showed that VIPLA$_2$ can bind to vesicle-associated membrane proteins A (VAMP-A) and is transported to the nuclear membrane, where it causes major changes in genes known for their role in plant immunity. In conclusion, we have shown that this phospholipase is a virulence factor that co-regulates the induction of virulence factors in fungal cells. It also appears to hijack host VAMP-A proteins to facilitate entry into the nucleus, where it hydrolyzes phospholipids from the nuclear membrane. This enzymatic action may act as a signaling cascade that suppresses basal plant immune responses, suggesting a critical role in early stages of the fungal infection process.

CS 2.1.3 - Functional analysis of secreted long non-coding RNA contributing to the virulence of the corn smut fungus *Ustilago maydis*

Y. Tanaka[1], H. Ohtani[1], F. Ishida[1], M. Yamaguchi[1], R. Yoshimoto[1], S. Tanaka[1]
[1]Setsunan University, Faculty of Agriculture, Hirakata, Japan

Plant pathogenic fungi secrete and deliver effector molecules into plant cells to establish parasitism. To date, most studies have focused primarily on proteinaceous molecules. Here, we report that the corn smut fungus, *Ustilago maydis*, secretes hundreds of long non-coding RNAs (lncRNAs) via extracellular vesicles. These lncRNAs are transcribed from intergenic regions of the genome and are found to be highly expressed. Many of them appear to be non-polyadenylated. The expression of some lncRNAs is specifically upregulated during plant infection. We created knockout strains for a group of highly expressed lncRNAs and performed plant inoculation experiments. Among these, the deletion mutant of *lncRNA_5109* exhibited the most significant reduction in pathogenicity. The complementation strain, created by introducing a genomic fragment of the intergenic region containing *lncRNA_5109*, showed transcript accumulation and restored pathogenicity. When the upstream region of *lncRNA_5109* was deleted, its expression significantly decreased, indicating that this region functions as a promoter. To clarify the function of *lncRNA_5109*, we conducted a pull-down assay using *in vitro*-transcribed RNA and cell lysate extracted from infected plant leaves. Through proteomic analysis of the precipitates, we identified maize LSm4, which may play a role in pre-mRNA splicing, as the most significant interaction partner of *lncRNA_5109*. Furthermore, the interaction was also verified *in vitro* through a pull-down assay using recombinant LSm4 protein and *lncRNA_5109*. These results suggest that *U. maydis* utilizes lncRNA to modulate host mRNA metabolism to promote virulence.

CS 2.1.4 - *Zymoseptoria tritici* effector diversity and interactions wheat resistance proteins

S. Karki[1], P. Pilo[1], C. Lawless[1], N. Mastrodimos[1,2], A.M. Tiley[1,3], E. Curran[1,4], D. Dutta[5], H. Gibriel[1], J. Burke[1], A. Feechan[5,1]
[1]University College Dublin, Dublin, Ireland, [2]University of California, Davis, California, United States, [3]Plant Science Division Department of Agriculture, Food and the Marine, Ireland, Dublin, Ireland, [4]South East Technological University, Carlow, Ireland, [5]Heriot-Watt University, Edinburgh, United Kingdom

Zymoseptoria tritici is an ascomycete fungus and the causal agent of the economically important disease of Septoria tritici leaf blotch (STB), which can cause significant yield losses in wheat. *Z. tritici* secretes an array of fungal effector proteins that facilitate host infection, colonisation and pycnidia production. The *Z. tritici* effector *Zt-11* is a small secreted protein upregulated during the transition of the pathogen from the biotrophic to necrotrophic phase of wheat infection. Deletion of *Zt-11* delayed disease development in wheat and reduced the number and size of pycnidia, as well as the number of macropycnidiospores produced by *Z. tritici*. Therefore, Zt-11 may play a role in *Z. tritici* aggressiveness and STB disease progression possibly via a salicylic acid associated pathway. Zt11 was previously shown to interact with the wheat small secreted proteins TaSSP6 and TaSSP7 and TaSRTRG6 (*Triticum aestivum* Septoria Responsive Taxonomically Restricted Gene) required for resistance. Collection and analysis of UK field isolates demonstrates that *Zt-11* is diverse. Currently we are investigating the Zt-11 haplotypes and isoforms and how Zt11 isoforms might impact the interaction with wheat host resistance proteins.

CS 2.1.5 - A fungal transcription factor *CtBOT6* converts a beneficial root endophyte *Colletotrichum tofieldiae* into an anthracnose leaf pathogen

R. Ujimatsu[1], *J. Takino*[2], *S. Aoki*[3], *M. Nakamura*[1], *H. Haba*[1], *A. Minami*[4], *K. Hiruma*[1]
[1]The University of Tokyo, Department of Life Sciences, Multidisciplinary Sciences, Tokyo, Japan, [2]Hokkaido University, Department of Chemistry, Sapporo, Japan, [3]The University of Tokyo, Department of Integrated Biosciences, Chiba, Japan, [4]Institute of Science Tokyo, Department of Chemistry, Tokyo, Japan

Plant endophytic fungi inhabit their host plants asymptomatically for most of their lifecycle; however, under certain host environments, they can trigger disease, implying their ability to switch lifestyles by modulating virulence factors. The root endophytic fungus *Colletotrichum tofieldiae* (Ct) promotes plant growth under low phosphate conditions, whereas a certain strain inhibits plant growth. Such pathogenic lifestyle is partly attributed to the specific activation of a secondary metabolite biosynthesis gene cluster, designated ABA-BOT cluster, during root colonization. However, the mechanisms by which Ct regulates its virulence, both through the ABA-BOT and the others, and how plants respond to this regulation, remain enigmatic. Here, we identify *CtBOT6*, a transcription factor within the ABA-BOT, as a pivotal regulator of fungal virulence and an inducer of extensive host gene reprogramming. Using Ct*Δbot6* knockout and transgenic lines with varying levels of *CtBOT6* expression in pathogenic and/or beneficial Ct, we show that *CtBOT6* regulates gene expression not only the ABA-BOT but also other virulence-associated genes, such as effectors and carbohydrate-active enzymes. Importantly, *CtBOT6* expression levels are strongly correlated with virulence, and its activation is sufficient to convert the beneficial Ct strain into a leaf anthracnose pathogen, facilitating the completion of its asexual lifecycle. In strong correlation with the observed virulence levels, Ct triggers plant responses, including defense and senescence in both roots and shoots —hallmarks of necrotrophic fungal infection—partially dependent on the host's abscisic acid pathway. Our findings suggest that *CtBOT6* regulates Ct lifestyles along the mutualistic-necrotrophic continuum.

CS 2.1.6 - When timing is everything: coordination of pathogenic development by feedback regulation between unfolded protein response and mating-type signaling in *Ustilago maydis*

K. Heimel[1]
[1]University of Göttingen, Microbial Cell Biology, Göttingen, Germany

Pathogenic development of the corn smut fungus *Ustilago maydis* is controlled by mating-type-regulated networks and involves a complex series of morphogenetic transitions. A prerequisite for plant infection is the fusion of two compatible haploid sporidia to form a dikaryotic filament that enters plant cells via appressoria. While the initial mating process is regulated by the pheromone/receptor system encoded by the *a*-mating type locus, all subsequent steps of pathogenic development are controlled by a transcription factor network downstream of the heterodimeric bE/bW transcription factor encoded by the *b*-mating type locus. This includes filamentous growth, appressoria formation, effector gene expression and also subsequent fungal proliferation inside of the host plant. The latter step, however, requires modification of this regulatory network by the Clp1 protein, which accumulates specifically after successful plant penetration, thereby linking fungal development to the plant environment. We showed that Clp1 is stabilized upon interaction with the central regulator of the unfolded protein response. The UPR is thought to be activated in response to the massive induction of more than 200 effector-encoding genes at this stage of pathogenic growth. Hence, UPR activation links effector gene expression to coordinated fungal proliferation inside the plant. Conversely, premature UPR activation blocks pathogenic development prior plant penetration. This is based on the inhibition of MAP kinase signaling within the pheromone response cascade, ultimately reducing activity of the regulatory a- and b-mating type network. We propose that the negative UPR-to-mating type feedback alleviates effector gene expression during fungal proliferation in planta to ensure ER homeostasis.

CS 2.1.7 - X-ray microtomography and MAP kinase signaling reveal distinct stages of *Fusarium oxysporum* root invasion and colonization patterns

V. Guastaferro[1], *S. Vitale*[1], *L. D'Amico*[2], *E. Longo*[2], *G. Tromba*[2], *N. Minc*[3], *M. Delarue*[4], *L. Di Costanzo*[1], *D. Turra*[1]
[1]University of Naples "Federico II", Department of Agricultural Sciences, Portici, Italy, [2]Elettra – Sincrotrone Trieste S.C.p.A., AREA Science Park, Basovizza, Italy, [3]Institut Jacques Monod, Paris, France, [4]LAAS-CNRS, Université de Toulouse, CNRS, Toulouse, France

Soil-borne fungal vascular pathogens are major agricultural threats, yet their precise root invasion mechanisms remain poorly understood. Here, we employed fluorescence microscopy, X-ray micro-computed tomography (micro-CT), nanofabrication, and reverse genetics approaches to investigate how *Fusarium oxysporum* invades and colonizes plant roots. Using chemically inert biomimetic platforms, we demonstrate that fungal hyphae can both follow surface ridges and adapt to confined spaces through thigmodifferentiation, forming extremely thin filaments similar

to those observed growing between plant epidermal cells. This invasion process is regulated by distinct MAP kinase signaling cascades: the Fmk1 pathway controls initial root penetration, while the High-Osmolarity Glycerol (HOG) pathway facilitates apoplastic colonization, likely in response to varying osmotic conditions across root tissues. We show that hyphae can penetrate and extend through sub-micrometric pores characteristic of the plant apoplast, ultimately accessing xylem vessels. Notably, X-ray microtomography enabled non-destructive visualization of intricate fungal colonization patterns typically challenging to observe through conventional imaging methods. These findings advance our understanding of the physical and molecular mechanisms governing soil-borne pathogen-root interactions and may inform targeted disease management strategies.

CS 2.1.8 - Remaining undetected: balancing plant cell wall degradation and elicitors of host immunity by the wheat pathogen *Z. tritici*

A. Sanchez Vallet[1], *C. Carrasco Lopez*[1], *S. Lopez Cobos*[1], *L. Meile*[1], *I. Vegas*[1], *D. Rebaque*[2]
[1]Polytechnic University of Madrid, Center for Plant Biotechnology and Genomics, Madrid, Spain, [2]University of Leon, Leon, Spain

Plant cell walls constitute a major defense barrier against pathogens; however, how specific cell wall components impact pathogen colonization is unclear. Pathogens secrete cell wall degrading enzymes to facilitate colonization, but damaged cells are often a source of cell wall-derived oligosaccharides that trigger host immunity and hinder pathogen infection. Here we functionally characterized a glycosyl hydrolase (*Zt*GH54) from the wheat pathogen *Zymoseptoria tritici*. *ZtGH54* is expressed during the necrotrophic phase and presumably contributes to nutrient acquisition. However, disruption of *ZtGH54* enhances *Z. tritici* virulence. The potential oligosaccharides released by ZtGH54 from the wheat cell wall activate wheat immune responses and hinder pathogen infection. The results demonstrate that tight regulation of *ZtGH54* is critical for the infection process to prevent early accumulation of plant cell wall-derived elicitors that would prematurely induce host immunity and impair fungal virulence. We suggest that the balance between plant cell wall degradation and the release of immunogenic wall-derived oligosaccharides by fungal cell wall degrading enzymes governs the outcome of plant-pathogen interactions.

CS 2.1.9 - Undermining the “cry for help”: the phytopathogenic fungus Verticillium dahliae secretes a novel antimicrobial to undermine host recruitment of antagonistic Pseudomonas bacteria

W. Punt[1], *A. Kraege*[1], *A. Doddi*[1], *J. Zhu*[1], *K. Wippel*[2], *N. Schmitz*[1], *N. Snelders*[1], *B. Thomma*[1]
[1]University of Cologne, Institute for Plant Science, Cologne, Germany, [2]University of Amsterdam, Swammerdam Institute for Life Sciences, Amsterdam, Netherlands

Plant pathogens secrete small cysteine-rich proteins called effectors during host colonization to promote disease development through various mechanisms. While effectors were typically thought to target host physiology, including host immune responses, we discovered that some

effectors target the microbes that live on and in the plant to promote host colonization. More specifically, the soil-borne fungal plant pathogen *Verticillium dahliae* secretes several effectors with selective antimicrobial activity that target microbial antagonists in the microbiota of its plant hosts. We now demonstrate the functional characterization of a novel effector that displays antimicrobial activity, named Av2. Deletion of *Av2* compromises the virulence of *V. dahliae in planta*, as tomato plants inoculated with the wild-type strain developed stronger symptoms of disease than plants inoculated with the deletion strain. Intriguingly, inoculation experiments using a gnotobiotic plant cultivation system revealed that the virulence contribution of Av2 is microbiota-dependent. To investigate the effect of Av2 during host colonisation we conducted amplicon sequencing and found that Av2 modulates the host microbiota by specifically supressing the recruitment of Pseudomonadales. Co-cultivation experiments revealed that *Av2* deletion leads to a reduction of *V. dahliae* growth in the presence of particular plant-associated *Pseudomonas spp.* Furthermore, the same antagonistic *Pseudomonas* spp. bacteria display sensitivity to Av2 *in vitro*. Altogether, we propose that *V. dahliae* secretes Av2 to manipulate the microbiota of its host plants by undermining the "cry for help" recruitment of beneficial *Pseudomonas spp.* to ultimately promote disease development.

CS 2.1.10 - Molecular mimicry of plant cell-surface immune receptors by fungal secreted leucine-rich repeat proteins

G. Kettles[1], *H. Ali*[1], *E. Thynne*[2], *E. Stukenbrock*[2]
[1]University of Birmingham, Birmingham, United Kingdom, [2]Christian-Albrechts University, Kiel, Germany

The wheat-infecting fungus *Zymoseptoria tritici* undergoes a lengthy symptomless infection phase before the appearance of disease lesions. It deploys secreted proteins (effectors) throughout infection to manipulate host physiology. In a recent Agroexpression screen of candidate effectors in *N. benthamiana*, we demonstrated that numerous effector candidates were able to (i) suppress cell death, (ii) suppress ROS production, or (iii) interfere with normal stomatal dynamics, suggesting how this fungus might suppress host immunity during early colonisation. One of these effectors (ZtLRR) was notable for being able to suppress all tested immune responses and for inducing stomatal closure. ZtLRR encodes a single leucine-rich repeat (LRR) domain and structural predictions for this effector are strikingly similar to the ectodomains of several well-characterised cell-surface plant immune receptors. In addition, targeted yeast two-hybrid experiments indicated that ZtLRR was able to interact with the ectodomain of the co-receptor BAK1, suggesting that by mimicking the structure of a plant immune receptor, ZtLRR is able to interfere with immune complex formation at the cell-surface/apoplast interface. Recently, we generated transgenic wheat lines expressing ZtLRR and found that immune suppression was also observed in wheat. Secreted LRR proteins are found in many fungal species, therefore we cloned and expressed ZtLRR orthologs from 15 other fungi and found that several of these proteins also had potent immune suppressing properties. This indicates that mimicry of host immune receptors by fungal LRR effectors may be an evolutionary ancient strategy for host manipulation.

CS 2.1.11 - Glutathione mediated redox state is important for *Ustilago maydis* growth, melanin formation and virulence

M. Kretschmer[1], *D. Damoo*[1], *K. Heimel*[2], *J. Kronstad*[1]
[1]University of British Columbia, MSL, Vancouver, Canada, [2]Georg-August-University, Goettingen, Germany

Ustilago maydis is the causal agent of corn smut disease. We recently showed that a combination of glucose plus the organic acid malate (G+M) activated the genetic biotrophy program in *U. maydis*. Observed phenotypic changes in G+M included accelerated growth, extracellular matrix and melanin formation usually only observed during sporulation. The glutathione biosynthesis pathway and the iron-regulon regulator Grx4, which has iron-sulfur clusters and GSH as cofactors, were differently regulated in G+M. Thus, we deleted the glutathione synthetase (Gsh2) and analyzed its impact on growth, melanin formation, stress response and virulence of *Ustilago maydis*. *Δgsh2* showed a growth defect on glucose, which could partially be rescued by addition of iron, malate and reduced GSH (GSHred). This links iron and glutathione metabolism with growth on G+M. Surprisingly, 0.5 mM GSHred was toxic to *Ustilago* and induced oxygen dependent melanin formation in glucose medium comparable to Gsh1 inhibition by BSO. The *Δgsh2* mutant further showed sensitivity to heat, high pH and ROS. ROS is mainly formed during respiration and the toxicity of GSHred could be linked via Antimycin A to the mitochondrial electron transport chain complex III, while *Δgsh2* itself showed sever sensitivity to KCN induced complex IV inhibition. The *Δgsh2* mutant further showed reduced virulence which could be rescued by low concentration of GSHred. Interestingly, GSH metabolism inhibition with BSO as well as high concentrations of GSHred also reduced virulence of *Ustilago maydis*. In conclusion we link redox state via ETC functions to growth, melanin formation and virulence in *U. maydis*.

CS 2.1.12 - Effectoromics and glucosinolate resistance in Botrytis cinerea

M. Levy[1], *D. Aditya Srivastava*[1], *D. Vela-Corcia*[1,2], *A. Dafa-Berger*[1]
[1]Hebrew University of Jersalem, Plant Pathology and Microbiology, Rehovot, Israel, [2]University of Málaga, The Institute for Mediterranean and Subtropical Horticulture, Malaga, Spain

Glucosinolates (GSs) are secondary metabolites that accumulate mainly in cruciferous plants. They are relatively nonreactive, hydrophilic, nonvolatile compounds that are stored in the plant vacuole. Upon plant injury, GSs are rapidly hydrolyzed by myrosinases to a multitude of physiologically active products, including isothiocyanates (ITCs), thiocyanates and nitriles. GS hydrolytic products have antifungal properties and play a role in plants' resistance against pathogens. *Botrytis cinerea*, a necrotrophic pathogen, has variable sensitivity to glucosinolates. *Botrytis cinerea* is a major plant pathogen known to infect more than 200 plant species including agricultural important crops. Transcriptomic analysis of *B. cinerea* after exposure to ITC revealed unique mechanisms utilize by the fungi to detoxify ITCs using specific MFS transporter, the enzyme b-lactamase and several putative effectors. We characterize the *mfsG* transporter protein and show that it is essential for ITCs efflux and pathogenicity in cruciferous plants. We also

characterize specificity and activity of β-lactamase enzyme *in- vitro* and demonstrated reduction in pathogenicity of *B. cinerea* on crucifer plants due to loss of this enzyme. We further showed that activation of genes involved in oxidative stress response of *B. cinerea* due to presence of ITCs correlated with increasing in reactive oxygen species and triggering of programed cell death. Additionally, we could identify and characterized several putative effectors that involved in GS detoxification. Understanding the molecular basis of *B. cinerea* resistance to overcome glucosinolate is essential for the development of new strategies for plant defense.

CS 2.2.1 - Unlocking genetic potential in *Trametes versicolor* to optimize mycelium-based biomaterials

J. Saluena[1], *E. Schunselaar*[2], *S. Vandelook*[1], *L. Lugones*[2], *H. Wösten*[2], *E. Peeters*[1]
[1]Vrije Universiteit Brussel, MICR, Ixelles, Belgium, [2]Utrecht University, Microbiology, Utrecht, Netherlands

Mycelium materials are gaining traction as sustainable alternatives to conventional materials (e.g., leather, polyesters, building materials) due to their versatility and novel range of properties. Despite growing industrial interest, scalability remains a key challenge for their widespread adoption. A major hurdle for their implementation is the limited understanding of the genetic mechanisms that influence the physiological traits underlying material properties. Along with growth conditions and downstream treatment, these properties heavily depend on the selected fungal strain. High-performing strains for material production often belong to the Polyporales order, where genomic knowledge is scarce and molecular tools are underdeveloped.
In this study, we aim to develop a genome-editing system for *Trametes versicolor* using CRISPR-mediated transformation of protoplasts. Protoplasts are generated via enzymatic digestion, and long homology arms are employed to target genes from the Ku family, which are crucial to the Non-Homologous End-Joining (NHEJ) DNA repair pathway. With enhanced relative frequency of homologous recombination, the resulting knockouts will enable more accurate and reproducible genetic modifications. This strain with improved engineerability will serve as a robust platform for studying molecular genetics in Polyporales species. Future research will focus on identifying genes that contribute to material properties, with the goal of not only improving material performance, but also enabling the fabrication of mycelium materials with new functionalities.

CS 2.2.2 - Development of a YTK-compatible secretion toolkit for the industry-relevant yeast *Kluyveromyces marxianus*

C. Belloch Molina[1,2], *A. Kishkevich*[3], *R. Romano*[1,4], *T. Ellis*[3], *M. Sousa Gallagher*[2,4], *J. Morrissey*[1,4]
[1]University College Cork, School of Microbiology, Cork, Ireland, [2]University College Cork, School of Engineering & Architecture, Cork, Ireland, [3]Imperial College London, Imperial College Centre for Synthetic Biology (IC-CSynB), London, United Kingdom, [4]University College Cork, Centre for Sustainable Fermentation and Bioprocessing Systems for Food and the Bioeconomy (SUSFERM), Cork, Ireland

In the context of sustainable biotechnology there is great interest in the industrial production of heterologous proteins. Secreted proteins are preferred since their recovery during downstream processes is easier. In many microorganisms, including fungi, secretion is mediated by short amino acid sequences that precede a protein, namely secretion signals. Synthetic biology allows the design and engineering of biological systems and can be applied to optimise protein secretion. Different synthetic biology tools allow the heterologous expression of proteins in fungi and yeasts. In particular the Golden Gate Cloning (MoClo system) and the Yeast Tool Kit (YTK) are seeing increasing use and many expansions to the YTK are being developed to increase *Saccharomyces cerevisiae* versatility. However, this yeast presents limitations for protein production. In contrast, the non-conventional yeast *Kluyveromyces marxianus* is a valuable candidate as microbial platform for industrial applications due to its unique features: thermotolerance, substrate versatility, GRAS (Generally Regarded as Safe) and QPS (Qualified Presumption of Safety) status. In this research, a YTK-compatible *K. marxianus* secretion toolkit was developed and implemented. Detection systems based on enzymatic activity and surface display were adapted to *K. marxianus* to rate secretion efficiency. Both methods proved to be useful in identifying and characterising efficient secretion signals although some interesting differences between the systems were also observed. This will be discussed in the context of designing high efficiency, tailored systems for production of secreted proteins from industrial feedstocks.

CS 2.2.3 - *Aspergillus oryzae: c*hallenges in bio-based industries through synthetic biology approach

C. Chutrakul[1], *S. Jeennor*[1], *S. Panchanawaporn*[1], *J. Anantayanon*[1], *T. Vorapreeda*[2], *N. Rattanaphan*[1], *K. Laoteng*[1]
[1]National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Pathum Thani, Thailand, [2]National Center for Genetic Engineering and Biotechnology (BIOTEC) At King Mongkut's University of Technology Thonburi, National Science and Technology for Development Agency (NSTDA), Bangkok, Thailand

Aspergillus oryzae has a long history of use in biotechnology and as a research model for functional gene analysis and cell metabolisms. It is listed as a Generally Recognised as Safe (GRAS) strain and has become of particular interest for various industrial applications due to its available genomic data and genetic manipulability, rapid growth, stress tolerance, high capacity to assimilate a wide range of substrates, and ability to secrete proteins and bioactive metabolites. Despite the significance of these traits, the industrial sector has not yet fully harnessed the potential of this fungus. Therefore, the development of an *A. oryzae* production system for industrial applications is necessary. We employed synthetic biology (SynBio) technology focused on upstream process development to design and generate a robust chassis cell of an industrial *A. oryzae* with multiple auxotrophies and controlled morphological traits for submerged fermentation. Furthermore, we established important genetic toolboxes, including an efficient expression system with strong promoters and auxotrophic markers, as well as functional RNA polymerase III promoters applied for CRSIPR-Cas9-mediated gene editing. The combination of SynBio fungal host and genetic toolboxes highlights the potential of the developed strain as a cell

factory for bio-based production through reconstitution of metabolic pathways, activation of cryptic antibiotic precursor gene or gene disruption/overexpression. So far, SynBio has not only opened up new perspective for advanced research in fungal biotechnology but has also facilitated the rational development of production processes for valuable functional ingredients applicable in various industrial sectors, including food, nutraceuticals, and cosmetics.

CS 2.2.4 - Development of new and highly efficient *Agrobacterium*-mediated platforms for genetic manipulation in some important filamentous fungi

V.-T. Tran[1,2], *H.-D. Thai*[1], *M.T. Trinh*[1], *L.T.D. Mai*[2], *T.X. Vu*[1,3]
[1]University of Science, Vietnam National University, Hanoi, National Key Laboratory of Enzyme and Protein Technology, Ha Noi, Vietnam, [2]University of Science, Vietnam National University, Hanoi, Faculty of Biology, Hanoi, Vietnam, [3]National Center for Technological Progress, Ministry of Science and Technology of Vietnam, Center for Experimental Biology, Hanoi, Vietnam

Fungi play important roles in nature and human life. Many beneficial species such as *Aspergillus oryzae*, *Aspergillus niger*, *Penicillium rubens* (*P. chrysogenum*), *Cordyceps militaris*, etc. are industrially exploited for the production of antibiotics, enzymes, organic acids, and other bioactive substances. Meanwhile, fungal plant pathogens including the citrus postharvest pathogen *Penicillium digitatum* represent as main causes of serious losses in agricultural production. Therefore, new approaches for molecular inspections of the fungi can help improve the production of the desired products or identify specific targets for controlling the fungal pathogen on citrus. We have developed highly efficient systems for genetic manipulation in some important filamentous fungi using *Agrobacterium tumefaciens*-mediated transformation (ATMT). We report for the first time new ATMT systems based on the uridine/uracil auxotrophy for *A. oryzae*, *A. niger*, *P. rubens*, and *C. militaris*. Our ATMT systems achieve high transformation yields of over 1000 transformants per 10^6 spores. We have also succeeded in developing the dual auxotrophic marker ATMT systems based on the uridine/uracil and histidine auxotrophy in *A. oryzae*, *A. niger*, and *C. militaris*. These systems are effective for gene targeting with a gene deletion efficiency of over 90%, especially in *A. oryzae* and *A. niger*. Additionally, we have also improved the ATMT systems based on antibiotic resistance markers for *A. niger*, *P. rubens*, and *P. digitatum* to serve gene function studies in wild-type fungi. Our developed ATMT systems provide new genetic platforms to construct fungal mutants for producing beneficial metabolites and gene function characterization in the relevant filamentous fungi.

CS 2.2.5 - Increasing fungal natural product titer with heterologous overexpression using MycoDrive genome engineering

M. Rawa[1], *R. Stankey*[2], *D. Johnson*[2], *K. Throckmorton*[2], *D. Mead*[2], *N. Keller*[1], *B. Knox*[2]
[1]University of Wisconsin-Madison, Madison, United States, [2]Terra Bioforge, Middleton, United States

Fungal natural products (NPs) are valued for their unique and diverse bioactivities – with vast potential impact to broad industries ranging from AgTech to healthcare and more. Usually, economic production for biotechnological applications requires significant increases in NP production. Incumbent methods to increase yield include mutagenesis which can be laborious and time intensive or, more recently with advances in synthetic biology, heterologous production which often requires genetically refactoring multi-gene biosynthetic gene cluster (BGC) pathways. Here, we offer CRISPR-based enzymes to precisely capture intact BGCs regardless of sequence content from host organisms to clone into custom MycoDrive™ fungal genome engineering vectors – bypassing limitations of a suite of PCR and homology based cloning approaches. MycoDrive engineering vectors are flexible enough to be transformable into competent protoplasts of any species and overexpress BGC payloads via multicopy integration stably throughout a genome. The pigment bikaverin from *Fusarium oxysporum* was expressed with MycoDrive in *Aspergillus nidulans* and achieved production titer 30x over standard genome engineering approaches with maximum expression seen with at least ~8 bikaverin BGC copies. To date, MycoDrive engineering has multicopy integrated 4 different BGCs in a single round of strain engineering – in progress is characterization of the pestalamide, enfumafungin, and cyclosporine metabolites.

CS 2.2.6 - Application of multi-omics analyses and heterologous expression to study lichen natural products

T.K. Shishido Joutsen[1,2], A.L. Silva da Costa[3], M. Wahlsten[4], W. Kim[5], P. Laine[1], J. Rikkinen[6,7], D.P. Fewer[4], T. Lundell[4], J.-S. Hur[8], L.T. Dall'Agnol[3], J. Collemare[2], P. Auvinen[1]
[1]University of Helsinki, Institute of Biotechnology, Helsinki, Finland, [2]Westerdijk Fungal Biodiversity Institute, Fungal Natural Products, Utrecht, Netherlands, [3]Federal University of Maranhao, Department of Biology, Sao Luis, Brazil, [4]University of Helsinki, Department of Microbiology, Helsinki, Finland, [5]Chonnam National University, Department of Applied Biology,, Gwangju, Korea, Republic of, [6]University of Helsinki, Finnish Museum of Natural History, Helsinki, Finland, [7]University of Helsinki, Organismal and Evolutionary Biology Research Programme, Faculty of Biological and Environmental Sciences, Helsinki, Finland, [8]Sunchon National University, Sunchon National Korean Lichen Research Institute, Sunchon, Korea, Republic of

Lichens are diverse microbial communities formed by a filamentous fungus (mycobiont), and a phototrophic symbiont of green algae and/or cyanobacteria, together with associated bacteria as well as yeast-type fungi. Most of the characterized lichen mycobionts belong to *Ascomycota*, and lichen systematics is thereby following fungal taxonomy. Natural products synthesized by the lichen symbionts often exhibit biological activities and most of the over 1000 natural products described from lichens are produced by the fungal partner. Only a few of them have their biosynthetic pathway characterized. Here, we use a combination of metagenomics, metatranscriptomics, metabolomics and heterologous expression to link the natural lichen product protocetraric acid to its biosynthetic gene cluster. Lichens were sampled from Finland and New Caledonia in the Pacific Ocean (n=18). A putative biosynthetic gene cluster could be assigned to protocetraric acid based on mass spectrometry detection aiding in correlation to gene presence and their expression in natural lichen systems. We successfully cloned a synthesized

polyketide synthase gene based on a *Cladonia rangiferina* sequence and used *Aspergillus oryzae* as a host to produce the first intermediate compound in the biosynthesis of protocetraric acid. Our results indicate that codon optimization and gene synthesis can be used for cloning and heterologous expression of a polyketide gene from lichen-forming fungi. Results from this study can facilitate production of protocetraric acid under laboratory conditions. This study showcases how cultivation-free methods and omics data analyses link biosynthetic pathways to their final products in organisms that are not amenable in the laboratory.

CS 2.2.7 - Regulation of starch-degrading enzymes in *Thermothelomyces thermophilus*

J. van den Brink[1], *T.X. Mascarenhas de Sousa*[1], *M. Arentshorst*[1], *A.F. Ram*[1]
[1]Leiden University, Institute Biology Leiden, Microbial Sciences, Leiden, Netherlands

The thermophilic fungus *Thermothelomyces thermophilus* possesses valuable industrial properties, including its ability to grow at higher temperatures and produce thermostable enzymes. However, there is still a significant gap in our understanding regarding the regulatory mechanisms that control the production of starch-degrading enzymes (SDEs). The aim of our study is to identify the transcription factors involved in the regulation of SDEs. Our strategy includes performing a phylogenetic analysis, creating marker-free mutants, and analyzing their physiology.
Functionally studied SDE-regulating transcription factors (AmyR, MalR and Col26) were blasted against 40 ascomycetes genomes and 130 homologs were used to make a phylogenetic comparison. Within *T. thermophilus*, three proteins were identified as potential regulators of SDE: Spoth2_2301920 groups with Col26 from *N. crassa*, Spoth2_68580 groups with MalR from *A. oryzae*, and Spoth2_2303067 was homologous to a group of uncharacterized transcription factors. To study the functions of the putative SDE regulators, the transcription factor encoding genes were deleted using a newly developed CRISPR/Cas9 system allowing iterative gene deletions. The single plasmid Cas9 system consists of a Cas9 expression construct, the hygromycine-resistance gene as a selection marker, a exchangeable sgDNA expression cassette, and human telomere regions functioning as a plasmid replicator. The addition of the telomer sequence turns the vector into a self-replicating vector, which can be lost effectively in the absence of selection pressure, allowing iterative cycles of genome modifications. The results on deleting the putative SDE-related transcription factor either as single mutants, or in combinations, will be presented.

CS 2.2.8 - A CRISPR/Cas9-based multicopy integration system for increased glucoamylase production in *Aspergillus niger*

J.L. van Dam[1], *S. Forrer*[1], *M. Arentshorst*[1], *P.K. Valappil*[1], *J. Visser*[1], *R.J.M. Lubbers*[1], *A.F.J. Ram*[1]
[1]Leiden University, Microbial Sciences, Leiden, Netherlands

The filamentous fungus *Aspergillus niger* is well known for its high protein secretion capacity and is therefore a preferred host for protein production. Glucoamylase is one of the highest expressed genes in *A. niger* and the promoter and terminator regions of glucoamylase are often used to drive the expression of (heterologous) genes of interest. Moreover, the introduction of multiple copies of such gene constructs is known to further boost product yields.
To increase glucoamylase production in *A. niger*, we have designed a CRISPR/Cas9-based gene targeting method to integrate up to six copies of the *glaA* gene to predetermined sites in the genome. Genes encoding extracellular enzymes such as alpha-amylase and alpha-glucosidases or proteases (PepA and PepB), were deleted and replaced by a Glucoamylase Landing Site (Gla_LS). Each Gla_LS consists of the *glaA* promoter and the *glaA* terminator region. In between the *glaA* promoter and *glaA* terminator regions a unique DNA sequence was introduced for which a unique Cas9 compatible guide RNA was designed. A strain lineage in a non-homologous end joining mutant background was made in which up to six Gla_LS were constructed. As a proof of principle, an *A. niger* strain in which six copies of the glucoamylase gene were introduced was subsequently analyzed for glucoamylase production.
We successfully used the expression platform to generate glucoamylase hyperproducing strains of *A. niger*. The expression platform is currently exploited for the expression of heterologous proteins.

CS 2.2.9 - Precise control of *Aspergillus niger* pellet size, heterogeneity and core architecture during shake flask cultivation

K. Engelbert[1], T.C. Cairns[1], T. Kheirkhah[2], C. Deffur[3], F. Zhang[3], H. Winter[3], S. Junne[4], H. Briesen[3], P. Neubauer[2], V. Meyer[1]
[1]Technische Universität Berlin, Applied and Molecular Microbiology, Berlin, Germany, [2]Technische Universität Berlin, Bioprocess Engineering, Berlin, Germany, [3]Technical University of Munich, Process Systems Engineering, Munich, Germany, [4]Aalborg University, Chemistry and Bioscience, Aalborg, Denmark

In submerged culture, filamentous fungi grow in three macromorphological forms: dispersed mycelium, clumps and pellets. The underlying processes that lead to their formation are not yet completely understood and require systematic investigation, as heterogeneous cultures limit the optimization of biotechnological processes.
Therefore, the cell factory *Aspergillus niger* was cultivated under 48 conditions in technical triplicate. The studied process parameters included spore densities, concentration of talc microparticles, stirrer speeds, and flask shape (+/- baffles). A high-throughput image analysis pipeline was used to analyse thousands of pellets from the resulting populations with pellet diameter, culture heterogeneity, and inter-replicate variation quantified. Newly developed µ-CT technology was used to investigate the pellet's inner architecture in remarkable detail. We used regression modelling to identify multiple parameters that can be used for precise, highly reproducible control of filamentous fungal shake flask growth. Additionally, we reveal three distinct pellet types and propose a new pellet classification system: (i) pellets formed by a single spore core, (ii) pellets formed by multiple spore cores and (iii) pellets formed by multiple mature pellets in later growth phases.
The study analyses the influence of process parameters on pellet populations in a systematic way.

The comprehensive data set allowed the identification of simple influencing process parameters. The strength additionally lies in analysing internal pellet architecture, which expands the understanding of pellet formation into three distinct classes. Together, these findings will drastically improve the control and reproducibility of shake flask assays, thus making them accurate models of larger vessel fungal fermentation.

CS 2.2.10 - *Trichoderma reesei* strain breeding by micro-droplet screening

Y. Shida[1], *H.A. Nguyen*[2], *W. Ogasawara*[2,1]
[1]Nagaoka University of Technology, Department of Materials Science and Bioengineering, Nagaoka, Japan, [2]Nagaoka University of Technology, Department of Science of Technology Innovation, Nagaoka, Japan

The filamentous fungus *Trichoderma reesei* is a potent producer of cellulases and is widely recognised for its ability to produce proteins in large quantities, which makes it a promising host for the expression of recombinant proteins. However, the presence of extracellular proteases in the large amount of native secreted proteins has been a limitation to its use for heterologous protein production. Nevertheless, the mechanisms of protease production, particularly in areas such as regulatory networks and interactions with other secreted proteins, remain partially understood, in contrast to the well-characterised cellulolytic enzymes of *T. reesei*. In this study, a droplet-based microfluidic high-throughput screening system was used to screen *T. reesei* mutants with altered protease productivity. The energy of the UV irradiation as a mutagen was adjusted to a viability of 70 per cent. For the droplet screening of mutants, key factors such as protease induction medium, droplet cultivation time, droplet fluorescence signal detection and droplet sorting were investigated. The screening was successful with the isolation of two mutants showing up to 30% and 40% reduction in secreted protease activities in 2% glucose medium compared to the parent strain. The analysis of these mutants will improve our understanding of the mechanisms of protease production in *T. reesei* and thus contribute to the broadening of its applications in the field of quality control of secreted cellulases and the production of recombinant proteins.

CS 2.2.11 - Investigating UPR and RESS mechanisms in trichoderma reesei: transcriptome insights, calcium-calmodulin signaling, and morphological implications under stress conditions

R. Jadhav[1], *R.L. Mach*[1], *S. Barends*[2], *I. Nikolaev*[2], *A. Mach-Aigner*[1]
[1]Vienna University of Technology, Institute of Chemical, Environmental and Bioscience Engineering, Vienna, Austria, [2]International Flavors & Fragrances, Leiden, Netherlands

In *Trichoderma reesei*, a hyper-secreting cellulase strain, we investigated the unfolded protein response (UPR) and repression under secretion stress (RESS) mechanisms to better understand their roles in protein secretion. UPR and RESS were induced by dithiothreitol (DTT), but cycloheximide failed to reverse the UPR, indicating a robust activation mechanism that is

independent of translation inhibition. The presence of RESS led to the hypothesis that it may occur at transcription initiation or mRNA degradation level, though further experiments are required for confirmation. Interestingly, transcriptome analysis under cellulase production-inducing conditions revealed the presence of UPR but not RESS, suggesting that RESS may not be as prominent under the conditions tested. Future analyses of enriched KOG categories at 72 and 96 hours will aim to identify novel UPR targets and mediators. The role of calcium-calmodulin signaling in UPR regulation was assessed, with no significant impact observed on UPR activation, indicating that this pathway is not directly involved in UPR in *T. reesei.* Morphological changes associated with UPR will be investigated using Nanolive imaging to provide a deeper understanding of how stress responses alter cellular architecture in this strain. These findings advance our understanding of UPR and RESS in *T. reesei* and lay the foundation for future optimization of industrial protein production.

CS 2.2.12 - Optimization of laccase from *Trametes versicolor* for improving the aflatoxin B1 detoxification

M. Zaccaria[1,2], *M. Beccaccioli*[3], *M.C. Bonaccorsi di Patti*[4], *V. Cristiglio*[5], *N. Sandlin*[2], *W. Dawson*[6], *T. Nakajima*[6], *B. Chan*[7], *L. Genovese*[1], *M. Reverberi*[3]
[1]CEA/INAC-MEM/L-Sim, University Grenoble Alpes, Grenoble, France, [2]Boston College, Department of Biology, Chestnut Hill, United States, [3]Sapienza University of Rome, Department of Environmental Biology, Rome, Italy, [4]Sapienza University of Rome, Department of Biochemical Sciences "Alessandro Rossi Fanelli", Rome, Italy, [5]Institut Laue-Langevin, Grenoble, France, [6]RIKEN Center for Computational Science, Kobe, Japan, [7]Nagasaki University, School of Engineering, Nagasaki, Japan

Laccase is an enzyme of general interest in biotechnology with potential in aflatoxin decontamination; however, the most active natural isoforms cannot provide the necessary efficiency to substantially benefit the industry. Previous research has focused on optimizing laccase through rational design or directed evolution. In the context of aflatoxin degradation, molecular docking has provided mechanistic insights, 3D structures analysis of different isoforms assessed interaction with aflatoxins, and mutational analysis explored beneficial changes. We employ laccase from *T. versicolor*, a fungal species whose ecological niche is tailored around laccase-mediated lignin degradation. Previous data from our lab indicates that performance improvement cannot be achieved by specializing laccase towards general categories of compounds (e.g. hydrocarbons, aromatic nonphenolic structures, or even aflatoxins as a category). We therefore perform an extensive, full quantum mechanical (QM) characterization on the entire *T. v*ersicolor laccase structure bound to aflatoxin B1 and aflatoxin G2 (~7,000 atoms). We mechanistically characterize the role of single amino acid residues in interacting with aflatoxin B1 and identify theoretical variants expected to have better performance on aflatoxin B1. These laccase variants were then cloned and transformed into *Pichia pastoris*, expressed, purified, and tested for aflatoxin B1 detoxification. Results indicate that laccase affinity for the substrate was enhanced by replacing specific amino acids as predicted by the model's simulations. These findings confirm the prediction that π-π interactions are important for substrate binding, and corroborates the use of full QM models as an effective decision-making tool in designing laccase variants targeted at efficient aflatoxin detoxification.

CS 2.3.1 - Sustainable food innovation with filamentous fungi

L. van Dam[1], *L.J. Jahn*[1]
[1]DTU, Biosustain, 2800 Kgs. Lyngby, Denmark

Climate change, environmental damage, and biodiversity loss rank among the most pressing challenges humanity faces today. The food system is a major contributor to these issues, making it crucial to rethink how we produce and consume food. Specifically, we must reduce the environmental impact of industrial animal farming and enhance resource efficiency by minimizing food waste. Filamentous fungi offer significant potential in addressing both of these challenges simultaneously. These fungi can utilize a diverse range of substrates, including lignocellulose-rich materials that are often discarded due to their fibrous and unpalatable nature. Through solid-state fermentation, they can transform these ingredients into flavorful and savory foods that resemble meat. My research focuses on exploring the gastronomic potential of fungi by screening fungal biodiversity for their gastronomic potential and safety, as well as optimizing fermentation conditions to understand the relationship between growth environments and fungal flavors and textures. This knowledge could pave the way for sustainable, nutritious, and delicious foods that serve as healthy alternatives to traditional animal products.

CS 2.3.2 - *Rhizopus oryzae* efficiently converts barley proteins into mycoprotein in solid state fermentation and improves food functionality of brewer's spent grain

M. Terp[1], *C.E. Rusbjerg-Weberskov*[1], *L. Yang*[2], *S. Gregersen Echers*[1], *J.D. Foley*[3], *V. Orlien*[3], *M. Lübeck*[1,2]
[1]Aalborg University, Aalborg, Denmark, [2]Myco4Food Aps, Copenhagen, Denmark, [3]University of Copenhagen, Copenhagen, Denmark

Vast amounts of beer is produced worldwide resulting in a massive side stream of Brewer's spent grain (BSG), which is a protein-rich biomass with high fiber content and an optimal moisture content for fungal solid-state fermentation (SSF). BSG is mainly used for ruminant feed and biogas production but can and should – from a sustainability viewpoint – be used as food instead. Progress has been made towards making BSG palatable as flour or crackers, however its minimal food functionality limits its use in food. We have utilized the "tempeh" fungus *Rhizopus oryzae* for SSF of BSG. We have shown that SSF with *R. oryzae* makes it possible to increase the protein content and at the same time introduce an increased degree of functionality. Our results showed a significant change in protein composition after 90 hours of fermentation in a controllable bioreactor with 95 % of the protein being mycoproteins and only 5 % being barley proteins. We have applied an optimized protocol for protein extraction to produce protein-rich extracts from the fermented BSG. The protein-rich extract exhibited enhanced functionality compared to crude fermented-BSG, highlighting its potential as a sustainable and versatile ingredient for food applications. Furthermore, we sequenced our strain via nanopore sequencing to better identify biosynthetic gene clusters coding for mycotoxin production. No mycotoxin clusters were found confirming the safety of using *R. oryzae* for food applications. The protein-

rich extract exhibited enhanced functionality compared to crude fermented-BSG, highlighting its potential as a sustainable and versatile ingredient for food applications.

CS 2.3.3 - Valorization of whiskey side-streams for sustainable mycoprotein production and waste reduction

T.-T. Bui[1], *Ö. Bayram*[1], *J.-C. Jacquier*[2], *Ö.S. Bayram*[1]
[1]Maynooth University, Biology, Maynooth, Ireland, [2]University College Dublin, School of Agriculture and Food Science, Institute of Food and Health, Dublin, Ireland

The whiskey industry is vital to Ireland's economy, contributing to jobs, exports, and preserving cultural heritage. However, whiskey production generates significant waste, including liquid byproducts like spent wash and solid residues like spent grains, which can cause environmental pollution if not managed properly. Poor waste management increases pollution risks and adds operational costs, while missing opportunities for resource recovery. Whiskey production waste is nutrient-rich, containing proteins, fibers, vitamins, and minerals, offering potential as animal feed or soil enhancement, though not suitable for direct use. We screened various edible mushroom and mold strains for their ability to grow in whiskey waste and identified several strains that effectively convert whiskey side streams into edible fungal mycoprotein. These strains produced wet biomass (10-15% weight/volume), which, when dried, yielded 3-5%. The fungal biomass contained over 25% protein by dried weight, as measured by the Kjeldahl method. Additionally, fungal growth reduced the chemical oxygen demand (COD) of the waste, lowering its environmental impact. These findings suggest that fungal biomass from whiskey waste could be repurposed as a nutritious food source for humans and livestock, offering sustainable waste management solutions while addressing environmental challenges.

CS 2.3.4 - Development of an automatically controlled modular bioreactor for Solid State Fermentation for research and industrial scale

P.S. Lübeck[1], *L. Yang*[2], *M. Lübeck*[1]
[1]Aalborg University, Chemistry and Bioscience, Aalborg, Denmark, [2]Myco4Food Aps, DTI Center for Bioresources, Taastrup, Denmark

Solid-state fermentation (SSF) is a well-known process developed and adopted in human society to produce traditional foods such as cheese, bread, soy sauce, and tempeh. It is also being explored for new applications, including the production of enzymes, new food and food ingredients as well as bio-based materials.
This has resulted in an increasing demand for SSF solutions on an industrial scale. However, to date, success with large-scale SSF has been limited to specific substrates and microorganisms, with fermentation conditions and setups determined empirically and not fully applicable to new process development and scaling of these new processes.
Myco4Food ApS (M4F) is a spin-out from Aalborg University (AAU), which aims to develop an innovative platform technology to solve these challenges in laboratory- and large-scale SSF,

which can be used both for research and industrial applications.
M4F has developed SSF bioreactors that facilitate the upcycling of bio-residuals into value-added products through fungal fermentation. These bioreactors have a unique design that includes a monitoring and controlling system of the fermentation. The bioreactor design is scalable and adjustable for different SSF processes and applications.
Our technology consiss of two main components:
1. Novel bioreactor design: This includes advanced sensors and control units to collect fermentation data and provide homogeneous fermentation conditions and effective microclimate control.
2. Backend System: This system processes data collected during SSF to predict fungal physiological variables and adjust operational parameters.
Our modular design allows customization to meet the requirements of different processes, providing improved compatibility and scalability.

CS 2.3.5 - Biosustainable production of indigoidine dye from polystyrene and polyethylene plastic waste

Z. Bowers[1], *Y. Chen*[2], *E. Oakley*[3], *B.R. Oakley*[3], *T.J. Williams*[2], *C.C.C. Wang*[1]
[1]University of Southern California, Department of Pharmacology and Pharmaceutical Sciences, Los Angeles, United States, [2]University of Southern California, Department of Chemistry, Los Angeles, United States, [3]University of Kansas, Department of Molecular Biosciences, Lawrence, United States

Plastic waste is an acute threat to the environment and human health. The complete elimination of plastics, however, is an inconceivable solution due to their revolutionary impact on daily life and immense economic contribution. Consequently, long-term plastic sustainability is an urgent matter that requires new approaches to achieve, such as the utilization of microorganisms. We previously demonstrated the conversion of polystyrene (PS) and polyethylene (PE), two common plastic polymers with poor recycling rates, into metabolically relevant fungal substrates. Further, the sustainable impact of fungi can be broadened by their ability to create complex secondary metabolites that have the potential to replace our current environmentally hazardous synthetic processes like those used in dye manufacturing. Thus, fungal production of the natural blue pigment, indigoidine, from plastic waste was of interest. We hypothesized that *Aspergillus nidulans* can produce notable titers of indigoidine from post-consumer PS and PE substrates. To accomplish this, the non-ribosomal peptide synthetase (NRPs) responsible for indigoidine biosynthesis was expressed in *A. nidulans*. The engineered strain's ability to utilize PS and PE substrates as sole carbon sources for growth and indigoidine production was determined. Metabolism of PS and PE substrates resulted in indigoidine titers of 624 mg L^{-1} ± 118 mg L^{-1} and 980 mg L^{-1} ± 315 mg L^{-1}, respectively. This study demonstrates the robust nature of *A. nidulans* as a heterologous host, expands the chemical diversity of fungal secondary metabolites from post-consumer plastic substrates, and contributes to the promising potential of fungi for global long-term plastic sustainability.

CS 2.3.6 - Carbon fiber reinforced polymers composite recycling and thermoset matrix up-cycling utilizing an engineered strain of Aspergillus nidulans

C. Wang[1], *C. Olivar*[2], *Z. Yu*[3], *B. Miller*[1], *M. Tangalos*[1], *C. Jenkinson*[4], *S. Nutt*[3], *B. Oakley*[4], *T. Williams*[2]
[1]University of Southern California, Pharmacology and Pharmaceutical Sciences, Los Angeles, United States, [2]University of Southern California, Chemistry, Los Angeles, United States, [3]University of Southern California, Chemical Engineering, Los Angeles, United States, [4]University of Kansas, Molecular Biosciences, Lawrence, United States

Carbon fiber reinforced polymers (CFRPs, or composites) are increasingly replacing traditional manufacturing materials used in automobile, aerospace, and energy sectors. With this shift, it is vital to develop end-of-life processes for CFRPs that retain the values of both the carbon fibers and the polymer matrix. Here we demonstrate a strategy to upcycle pre- and postconsumer polystyrene-containing CFRPs, cross-linked with unsaturated polyesters or vinyl esters, to benzoic acid. The thermoset matrix is upgraded via biocatalysis utilizing an engineered strain of the filamentous fungus Aspergillus nidulans, which gives access to valuable secondary metabolites in high yields, exemplified here by (2Z,4Z,6E)-octa-2,4,6-trienoic acid. Reactions are engineered to preserve the carbon fibers with much of their sizing so that the isolated carbon fiber plies are manufactured into new composite coupons that exhibit mechanical properties comparable to those of virgin manufacturing substrates. In sum, this represents the first system to reclaim a high value from both the fiber fabric and polymer matrix of a CFRP.

CS 2.3.8 - Distinct functions of the cell wall synthesis regulators in *Pleurotus ostreatus*: a prelude to cell wall engineering for future mushroom material improvements

M. Kawauchi[1], *H. Kojima*[1], *T. Izumi*[1], *Y. Otsuka*[1], *K. Schiphof*[1], *K. Tsuji*[1], *A. Yoshimi*[2,1], *C. Tanaka*[2,1], *S. Yano*[3], *T. Nakazawa*[1], *Y. Honda*[1]
[1]Kyoto University, Graduate School of Agriculture, Kyoto, Japan, [2]Kyoto University, Graduate School of Global Environmental Studies, Kyoto, Japan, [3]Yamagata University, Graduate School of Sciences and Engineering, Yonezawa, Japan

Mushroom materials have received increased attention as new sustainable materials, and recent studies suggest that these material properties are determined by the cell wall structures of mycelia. In the previous study, the cell wall structure of *Pleurotus ostreatus* (basidiomycete) and *Aspergillus nidulans* (ascomycete) was visualized together with measurements of the cell wall polysaccharide composition. From these analyses, we proposed a major difference in the cell wall structure between ascomycete and basidiomycete. Therefore, we are starting to analyze transcription factors involved in the cell wall integrity signal transduction pathway in *P. ostreatus* to understand how such cell wall structural differences are generated at the regulatory level. First, phylogenetic analysis identified two putative clade A APSES family transcription factors, Mbp1 and Swi6, in *P. ostreatus*. *mbp1* disruption caused a decrease in cell wall thickness, the

expression of β-glucan synthase genes, and the relative percentage of β-glucan, indicating that Mbp1 is required for normal β-glucan synthesis. In contrast, *swi6* disruption resulted in an abnormal distribution of cell wall thickness, expression changes in certain chitin synthase genes, and slightly higher relative percentage of chitin, suggesting a role in regulating chitin synthesis, rather than β-glucan synthesis. Interestingly, Δ*mbp1* and Δ*swi6* mycelial mats were easier to break, whereas the wild-type mat was flexible, suggesting their mechanical property changes due to their abnormal cell wall structures. In conclusion, this study clarified the respective role of Mbp1 and Swi6 in cell wall synthesis regulation and more importantly, demonstrated the potential of cell wall engineering to improve mushroom materials.

CS 2.3.9 - Uncovering the transcriptional landscape of *Fomes fomentarius* during fungal-based material production through gene co-expression network analysis

T. Cairns[1], *C. Freidank-Pohl*[1], *A.S. Birke*[1], *C. Regner*[1], *S. Jung*[1], *V. Meyer*[1]
[1]Technische Universität Berlin, Chair of Applied and Molecular Microbiology, Berlin, Germany

Fungal-based composites are high-performance biomaterials produced from forestry and agricultural waste streams. Production at scale can revolutionize the material world by generating low emission, non-toxic and biodegradable construction, packaging, textile, and other substrates. The basidiomycete *Fomes fomentarius* is one leading species for biomaterial production, yet little is known about the transcriptional basis of fungal growth during composite formation. Co-expression network analysis based on RNA-Seq profiling has enabled accurate predictions of gene function in a range of fungi, and we thus aimed to develop such resources for *F. fomentarius*. We profiled gene expression from a numerous of lab culture (n = 9) or biomaterial formation (n = 18) to identify genes important for guiding mycelium formation through hyphal growth and cell wall biosynthesis. Probing co-expression networks revealed a fungal-specific transcription factor named CacA strongly co-expressed with multiple chitin and glucan biosynthetic genes or Rho GTPase encoding genes, suggesting this protein is a high-priority target for engineering adhesion and branching during biomaterials growth. We identified entirely new types of co-expressed contiguous clusters not previously described in fungi, including genes predicted to encode hydrophobins, kinases, lipases, F-box domains, chitin synthases, amongst others. The co-expression data generated in this study will enable us to understand the transcriptional basis of *F. fomentarius* biomaterial formation in unprecedented detail. We confirm accurate network-derived predictions of gene function in *F. fomentarius* and generate the necessary data and scripts for analysis by any end user.

CS 2.3.10 - Effective bioremediation of toxic-waste cooking oil using lipases produced by mycotoxin-free strain of Aspergillus foetidus

M. Espinoza-López[1], *S. Patrón-Herrera*[1], *D. R. Alemán*[1], *J. Alemán S.*[1], *C. Cruz C.*[1], *R. Trejo-Valencia*[1], *M. Reyes-Rodríguez*[1], *D. Magaña-Ortíz*[1]
[1]Tecnológico Nacional de México, Campus Mérida, Chemical, Biochemical and Environmental Engineering Department, Mérida, Mexico

The inadequate disposal of residues derived from waste cooking oil (WCO) could cause hypoxia in water reserves and profounds alterations in productive soils and ecosystems. Moreover, consumption of burning oil cause coronary heart disease, and it is related to acrylamide formation that produces muscle weakness, lack of coordination, reduces reproductive capacity and cancer. It is reported that *Aspergillus* genera can degrade oils through the lipase activity, however most of these species are mycotoxin producers representing a major biological risk. In contrast, *Aspergillus foetidus* ATCC 10061 is mycotoxin-free strain well-used in laboratory and industries; nevertheless, its potential in degradation of WCO remains unexplored. Therefore, the main purpose of this work was to determine the parameters related to degradation of WCO employing *Aspergillus foetidus* strain, also, composition of this toxic waste was analyzed before and after treatment. As a result, microorganism was able to degrade up to a 95% of WCO and thus, the fungal biomass in treatments was 15-fold in comparison to controls grown in absence of WCO. On the phytotoxicity tests using Habanero pepper (*Capsicum chinense*) was established that the use of the medium recuperated after the bioremediation showed up benefits on the plant growing getting more than two-fold size in comparison with the ones exposed directly to WCO without treatment. Finally, fatty acid components were analyzed using mass-spectrometry.
These results could apply in environmental cleanup and agriculture reducing the adverse effects of WCO. Future research could refine degradation parameters and expand applications to enhance its bioremediation capabilities.

CS 2.3.11 - Understanding anaerobic fungi carbohydrate degrading enzyme induction for agricultural waste valorisation

J.L. Matthews[1,2], S.C. Fry[2], S. Galgano[3], M. Blow[4], K. LaButti[4], V. Ng[4], I. Grigoriev[4], J.M. van Munster[1]
[1]SRUC, Plant and Soil Sciences, Edinburgh, United Kingdom, [2]University of Edinburgh, Institute of Molecular Plant Sciences, Edinburgh, United Kingdom, [3]SRUC, Monogastric Science Research, Edinburgh, United Kingdom, [4]U.S Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, United States

Anaerobic fungi are powerful lignocellulose degraders, they have been observed to be amongst the primary colonisers of lignocellulose in the rumen and preferentially recognise damaged plant tissues. However, how these fungi recognise individual plant polysaccharides and respond to their presence is not fully resolved.
The growth of two anaerobic fungi species with distinct characteristics has been screened on different monosaccharides and polysaccharides derived from wheat straw, a major biofuel stock and agricultural waste. Both anaerobic fungi species could only utilise a few of these substrates for growth, however, the presence of a range of non-metabolizable sugars can inhibit or aid fungal growth on these substrates.
To investigate further the fungal enzymatic responses to polysaccharides of potential industrial interest (e.g., cellulose, arabinoxylan, and galactomannan), the products from fungal digestion have been visualised using thin-layer chromatography. RNA-SEQ will be used to identify the enzyme expression of both fungal species.
Together, these studies enable comparison of strategies that both anaerobic fungal species may use for lignocellulose recognition and degradation. Furthermore, it will also aid in identifying

enzymes anaerobic fungi employ as part of their degradative mechanisms and how their production is affected by lignocellulose-derived inducers.
This knowledge may form the basis for further exploitation of these fungi and their enzymes in biotechnology and agricultural waste valorisation.

CS 2.3.12 - Exploring filamentous fungi as eco-friendly tools for reducing CO2 emissions in the concrete industry

C. Danner[1], *J. Charest*[1], *J. Lederer*[1], *R.L. Mach*[1], *A.R. Mach-Aigner*[1]
[1]TU Wien, Institute of Chemical, Environmental and Bioscience Engineering, Wien, Austria

Concrete is the second most widely used material in the world after water, consuming large amounts of natural resources like limestone, gravel, and sand. The global production of cement, which is the key ingredient for concrete depicts, with around 1.6 billion metric tons of carbon dioxide, one of the top three sources of anthropogenic CO2 emissions. To achieve the sustainable development goals, it is important to reduce the carbon and material footprint of the concrete industry while ensuring the global demand is still met. To address this challenge, the development of new sustainable approaches is required to produce concrete, enhance its durability, and develop recycling methods that do not compromise quality. Recently, the usage of microorganisms has emerged as an area of increasing interest for an eco-friendlier concrete industry. For instance, microbial-induced calcium carbonate precipitation in traditional concrete can improve the durability or recyclability of concrete. A prerequisite for this is detailed knowledge of the behavior of microorganisms in concrete. This study aimed to explore the applicability of filamentous fungi within this context, inspired by a microbial community composition analysis that showed the natural colonization of various fungal species on concrete samples. Subsequently, we tested the tolerance of two filamentous fungi, *Trichoderma reesei* and *Mortierella alpina,* towards concrete in different media compositions and studied their ability to grow within and on the surface of concrete. Our findings will be presented and discussed in the context of application possibilities for these filamentous fungi as a biotechnological tool to improve concrete sustainability.

CS 2.4.1 - Developmentally regulated transposon activity as a mechanism for rapid adaptation in the clonally evolving fungal pathogen *Fusarium oxysporum*

A. Rodríguez López[1], *J. Anglada Delgado*[1], *C. López Díaz*[1], *A. Di Pietro*[1]
[1]Universidad de Córdoba, Departamento de Genética, Córdoba, Spain

Transposable elements (TEs) represent an important source of genetic variation and are considered major drivers of genome evolution. The clonally propagating fungus *Fusarium oxysporum* (*Fo*) causes devastating vascular wilts in a wide range of crops and life-threatening opportunistic infections in humans. Its ability to infect organisms from different kingdoms makes *Fo* an ideal model for studying the genetic mechanisms underlying adaptation to diverse host environments. Previous studies revealed a pivotal role of DNA transposons in the rapid

adaptation of the tomato pathogenic isolate Fol4287 during serial passages conducted under different environmental conditions. More than 80% of the TE insertions detected in experimentally evolved lines were attributed to *Hormin*, a non-autonomous miniature DNA element derived from the hAT superfamily TE *Hornet*. Here we investigated the expression of *Hornet* transposase, responsible for *Hormin* mobilization, during different stages of fungal development. RT-qPCR performed during synchronized growth in liquid medium demonstrated a marked upregulation of *Hornet* transcript levels during the early stages of asexual sporulation, with a peak of expression observed in freshly produced microconidia. Furthermore, experimental evolution on solid complete medium revealed a higher rate of phenotypic variability in populations evolved from microconidia compared to those evolved from mycelium. Our findings suggest that conidiation-specific activation of a TE promotes environmental adaptation in a clonally evolving fungal pathogen.

CS 2.4.2 - Histone deacetylase-1 is required for epigenomic stability in *Neurospora crassa*

F. Ebot-Ojong[1], *A. Deaven*[1], *A. Ferraro*[1], *F. Kaddar*[2], *C. Hull-Crew*[2], *A. Scadden*[2], *A. Klocko*[2], *Z. Lewis*[1]
[1]University of Georgia, Microbiology, Athens, United States, [2]University of Colorado Colorado Springs, Department of Chemistry & Biochemistry, Athens, United States

Facultative heterochromatin is a specialized chromatin structure that controls context-specific gene expression. In *Neurospora crassa*, the shift from mycelial growth to perithecial development marks a major lifestyle transition in which fruiting body production is prioritized over substrate colonization. This transition must be tightly regulated, occurring only under suitable conditions. We found that Polycomb Repressive Complex 2 (PRC2) plays a crucial role in repressing the mycelia-to-perithecia transition by ensuring that key conditions, such as nutritional stress and mating partner availability, are met before development proceeds. Loss of PRC2 activity results in premature perithecial development, even in the absence of a mating partner. PRC2 represses perithecial-specific genes by trimethylating histone H3 on lysine-27 across large, multi-gene regions encompassing 7% of the *N. crassa* genome. Through a genetic screen, we identified regulators of PRC2 and its product, H3K27me3. Notably, we found mutations in constitutive heterochromatin components, including *histone deacetylase-1* (*hda-1*), cause de-repression of PRC2 target genes. We show HDA-1-deficient cells undergo progressive epigenome instability over hundreds of nuclear divisions, characterized by hyperacetylation of histones, local loss of H3K9 methylation, and global redistribution of H3K27me3. Together, our findings suggest that histone deacetylase-1 is a key guardian of the epigenome.

CS 2.4.3 - Conserved DNA methylation within the Neurospora genus

J. Svedberg[1], *J. Reimegård*[2], *H. Johannesson*[1]
[1]Stockholm University, Department of Ecology, Environment and Plant Sciences, Stockholm, Sweden, [2]Science for Life Laboratory, ScilifeLab, Department of Cell and Molecular Biology, National Bioinformatics Infrastructure Sweden, Uppsala, Sweden

5-methylcytosine DNA methylation is mostly found in repetitive regions in *Neurospora*, where repeat induced point-mutations lead to the formation of H3K9me3 mediated heterochromatin which in turn induces DNA methylation. There has been little evidence of methylation of genic sequences and of control of gene expression through dynamic methylation throughout the life cycle. Most previous studies on methylation in *Neurospora* have focused on *N. crassa*, and we have gained significant knowledge of the molecular underpinnings of DNA methylation based on work done in this species, but little has been known of DNA methylation in the rest of the genus. In a previous study, we investigated DNA methylation patterns by performing bisulfite sequencing of 10 strains from 5 different *Neurospora* species. Here we use this dataset to investigate patterns of conservation of methylation between these species, and identify an excess of sites where methylation is highly conserved in all species. Unlike most methylated sites, which are found in repeats, these are instead mostly located within genes and they are also associated with a specific G-rich sequence motif. Using transcriptomic data we investigate the association between methylation and gene expression in several different tissues and under several different growth conditions and we also investigate the causes of methylation at conserved sites and to what extent it differs from methylation caused by the canonical pathway described in *N. crassa* through the use of deletion mutants.

CS 2.4.4 - Exploring the impact of DNA Methylation on three-dimensional genome architecture in a major wheat pathogen

C. Lorrain[1], *I. Glavincheska*[1]
[1]ETH Zurich, Institute of Integrative Biology - Plant Pathology, Zurich, Switzerland

Genome structure and maintenance are key determinants of the evolvability of organisms. In eukaryotes, including fungi, genomes are organized within the three-dimensional (3D) nuclear space, where chromosomes occupy distinct regions. The genome of the wheat pathogen *Zymoseptoria tritici* is partitioned into gene-rich, transposon-poor core compartments and accessory compartments enriched in species-specific genes and transposable elements (TEs). We recently uncovered the 3D conformation of the *Z. tritici* reference genome, revealing topologically associating domain (TAD)-like structures associated with distinct epigenetic and transcriptomic landscapes. Notably, TAD-like boundary regions were enriched in specific histone modifications, depleted in cytosine DNA methylation, and showed low TE content. Cytosine DNA methylation plays a critical role in silencing TEs and maintaining genome integrity. In natural populations of *Z. tritici*, the loss of the DNA methyltransferase DIM2 led to a nearly complete loss of cytosine DNA methylation, resulting in TE reactivation[1]. However, the influence of DNA methylation variation on 3D genome conformation remains poorly understood. In this study, we combined Hi-C, genome, transcriptome, and methylome sequencing of two pairs of *Z. tritici* strains with active and inactive DIM2 to investigate how variation in DNA methylation affects genome architecture and 3D organization.

1. Möller, M. *et al.* Recent loss of the Dim2 DNA methyltransferase decreases mutation rate in repeats and changes evolutionary trajectory in a fungal pathogen. *PLoS Genet* 17, e1009448 (2021)

CS 2.4.5 - Epigenetic regulation as a mechanism for gain of virulence in rust fungi

R. Domingues Carvalho[1,2], *A. Korolev*[1], *C. Stephens*[1], *A. Bryan*[1], *J. Rodriguez Algaba*[3], *A. Justesen*[3], *M. Støvring Hovmøller*[3], *D. G.O. Saunders*[1]
[1]John Innes Centre, Crop genetics, Norwich, United Kingdom, [2]Instituto Superior de Agronomia, Patologia Vegetal, Lisboa, Portugal, [3]Aarhus University, Agroecology, Slagelse, Denmark

Yellow rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), poses a major threat to global wheat production. New fungal variants capable of virulence on previously resistant varieties keep emerging due to evolutionary pressure on pathogen effector proteins with avirulence function, favouring their modification to evade host immunity. One understudied mechanism for these changes is epigenetic switching leading to expression polymorphisms.
We recently found a promising avirulence gene candidate (*PST_425*) for *Pst*. RNA-seq analysis illustrated loss of expression of *PST_425* occurring in a subset of *Pst* isolates that recently evolved to evade recognition in several European wheat cultivars. With no sequence variation in the promoter region between *Pst* isolates differing in *PST_425* expression, suppression of expression could be due to epigenetic factors. Moreover, low expression of *PST_425* occurs among *Pst* isolates collected on wheat cultivars with a shared genetic lineage, suggesting that this lineage may contain the corresponding host resistance protein effective against PST_425.
PST_425 has all the classic features of an effector; it is short, contains a secretion signal and is highly expressed in haustoria. A Yeast Signal Trap assay confirmed that PST_425 can be secreted. Through yeast-two-hybrid analysis, we also identified cystathionine beta-lyase as a host target of PST_425. This protein catalyses the penultimate step in methionine biosynthesis, a process linked to plant defence-related genes. The next step in this study is to determine whether PST_425 could be epigenetically regulated, thereby establishing the potential for epigenetic switching in virulence gains for wheat rust fungi.

CS 2.4.6 - DNA damage recognition and ADP-ribosylation signalling by fungal PARPs

A.G. Bainbridge[1], *C. Parkin*[1], *J.G.M. Rack*[1]
[1]University of Exeter, MRC Centre for Medical Mycology, Exeter, United Kingdom

In fungi, accumulation of genetic mutations can drive in-host microevolution and the emergence of antifungal drug resistance. However, exposure to high levels of genotoxic agents, most prominently reactive oxygen species (ROS), damages many cellular components including DNA, which uncontained leads to loss of genomic integrity and ultimately cell death. ADP-ribosylation is one of the key signalling pathways that controls the DNA damage response (DDR) by sensing damaged DNA, stalling cell cycle progression, and initiating DNA repair and was shown to be crucial for virulence in several fungal species. Here we report on the structural and biochemical characterisation of ADP-ribosylation signalling in *Aspergillus fumigatus*, a critical fungal pathogen associated with high morbidity and mortality. We show that damaged DNA is directly recognised by the ADP-ribosylation signal ‘writer’, *Af*-PARP, leading to its activation and

creation of a localised ADP-ribosylation signal. The activation of *Af*-PARP's enzymatic activity release on a complex intramolecular interaction that is distinct from closely related homologues in *Animalia* and *Plantae*. This work creates the basis for further investigations into DNA damage-associated ADP-ribosylation signalling and will further our understanding of how (pathogenic) fungi coordinate their DDR, maintain genomic stability, and survive within the host niche. As such, insights gained by this work may promote the development of new strategies to treat fungal infections and combat rising antifungal resistance.

CS 2.4.7 - Epigenetic modifications involved in the evolution of the Ascomycete Pseudogymnoascus destructans: role in the repression of transposable elements

O. Mion[1], *L. Sastre*[1], *M. Lambert*[1], *A.-S. Fiston-Lavier*[1,2], *S.J. Puechmaille*[1,2]
[1]University of Montpellier, Institut des Sciences de l'Evolution, Montpellier, Montpellier, France, [2]Institut Universitaire de France, Paris, France

Methylation is a conserved epigenetic marker found across all domains of life, including fungi. DNA methylation, particularly 5-methylcytosine (5mC) plays a critical role in regulating transposable elements (TEs), repeated elements known as actors of genome evolution, by silencing them.
Previous comparative studies have revealed significant differences in 5mC prevalence between Ascomycota and Basidiomycota, with 5mC being widespread in Basidiomycota but rare in Ascomycota. However, the Ascomycota bat pathogen *Pseudogymnoascus destructans* (*Pd*), is an exception showing a high abundance of 5mC.
Our study aimed to test and characterize this pattern by comparing a set of *Pd* (N=11) and close relatives' genomes (N=15 outgroups). After generating long-read sequencing and methylation data using Oxford Nanopore Technologie for these genomes, we assembled and annotated them. Then, we
(1) assessed whether *Pd* exhibits distinct 5mC patterns compared to its close relatives,
(2) examined the correlation between 5mC and TEs,
(3) identified which TE classes are most influenced by 5mC, and
(4) investigated potential associations between 5mC and TE age.
Our findings revealed significant differences in TE content and 5mC methylation between *Pd* and its outgroups, underscoring the dynamic role of TEs. Notable variability within TE classes, particularly LINEs, was observed among individuals of *Pd*. Additionally, TE age analysis indicated a recent burst of transposition in *Pd*, in contrast with the outgroups, which possess fewer and older TEs. Although no direct relationship between TE age and methylation was found, the bimodal distribution of 5mC methylation suggests a potential association with genomic context that warrants further investigation.

CS 2.4.8 - A selfish Spore Killer element contributes to mitotic stability of a dispensable fungal chromosome

G. Puebla Planas[1], *D. Hazal Ayhan*[2], *P. Gutierrez Escrivano*[1], *L. Gomez Gil*[1], *C. Lopez Díaz*[1], *S. Janevska*[3], *M. Sanchez Lopez Berges*[1], *L.J. Ma*[2], *A. Di Pietro*[1]

[1]University of Cordoba, Cordoba, Spain, [2]University of Massachusetts Amherst, Massachusetts, United States, [3]Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Jena, Germany

Fungi carry dispensable genomic regions, including entire chromosomes, which are not essential for survival and can be lost at high frequency under certain environmental conditions. One unanswered question is how such dispensable regions persist in the population despite their mitotic instability. Here we studied the mechanisms promoting stability of a dispensable chromosome in the fungal pathogen *Fusarium oxysporum* (*Fo*), which causes vascular wilt disease in more than a hundred different crop species and opportunistic infections in humans. We found that the conserved core chromosome 12 (chr12) is frequently lost during serial passaging on plates in the clinical keratitis *Fo* isolate MRL8996, but not in the tomato pathogenic isolate Fol4287. Sequence analysis revealed that chr12 of MRL8996 lacks a 90 kb region present in Fol4287, which harbors a single copy of Spore Killer (Spok). Spoks are a class of genetic elements that act as meiotic drivers by killing neighboring cells lacking the element. Strikingly, transfer of the single Spok element from chr12 of Fol4287 to fluorescently labelled chr12 of MRL8996 led to a significant reduction in spontaneous chromosome loss events, as determined by flow cytometry. Importantly, no stabilizing effect was observed upon transfer of a Spok allele carrying a point mutation previously shown to abolish the killer activity of Spok *in Podospora anserina*. Our results suggest that selfish meiotic drivers such as Spoks contribute to mitotic stability of dispensable chromosomes in fungi.

CS 2.5.1 - Interrogating yeast biodiversity with “silent” synonymous codon bias

A. LaBella[1], L. Dineen[1], S. Lee[1,2], B. Zavala[1], S. Clemens[1], C. West[1], S. Lo[3], T. Y1000plus Project[4,5]
[1]UNC Charlotte, Department of Bioinformatics and Genomics, Charlotte, United States, [2]Cornell University, Ithaca, United States, [3]Unafilliated, Charlotte, United States, [4]Vanderbilt University, Nashville, United States, [5]University of Wisconsin-Madison, Madison, United States

Why are some Saccharomcyotina yeasts human pathogens while others make great cheese? Genetic differences contribute to the vast biodiversity in yeast ecology, metabolism, and other growth phenotypes in our sample of 1,154 yeasts. Some of this diversity is associated with gene gain and loss, which is relatively straightforward to detect in genomes. Much of this diversity, however, is due to altered gene expression, which is difficult to assess across more than one thousand non-model yeasts. Despite the moniker “silent,” synonymous genetic changes that do not alter the protein sequence can impact both transcription and translation rates. In our work, we leverage changes in synonymous codon usage as a proxy for gene expression to identify pathways up- or down-regulated in yeasts that share common phenotypes. We find that codon usage is highly informative in studying yeast metabolism, pathogenicity, horizontal gene transfer, and ecology. For example, codon usage in ribosomal biogenesis genes is highly predictive for growth at 37°C. We also deploy experimental methods to further investigate how tRNA abundance and codon usage bias directly impact expression. Codon usage bias is a treasure trove

of genetic information that has been broadly overlooked and may have widespread applications to fungal biodiversity studies.

CS 2.5.2 - Evolution of maltose utilisation in Irish isolates of Saccharomyces eubayanus

S. Bergin[1], *J. Scully*[1], *M. Osborne*[2], *K. Wolfe*[2], *G. Butler*[1]
[1]University College Dublin, School of Biomolecular and Biomedical Science, Dublin, Ireland,
[2]University College Dublin, School of Medicine, Dublin, Ireland

Saccharomyces eubayanus is, along with *Saccharomyces cerevisiae*, one parent of the hybrid lager yeast *Saccharomyces pastorianus.* Despite lager brewing having been developed in Germany, and despite extensive sampling, *S. eubayanus* has never been isolated from the European mainland. In 2019, we discovered two *S. eubayanus* strains in the University College Dublin campus in Ireland, the first European isolates, and showed that they belong to the same clade (Holarctic) as the *S. pastorianus* parent strain. Here, we report the isolation of five additional strains from various woodlands in Ireland. All seven Irish strains belong to an Irish subpopulation within the Holarctic clade.
The ability to ferment maltose is animportant trait for brewing yeast, with maltose comprising ~60% of the fermentable sugars in wort. In the majority of *Saccharomyces spp.,* the genes involved in maltose utilisation are found clustered in MAL loci; including a maltase (MALS), a maltose transporter (MALT), and a regulatory gene (MALR). Unlike *S. pastorianus* and the *S. eubayanus* type strain, the Irish strains are poor fermenters of maltose.
An experimental evolution approach, in which two Irish *S. eubayanus* strains (UCD650 and UCD926) were grown in media containing maltose as the sole carbon source, was performed to improve brewing capability. The evolved lineages were able to metabolise maltose, and both lineages acquired different homozygous substitution mutations in the same MALR gene. Additionally, an evolved strain derived from UCD650 gained a 30-kb duplication of a genomic region containing a MAL locus. This duplication was already present in the wild UCD926.

CS 2.5.3 - The evolutionary landscape of primary carbon metabolism across the *Aspergillus* genus

J. Li[1], *F. Dekker*[1], *M. Peng*[1], *R.P. de Vries*[1]
[1]Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands

Sugar conversion through primary carbon catabolism in fungi is a complex progress that involves many pathways. As such, detailed insight into sugar metabolism of different species is highly relevant for our understanding of the role of fungi in their natural environment as well as for engineering the sugar metabolic pathways for the production of interesting biochemicals. In a previous FICUS project, we evaluated sugar metabolic models of six taxonomical distant species and revealed that transfer of the sugar metabolic model to other Eurotiomycetes is highly reliable. However, we also noticed significant differences in the transcriptomic response of metabolic genes in Aspergillus niger and Aspergillus nidulans.

As part of the whole genus genome project of Aspergillus, genome sequences for nearly 300 Aspergillus species have been generated. In this project we generated models for primary carbon metabolism for all these species and used them to generate a detailed evolutionary map of this important biological process across the genus, as well as compared to a set of non-Aspergillus reference fungi. This demonstrated the high diversity of primary carbon metabolism among fungi, with frequent gene duplications and losses of genes encoding enzymes catalyzing individual metabolic steps.

CS 2.5.4 - Unveiling the diversity of *Trichoderma reesei*: new paths for biotechnological advances

T. Benocci[1,2], *C. Fiorenzani*[2], *F. Baltar*[1], *R. Baroncelli*[2]
[1]University of Vienna, Functional and Evolutionary Ecology, Vienna, Austria, [2]University of Bologna, Department of Agricultural and Food Sciences (DISTAL), Bologna, Italy

Trichoderma reesei is a well-studied saprotrophic fungus from tropical rainforests, known for its exceptional enzyme secretion. This trait has made it a cornerstone of industrial biotechnology, particularly in the production of lignocellulolytic enzymes (e.g. cellulases) used to convert plant biomass into sustainable fuels and chemicals. Despite significant advances in developing high-performance industrial strains, most are derived from a narrow genetic base, primarily the QM6a strain, leaving much of *T. reesei*'s genetic diversity unexplored.
In this study, we investigate the genetic and phenotypic diversity of nine natural *T. reesei* strains, including the reference strain QM6a, collected from diverse environments worldwide, with one rare strain originated from a marine habitat.
Through comparative genomics and phenotypic screening, we explore the genetic diversity and adaptive mechanisms these strains employ to thrive in their unique environments. Our analysis focuses on nutrient recycling, biomass degradation, Carbohydrate-Active Enzymes (CAZymes) repertoire, regulatory pathways, and salinity tolerance.
This dataset offers valuable insights into the evolution and adaptation of *T. reesei*, uncovering potential for strain improvement in industrial biotechnology. Our findings identify promising leads for developing optimized enzyme cocktails for more efficient biomass conversion, underscoring the importance of exploring natural genetic diversity to fully unlock *T. reesei*'s potential for sustainable industrial applications.

CS 2.5.5 - A taxonomic revision of the *Fusarium sambucinum* species complex

M. Sandoval-Denis[1], *M. Costa*[1], *R. Schumacher*[2], *A. Kermode*[3], *A. Buddie*[3], *M. Ryan*[3], *Y. Baker*[4], *W. Meyer*[5], *J. Groenewald*[1], *P. Crous*[1]
[1]Westerdijk Fungal Biodiversity Institute, Evolutionary Phytopathology, Utrecht, Netherlands, [2]retired, Fürstenwalde/Spree, Germany, [3]CABI, Egham, Surrey, United Kingdom, [4]Julius Kühn Institute Institute, Epidemiology and Pathogen Diagnostics, Braunschweig, Germany, [5]Julius Kühn Institute, Epidemiology and Pathogen Diagnostics, Braunschweig, Germany

Devastating plant pathogens, endophytes, rare human opportunistic pathogens and saprotrophs, are some known lifestyles of the *Fusarium sambucinum* species complex (FSAMSC). The FSAMSC represents a clade of morphologically diverse, globally distributed, mycotoxigenic fungi. It is of central relevance for *Fusarium* taxonomy as it includes the conserved generic type of *Fusarium* (*F. sambucinum*), and that of the sexually typified *Gibberella* (*G. pulicaris*). We tested recent phylogenetic circumscriptions in FSAMSC using multigene phylogenetics, traditional cultural and morphological analyses, and coalescent-based species tree estimations methods using a large set of strains from four culture collections (*i.e.*, BBA, CBS, IMI and NRRL) plus recent isolations from diverse substrates. Seventy-five species are resolved in FSAMSC making it one of the most speciose groups in *Fusarium*. However, only 40 phylospecies are currently linked to types and Latin binomials. Thirty-four novel species are described, and illustrations are provided for all the species studied. Differences between coalescent-based methods and genealogical concordance results challenge the current species delimitations within *F. graminearum sensu lato* (*F. graminearum* species complex, a species aggregate within FSAMSC), and lesser-studied species like *F. armeniacum*, *F. longipes*, *F. sibiricum*, and *F. sporotrichioides*. In line with these results, narrower species circumscriptions are proposed, which correlate with distinctive morphological features. Fresh material of *F. sambucinum* was collected from its original substrate and location (*Sambucus nigra*, Germany), and its identity confirmed by morphological and mating experiments. An epitype is designated, thus finally stabilising the taxonomy of this important taxon, now circumscribed within a genetically and morphologically well-characterised phylogenetic clade.

CS 2.5.7 - Exploring fungal diversity in the North Sea and Atlantic Ocean

R. Ledo Doval[1], *R. de Vries*[1], *J. Houbraken*[2], *A. Gęsiorska*[1], *A. Wiebenga*[1]
[1]Westerdijk Fungal Biodiversity Institute, Fungal Physiology, Utrecht, Netherlands, [2]Westerdijk Fungal Biodiversity Institute, Food and Indoor Mycology, Utrecht, Netherlands

Marine fungi play vital roles in the ocean's ecosystems, shaping microbial diversity, driving ecological interactions, and influencing molecular processes. Despite recent advances in documenting the phylogeny, physiology, and ultrastructure of marine fungi, our understanding of their diversity and distribution remains incomplete. The ocean’s size and variety of substrates provide a largely unexplored source of fungal communities.
During the NICO expedition (2018), biological samples were collected from various marine stations across the North Sea and Atlantic Ocean, providing material for the identification and isolation of marine-derived fungi. A total of 240 fungal isolates were successfully recovered, identified, and classified, most to the species level, using a combination of sequencing approaches and detailed growth profiling. This growth analysis was designed to assess the substrate utilization preferences and growth patterns of the identified species.
By mapping each fungal species to the specific substrates, environmental conditions and locations from which they were collected, this study offers new insights into the distribution of marine fungal species. It not only expands the known diversity of marine fungi but also deepens our understanding of their distribution and substrate utilization abilities.

CS 2.5.8 - Fungi as a sustainable solution for *Striga* eradication

K. Mehta Bhatt[1], *N.v.d. Steeg*[1], *W.T. Pan*[1], *J. Dijksterhuis*[1], *T. Mostert*[1], *J. Maciá-Vicente*[2], *E. Kuramae*[2,3], *J. Groenewald*[1], *P. Crous*[1,3], *J. Collemare*[1]
[1]Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands, [2]Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, Netherlands, [3]Utrecht University, Utrecht, Netherlands

The root parasitic weed *Striga* causes yield losses of 30–100% in *Sorghum* and other cereal crops in sub-Saharan Africa. *Striga* infestation therefore negatively impacts the livelihood of smallholder farmers. Our aim in Promise-II project (https://promise.nioo.knaw.nl/en) is to explore potential of beneficial soil microbes as biocontrol agents to control *Striga*. Previously, approximately 2,700 fungal strains were isolated from Ethiopian field soils, *Sorghum* roots and *Striga* seeds. About 10% of 1,262 fungal strains showed a negative interaction with *Striga* - seed degradation and germination inhibition. In the present work, our aim is to characterize the mode of action of selected fungal strains, using comparative genomics and transcriptomics approaches to identify the molecular determinants responsible for the observed interactions. We sequenced the genomes of 36 fungal strains that belong to diverse genera across the fungal taxonomy. We hypothesized that secondary metabolites could be responsible for the observed phenotype. Using phylogenetic dereplication of genes encoding core biosynthetic enzymes and comparative genomics, we identified few candidate biosynthetic pathways. Further, the mycotoxigenic potential of the candidate biocontrol fungal strains were tested. Based on those results, we are now focusing on candidate fungal strains from three genera for Cryo-SEM and transcriptomics analyses during fungus-*Striga* interaction. This will allow us verifying the expression of candidate biosynthetic pathways, and identifying other candidate genes, including protein effectors and carbohydrate-active enzymes. This knowledge will aid the development of selected fungal species as biocontrol agents towards a better protection of *Sorghum* fields against *Striga* infestations in sub-Saharan Africa.

CS 2.5.9 - Fusarium pathogen population and mycotoxin diversity in barley grains from commercial fields in the Canadian prairies

X. Wang[1]
[1]Morden Research and Development Centre, Agriculture and Agri-Food Canada, Morden, Canada

Fusarium head blight (FHB) is a serious fungal disease of small cereal crops. In addition to the reductions in grain yield and grade, this disease can also result in the accumulation of harmful mycotoxins in infected grains. Between 2018 and 2023, FHB surveys for *Fusarium* pathogen complex and mycotoxin in barley grains were conducted in over 250 commercial fields across Canadian prairies. Results of the FHB survey show that *F. poae* and *F. graminearum* are the two most common *Fusarium* species affecting barley. Other *Fusarium* species, including *F. sporotrichioides*, *F. avenaceum*, and *F. culmorum*, are also found. Deoxynivalenol (DON) and nivalenol (NIV), type B trichothecenes, are the most abundant *Fusarium* mycotoxins in barley grains from producer's fields. The principle component analysis of *Fusarium* DNA and

mycotoxin concentration demonstrates close clustering of *F. poae* DNA and NIV, *F. graminearum* DNA and DON, as well as *F. sporotrichioides* and HT2/T2. The prevalence of *F. poae* infection in barley grains and the ability of this pathogen to produce NIV highlights its importance in the *Fusarium* pathogen complex. A dataset of *F. poae* strains from different regions of Canada is subjected to the population study using restriction-site associated DNA sequencing. This study will provide new insights into the complex genetics of *F. poae* and lay the groundwork for future research of this pathogen.

CS 2.5.10 - Fungal diversity and biodeterioration of archaeological carvings in badami Cave Temples: an in-depth microcosm study

S. Agrawal[1], *P. Scanlan*[1]
[1]University College Cork, APC Microbiome Ireland and School of Microbiology, Cork, Iceland

The Badami Caves represent a remarkable example of ancient Indian rock-cut architecture, dating back to the 6th century. Located in the Malaprabha River valley, these caves are part of the candidate UNESCO World Heritage Site known as the "Evolution of Temple Architecture—Aihole-Badami-Pattadakal," which is considered the cradle of temple architecture in India. Our study aimed to investigate the diversity, distribution, and biodeterioration of fungal communities present on the cave surfaces, alongside a comprehensive analysis of fungal biodeterioration affecting the cave carvings. Employing specialized techniques, we monitored the dissolution of calcite, changes in pH levels, and the biomineralization capabilities of isolated fungal strains. We also analyzed fungal acid production using high-performance liquid chromatography (HPLC). Our findings revealed that the primary genera of fungi found on the cave surfaces included *Acremonium*, *Curvularia*, *Cladosporium*, *Penicillium*, and *Aspergillus*. These isolated fungi were observed to produce acids that contributed to the dissolution of calcium carbonate and a subsequent decrease in pH values. Notably, the dominant genus responsible for acid production and biomineralization promotion was *Aspergillus*. These discoveries offer valuable insights into the ecology and functions of fungi inhabiting stone surfaces, enhancing our understanding of how to preserve and protect sculptures from biodeterioration.

CS 2.5.11 - The enigmatic role of mini-chromosomes in *Fusarium verticillioides*

M. Reverberi[1], *A. Grottoli*[2], *V. Scala*[2], *M. Beccaccioli*[1], *M.C. Ferrara*[1], *A. Doddi*[1], *L. Faino*[1]
[1]University of Rome, Department of Environmental Biology, Roma, Italy, [2]Council for Agricultural Research and Economics, Research Centre for Plant Protection and Certification, Rome, Italy

Fusarium verticillioides (*Fv*) is a maize pathogen that mycotoxins like fumonisins. To better understand *Fv* virulence, the genome of *Fv*10027 was sequenced, assembled and compared to the genome of *Fv*7600. Comparative genomics between *Fv*7600 and *Fv*10027 showed a difference in genome size of about 1.4 Mb despite 99% nucleotide identity. Additionally, the genome of *Fv*10027 add two mini-chromosomes of about 1 Mb and 750 Kb not present in *Fv*7600. To

determine the presence of these two mini-chromosomes in the Italian *Fv* population, 24 strains were sequenced, and presence/absence analysis showed that only three *Fv* strains had those additional chromosomes. The analysis of *Fv*10027 dispensable chromosomes showed enrichment of repetitive elements but we were not able to detect any enrichment on secreted proteins and or highly induced genes after infection. Additionally, we found that the 1Mb chromosome does not have any virulent effect on the Fv10027 infecting mays. Intriguingly, BLAST analysis on the *Fv*10027 proteins codified on mini-chromosomes have the best identity with *F. oxysporum* proteins located at dispensable chromosome 3 and 6 rather than *Fv*7600. Moreover, synonyms substitution analysis suggests that mini-chromosomes of *F. verticillioides* were probably not acquired through a horizontal chromosomal transfer from *F. oxysporum* but rather originated before the split of the two species. Currently, we are testing whether additional chromosomes of *F. verticillioides* can be useful in the interaction with other organisms like humans. Now, we are testing 20 *F. verticillioides* strains isolated from human infection of mays to understand whether there is any host specificity.

CS 2.5.12 - Novel pore-forming aegerolysins and their MACPF partners from fungi

N. Kraševec[1], *L.L. Popovšek*[2], *Č. Pantner*[3], *M. Skočaj*[2], *K. Sepčić*[2]
[1]National Institute of Chemistry, Department of Molecular Biology and Nanobiotechnology, Ljubljana, Slovenia, [2]University of Ljubljana, Biotechnical Faculty, Department of Biology, Ljubljana, Slovenia, [3]University of Ljubljana, Biotechnical Faculty, Department of Microbiology, Ljubljana, Slovenia

The aegerolysin family has received increasing attention in recent years due to its potential applications in biomedicine and biotechnology, despite the limited knowledge of its function. For example, some fungal aegerolysins can serve as probes for the detection, labelling, and imaging of specific membrane lipids, lipid rafts, cancer cells, invertebrates, or parasites. Their genes and their expression, or antibodies produced against aegerolysins, can serve as biomarkers or immunodiagnostic tools for the progression of fruiting body differentiation, fungal pathogens exposure, or infectious disease progression. In combination with larger protein partners, some of them can form pore-forming complexes that can be used to selectively eliminate insect pests or treat certain types of cancer cells.
Although aegerolysins are most abundant in fungi, some of them are also found in other kingdoms of the tree of life. The highly conserved beta-sandwich structure of these low molecular weight proteins is based on low identity primary sequences. Their sequences and copy numbers appear to be species and strain-specific. We have compared their phylogenetic tree with taxonomic distribution of the species. Different combinations of lipids are involved in the interactions of aegerolysins with different target organisms and may involve further interactions with various larger non-aegerolysin partners that can lead to pore formation. We biochemically characterized four novel aegerolysins and their MACPF protein partners from mushrooms *Heterobasidion irregulare*, *Trametes versicolor*, *Mucidula mucida* and *Lepista nuda* and compared them with the best-studied aegerolysins from the fungal genus *Pleurotus* and the previously studied aegeroysins from *Beauveria bassiana* and *Aspergillus niger*.

CS 2.6.1 - The extracellular transcriptome in Cryptococcus neoformans

G. Janbon[1]
[1]Institut Pasteur, Mycology, Paris, France

Extracellular vesicles (EVs) are now recognized as key players in the biology of numerous organisms, including pathogenic fungi. However, studying EVs in these organisms remains challenging. The recent implementation of new protocols to purify EVs in the pathogenic yeast *Cryptococcus neoformans* has resulted in a more detailed description of their structure and protein composition. Although a few publications describing RNA molecules associated with EVs have already been published, we reasoned that these new protocols would be beneficial for gaining a deeper understanding of the EV transcriptome. We thus purified EVs and confirmed that some RNAs were associated with these EV extracts. Iodixanol gradient analyses also revealed that these RNAs co-sedimented with EVs. We then sequenced these RNAs in parallel with RNAs extracted from the very cells producing these EVs using different types of sequencing libraries. Our data confirm the presence of siRNAs and tRFs associated with EVs, some of which are enriched. We also identified some snoRNAs, which in *Cryptococcu*s are mostly borne by coding gene or lncRNA introns.

CS 2.6.2 - Breaking the model – exploring the tRNA biology of non-model fungi

L. Dineen[1], *D. Wilson*[1], *L. Bodducherla*[1], *A. LaBella*[1]
[1]UNC Charlotte, Bioinformatics and Genomics, Charlotte, United States

The field of tRNA biology has developed exponentially over the past decade. Traditionally, tRNA molecules were placed passively at the centre of the translation machinery. More recently, research has pinpointed tRNA as having a major active role in mediating stress response in eukaryotes. Despite the significant advances in this field, we still know very little about tRNA dynamics and the role of tRNA gene diversity as tRNA sequencing has been challenging historically. Furthermore, there is a distinct lack of cross-species data, leading to a gap in our fundamental understanding of tRNA in fungi. Using direct RNA sequencing, we have carried out tRNA sequencing in a set of non-model yeasts for the first time. We present this data along with novel analysis methods to improve data capture from tRNA-seq experiments. Additionally, tRNA undergo extensive pre-possessing and are highly modified by tRNA modification enzymes. These enzymes act in site and base specific manners and require multi-enzymatic biosynthesis pathways. Furthermore, tRNA modifying enzymes have been previously shown to have antifungal target potential. Despite their importance, the diversity of modifying enzyme repertoires across several fungal species has not yet been explored. We employ machine learning techniques to predict tRNA modification enzyme annotations across an entire fungal subphylum, the Saccharomycotina. We observe the tRNA modification enzyme diversity on a previously unseen scale and, as a result, provide new insights into tRNA biology. Together our findings raise

new questions concerning the evolution of tRNA modification enzyme repertoires and tRNA diversity in fungi.

CS 2.6.3 - Transcriptional adaptation is conserved in the filamentous fungus *Neurospora crassa* and plays a considerable role in fungal fitness

H. Haj Hammadeh[1], *D. Stainier*[1]
[1]Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany

Transcriptional adaptation (TA) is a cellular response to certain genetic perturbations, where mRNA destabilizing mutations in one gene trigger the transcriptional modulation of other genes, known as adapting genes. At the molecular level, the mutant mRNA, rather than the loss of protein, leads to the transcriptional changes in the adapting gene(s).
Despite the significance and growing interest in genetic compensation, transcriptional adaptation has only been investigated in higher eukaryotes. Consequently, it remains unclear whether this phenomenon occurs in basal eukaryotes as well.
Here, we report transcriptional adaptation in the filamentous fungus *Neurospora crassa* and find that this process requires factors involved in mutant mRNA decay, similar to findings in *C. elegans* and zebrafish as well as in mouse cells in culture. Specifically, premature termination codon (PTC) but not full-locus deletion (FLD), alleles of *camk-1* display upregulation of *vsd-8*. A PTC allele in the gene *camk-1* exhibits a milder phenotype than the FLD allele. Notably, knocking out the adapting gene *vsd-8* in the *camk-1* PTC allele leads to a phenotype similar to that observed in the *camk-1* FLD allele. In addition, knocking out the nonsense-mediated decay (NMD) factors *upf-3* and, *upf-2* in the *camK-1* PTC allele also leads to a phenotype similar to that observed in the *camk-1* FLD allele.
Altogether, these results provide evidence for transcriptional adaptation in *N. crassa*, establishing it as a powerful model for further investigation into the underlying molecular mechanisms.

CS 2.6.4 - Alternative polyadenylation site usages in Cryptococcus neoformans (and its role in stress adaptation)

S. Delfosse[1], *N. Krid*[1], *F. Moyrand*[1], *C. Maufrais*[1,2], *G. Badis*[3], *G. Janbon*[1], *J. Colin*[1,4]
[1]Institut Pasteur, Mycology, Paris, France, [2]Institut Pasteur, HUB Bioinformatique et Biostatistique, Paris, France, [3]Institut Pasteur, Genomes & Genetics, Paris, France, [4]Ecole Pratique des Hautes Etudes, Paris, France

Cryptococcus neoformans, a human fungal pathogen, exhibits remarkable adaptability to extreme environments, including fluctuations in temperature, pH, and nutrient availability. These conditions necessitate rapid transcriptional and post-transcriptional changes to ensure *C. neoformans'* survival, proliferation, and virulence. Indeed, our previous analyses revealed that the transcriptome structure in this yeast is very complex and dynamic. More recently, the analysis of RNA-Seq, 3UTR-Seq, and Oxford Nanopore direct RNA sequencing data suggest that alternative polyadenylation (APA) site usage is also prevalent in this yeast. In mammals, APA is very

common and can influence mRNA stability, localization, and translation.
In this study, we focus on the usage of APA in *C. neoformans*, with particular interest in the *arg5,6* gene. This gene encodes a bifunctional protein, and intriguingly, its alternative polyadenylation site is located precisely between the two enzymes produced. Interestingly, this alternative polyadenylation site seems to be evolutionarily conserved. To understand the potential role of this short transcript, we constructed different types of mutants and performed a large variety of phenotypic analyses. The results of these analyses will be presented.

CS 2.6.5 - An improved description of the untranslated regions of poly(A)-tailed RNA from Aspergillus fumigatus

L. Schrettenbrunner[1], *C. Maufrais*[2], *G. Janbon*[2], *M. Blango*[1]
[1]Leibniz-HKI, RNA Biology of Fungal Infections, Jena, Germany, [2]Institute Pasteur, Paris, France

The filamentous fungus *Aspergillus fumigatus* is an opportunistic human pathogen and is categorized in the critical group of the WHO fungal pathogen priority list. Despite its intensely studied genome, relatively little is known about its transcriptome, including transcriptional start/end sites and the functional relevance of regulatory elements like untranslated regions (UTRs). With this project we aim to improve the map of *A. fumigatus* UTRs by experimentally validating and precisely defining the 5’ and 3’ ends of poly(A) transcripts. After enrichment of poly(A)-tailed RNA, we performed specialized 5’ and 3’ RNA-end sequencing. Initial screening of aligned reads showed that these were clearly enriched at the respective transcript ends. Peaks were called on the extracted 5’ and 3’ ends of each read of each method, respectively. High confidence sites were denoted as positions that were found in at least 3 replicates. These sites were then assigned to the closest gene on the reference genome. Finally, we performed manual curation to improve the overall annotation of end sites, which ultimately led to 67% of all genes with an associated high confidence 3’ end and 27% of all genes with a high confidence 5’ end. In addition to the mapped primary transcriptional ends, we also identified alternative end sites, sites with potential early termination, and 5’/3’ end sites within the coding sequences of genes. We hope that this data set will ultimately serve the *A. fumigatus* community as a resource to generate additional hypotheses and facilitate future investigations of the *A. fumigatus* transcriptome.

CS 2.6.6 - A near-terminal double-stranded RNA element is associated with near-identical [D1,2] stwintrons integrated in different, unlinked genes in species of *Xylariales*

E. Fekete[1], *V. Ág-Rácz*[1], *A. Márton*[1], *N. Ág*[1], *M. Flipphi*[1], *L. Karaffa*[1]
[1]University of Debrecen, Debrecen, Hungary

Spliceosomal U2 introns are ubiquitous in nuclear transcriptomes. Stwintrons consist of nested U2 introns. In a [D1,2] stwintron, an internal intron splits the 5'-donor of an external intron between its first and second nucleotide (nt). Almost all stwintrons described to date are of the [D1,2] type suggesting unique means for their duplication. Sequence-similar [D1,2] stwintrons –

sister stwintrons– are typically integrated at new intron positions in one species. Here, 285 sequence-similar [D1,2] stwintrons were cross-identified in 14 *Xylariales* genomes. Occasional missplicing was apparent, where almost the entire stwintron was excised by one splicing reaction. Clades of near-identical sister stwintrons were identified in *Xylaria* sp. MSU SB201401 and *Xylaria longipes* which differ only by one-to-five nt albeit integrated in completely different genes. These species-specific, near-identical stwintrons share near-terminal inverted repeat elements of 10-nt length we named 5'-NTIRE-10 and 3'-NTIRE-10, which are fully complementary as RNA. This characteristic suggests the crucial involvement of an RNA intermediate during [D1,2] stwintron duplication. The topical 5'-NTIRE-10 sequence is 5'-GUAUAAAAAC. Both elements are always located at very short distance from the RNA termini: four and five nt, respectively. 5'- and 3- NTIRE-10 partners can form a near-terminal stem structure that brings in close proximity the terminal G's of the [D1,2] stwintron and of its alternative misspliced intron. The folding of the interior intron RNA appears irrelevant. Ten of the 21 stwintrons with fully complementary NTIRE-10's are present in *Xylaria* sp. MSU SB201401 implying that [D1,2] duplication has most recently occurred more frequently in this species.

CS 2.6.7 - *Alternative evolutionary trajectories following RNAi loss in Cryptococcus*

J. Heitman[1]
[1]Duke University, Molecular Genetics and Microbiology, Durham, United States

Cryptococcus infections cause ~15% of AIDS-related deaths due to limited antifungal therapies and drug resistance. Clinical and environmental *Cryptococcus* isolates were assayed for increased mutation rates, and two hypermutator clinical isolates with increased mutation to rapamycin+FK506 resistance were identified. In contrast to hypermutators that result from mutations in mismatch repair or DNA polymerases, in these novel hypermutators Cnl1 transposon insertions conferred the majority of resistance and could also independently cause resistance to the clinically relevant drug 5-flucytosine. Whole-genome sequencing revealed both hypermutators harbor a nonsense mutation in the RNA-interference component Znf3 and hundreds of Cnl1 transposons organized into massive sub-telomeric arrays on each chromosome. QTL mapping of hypermutator X wild-type progeny identified a locus associated with hypermutation that included *znf3*. CRISPR editing of the *znf3* nonsense mutation abolished hypermutation and restored small-interfering-RNA production against Cnl1 transposons. Thus, hypermutation and drug resistance in these clinical isolates results from loss of RNAi and accumulation of Cnl1 transposons. Next two bioinformatic pipelines were developed and employed to identify other naturally occurring RNAi-loss isolates. These isolates are not hypermutators and have not accumulated a transposon burden. By CRISPR genome editing we confirmed that RNAi loss mutations in these isolates indeed result in loss of RNAi function. Following a genetic cross of these RNAi loss isolates with a mating partner bearing a transposon burden, F1 progeny were obtained exhibiting a marked hypermutator phenotype. These studies illustrate distinct evolutionary trajectories following loss of RNAi, and alternative routes via which RNAi loss can lead to hypermutator phenotypes.

CS 2.6.8 - RNAi drives heritability of epimutational antimicrobial resistance

C. Pérez-Arques[1], *M.I. Navarro-Mendoza*[1], *Z. Xu*[1], *G. Walther*[2], *J. Heitman*[1]
[1]Duke University, Department of Molecular Genetics and Microbiology, Durham, United States, [2]Hans Knöll Institute, National Reference Center for Invasive Fungal Infections, Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany

Epigenetic modifications that alter gene expression without changing the DNA sequence –known as epimutations– are a widespread phenomenon in eukaryotic organisms. Epimutations may arise from RNA interference, DNA methylation, and/or chromatin modifications, contributing to antifungal drug resistance and affecting virulence traits in fungal pathogens. Our research identifies RNAi epimutations in fertile *Mucor* phylogenetic species within the *Mucor circinelloides* species complex, a neglected group of human pathogens. These RNAi epimutations arise spontaneously upon an external stress and can be transmitted to the next generation in the absence of the initial insult. The inheritance pattern observed for these epimutations is DNA sequence-independent and non-Mendelian.
The absence of other repressive chromatin marks typically associated with epigenetic inheritance highlights that small RNA (sRNA) molecules act as the sole determinants of inheritance. This conclusion is further supported by unique sRNA signature patterns shared between epimutant parents and their progeny. Our findings demonstrate that epimutations are broadly present across the *Mucor* species complex and act exclusively through posttranscriptional gene silencing to control gene expression, advancing the understanding of genetic and epigenetic inheritance mechanisms in eukaryotes. Although epimutations are stable through both mitosis and meiosis, their detection may pose a challenge in typical culture methods employed in clinical diagnostics given that these frequently involve growth in the absence of drug selective pressure. Understanding how epimutations arise and modify gene expression may enable their detection in clinical settings and provide solutions for the challenges posed by rising antimicrobial drug resistance.

CS 2.6.9 - Spray-induced gene silencing: key factors for a successful implementation in the control of fungal plant pathogens

S. Mosquera[1], *M. Ginesy*[1], *I.T. Bocos Asenjo*[1], *H. Amin*[1], *S. Díez Hermano*[1], *J.J. Diez Casero*[1], *J. Niño Sánchez*[1]
[1]University of Valladolid, Plant Production and Forest Resources / Institute for Research in Sustainable Forest Management (iuFOR), Palencia, Spain

In recent years, RNA interference (RNAi)-based control has emerged as a viable alternative to traditional crop protection products in managing fungal plant pathogens. Among these methods, Spray-Induced Gene Silencing (SIGS) shows great promise due to its effectiveness, environmental friendliness, ability to prevent resistant strains, and high development potential. SIGS involves applying double-stranded RNA (dsRNA) to plants, targeting essential pathogen genes, which triggers gene silencing and inhibits disease development. Our laboratory trials have

demonstrated its success against pathogens such as *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Verticillium dahliae*, *Rhizoctonia solani*, *Fusarium graminearum*, *Fusarium circinatum*, and oomycetes like *Phytophthora capsici and Phytophtora cinnamomi*. However, when moving from laboratory to greenhouse and field conditions, challenges such as dsRNA stability and effective delivery to pathogens must be addressed.
To optimize SIGS, several factors must be considered in dsRNA design, including the selection of target genes (essential vs virulence genes), gene conservation, expression levels, turnover rate, dsRNA length, and mRNA accessibility. Additionally, optimizing siRNA populations by predicting effective guide RNAs, and minimizing off-target effects are critical, as is understanding how pathogens or host plants uptake dsRNA.
Drawing from our experience with nanotechnology, particularly double-layer hydroxides and liposomal vesicles, we assess options for enhancing SIGS efficiency, dsRNA protection, delivery and cost-effectiveness. This study highlights the knowledge gaps that require further research and serves as a guide for researchers new to SIGS technology, advancing its potential to control a broad range of fungal pathogens.

CS 2.6.10 - Advancing plant defense: RNAi-based control of fungal pathogens in grapevine

M. Karas[1], *F. Negrini*[1], *R. Baroncelli*[1], *E.G. Kabeto*[1], *X. Chen*[1], *L. Capriotti*[2], *S. Sabbadini*[2], *B. Mezzetti*[2], *E. Baraldi*[1]
[1]Alma Mater Studiorum - University of Bologna, Department of Agricultural and Food Sciences, DISTAL, Bologna, Italy, [2]Marche Polytechnic University, Department of Agricultural, Food, and Environmental Sciences, Ancona, Italy

The increasing incidence of fungal pathogens in viticulture, including *Plasmopara viticola* and *Erysiphe necator*, poses a significant threat to grapevine production and sustainability. Traditional control methods rely heavily on chemical fungicides, which pose environmental risks. In response, control strategies based on RNA interference (RNAi) and the application of double strand RNA (dsRNA) present a novel, eco-friendly alternative for managing fungal diseases. This study focuses on the development and application of Spray-Induced Gene Silencing (SIGS) to combat these key pathogens.
We successfully produced three pathogen-specific target molecules, focusing on effectors involved in fungal infection. Additionally, *CYP51* was used as a positive control based on existing literature, while a non-specific molecule served as a negative control. Promising results were observed for two of the specific molecules, which significantly reduced *Erysiphe necator* (powdery mildew) infection, demonstrating strong pathogen suppression. For *Plasmopara viticola* (downy mildew), we plan to target newly selected housekeeping genes, following a similar approach to that used in the powdery mildew trials.
The administration of dsRNA through SIGS against these targets offers a potentially effective strategy for controlling fungal pathogens without the environmental impact of chemical treatments. This approach lays the foundation for more sustainable crop protection methods in pesticide-dependent systems such as viticulture, and for broader applications in other crops vulnerable to fungal diseases.
This study was carried out within the Agritech National Research Center and received funding

from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

CS 2.6.11 - Participation of miRNA in the multidirectional communication between wheat and pathogenic *Fusarium culmorum*, and wheat and beneficial *Trichoderma atroviride*

S. Salamon[1], *L. Błaszczyk*[1], *P. Banachewicz*[1], *P. Havrysh*[1], *A. Chojnacka*[1]
[1]Institute of Plant Genetics PAS, Department of Plant Microbiomics, Poznań, Poland

Plants have developed complex gene regulation mechanisms in response to continuous exposure to beneficial or harmful microorganisms. The key elements of this intricate network of gene regulatory pathways are the endogenous small RNAs (sRNAs). These non-coding RNA molecules, typically 20-30 nucleotides long, function as precise repressors of gene expression. They may exert their effects at the transcriptional level through mechanisms such as DNA methylation and histone modification or at the post-transcriptional level by cleaving the target transcript or inhibiting translation. The largest class of regulatory molecules of gene expression are miRNAs. The main goal of the study is to decipher the role of RNA interference (RNAi) in biotic wheat-fungi interaction by understanding the miRNA-mediated communication between wheat and pathogenic *Fusarium culmorum* and symbiotic *Trichoderma atroviride*. Using the high throughput small RNA sequencing, the temporal (time course) and spatial (above- and under-ground plant organs) miRNA expression patterns in wheat inoculated with two different species of fungi in complex interaction systems: wheat – *F. culmorum*, wheat – *T. atroviride*, wheat – *T. atroviride* – *F. culmorum* was determined and compared in order to study the effects of fungal species, host organs, and interaction phase on the miRNA molecule compositions and distributions in wheat plants. We observed different expression profiles of the following miRNAs: tae-miR156, tae-miR9772, ata-miR393-5p, bdi-miR396a-5p, tae-miR9772, tae-miR395b. The role of the indicated particles in the wheat biotic interactions requires further investigation.
This research was funded by the Polish National Science Centre (project No. 2022/47/B/NZ9/01282).

CS 2.7.1 - Mitochondrial rRNA Methyltransferase MRM1 sustains the functionality of mitochondria by regulating translation elongation of all mitochondrial mRNAs independent of its methyltransferase activity

L. Xie[1], *L. Zhang*[1], *S. Ren*[1], *T. Wu*[2], *Z. Wang*[1], *C. He*[2], *Y. Liu*[3], *Z. Zhou*[1]
[1]Huazhong Agricultural University, College of Lifescience and Technology, Wuhan, China, [2]The University of Chicago, Department of Chemistry, Chicago, United States, [3]The University of Texas Southwestern Medical Center, Department of Physiology, Dallas, United States

Mitochondria generate ATP through OXPHOS and are the powerhouses of cells. They have their own genetic system. Mitochondrial translation is essential for OXPHOS production, and the

process of translation elongation is key for protein folding and complex assembly, though its regulatory mechanisms remain unclear. *Neurospora crassa*, a model for genetic and biochemical research, has been instrumental in mitochondrial studies, particularly OXPHOS, with a composition more similar to humans than yeast. Here we identified the methyltransferase MRM1 as a key factor for mitochondrial biogenesis and respiration in *Neurospora crassa*. MRM1 catalyzes Gm2453 on mitochondrial 23S rRNA, yet its role in mitochondrial function is independent of its enzymatic activity. MRM1 interacts with mitoribosomes and all mitochondrial mRNAs simultaneously through its N-terminal intrinsically disordered region (IDR). Positional analysis showed that MRM1 binds to non-optimal codon regions of mitochondrial mRNAs. Deletion of the interaction of either mitoribosome or mitochondrial mRNA led to increased mitochondrial translation, but reduced protein stability and disrupted complex assembly. These indicates that MRM1 inhibit translation elongation speed to ensure the proper folding of mitochondrial-encoded proteins and the accurate assembly of complexes and supercomplexes. Notably, the IDR of MRM1 is conserved only in filamentous fungi. Deletion of MRM1 homolog in *Magnaporthe oryzae* significantly reduced its infectivity to hosts, making it a promising target for antifungal drug development. In conclusion, we identified MRM1 as a general regulator for mitochondrial translation elongation in *Neurospora crassa*, elucidated its role in maintaining mitochondrial respiration, and highlighted its potential in antifungal drug development.

CS 2.7.2 - Iterative CRISPR/Cas9 genome editing to reduce extracellular protease activity for heterologous protein production in *Aspergillus niger*

M. Arentshorst[1], *J. van Dam*[1], *M. Raats*[2], *T. Verschuren*[2], *M. Zhou*[2], *J. Visser*[1], *R. Lubbers*[1], *A. Ram*[1]
[1]Leiden University, Institute Biology Leiden, Fungal Genetics and Biotechnology, Leiden, Netherlands, [2]Avans University of Applied Sciences Breda, Center of Expertise Materials and Energy Transition, Breda, Netherlands

The high protein secretion capacity of *Aspergillus niger* has been well recognized and exploited for the production of homologous and heterologous enzymes and other proteins. Genome mining revealed many extracellular or secretion pathway related (Golgi/vacuolar localized) proteases that are potentially harmful for heterologous protein yields. Inferred from experimental data and computational predictions, 60 possible secretion related proteases were identified.
To build a protein expression platform in *A. niger* and to identify proteases harmful for the production of heterologous proteins, a strain lineage of *A. niger* was developed to effectively express the gene of interest (GOI) combined with reduced protease activity. We used iterative CRISPR/Cas9-based genome editing to first delete genes encoding the most abundant secreted proteins (glucoamylase, acid amylase and alpha-glucosidase A), and genes involved in acidification (glucose oxidase and oxaloacetate hydrolase). In this non-acidifying background predicted secretion related proteases were deleted. Here we report a strain lineage consisting of 34 strains in which a total of 60 protease encoding genes were deleted. Gene deletions we verified by diagnostic PCR and representative strains from the lineage were genome sequenced to verify the deletions and to asses chromosome stability. Initial gene deletions were made by replacing the gene with a glucoamylase landing site, allowing targeted integration of the GOI.

Using this integration system, up to 10 copies of the GOI can be integrated effectively. The strain lineage is a powerful tool to identify secretion related proteases that are harmful for the production of heterologous proteins prone to proteolytic degradation.

CS 2.7.3 - Enhancing *impala* transposon-mediated insertional mutagenesis in *Zymoseptoria tritici*, a fungal pathogen of wheat

M.-H. Lebrun[1], *Y. Petit*[2], *C. Delude*[3], *A. Pitarch*[2], *G. Scalliet*[3]
[1]Université Paris-Saclay, CNRS, CEA, UMR I2BC, Genomics, Gif sur Yvette, France, [2]Université Paris-Saclay, INRAE, UR BIOGER, Palaiseau, France, [3]Syngenta Crop Protection AG, Stein, Switzerland

An improved insertional mutagenesis protocol was developped using the fungal transposable element (TE) *impala* from *TC1-mariner* family. *impala* transposase is necessary to excise and reinsert the TE at a TA site. *impala* was identified in *Fusarium oxysporum*, and it transposed successfully in other fungi (Dufresne and Daboussi 2010 Methods Mol. Biol. 638:41-54). Excision vectors were constructed with different *impala* haplotypes inserted in the 5'UTR of *Aspergillus nidulans* nitrate reductase gene. They were used to select *impala* excision events in *Zymoseptoria tritici* on nitrate synthetic media. Native *impala* had high excision (85%) and re-insertion (90%) rates, but a low frequency of excision (10^{-6}). *impala* displayed a positive bias toward genes (90%), and it inserted preferentially close to transcription starting sites (55%). This pattern of insertion suggested that *impala* could be used for activation tagging. To reach this goal, we developed vectors for the controlled transposition of *impala*. We showed that a defective *impala* (d-*impala*) could be trans-activated by an *impala* transposase expressed under the control of *Z. tritici* nitrate reductase promoter. We added a strong constitutive promotor (*pGdpA* from *A. nidulans*) in d-*impala* for activation tagging. The chimeric d-*impala*:*pGpdA* was able to excise and re-insert in *Z. tritici* genome with the same pattern as native *impala*. Optimization of *impala* transposase according to *Z. tritici* codon usage greatly improved its excision frequency (x1000) suggesting that *impala* transposition is controlled by codon usage. These improved tools provided new methods for mutagenesis in *Z. tritici* that could be applied to other fungi.

CS 2.7.4 - Efficient and targeted loss of heterozygosity in Candida albicans using CRISPR-Cas9

P. C. Despres[1,2], *N. C. Gervais*[2], *M. Fogal*[2], *R. K.J. Rogers*[2], *C. A. Cuomo*[3,1], *R. S. Shapiro*[2]
[1]Broad Institute, Boston, United States, [2]University of Guelph, Guelph, Canada, [3]Brown University, Providence, United States

The diploid genome of Candida albicans is highly heterozygous, with most allele pairs diverging at either the coding or regulatory level. When faced with selection pressure like antifungal exposure, this hidden genetic diversity can provide a reservoir of adaptive mutations through loss of heterozygosity (LOH) events. Validating the potential phenotypic impact of LOH events observed in clinical or experimentally evolved strains can be difficult due to the challenge of precisely targeting one allele over the other. Here, we show that the CRISPR-Cas9 system can be

used to overcome this challenge. By designing allele-specific guide sequences, we can induce targeted, directed LOH events. Additionally, the resulting recombination tracts have similar lengths to those observed naturally. Using this approach, we efficiently recapitulate a recently described LOH event that increases fluconazole resistance and assess off-target effects using whole-genome sequencing. To facilitate future use of this method, we provide databases of allele-specific guide sequences for Cas9 and Cas12a for C. albicans, as well as tools to generate custom databases for any diploid species or targeted nuclease of interest. This approach will therefore be useful in probing the adaptive role of LOH events in this important human pathogen.

CS 3.1.1 - The cytosolic interactors of NsdD GATA factor control sexual development in *Aspergillus nidulans*

S.-C. Jun[1], *K.-H. Han*[1]
[1]Woosuk University, Department of Pharmaceutical Engineering, Jeonbuk, Korea, Republic of

Sexual development is one of the main pathways through which fungi propagate and adapt to harsh environmental conditions. Previously, the *nsdD* gene was identified as a global regulator of sexual development in the homothallic fungus *Aspergillus nidulans*. Additionally, its interacting proteins, IndB and IndD, were found in *A. nidulans*; these proteins physically interact with NsdD, a GATA-type transcription factor, and are thought to play a role in regulating sexual development in this fungus. However, the exact regulatory mechanisms of IndB and IndD in sexual development and their specific functional domains remain uncharacterized. Using the Split-YFP/BiFC method, we analyzed the intracellular localization of interactions among NsdD, IndB, and IndD in *A. nidulans*. Fluorescent YFP signals for these interactions were primarily observed in the cytoplasm, especially within the vesicle domes and metulae of the conidiophore, an asexual reproductive structure, with additional signals in the septum and hyphal cell wall. To investigate potential redundancy in the functions of IndB and IndD, we constructed both single and double knockout mutants of these genes and analyzed their roles in sexual development. Single knockout mutants showed normal sexual development similar to the wild type. In contrast, the double knockout mutants exhibited uncontrolled formation of both mature and immature cleistothecia during sexual development and showed increased sensitivity to a cell wall lysis enzyme compared to the wild type and single mutants. These findings suggest that IndB and IndD act as negative regulators in sexual development with overlapping functions and may also play a role in cell wall integrity signaling in *A. nidulans*.

CS 3.1.2 - Polycomb repressive complex II balances vegetative growth and perithecial development in *Neurospora crassa*

A. Deaven[1], *A. Ameri*[1], *M. Lane*[1], *A. Abduljalil*[2], *Z. Lewis*[1]
[1]University of Georgia, Microbiology, Athens, United States, [2]University of Nevada, Reno, Microbiology & Immunology, Reno, United States

In most branches of the fungal kingdom, sexual development marks a dramatic shift from simple multicellular hyphae to large fruiting bodies composed of multiple layers of unique tissue types.

It is essential to tightly regulate and coordinate signals to prevent unnecessary energy investment into building these structures. In *Neurospora crassa*, we have found that Polycomb Repressive Complex II (PRC2) establishes domains of repressive chromatin across genes that are uniquely expressed during sexual development. PRC2 is a highly conserved regulator of multicellular development that represses genes by establishing domains of histone H3 lysine 27 tri-methylation (H3K27me3) across conditionally activated genes. Loss of PRC2 activity results in the precocious formation of perithecia-like structures in the absence of a compatible mating-type partner. However, bulk ChIP-seq has revealed that only subtle changes to the distribution of H3K27me3 occur in perithecia, even at genes that are strongly induced during sexual development. In contrast, we identified one intergenic region adjacent to the coding sequence of a predicted forkhead transcription factor, *vsd-1*, that loses enrichment of H3K27me3. *vsd-1* is essential for female development and suppresses the development of false perithecia in an H3K27me3-deficient background. Together, we have found that histone methylation is a key mediator of signals that promote sexual development and prevents aberrant perithecia development. We propose that upregulation of sexual development genes occurs via subtle, tissue-specific modifications to the epigenome as well as coordinated activity of specific transcription factors.

CS 3.1.3 - Exploring selective autophagy in the filamentous fungus *Sordaria macrospora*: identifying interactors of the pexophagy receptor SmNBR1 via BioID

S. Groth[1], *A. Werner*[2], *K. Schmitt*[3], *O. Valerius*[3], *S. Pöggeler*[1]
[1]Georg-August-University of Göttingen, Department of Genetics of Eukaryotic Microorganisms, Göttingen, Germany, [2]Georg-August-University of Göttingen, Department for Molecular Structural Biology, Göttingen, Germany, [3]Georg-August-University of Göttingen, Department of Molecular Microbiology and Genetics, Göttingen, Germany

Selective autophagy is a conserved subcellular process that uses receptors to target specific cargo for degradation in the vacuole. In the filamentous ascomycete *Sordaria macrospora, neighbour of BRCA1 (SmNBR1) was identified as receptor for the* selective autophagy of peroxisomes (pexophagy). However, little is known about further players in the pexophagy of *S. macrospora*. Thus, we aimed to identify SmNBR1-interactors by employing the Biotin Identification (BioID) method. Hereby, SmNBR1 is fused to a promiscuous biotin-ligase, which labels neighbouring proteins with biotin. Biotin-labelled proteins are then enriched by affinity purification and identified by liquid chromatography coupled to mass spectrometry (LC-MS). In these experiments, we identified several proteins to be putative SmNBR1 interactors. Among them are the core autophagy protein SmATG11 and a coupling of ubiquitin to ER degradation (CUE) domain-containing protein, which we termed SmCUE3. Since ATG11 homologs in other organisms are scaffold proteins crucial for various selective autophagy processes, SmATG11 is a reasonable candidate, and we are currently characterizing its role in the life cycle of *S. macrospora*. The enrichment of the ubiquitin-binding protein SmCUE3, however, could explain a previously unexplored difference between mammalian/plant NBR1 homologs and fungal NBR1 homologs. While the former exhibit an ubiquitin-associated (UBA) domain, this sequence motif is lacking in fungal homologs. Therefore, we speculate that SmCUE3 might serve as an adaptor

between SmNBR1 and its ubiquitinated cargo by replacing the missing UBA domain. To test this hypothesis, we are currently verifying the interaction of SmNBR1 with SmCUE3 and its function in selective autophagy.

CS 3.1.4 - The Cryptococcus neoformans STRIPAK complex controls genome stability, sexual development and virulence

P.P. Peterson[1], J.-T. Choi[2], C. Fu[3], L.E. Cowen[3], S. Sun[1], Y.-S. Bahn[2], J. Heitman[1]
[1]Duke University Medical Center, Molecular Genetics and Microbiology, Durham, United States, [2]Yonsei University, Department of Biotechnology, Seoul, Korea, Republic of, [3]University of Toronto, Department of Molecular Genetics, Toronto, Canada

The eukaryotic serine/threonine protein phosphatase PP2A interacts with the highly conserved multi-protein striatin-interacting phosphatase and kinase (STRIPAK) complex. Orthologs of STRIPAK components were identified in *Cryptococcus neoformans*, namely PP2AA/Tpd3, PP2AC/Pph22, PP2AB’’’/Far8, STRIP/Far11, SLMAP/Far9, and Mob3. Structural modeling, protein domain analysis, and detected protein-protein interactions suggest *C. neoformans* STRIPAK is assembled similarly to human and fungal orthologs. Here, five of the STRIPAK components, Pph22, Far8, Far9, Far11, and Mob3, were functionally characterized. Whole-genome sequencing revealed that mutations in STRIPAK complex subunits lead to increased segmental and chromosomal aneuploidy, suggesting STRIPAK functions in maintaining genome stability. STRIPAK component deletion mutants exhibit defects in mating and sexual differentiation, including impaired hyphae, basidia, and basidiospore production. Loss of *PPH22*, *FAR8*, *FAR9*, or *FAR11* leads to severely reduced growth at elevated temperature, abnormal morphology, and impaired virulence. The *pph22*Δ, *far8*Δ, *far9*Δ, *far11*Δ mutants are also unable to grow in the presence of calcineurin inhibitors cyclosporine A or FK506, demonstrating these mutations are synthetically lethal with loss of calcineurin activity. Conversely, *mob3*Δ mutants display increased thermotolerance, capsule production, and melanization, and are hypervirulent in a murine infection model, showing significant lung inflammation and brain dissemination as early as seven days post infection. Transcriptome analyses indicated that loss of STRIPAK subunits significantly affects expression of genes involved in energy metabolism, transmembrane transport, RNA processing, and virulence. Taken together, these findings reveal that the *C. neoformans* STRIPAK complex plays an important role in genome stability, vegetative growth, sexual development, and virulence in this prominent human fungal pathogen.

CS 3.1.5 - Dynamic molecular dialogues in *A. nidulans* development: Interplay between pheromone producing enzymes and transcriptional regulators

E.S. Xylakis[1], A. Zhornyak[1], L. Moorkamp[1], E. Bastakis[1], B. Popova[1], K. Schmitt[1], O. Valerius[1], G.H. Braus[1]
[1]Georg August University, Department of Molecular Microbiology & Genetics, Goettingen, Germany

Internal as well as external stimuli determine the ratio of the further development of vegetatively growing *Aspergillus nidulans* hyphae to more asexual or more sexual development. The timing and balance between sexual and asexual development are controlled by the products of the fatty acid oxygenases PpoA (**P**si (precocious sexual inducer)-**p**roducing **o**xygenase **A**) and PpoC (**P**si-**p**roducing **o**xygenase **C**). Specific cellular Psi factor accumulation represents the molecular cues, which channel the ratio between the distinct transcriptional programs for the asexual or sexual developmental pathways. Therefore PpoA and PpoC indirectly regulate the transcriptomic landscape by producing specific gene expression patterns for either sexual or asexual development. Transcriptional changes driven by Psi factors are essential for ensuring the controlled and regulated progress of each developmental pathway in response to environmental signals. We have analyzed the physical interactions of the Psi-producing oxygenases and important players of fungal transcription. The current status of our study in exploring the interaction dynamics between metabolic enzymes and transcriptional regulators and their consequences for fungal development will be presented

CS 3.1.6 - Antagonistic Pathways of Lipid Transfer Proteins (LTPs) for *de novo* Membrane Assembly in Fission Yeast

M. Basante-Bedoya[1], *S.G. Martin*[1]
[1]University of Geneva, Department of Molecular and Cellular Biology (MOCEL), Geneva, Switzerland

Fungal sporulation involves *de novo* synthesis of the spore plasma membrane, named the forespore membrane (FSM), which surrounds each meiotic nucleus. Biomembrane lipid composition is regulated by synthesis, degradation, and lipid transport. Lipid transfer proteins (LTPs) facilitate non-vesicular lipid transport. In *Schizosaccharomyces pombe*, Ltc1, from the LTP anchored at membrane contact sites (LAM) family, transports ergosterol from the plasma membrane (PM) to the endosomes, and Osh41, from the oxysterol-binding homology (Osh) protein family, conversely promotes PM ergosterol levels [1]. However, neither LTP is essential, suggesting redundancy with other transport mechanisms, and their role in sporulation remains unknown.
We have undertaken a systematic exploration of the function of LTPs in fission yeast. Our findings on Osh-family LTPs show that: (i) Concurrent Ltc1/Osh3 or Osh41/Osh2 deletions are synthetically lethal, indicating redundancy; (ii) Deletion of neither *osh41* nor *osh42* causes major effect on sporulation, yet *osh41*Δ*osh42*Δ double mutant exhibits complete failure of spore formation; (iii) *osh3* deletion partially restores *osh41Δ* mutant PM-ergosterol levels and circumvents the sporulation failure of *osh41*Δ*osh42*Δ cells, suggesting antagonistic functions between Osh41/Osh42 and Osh3. Our results highlight both functional redundancy and antagonistic functions among lipid transfer protein families.
[1] Marek, M., Vincenzetti, V. & Martin, S. G., J. Cell Biol. 219, e202001147 (2020).

CS 3.1.7 - Fungal feature tracker 2.0: unleashing the power of image analysis in fungal research

G. Vidal-Diez de Ulzurrun[1], *T.-Y. Huang*[2], *Y.-P. Hsueh*[1,2]
[1]Max Planck Institute for Biology, Complex Biological Interactions, Tuebingen, Germany, [2]IMB Academia Sinica, Taipei, Taiwan, China

The development of filamentous fungi may seem simple, but through processes like branching and elongation, these organisms create extensive mycelial networks that can span kilometers and endure for years. These networks swiftly adapt to environmental changes, serve as communication systems, and transport nutrients across long distances. However, translating these complex structural dynamics into quantifiable data remains a significant challenge in fungal research.
To address this, we developed the Fungal Feature Tracker—a tool designed to provide robust mathematical analyses of fungal morphological traits. While our original software required manual input for image processing and provided basic measurements on individual images, the updated version offers significant improvements. Image processing is now fully automated through machine learning, eliminating the need for user interaction. Furthermore, the new algorithm enhances the analysis by processing entire series of images over time, capturing dynamic growth patterns with greater precision.
Additionally, the tool improves the analysis of growth patterns and allows for localized investigations of mycelial structures, providing a more detailed view of fungal development. Using nutrient variation as a case study, we observed distinct growth patterns in the nematode-trapping fungus *Arthrobotrys oligospora* responding to different nutrient sources, further showcasing the software's potential for revealing new insights into fungal biology.

CS 3.1.8 - Five successive transcriptomic waves control sexual reproduction in Podospora anserina

F. Bidard[1], *P. Grognet*[2], *G. Lelandais*[3], *M.-H. Muchielli*[4], *R. Debuchy*[5], *V. Berteaux-Lecellier*[6], *F. Malagnac*[5]
[1]IFP Energies Nouvelles, Rueil-Malmaison, France, [2]University Paris Saclay, I2BC, Gif sur Yvette, France, [3]University Paris Saclay, Gif sur Yvette, France, [4]University Paris Saclay, IPS2, Gif sur Yvette, France, [5]University Paris Saclay, I2BC, Gif Sur Yvette, France, [6]Ecologie Marine Tropicale dans les Océans Pacifique et Indien, Nouméa, France

Despite the inherent challenge of finding suitable mating partners, most eukaryotes use sexual reproduction to produce offspring with increased genetic diversity and fitness. The persistence of this mode of reproduction is a key question in evolutionary biology. Fungi offer valuable insights due to their diversity and short life cycle. This study focuses on Podospora anserina, a pseudohomothallic fungus that circumvents self-sterility by maintaining two compatible nuclei in one mycelium. We performed genome-wide gene expression profiling during ten stages of P. anserina sexual reproduction and identified five major expression patterns. Our expert annotation approaches identified differentially expressed genes related to secondary metabolite production,

fungal vegetative incompatibility, programmed cell death, and epigenetic regulation. In addition, master transcriptional regulators and their target networks were uncovered. This study provides a comprehensive database for future functional genomics experiments and novel pathway characterization during sexual reproduction.

CS 3.2.1 - Flotillin-containing lipid raft microdomains are linked to calcium in *Aspergillus nidulans*

M. Kawashima[1,2], *T. Krüger*[1], *A.A. Brakhage*[1,2]
[1]Leibniz Institute for Natural Product Research and Infection Biology, Department of Molecular and Applied Microbiology, Jena, Germany, [2]Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany

Lipid rafts are tight assemblies of proteins and lipids in a biological membrane and are thought to be involved in many physiological processes such as immune signalling and host-pathogen interactions. However, due to their small sizes exceeding the resolution limit of conventional light microscopy, direct measurement and characterisation of lipid rafts in living membranes remains to be a challenge. While most studies on lipid rafts have been carried out on mammalian cells, here we use the genetic model fungus *Aspergillus nidulans* to allow a more versatile characterisation of lipid rafts at both molecular and organismic levels. In particular we investigate the role of flotillin (FloA), a lipid raft marker conserved across many organisms. Using a nanoluciferase reporter strain, we demonstrate that the *A. nidulans* flotillin FloA is highly expressed when the fungus is confronted with high calcium stress. Transcriptomic analysis further reveals that repression of *floA* under these conditions leads to the upregulation of numerous mitochondrial genes, suggesting a functional connection between FloA and mitochondrial activity. In addition, in vivo protein-proximity labelling is performed to assess the physical interaction partner proteins of FloA. We also explore the role of FloA in microbial communication through co-cultivation experiments with the soil bacterium *Streptomyces iranensis* and show that the bacterium is able to induce the high expression of FloA, most likely by secreting a natural product. These findings contribute to a deeper understanding of lipid raft dynamics and the organisation of eukaryotic membranes.

CS 3.2.2 - Endocytosis in Aspergillus nidulans

M. Pinar[1], *M. Delgado*[1], *P. Polonio*[1], *S. Fandiño*[1], *E. Espeso*[1], *M.A. Penalva*[1]
[1]CSIC Centro de Investigaciones Biologicas Margarita Salas, Madrid, Spain

Endocytic recycling is crucial for the hyphal mode of life. However, the process of endocytic internalization (referred to as endocytosis), which mediates the first step in this pathway, is insufficiently understood. We report a detailed investigation of endocytosis in Aspergillus nidulans, arguably the most thorough analysis ever carried out in a filamentous fungus. Endocytosis occurs at cortical actin patches, reflecting the fact that vesicle internalization is powered by actin polymerization. We have developed an extensive set of fluorescent chimeric reporters of F-actin and endogenously FP-tagged several of the components of the so denoted, by

Drubin and Kaksonen, actin polymerization module of endocytosis. We have determined, using kymographs derived from movies acquired with a time resolution of 5 fps, that the time of residence of F-actin in cortical patches is 13 seconds. We have investigated the role of Arp2/3 and the mechanism by which this complex, that gives rise to highly branched actin structures, is seeded with preformed linear actin filaments whose nucleation is formin-independent. We have studied how actin polymerization in endocytic patches is terminated by the concerted action of capping protein and accessory factors, and how subsequent F-actin severing by cofilin and associates plays a key role in endocytosis. For each and every protein predicted to have a crucial role in endocytic internalization, we demonstrate, using heterokaryon rescue techniques, that ablation of the corresponding gene is lethal. For those genes which play a non-essential role we demonstrate that the extent of their involvement correlates with efficiency of endocytic recycling.

CS 3.2.3 - Cross-species comparison of AlphaFold-derived G protein-coupled receptor structures reveals novel melatonin-related receptor inNeurospora crassa

C. Maienza[1], *G. Lamoureux*[1,2], *K. Lee*[1,3]
[1]Rutgers University - Camden, Center for Computational and Integrative Biology, Camden, United States, [2]Rutgers University - Camden, Department of Chemistry, Camden, United States, [3]Rutgers University - Camden, Department of Biology, Camden, United States

Melatonin, a molecule with diverse biological functions, is ubiquitously present in living organisms. There is significant interest in understanding melatonin signal transduction pathways in humans, particularly due to its critical role in regulating the sleep-wake cycle. However, a knowledge gap remains in fully elucidating the mechanisms by which melatonin influences circadian regulation. To bridge this gap, there is a growing need for a model system to study the role of melatonin in circadian clocks, with *Neurospora crassa* being a promising candidate. As a first step in this investigation, we focused on identifying melatonin receptors in *N. crassa*. Given the lack of sequence similarity between potential receptors in this fungus and known human melatonin receptors, we utilized structural similarity analysis through AlphaFold2. This approach led to the identification of a strong candidate gene, *gpr-3*, which shares structural similarities with human melatonin receptors. Experimental validation confirmed that the removal of GPR-3 from cells results in the absence of melatonin signaling. This proof-of-concept study underscores the potential of *N. crassa* as a model organism for circadian research and demonstrates the broader applicability of using AlphaFold2, especially when sequence similarity does not lead to candidate genes, for identifying novel receptors across different species.

CS 3.2.4 - Investigating the role of the Nuclear Dbf2-related (NDR) kinase Cot1 in appressorium morphogenesis and polarized growth in the blast fungus *Magnaporthe oryzae*

R. Taylor[1], *A. Rogers*[1], *R. Proko*[1], *J. Stajich*[2], *M. Egan*[1]
[1]University of Arkansas System Division of Agriculture, Department of Entomology and Plant Pathology, Fayetteville, United States, [2]University of California Riverside, Department of Microbiology and Plant Pathology, Riverside, United States

The filamentous fungus *Magnaporthe oryzae*, which causes blast disease in rice, wheat, and numerous other grasses, produces a specialized pressure-generating infection cell called an appressorium to rupture the host leaf cuticle and initiate biotrophic infection. As part of a fluorescence microscopy-based screen for mutants perturbed in cytoskeleton-mediated appressorium repolarization, we isolated a mutant (M41) that exhibited severely attenuated polarized growth and produced malformed appressoria with aberrant subcellular architecture. Comparative whole-genome sequencing of M41 revealed a non-synonymous point mutation within a gene encoding a Nuclear Dbf2-related (NDR) kinase, and Cot1 ortholog, resulting in a G402A substitution within the predicted kinase domain. Cot1 belongs to a subfamily of AGC kinases known to regulate diverse processes, including cell growth and proliferation. Genetic complementation of M41 with the wild-type *COT1* allele rescued all mutant phenotypes. Strikingly, we found that Cot1 undergoes bi-directional trafficking along microtubules in a Hook1-dependent manner, suggesting that it likely localizes to motile early endosomes. In other model fungi, certain mRNA-binding proteins are known substrates of NDR kinases, which control their cellular localization and the fate of their associated mRNA. Consistent with this, we found that the *M. oryzae* ortholog of the mRNA-binding protein Gul1 co-traffics with Cot1. Furthermore, in Δ*cot1* mutants, Gul1 trafficking is largely abolished, and the mRNA-binding protein instead localizes to discrete foci resembling P-bodies. Taken together, our data suggest a potential role for Cot1-mediated localized mRNA translation in appressorium morphogenesis and plant infection.

CS 3.2.5 - Role of phenotypic switching in the regulation of *Fusarium oxysporum* development and pathogenicity

P. Gutierrez-Escribano[1], *A. Di Pietro*[1]
[1]Universidad de Córdoba, Genetics, Córdoba, Spain

Phenotypic plasticity ensures rapid adaptation to ever-changing environments. Non-genetic phenotypic heterogeneity is widely observed in various biological systems and poses a significant challenge in cancer therapy. In fungal pathogens, epigenetically determined phenotypic switching has been linked to differentiation, host colonisation and the emergence of drug resistance. *Fusarium oxysporum* is a large species complex (FOSC) of ascomycete lineages that causes vascular wilt disease in over one hundred different plant hosts and is also an important emerging human pathogen. We report that the tomato wilt isolate *F. oxysporum* f. sp. *lycopersici* Fol4287 as well as the human pathogenic isolate MRL8996, can stochastically activate heritable and

reversible switches between different phenotypic states, referred to as “white” and “pink” based on the colour of the colonies when grown in the presence of vital stains. These switch phenotypes differ in their morphology, physiology and pathogenicity traits. We investigated the role of various environmental signals in triggering the phenotypic transition as well as the transcriptional circuits involved in the establishment and maintenance of phenotypic variants. Our data suggest that phenotypic switching plays an important role in the adaptive response of *F. oxysporum* and is critical for understanding fungal microevolution within the host-pathogen context.

CS 3.2.6 - C. albicans tolerance to the antifungal drug fluconazole is regulated by cytoplasmic crowding

E. Plumb[1], *A. Serrano*[1], *J. Elferich*[2], *N. Grigorieff*[2], *M. Bassilana*[1], *R. Arkowitz*[1]
[1]University Côte d'Azur - CNRS - INSERM, Institute of Biology Valrose, Nice, France,
[2]University of Massachusetts Chan Medical School, RNA Therapeutics Institute, Worcester, United States

Candida albicans is a major cause of both persistent mucosal and life-threatening infections. A subpopulation, referred to as tolerant cells, grows at reduced rates in inhibitory concentrations (10 x MIC) of the most widely used antifungal drug, fluconazole [1]. Tolerance likely contributes to recurrent and persistent fungal infections, hence we set out to investigate cellular responses to prolonged exposure to high antifungal concentrations. We took advantage of a passive microrheological probe, genetically encoded multimeric nanoparticles (GEMs) that are 40 nm in diameter (in the mesoscale) and analyzed their diffusivity as a readout of cytoplasm crowding/viscosity [2]. Our results reveal a dramatic increase in cytoplasmic crowding upon inhibition of the fluconazole target: ergosterol biosynthesis. Conversely, we observed that decreasing cellular ribosome levels, either genetically or chemically, recovers this fluconazole effect and concomitantly reduces drug tolerance. Together, these results suggest that changes in ribosome levels mediate the dramatic increase in cytoplasmic crowding that occurs upon prolonged inhibition of ergosterol biosynthesis. To directly test this hypothesis, we have recently begun to quantify cytoplasmic ribosome levels using cryo-electron microscopy [3]. We speculate that tuning cytoplasmic ribosome levels may play a role in tolerance to azole antifungal drugs and hence be important in treating persistent *Candida albicans* infections.

1) Rosenberg *et al. Nature Communications* 2018 **9**:2470
2) Delarue *et al. Cell* 2018 **174**:338
3) Bronwyn *et al. eLife* 2022 **11**:e79272

CS 3.2.7 - Mucoromycete germlings undergo regulated cell death mediated by adenylyl cyclases in the innate immune response to bacterial perception

D. Tota[1], *M.L. Gaspar*[2], *C. Lax*[3], *V. Garre*[3], *L. Esipov*[2], *T. Pawlowska*[2]
[1]Cornell University, Graduate Field of Microbiology, Ithaca, United States, [2]Cornell University, Plant Pathology & Plant-Microbiology, Ithaca, United States, [3]Universidad de Murcia, Departamento de Genética y Microbiología, Murcia, Spain

Microorganisms' survival is dependent on the ability to perceive and respond to the biotic environment. Innate immunity enables cells to sense the non-self, regulate interactions with potential pathogens, and maintain organismal integrity. Despite fungi being known to inhabit virtually all environments and interact with multiple kingdoms of life, the understanding of fungal defenses, especially that of early-divergent fungi, is in its infancy. Our work using wildtype and adenylyl cyclase mutants has revealed that germlings of *Rhizopus microsporus*, an emerging model for Mucoromycete-bacterial interactions, antagonistically respond to the bacteria *Mycetohabitans* sp. B13 and *Ralstonia pickettii* through adenylyl cyclase-mediated signaling. Response by germlings includes lipid peroxidation and regulated cell death, as characterized by fluorescence and transmission electron microscopy and flow cytometry. Our study system also includes *Mucor lusitanicus*, a model for Mucoromycete genetic manipulation. Growth inhibition assays of wildtype and adenylyl cyclase mutant strains of *R. microsporus* and *M. lusitanicus* exposed to various isolated bacterial MAMPs (microbe-associated molecular patterns) suggest that Mucoromycetes exhibit a generic stress response to the perception of bacteria, regardless of bacterial identity or activity. We hypothesize that Mucoromycotina fungi initiate a generalized innate immune response that is functionally similar to plant and animal innate immunity. As regulated cell death is a novel innate immune response in fungi, we plan to characterize the form of regulated cell death initiated by *R. microsporus* and *M. lusitanicus* in response to bacterial perception by quantifying the molecular hallmarks of regulated cell death using bioassays, fluorescence and transmission electron microscopy, and flow cytometry.

CS 3.2.8 - Atypical cell death phenomenon induced by cell fusion revealed by pairing diverse strains in the industrial fungus *Aspergillus oryzae*

Y. Zou[1], *T. Katayama*[1], *K. Iwashita*[2], *J.-I. Maruyama*[1]
[1]The University of Tokyo, Department of Biotechnology, Tokyo, Japan, [2]National Research Institute of Brewing, Hiroshima, Japan

In filamentous fungi, cell fusion between genetically incompatible strains results in cell death, a phenomenon referred to as heterokaryon incompatibility. In *Aspergillus oryzae*, an industrial filamentous fungus used in Japanese food fermentation, there are numerous diverse strains for different purposes such as sake, soy sauce, and *miso* production. Our previous study revealed that *A. oryzae* strains can be classified into compatible groups, and cell death was typically detected in both fused cells derived from incompatible strains. In this study, we extensively examined

diverse *A. oryzae* strains in heterokaryon incompatibility and assessed cell death phenomena. To explore the connection between the phylogeny and overall diversity in *A. oryzae*, multiple strains were selected from each of phylogenetic clades. Compatibility analysis by protoplast fusion revealed that compatible group classification exhibited a consistency with the phylogenetic clades. Additionally, co-culturing was performed to analyze heterokaryotic cell formation through cell fusion; strains from distinct phylogenetic clades did not form heterokaryon cells detected. However, unexpectedly, some of strain pairings within the same phylogenetic clade failed to produce heterokaryotic cells, which conflicted with the compatibility groupings by protoplast fusion. During cell fusion in such strain parings, either of the fused cells underwent cell death. These findings revealed an atypical cell death phenomenon induced by cell fusion under specific pairings of the diverse *A. oryzae* strains.

CS 3.3.1 - Discovery of penicillic acid as a chemical probe against tau aggregation in Alzheimer's Disease

J. Shyong[1], J. Wang[1], T.Q.D. Huynh[2], M. Fayzullina[1], B. Yuan[1], C.-K. Lee[2,3], T. Minehan[4], P. Seidler[1], C. Wang[1,5]
[1]University of Southern California, Pharmacology and Pharmaceutical Sciences, Los Angeles, United States, [2]Taipei Medical University, College of Pharmacy, Taipei, Taiwan, China , [3]Taipei Medical University, Ph.D. Program in Clinical Drug Development of Herbal Medicine, Taipei, Taiwan, China , [4]California State University, Northridge, Chemistry and Biochemistry, Northridge, United States, [5]University of Southern California, Chemistry, Los Angeles, United States

Alzheimer's Disease (AD) is a neurodegenerative disorder proven to be caused by the aggregation of protein tau into fibrils, resulting in neuronal death. The irreparable neuronal damage leads to irreversible symptoms with no cure; therefore, disaggregation of these tau fibrils could be targeted as a therapeutic approach to AD. Here we have developed a fungal natural product library through the genetic modification of global regulator *mcrA* to screen for secondary metabolites that have bioactive potential towards AD tau. Our initial screenings indicate that penicillic acid demonstrates anti-aggregation activity towards tau, while further *in vitro* experiments reveal that penicillic acid directly inhibits tau by disaggregating fibrils. Although penicillic acid possesses blood-brain barrier penetrability properties that are computationally predicted to be favorable, it is presumed to contain some mutagenic effects as well. To address this, we used the backbone of penicillic acid as a chemical probe to discover similar compounds that can inhibit AD tau aggregation with limited mutagenicity. This work suggests the potential of discovering chemical probes through natural product screening for small-molecule drug discovery of tauopathies

.

CS 3.3.2 - Early steps of the biosynthesis of the anticancer antibiotic pleurotin

J. Weaver[1], D. Alkhder[1,2], P. Prasongpholchai[1], M. Tadesse[1], E. de los Santos[1,3], L. Song[4], C. Corre[1,4], F. Alberti[1]
[1]University of Warwick, School of Life Sciences, Coventry, United Kingdom, [2]University of Leicester, Leicester Medical School, Leicester, United Kingdom, [3]UCB Biopharma, Slough, United Kingdom, [4]University of Warwick, Department of Chemistry, Coventry, United Kingdom

Pleurotin is a meroterpenoid secondary metabolite made by the Basidiomycota fungus *Hohenbuehelia grisea*, which was first identified as an antibiotic [1] and it is now also considered a lead anticancer molecule due to its irreversible inhibition of the thioredoxin-thioredoxin reductase system [2]. Bioactive pleurotin congeners made by *H. grisea* have been described, including the antiviral 4-hydroxypleurogrisein [3]. Total synthesis of pleurotin has been achieved, including through a stereoselective route [4], however its biosynthesis has not been characterised. In this study, we used isotope-labelled precursor feeding to show that the non-terpenoid quinone ring of pleurotin and its congeners nematoctone, pleurothiazole and 4-hydroxypleurogrisein is derived from phenylalanine. We sequenced the genome of *H. grisea* and used comparative transcriptomics to identify putative pleurotin biosynthetic genes. The heterologous expression of a UbiA-like prenyltransferase from *H. grisea*, alongside its FPP synthase for increased precursor supply, resulted in the accumulation of the first predicted pleurotin biosynthetic intermediate, 3-farnesyl-4-hydroxybenzoic acid [5]. This work sets the foundation to fully elucidate the biosynthesis of pleurotin and its congeners, with long-term potential to optimise their production for therapeutic use and engineer the pathway towards the biosynthesis of valuable analogues.

References:

[1] Robbins WJ *et al*. (1947) *Proc Natl Acad Sci USA 33*, 171.
[2] Welsh SJ *et al*. (2003) *Mol Cancer Ther 2*, 235.
[3] Sandargo B *et al.* (2018) *Molecules 23*, 2697.
[4] Gao Y *et al*. (2024) *J Am Chem Soc 146*, 18230.
[5] Weaver JA *et al*. (2024) *ACS Chem Biol* accepted article, https://doi.org/10.1021/acschembio.4c00599.

CS 3.3.3 - Transformation of *Alternaria dauci* demonstrates the involvement of two polyketide synthase genes in aldaulactone production and fungal pathogenicity

J.M. Bernardino[1], E. Neau[1], M. Gadras[1], J. Kocuiba[1], A. Rigaud[1], J. Courtial[1], J.-J. Helesbeux[2], S. Aligon[1], B. Hamon[1], J. Colou[1], J. Collemare[3], P. Poupard[1], N. Bataillé-Simoneau[1], R. Berruyer[1]
[1]Université d'Angers, Institut Agro, INRAE, UMR IRHS 1345, SFR 4207 QUASAV, Angers, France, [2]Université d'Angers, Substances d'Origine Naturelle et Analogues Structuraux, SFR 4207 QUASAV, Angers, France, [3]Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands

Chemical warfare between host and pathogen plays a crucial role in plant-necrotrophic pathogen interactions, but examples of its involvement in quantitative disease resistance in plants are poorly documented. In the *Daucus carota-Alternaria dauci* pathosystem, the novel toxin aldaulactone has been identified as a key factor in both fungal pathogenicity and the carrot's partial resistance to the pathogen. Bioinformatic analyses have pinpointed a secondary metabolism gene cluster that harbors two polyketide synthase (PKS) genes, *AdPKS7* and *AdPKS8*, that are likely responsible for the biosynthesis of aldaulactone. Here, we present the functional validation of *AdPKS7* and *AdPKS8* as the genes responsible for aldaulactone production in *A. dauci*. We generated knock-out *A. dauci* mutants for *AdPKS7* and *AdPKS8* by replacing essential domains with a hygromycin resistance gene, marking the first reported case of genetic manipulation in *A. dauci*. Following transformation, the mutants were analyzed for toxin production via HPLC-UV and assessed for pathogenicity *in planta*. Aldaulactone production was abolished in all PKS mutants, which also exhibited significantly reduced pathogenicity on H1-susceptible carrot leaves. These findings confirm the roles of *AdPKS7* and *AdPKS8* in aldaulactone biosynthesis and their contribution to fungal pathogenicity.

CS 3.3.4 - Ochratoxin A: current knowledge and future directions

M. Ferrara[1], *A. Gallo*[2], *L. Gambacorta*[1], *M. Solfrizzo*[1], *S. Baker*[3,4], *I. Grigoriev*[5,6], *G. Perrone*[1]
[1]Institute of Sciences of Food Production, CNR-ISPA, Bari, Italy, [2]Institute of Sciences of Food Production, CNR-ISPA, Lecce, Italy, [3]Pacific Northwest National Laboratory, Functional and Systems Biology Group, Environmental Molecular Sciences Division, Richland, United States, [4]DOE Joint Bioenergy Institute, Emeryville, United States, [5]Lawrence Berkeley National Laboratory, US Department of Energy Joint Genome Institute, Berkeley, United States, [6]University of California Berkeley, Department of Plant and Microbial Biology, Berkeley, United States

Ochratoxin A (OTA) is considered as the most toxic of the other ochratoxins synthesized by various fungal species belonging to the *Aspergillus* and *Penicillium* species. OTA commonly contaminates food and beverages, resulting in animal and human health issues. Initially, the study of the biochemical and molecular aspects of OTA production focused on the first known OTA producing species, such as *A. ochraceus* and *P. nordicum*. Later, great attention was centered on the OTA contamination of grapes and wine in the Mediterranean area due to *A. carbonarius* infection. Our team has made significant contributions to the molecular characterization of OTA cluster in *A. carbonarius*, elucidating the biosynthesis pathway. This was achieved over the years through the sequencing of *A. carbonarius* genomes and the production of several mutant strains. Recently, we have identified a new gene, *otaY*, encoding a cyclase involved in the ring closure step of the OTA polyketide backbone. A comparative analysis of 21 *Aspergillus* and *Penicillium* species evidenced a strong synteny in OTA core genes across the species. Furthermore, we conducted the genome sequencing of two *A. ochraceus* strains in collaboration with the DOE and JGI Institute. Although the mycotoxin tooks its name from this species, the analysis confirmed a large gene deletion in the biosynthetic gene cluster, and the incapability to produce OTA,. These findings confirm preliminary evidences by other authors.
Data presented emphasize the scientific efforts in understanding the OTA biosynthesis pathway

and highlight the need for further studies to prevent the misidentification of fungal toxigenic species.

CS 3.3.5 - Synthetic strategies to optimize octatrienoic acid production in Aspergillus nidulans

M. Tangalos[1,2], *C. Jenkinson*[3], *B. Miller*[1,2], *P.F. Chiu*[1], *C. Wang*[1], *B. Oakley*[3]
[1]University of Southern California, Pharmacy and Pharmaceutical Sciences, Los Angeles, United States, [2]University of Southern California, Chemistry, Los Angeles, United States, [3]Kansas University, Molecular Biosciences, Lawrence, United States

Aspergilli produce many commercially valuable and bioactive secondary metabolites (SMs). Because SM biosynthesis typically occurs at low levels, promoters from native housekeeping or primary metabolism genes have been used to upregulate target SMs. Optimizing current platforms and exploring new promoters are powerful opportunities to enhance SM production for the industrial scale.
We compared the use of simple promoter systems against positive feedback systems to upregulate (2Z, 4Z, 6E)-octa-2,4,6-trienoic acid (ZZE-OTA), an intermediate of the (+)-asperlin pathway. In the simple promoter systems, the inducible *alcA*(p) and the constitutive *gpdA*(p), from the alcohol dehydrogenase I and G3P dehydrogenase clusters, were used to directly drive ZZE-OTA biosynthesis. In the positive feedback system, *alcA*(p) and *gpdA*(p) are used to promote *afoA*, which encodes a transcription factor from the asperfuranone cluster. Promoters from the asperfuranone cluster were then engineered to drive ZZE-OTA biosynthesis, as well as more *afoA*, activated by the AfoA transcription factor.
RNA-seq analysis revealed additional strong promoter candidates to drive the AfoA system: a gene involved in thiamine biosynthesis (*nmtA*), the transcription elongation factor 1 gene (*tefA*), and a highly expressed gene that encodes metallothionein (*mtnA*). The constitutive AfoA feedback systems overwhelmingly outperform the simple promoter systems in ZZE-OTA output. Amongst the constitutive AfoA feedback systems, the novel *mtnA*(p) outperformed the others, revealing an optimized strategy for ZZE-OTA production.
Ongoing efforts include employing a palladium-acetate catalyst to isomerize ZZE-OTA to (2E, 4E, 6E)-octa-2,4,6-trienoic acid (EEE OTA), a known promoter of melanogenesis. We hope to next compare melanogenesis bioactivity between ZZE-OTA and EEE-OTA.

CS 3.3.6 - Cercosporin production improves *Cercospora beticola* virulence on sugarbeet plants grown in field soil

M. Natwick[1], *N. Wyatt*[2], *L. Rangel*[3], *S. Shrestha*[1], *G. Secor*[1], *M. Bolton*[2]
[1]North Dakota State University, Plant Pathology, Fargo, United States, [2]USDA-ARS, Sugarbeet Research Unit, Fargo, United States, [3]James Hutton Institute, Cell and Molecular Sciences, Dundee, United Kingdom

Sugarbeet (*Beta vulgaris*) is the second largest source of sucrose worldwide. *Cercospora beticola* is the causal pathogen of a foliar disease of sugarbeet called Cercospora leaf spot (CLS).

Cercospora spp. are well known for their ability to produce cercosporin; a photoactivated polyketide secondary metabolite effector (SME) that creates reactive oxygen species, causing nonspecific cell death. Cercosporin is suggested to play a role in *C. beticola* pathogenesis, but the exact function has yet to be defined. The keystone enzyme for cercosporin biosynthesis is CTB1. Here, we generated *C. beticola* mutants lacking *CTB1* to help elucidate the role of this SME during infection. Surprisingly, initial inoculations of *CTB1*-deficient *C. beticola* showed that the mutants exhibited increased virulence compared to the wild-type when the sugarbeet plants were grown in sterile potting mix under greenhouse conditions. Conversely, when the plants were grown in sugarbeet field soil under greenhouse conditions, *CTB1* mutants were less virulent than wild-type isolates. 16S and ITS deep-sequencing confirmed differential abundance of select phyllosphere microbiota during infection time-course experiments. We propose that cercosporin is utilized during infection as a tool to outcompete other microbes occupying the sugarbeet foliar community by acting as an antimicrobial agent. Thus, this research suggests that cercosporin acts as a virulence factor by manipulating the microbial community rather than solely by causing plant cell death as previously thought.

CS 3.3.7 - Arginoketides mediating cross-kingdom microbial interactions are modified by a fungal oxidase

M. Rosin[1,2], *L. Bulmann*[1], *M. Stroe*[1,3], *P. Hortschansky*[1], *T. Krüger*[1], *O. Kniemeyer*[1], *V. Schroeckh*[1], *A. Brakhage*[1,2]
[1]Leibniz Institut for Natural Product Research and Infection Biology - HKI, Molecular and Applied Microbiology, Jena, Germany, [2]Friedrich Schiller University Jena, Jena, Germany, [3]BASF SE Ludwigshafen, Present Adress, Ludwigshafen, Germany

Soil provides a habitat for various microorganisms living in close proximity to each other. The microbial interactions within such a soil community lead to the inclusion or exclusion of organisms in a microbiome. These interactions often occur *via* natural products (Brakhage, 2013; Krespach et al., 2023). An example is given by the arginoketide azalomycin F (AzF), produced by the soil-dwelling actinobacterium *Streptomyces iranensis*. AzF impacts the surrounding microorganisms, *e.g.*, it triggers the induction of otherwise silent biosynthesis gene clusters (BGC) in the fungi *Aspergillus nidulans* and *Aspergillus fumigatus* or acts as toxin towards the green alga *Chlamydomonas reinhardtii* (Krespach et al., 2023, 2020).
Here, we provide evidence that *A. fumigatus* modifies the arginoketide signal by secretion of an oxidase. The modified AzF is not able to induce the *ors* BGC in *A. nidulans* and showed much reduced toxicity against fungi and the green algae *C. reinhardtii*. Expression of the oxidase gene which we named *arkO* (arginoketide oxidase) is highly induced by presence of AzF and the ArkO protein is released by the fungus into the extracellular milieu only after exposure to azalomycin F. Purification of the enzyme and analysis of its activity indicated that the protein acts specific towards AzF and similar arginoketides as monazomycin, desertomycin A and linearmycin A.
Brakhage AA. Nat Rev Microbiol. 2013 Jan;11(1):21-32.
Krespach, *et al. ISME J* **14**, 2794–2805 (2020).
Krespach*, Stroe*, Netzker, Rosin, *et al. Nat Microbiol* **8**, 1348–1361 (2023).

CS 3.3.8 - Unlocking lichen biochemistry: genomic insights into BGC discovery and the road ahead

G. Singh[1,2]
[1]University of Padova, Department of Biology, Padova, Italy, [2]Botanical Garden of Padova, Padova, Italy

Biosynthetic research on symbiotic fungi, particularly lichens, has historically lagged behind that of non-symbiotic fungi due to significant experimental challenges. However, recent advancements in omics-assisted biosynthetic exploration and correlative metabolomics offer valuable insights for discovering and prioritizing biosynthetic gene clusters (BGCs) for drug discovery. Integrating progress from diverse fields has uncovered previously unknown aspects of the eco-evolutionary dynamics of secondary metabolites in lichen-forming fungi (LFF), paving the way for large-scale industrial applications by revealing new insights into lichen chemistry and biosynthesis.
Mining of 110 lichen genomes reveals that:
1) polyketide synthases (PKSs) constitute the dominant BGC class in LFF, followed by non-ribosomal peptide synthetases (NRPSs) and terpenes;
2) ribosomally synthesized peptides account for approximately 20% of the biosynthetic space in these organisms; and
3) supposedly rare BGCs, such as tIIIPKSs and isocyanide synthases, are more widespread in LFF than previously thought.
Additionally, BGC clustering shows that only a small fraction of LFF BGCs have been functionally characterized, with the majority remaining unexplored—representing structurally and functionally novel metabolites. Synteny analysis of known metabolites reveals species-specific features within these clusters, suggesting unique eco-evolutionary roles for LFF.
The genomic data generated thus far provides a solid foundation for unraveling the eco-evolutionary dynamics of lichen BGC landscapes. This data affirms lichens to be a treasure chest of metabolite diversity and assists in making omics-informed predictions about the functions of lichen BGCs, helping prioritize the most promising drug leads from a vast array of metabolites and biosynthetic genes.

CS 3.4.1 - RNAseq and targeted metabolomics implicate the guanine nucleotide exchange factor RIC8 in regulation of energy homeostasis, amino acid compartmentation and asexual development in *Neurospora crassa*

K. Borkovich[1], *M. Quinn*[1], *A. Carrillo*[1], *L. Halilovic*[1]
[1]University of California, Riverside, Microbiology and Plant Pathology, Riverside, United States

Heterotrimeric G protein signaling pathways control growth and development in eukaryotes. In the multicellular fungus *Neurospora crassa*, the guanine nucleotide exchange factor RIC8 regulates heterotrimeric Gα subunits. In this study, we used RNA-seq and Liquid Chromatography-Mass Spectrometry (LC-MS) to profile the transcriptomes and metabolomes of

N. crassa wild type, the Gα subunit mutants Δ*gna-1* and Δ*gna-3*, and Δ*ric8* strains. These strains represent a continuum of growth and asexual development (conidiation), with wild type and Δ*gna-1* mutants producing hyphae in submerged cultures, while Δ*gna-3* and Δ*ric8* mutants develop conidiophores, particularly in the Δ*ric8* mutant. RNAseq analysis showed that the Δ*gna-1* mutant possesses 159 mis-regulated genes, while Δ*gna-3* and Δ*ric8* strains have more than 1000 each. Many of the mis-regulated genes are involved in energy homeostasis, metabolism or conidiation. LC-MS revealed changes in levels of primary metabolites in the mutants, with several intermediates in the arginine biosynthetic pathway impacted in Δ*ric8* strains. The differences could not be fully explained by the expression or activity of pathway enzymes. However, transcript levels for two predicted vacuolar arginine transporters were affected in Δ*ric8* mutants. Analysis of arginine and ornithine levels in transporter mutants yielded support for altered compartmentation of arginine and ornithine between the cytosol and vacuole in Δ*ric8* strains. We also validated previous reports that arginine and ornithine levels are low in conidia. Taken together, our results suggest that RIC8 contributes to modulation of asexual sporulation in *N. crassa* at least in part through expression of vacuolar transporter genes.

CS 3.4.2 - Identification of novel Aspergillus fumigatus SIN kinase interactors through near-neighbor analysis

H. Thorn[1,2], *A. Martin-Vicente*[1], *J. Xie*[1,2], *A. Nandi*[1], *D. Bale*[1,2], *U. Perez-Cuesta*[1], *J. Fortwendel*[1,3]
[1]University of Tennessee Health Science Center, Clinical Pharmacy and Translational Medicine, Memphis, United States, [2]University of Tennessee Health Science Center, PhD Program in Pharmaceutical Sciences, Memphis, United States, [3]University of Tennessee Health Science Center, Microbiology, Immunology and Biochemistry, Memphis, United States

Aspergillus fumigatus is a leading cause of invasive fungal infections, and novel therapeutic strategies are urgently needed. Previous work in our laboratory suggests that septation inhibition may be one such strategy in combination with echinocandin treatment, as loss of septation renders *A. fumigatus* avirulent and unable to tolerate echinocandin stress at concentrations where the drugs would normally exert fungistatic activity. We have shown that the terminal member of the Septation Initiation Network (SIN) signaling complex, composed of the kinase SidB and its activator MobA, is essential for proper septation, virulence, and echinocandin response. However, the downstream effectors of this complex remain largely unknown. Proteins that interact with the SIN are likely important effectors for proper septation, so we have employed proximity-based labeling of the SidB/MobA kinase module using TurboID for the first time in A. fumigatus. LC-MS/MS of lysates from TurboID-tagged culture indicates that SidB and MobA have very similar interaction profiles, as expected based on our previous genetic analysis revealing that MobA is essential for SidB's role in septation. We found that samples from tagged strains shared 507 proteins overrepresented compared to the control. Of these, we have selected 17 candidate proteins for further characterization based on predicted roles in cell wall synthesis or cytoskeletal dynamics. Genes encoding candidate proteins were deleted, and several deletion mutants exhibited growth defects. This work demonstrates successful adaptation of the TurboID technology to *A. fumigatus*, which may be used in the future to delineate other molecular pathways involved in pathogenesis.

CS 3.4.3 - Identification of an a-factor-like peptide mating pheromone secreted by the heterothallic ascomycete *Aspergillus fumigatus*

S. Krappmann[1], *E. Gabl*[1], *S. Pöggeler*[2], *P. Dyer*[3], *S. Baker*[4], *M. Nowrousian*[5]
[1]University Hospital Erlangen and Friedrich-Alexander University (FAU) Erlangen-Nürnberg, Institute of Clinical Microbiology, Immunology and Hygiene, Erlangen, Germany, [2]Georg-August University (GAU) of Göttingen, Institute of Microbiology and Genetics, Department of Genetics of Eukaryotic Microorganisms, Göttingen, Germany, [3]University of Nottingham, School of Life Sciences, Nottingham, United Kingdom, [4]Pacific Northwest National Laboratory, Environmental Molecular Science Laboratory (EMSL), Microbial Molecular Phenotyping Group, Richland, United States, [5]Ruhr University Bochum (RUB), Department of Molecular and Cellular Botany, Bochum, Germany

In heterothallic ascomycetes, mating-type systems secure that compatible isolates fuse to enter the sexual phase. This process requires reciprocal secretion and recognition of pheromones, small peptides that are processed from precursors to become secreted. Identification of fungal mating pheromones with their cognate receptors is achieved by genome mining and homology searches, based on considerable conservation at the protein sequence level. For the Eurotiomycetes this approach had failed for **a**-factor-like pheromones due to their small size and low sequence conservation. Sexuality of the heterothallic mould *Aspergillus fumigatus* is genetically determined by a bipolar mating-type system encoding master regulators in an exclusive manner. By making use of transcriptional profiling data, we identified a candidate gene *ppgB* to encode the elusive **a**-factor pheromone of this opportunistic pathogen. The deduced peptide is 24 amino acids in length and comprises a canonical CaaX box motif. Inspection of *Aspergillus* genome sequences from members of the section *Fumigati* revealed complete conservation of the deduced PpgB as well as of the α-factor-like PpgA, indicating that other hyphal incompatibility factors than pheromone discrimination are at play for fungal speciation. Functional analyses employing pheromone-sensitive yeast cells support the hypothesis that PpgB serves as prototype for the long-sought **a**-factor-like pheromone of the aspergilli. An *A. fumigatus* strain deleted for the *ppgB* gene was unable to mate and form cleistothecia with a compatible partner. The identification of *A. fumigatus* PpgB and its encoding gene closes a substantial knowledge gap with respect to cellular recognition and sexual propagation of Eurotiomycete fungi.

CS 3.4.4 - A conserved cell-cell communication mechanism mediates interspecies interactions between *Neurospora crassa* and *Trichoderma atroviride*

N. Rotermund[1], *H. Haj Hammadeh*[1], *A. Serrano*[1], *J.S. Friedrich*[1], *U. Brandt*[1], *H. Knobel*[1], *A. Fleißner*[1]
[1]Technische Universität Braunschweig, Institut für Genetik, Braunschweig, Germany

Cell fusion is crucial for the development of eukaryotic organisms, including filamentous fungi such as *Neurospora crassa*. In many species, conidial germ tubes grow towards each other and fuse to form a supracellular network, which further develops into a mycelial colony. In *N. crassa*,

partner cells synchronize their behavior by taking turns in signal sending and receiving, indicated by the alternating recruitment of the MAP kinase MAK-2 and protein SO to the plasma membrane. This communication mechanism is conserved in the grey mold *Botrytis cinerea*, where the MAK-2 homologue, BMP1, and the SO homologue, BcPro40, demonstrate similar dynamics.
In mixed populations of *B. cinerea* and *N. crassa* spores, germ tubes of both species exhibit mutual attraction, implying a conserved communication mechanism. We hypothesized that this mechanism is also crucial for other fungal interspecies interactions, such as mycoparasitism. In the mycoparasitic fungus *Trichoderma atroviride*, knockout mutants of the *mak-2* (*tmk1*) and *so* homologous (*tso*) are completely deficient in intraspecies germling interactions, indicating the conservation of this cell-dialogue mechanism.
When wild-type *T. atroviride* and *N. crassa* spores are mixed, frequent interspecies interactions occur. However, during these interactions, SO-GFP mislocalized compared to intraspecies behaviors. Interestingly, rapid cell death of both partners was commonly observed following contact, indicating a non-self recognition mechanism that prevents interspecies fusion. Our findings suggest that while the cell dialogue mechanism is highly conserved, species specificity results from downstream mechanisms that inhibit interspecies fusion.

CS 3.4.5 - Plant stress perception alters inter-kingdom communication in the rhizosphere

S. Vitale[1], *M. Papaianni*[1], *M. Renesi*[1], *F. Salzano*[1], *I. Dilelio*[1], *F. Pennacchio*[1], *D. Turrà*[1], *S.L. Woo*[1], *G. Diretto*[2], *M. Lorito*[1], *N. Lombardi*[1]
[1]University of Naples "Federico II", Portici, Italy, [2]Italian National Agency for New Technologies, Energy and Sustainable Development, Portici, Italy

Root exudates (REs) released by plants are pivotal in mediating multitrophic interactions within the rhizosphere, acting as primary signaling blends that influence microbial community composition and function. Despite their importance, the mechanisms by which stressed plants alter RE chemical profiles and subsequently affect rhizospheric microbial dynamics remain poorly understood. Here we examined the impact of abiotic (mechanical wounding) and biotic stresses (infection by *Botrytis cinerea*, chewing by *Spodoptera littoralis*, and aphid infestation by *Macrosiphum euphorbiae*) on tomato plants. The resultant REs were subjected to chemotropic assays to assess their effects on the biological control agents (BCAs) *Trichoderma afroharzianum* and *Beauveria bassiana*, as well as the phytopathogen *Fusarium oxysporum*. All REs demonstrated inhibitory and repellent effects on the conidia and germ tubes of *Fusarium oxysporum*, while attractiveness of BCA germ tubes. Notably, REs from insect-stressed plants exhibited the highest chemotropic activity on BCAs and the strongest repellence to fungal pathogens. To elucidate the molecular underpinnings of this inter-kingdom signaling, we analyzed the root-secreted metabolome and conducted activity-guided fractionation of selected REs. Our findings suggest that low molecular weight proteins are implicated in the repellent activity against pathogens. This study reveals that both conserved and unique molecular signatures are involved in plant stress responses and their recognition by soil microbial communities. These insights advance our understanding of plant-microbe interactions and highlight the potential for developing stress-resilient crops with enhanced pathogen resistance.

CS 3.4.6 - Guardian of the cell: How cryptochrome balances the stress response in Aspergillus nidulans

A. Landmark[1], *R. Fischer*[1]
[1]Karlsruhe Institute of Technology, Karlsruhe, Germany

The perception of light by fungi plays a significant role in adapting to environmental changes like the time of day, rising temperature, or changes in the oxygen level. It relies primarily on the red- and blue-light spectrum and the corresponding light receptors, the red/far-red light-absorbing phytochrome, and the blue-light sensing *White Collar Complex*. These light receptors act independently or together to modulate gene transcription and/or protein activity and impact the fungal development, growth, and metabolite production in response to light stimuli. Members of another blue-light receptor group, the cryptochrome/photolyase family (CPF), play a key role in the processing and transduction of light signals in plants and animals. However, they seem to have only a minor impact on the blue light response in fungi.
Here, we show that the photolyase CryA from *Aspergillus nidulans* modifies the phytochrome activity. CryA, previously described as a cryptochrome-like photolyase, is involved in sexual and asexual development of *A. nidulans*. We show that it uses FAD and MTHF as chromophores and that CryA alters the transcription of blue- and red-light-dependent genes by physically interacting with the phytochrome FphA of *A. nidulans.* It thereby negatively regulates the red-light response. These results provide a deeper insight into the effect of light on fungal development, suggesting a more complex role for fungal CPF members and their coordination with other light receptors.

CS 3.4.7 - Cracking the code of infection: mapping the phosphorylation network behind infection-related development in rice blast fungus *Magnaporthe oryzae*

N. Cruz-Mireles[1], *P. Boateng Amoah*[1], *M. Oses-Ruiz*[1], *P. Derbyshire*[1], *E. Cawston*[1], *M.J. Bautista*[1], *J. Sklenar*[1], *X. Yan*[1], *L. Ryder*[1], *D. MacLean*[1], *N. Talbot*[1], *F. Menke*[1]
[1]Sainsbury Laboratory, Norwich, United Kingdom

Fungal pathogens continue to pose substantial threats to global food security, causing some of the most devastating crop diseases worldwide. We present a pioneering investigation into the infection-related development of the rice blast fungus, *Magnaporthe oryzae*, employing a quantitative mass spectrometry-based phosphoproteomic approach. Our study maps 8,005 phosphosites on 2,062 fungal proteins, providing a detailed landscape of the phosphorylation events that orchestrate plant infection. This reveals a profound re-wiring of phosphorylation-based signalling cascades during fungal infection, shedding light on the dynamic molecular responses elicited by *M. oryzae*. To unravel the broader significance of these findings, we undertook a comparative analysis of phosphosite conservation across 41 fungal species, revealing phosphorylation signatures linked to plant-associated fungal lifestyles, biotrophic and hemibiotrophic fungal infections. As the Pmk1 MAP kinase is a key orchestrator of plant infection in many plant pathogenic fungi, we then used parallel reaction monitoring to identify Pmk1 MAPK substrates. Our investigation defines 32 putative substrates of Pmk1, revealing a

complex network of regulatory interactions. This led to identification of a novel regulator, Vts1, which undergoes Pmk1-dependent phosphorylation and is indispensable for the manifestation of rice blast disease. Vts1 is a SAM domain protein that plays an essential function in appressorium morphogenesis, septin aggregation and re-polarisation. Using an analogue-sensitive Pmk1 mutant, parallel reaction monitoring, and subsequent site-specific mutagenesis, we show that a single Pmk1-dependent phosphorylation event is necessary for its function as a virulence determinant during rice blast disease.

CS 3.4.8 - Light dependent regulation of plant cell wall degradation and secondary metabolism via the HOG pathway in *Trichoderma reesei*

M. Schalamun[1,2], *W. Hinterdobler*[1,2], *J. Schinnerl*[3], *L. Brecker*[4], *M. Schmoll*[1,5]
[1]AIT Austrian Institute of Technology, Department Health and Bioresources, Tulln, Austria, [2]MyPilz GmbH, Vienna, Austria, [3]University of Vienna, Department of Botany and Biodiversity Research, Vienna, Austria, [4]University of Vienna, Department of Organic Chemistry, Vienn, Austria, [5]University of Vienna, Department of Microbiology and Ecosystem Science, Vienna, Austria

Nutrient sensing is crucial for gene regulation in fungi, with the High Osmolarity Glycerol (HOG) pathway, a key MAPKinase pathway, playing a central role in environmental stress responses in *Trichoderma reesei*. Previous studies showed that the MAPKinases TMK1, TMK2, and TMK3 regulate cellulase formation and secondary metabolism, though the role of light was not always considered. Given the influence of light on fungal physiology in general, we investigated light-dependent signal transmission via the HOG pathway.
We explored the role of the HOG pathway in regulating carbon and secondary metabolism using deletion mutants of MAPKKK (Δ*ttk3*), MAPKK (Δ*tkk3)*, and MAPK (Δ*tmk3*). Functional assays, transcriptome analyses, and network analyses revealed striking differences between light- and dark-associated functions as well as complex interrelationships with components of the G-protein pathway.
TMK3 was shown to be essential for cellulase formation in darkness. We now show regulatory similarities to gene regulation by the G-protein alpha subunit GNA1 and the beta subunit GNB1, suggesting an overlap between these pathways in darkness. Additionally, secondary metabolite analysis revealed that the HOG pathway affects the regulation of several compounds, including sorbicillinoids, reinforcing the connection between cellulase regulation and secondary metabolism. Gene expression analysis further showed that the expression of secondary metabolism encoding genes is light dependently regulated by the HOG pathway.
These findings emphasize the importance of the HOG pathway in integrating environmental signals to balance carbon metabolism and enzyme biosynthesis with metabolite production in *T. reesei*, providing valuable insights for optimizing enzyme production in industrial applications.

CS 3.5.1 - Mycombiome-based improvement of growth in urban farm plants

C.-Y. Chen[1], *N. Naqvi*[1,2]
[1]Temasek Life Sciences Laboratory, Singapore, Singapore, [2]National University of Singapore, Department of Biological Sciences, Singapore, Singapore

Most land plants live in beneficial association with symbiotic fungi and rely on such interactions to improve their growth and fitness. The efficacy of such fungi inducing plant growth has gained support from studies indicating their potential as biofertilizers and prospective agricultural applications. Here, we demonstrate that a root fungal endophyte, *Tinctoporellus* species isolate AR8, enables yield improvement in model plant systems and Brassicaceae leafy vegetables such as Choy Sum (*Brassica rapa* var. *parachinensis*). Mechanistically, AR8-plant interaction shifts the metabolic flux from core metabolites to phenylpropanoid biosynthesis, and such secondary metabolites are subsequently channeled to promote plant growth. The requirement of phenylpropanoids for AR8-mediated growth induction was confirmed by chemical complementation with *p*-coumaric acid, which restored the growth phenotypes in AR8-inoculated *pal1* mutant plants. More importantly, the phenylpropanoid network assumes the role of a regulator in auxin signaling for plant growth. AR8 enables local auxin biosynthesis in host roots and provides an auxin source as a long-distance signal carrier that regulates shoot biomass improvement. The disruption of auxin signaling in AR8-inoculated *pal1* mutant plants is restored by complementing with exogenous *p*-coumaric acid, suggesting that hydroxycinnamic acid and *p*-coumaric acid as major plant growth-promoting hubs bridging phenylpropanoid pathway and auxin signaling during beneficial interactions with AR8. Collectively, we uncovered a novel beneficial mycobiont with vast potential as a biofertilizer for boosting plant biomass and deciphered the molecular basis of a novel cross-kingdom beneficial interaction.

CS 3.5.2 - Evaluating the role of bacterial endosymbionts in evolution and diversity of plant associated Mucoromycota fungal communities

D. Gohar[1], *N. K. Reynolds*[2], *M. Napo*[3], *A. Kock*[3], *K. A. Alayande*[3], *K. J. Hoffman*[1], *E. J. Clark*[1], *R. A. Adeleke*[3], *T. E. Pawlowska*[4], *J. K. Uehling*[1]
[1]Oregon State University, Department of Botany and Plant Pathology, Corvallis, United States, [2]California State Polytechnic University, Department of Biological Sciences, Pomona, United States, [3]North-West University, Unit for environmental Sciences and Management, Potchefstroom, South Africa, [4]Cornell University, School of Integrative Plant Science, Ithaca, United States

Mucoromycota fungi are ecologically and economically significant, yet remain among the least understood fungal groups. As one of the earliest-diverging lineages, these fungi engage in mutualistic relationships with plants while also functioning as decomposers in various ecosystems. Environmental Mucoromycota fungi regularly harbor ancient and highly coevolved endosymbiotic bacteria. The presence of endohyphal bacteria (EHB) significantly impacts fungal biology, including metabolism, gene expression, and reproduction via sporulation. However,

knowledge on the impact of EHB interactions on Mucoromycota diversity and consequently on plant-associated communities remains limited. We hypothesize that such interactions can alter fungal symbioses with other organisms, including plants, soil microbes, and fungi, producing predictable community structures. To investigate the role of endosymbionts in the ecological community assembly and diversification of Mucoromycota, we collected soil samples from over 300 sites across California (USA) and South Africa. Leveraging the chitinolytic capabilities of Mucoromycota, we will use crabshell baiting to isolate and culture these fungi. The presence of EHB will be confirmed by amplifying the 16S rRNA region from DNA extracted from hyphae. The DNA will undergo high-throughput sequencing, allowing us to analyze the genomic heterogeneity of these isolates and evaluate how EHB influence fungal genomic evolution. Additionally, we will examine the selection pressures on bacterial genomes and their evolutionary trajectories, considering the history of Mucoromycota-EHB interactions. To achieve this, we will employ genome-scale phylogenetic and comparative analyses involving BRE and their free-living relatives. Ultimately, we aim to elucidate the functional implications of Mucoromycota-EHB interactions and their role in shaping community diversity.

CS 3.5.3 - Peculiarities in the genome maintenance of Arbuscular Mycorrhizal Fungi

D. Barua[1], *A. Muszewska*[1]
[1]Institute of Biochemistry and Biophysics Polish Academy of Sciences, Laboratory of Fungal Bioinformatics, Warsaw, Poland

Arbuscular Mycorrhizal Fungi (AMF) are a division of early diverging fungi (Glomeromycota) that establish symbiotic relationships with 71% of land plants. AMF absorb phosphorus and nitrogen from the soil and transfer them to host plants, in exchange for carbon sources. Recent studies have shown how nutrient transport occurs between the fungus and the host plant. Glomeromycota possess multinucleate cells with non-identical genetic material in individual nuclei. This leads to their nuclei behaving like a population rather than an individual in every species. The complexity of laboratory handling of symbiotic Glomeromycota representatives hinders molecular studies. Despite the economic and ecological significance, the genetic basis of many biological processes in this phylum are not known. In this study, we attempt to expand the search for nucleic acid processing components in AMF to explain the plausible mechanisms of genome maintenance and DNA repair in this lineage using an in-silico approach. Our findings reveal several gene losses, including the Rad9-Hus1-Rad1 (9-1-1) clamp complex, GINS 1, 3, and 4 along with Mcm10 of the CMGE helicase complex, RecQ and SecA helicases, FAN1 nuclease acting downstream of the Fanconi Anemia pathway, all of which are key cell cycle and DNA repair proteins conserved across eukaryotes. We also find losses of mitochondrial biogenesis (Pet127) and centromere (CENPM, CBF3) proteins essential for metabolism and DNA binding respectively. These recurrent loss of genome maintenance processes during the course of evolution points to the presence of alternative mechanisms that enable AMF to evolve and proliferate.

CS 3.5.4 - Characterizing the secretome of a lichenized fungus *Xanthoria parietina* and its role in the lichen symbiosis

G. Tagirdzhanova[1], *K. Scharnagl*[2], *N. Sahu*[1], *X. Yan*[1], *N.J. Talbot*[1]
[1]The Sainsbury Laboratory, Norwich, United Kingdom, [2]University of California Berkeley, The University and Jepson Herbaria, Berkeley CA, United States

Lichens – the textbook example of a symbiosis –are a diverse array of organisms centred around the relationship between a fungus and an alga. Nearly one fifth of known fungal species engage in lichen symbioses, yet the molecular mechanisms behind the symbiotic interactions remain largely unknown, in part owing to the recalcitrance that lichen fungi show towards laboratory experimentation. To uncover mechanics of lichen symbiosis, we combined metagenomic analysis with metatranscriptomics and protein structure prediction. Using *Xanthoria parietina* as our model system, we identified 168 genome sequences within a lichen thallus, including several fungi, algae and an extensive bacterial microbiome. This highlights the likely complexity of the symbiotic association. We also profiled gene expression of the lichen fungus in a thallus and compared this with the fungus in its aposymbiotic culture. In the predicted proteome, we identified potentially secreted proteins and profiled them based on sequence and by structural similarity to protein databases using Alphafold. By clustering the predicted secretome, we identified groups of proteins that show similarity to known effectors from phytopathogenic fungi. We have characterized several candidate effectors and explored their potential functions using a range of informatic and experimental approaches. We will report our analysis of the role of the extensive repertoire of secreted proteins in the lichen symbiosis.

CS 3.5.5 - Understanding the role of antimicrobial effector proteins secreted by the lichen-forming fungus *Peltigera rufescens*

C. Kelly[1], *H. Rövenich*[1], *B. Thomma*[1]
[1]University of Cologne, Institute for Plant Science, Cologne, Germany

Symbiotic associations are found across the fungal kingdom, among which lichens are one of the most successful mutualistic fungal symbioses. By classical dual definition, lichens are comprised of two primary partners. While one is a lichen-forming fungus, termed the mycobiont, the other is a photosynthetic partner, called the photobiont. We believe effector mediated microbiome manipulation previously discovered for pathogenic fungi is also fundamental to fungi with different lifestyles and therefore aim to understand the role of antimicrobial proteins for lichen-forming fungi. Initial analyses of a high quality *Peltigera rufescens* genome identified potential effectors with antimicrobial activity that might play a role in shaping the community structure of the lichen microbiota. A candidate, for which RNA-sequencing counts support the expression of the protein coding gene in the natural lichen, displays antimicrobial activity *in vitro* on bacteria and fungi from the lichen's natural environment. Beyond we recorded a reduction in photosynthetic activity when the protein is applied to phototrophic organisms. The selected ~18 kDa protein contains a ricin B lectin domain which in studies on the ricin protein, a plant

toxin, has been shown to bind simple sugars. In the future we will gather evidence for the secretion of our protein in the natural *Peltigera* lichen and uncover its mode of action.

CS 3.5.6 - The root endophyte *Colletotrichum tofieldiae* promotes plant growth in a manner dependent on nitrogen status and the composition of rhizosphere bacteria

T.A.N. Nguyen[1], *M. Nakamura*[1], *Y.D. Utami*[1], *K. Hiruma*[1]
[1]The University of Tokyo, Department of Life Sciences, Tokyo, Japan

Plants employ diverse strategies to adapt to nitrogen deficiency. The root endophytic fungus *Colletotrichum tofieldiae* (Ct) colonizes the roots of *Arabidopsis thaliana*, promoting growth by transferring phosphorus under phosphorus-limited conditions. Here, through comprehensive field and laboratory experiments, including gnotobiotic systems, we demonstrate that Ct enhances the growth of *A. thaliana*, several Brassicaceae vegetables, and *Lactuca sativa* under nitrogen-limiting conditions. This plant growth promotion (PGP) is regulated by the nitrogen status of the host, with Ct specifically enhancing growth under soluble nitrogen limiting conditions. During root colonization, Ct induces fungal genes encoding enzymes and transcription factors associated with nitrate and peptide utilization. Plant-side transcriptomic analysis revealed that Ct induces *A. thaliana* nitrate-related genes, including high-affinity nitrate transporters (*NRT2s*). Disruption of four major *NRT2* genes (*NRT2.1*, *NRT2.2*, *NRT2.4*, *NRT2.5*) impaired Ct-mediated PGP, indicating their essential role in this process. Interestingly, however, $^{15}NO_3^-$-labeled isotope analysis revealed that Ct transfers nitrogen to plants through its hyphae in a manner independent of these NRT2s, suggesting that NRT2s facilitate PGP independently of direct nitrogen transfer by Ct. Moreover, Ct modulates the root rhizosphere bacterial community in soils. Among them, a plant-growth-promoting bacteria with high amino acid utilization capabilities was recruited to the host via Ct's hyphae. Importantly, Ct works synergistically with the beneficial bacteria to further promote plant growth. These findings underscore Ct's capacity to enhance plant growth under nitrogen deficiency via both direct and indirect strategies, including complex interactions with other microbes, which may contribute to its consistent PGP effect under fluctuating field conditions.

CS 3.5.7 - Genomic characterization of fungal endophytes with biopesticide potential

J. Rojas[1,2], *C.E. Díaz*[3], *M.F. Andrés Yeves*[1], *A. Gónzalez-Coloma*[1], *J. Imperial*[4]
[1]Instituto de Ciencias Agrarias (ICA, CSIC), Madrid, Spain, [2]Universidad Politécnica de Madrid, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Madrid, Spain, [3]Instituto de Productos Naturales y Agrobiología (IPNA, CSIC), La Laguna, Spain, [4]Centro de Biotecnología y Genética de Plantas (UPM-INIA/CSIC), Pozuelo de Alarcón, Spain

There is an urgent demand for the development of greener and more sustainable pesticides due to the environmental and health concerns associated to the use of conventional synthetic pesticides. A promising approach is the exploration of biopesticides derived from endophytic fungi. Fungi

are well-known sources of secondary metabolites (e.g., polyketides, terpenes, alkaloids), many of which have important applications in medicine, industry, and agriculture, and it is believed that production of such metabolites by endophytic fungi could play a role in protecting the host plant against biotic stresses, thus justifying their existence as symbionts. Despite its potential, much of the metabolic and genomic diversity of endophytic fungi remains undiscovered. We have undertaken characterization of the biopesticide potential of endophytic fungi isolated from plants already known to produce active biopesticides: two Macaronesian endemic plants, *Persea indica* and *Bethencourtia palmensis,* and the common wormwood (*Artemisia absinthium*). In this study, we present the annotated genomes of six endophytic fungal isolates selected for their activity against common plant pests, including insects, parasitic nematodes, bacterial pathogens, and fungal pathogens. These isolates include *Stemphylium* sp. from *A. absinthium*, *Phyllosticta* sp. from *P. indica*, and *Stemphylium* sp., *Alternaria* sp., and *Epicoccum* sp. from *B. palmensis*. Their potential for biopesticide production has been evaluated through the identification of their biosynthetic gene clusters involved in secondary metabolite production. This genomic and biosynthetic information provides a valuable resource for further investigations into the molecular pathways and potential biotechnological applications of these endophytic fungi.

CS 3.5.8 - Structure of Mucoromycota fungal communities and their associated endosymbiotic bacteria across two different biomes in the U.S. and Israel

N. Reynolds[1], *K. Amses*[2], *J. Uehling*[3], *R. Adeleke*[4], *M. Sternberg*[5], *M. Branine*[6], *T. Pawlowska*[7]
[1]California Polytechnic University, Pomona, Biological Sciences, Pomona, United States, [2]University of Pennsylvania, Perelman School of Medicine, Philadelphia, United States, [3]Oregon State University, Department of Botany and Plant Pathology, Corvallis, United States, [4]North-West University, Unit for Environmental Sciences and Management, Potchefstroom, South Africa, [5]Tel Aviv University, School of Plant Sciences and Food Security, Tel Aviv, Israel, [6]Cornell University, Graduate Field of Microbiology, Ithaca, United States, [7]Cornell University, School of Integrative Plant Science, Ithaca, United States

Despite the ecological importance of Mucoromycota fungi as mycorrhizal symbionts, opportunistic human and plant pathogens, and post-harvest spoilage agents, they remain understudied compared to Dikarya. Fundamental aspects such as geographical distribution, dispersal patterns, and community structure remain unclear. The endosymbiotic bacteria (EB) that many Mucoromycota species harbor have generated new questions regarding their effects on fungal host evolution. EB have varying effects on the host fungi depending on the species, influencing asexual and sexual reproduction and metabolic functioning. To investigate communities, we collected rhizosphere soils from four sites in California and two in Israel representing two biomes (Desert and Mediterranean scrub). Metabarcoding data were generated using bacterial (16S rDNA) and fungal (28S rDNA) primers and show several OTUs unique to each habitat. A nested PCR approach was designed for 16S to enrich samples for known EB groups. Both biotic filtering and dispersal filtering significantly affected fungal and bacterial communities; however, dispersal filtering was only significant over larger distances (km scale). Desert samples had a higher proportion of fungal OTUs assigned to opportunistic human pathogenic species not detected from the coast and had higher proportions of Zoopagomycota.

On the other hand, coastal samples had greater diversity of mycorrhizal OTUs (Glomerales and Endogonales). Mediterranean scrub samples showed higher proportions of *Mycoplasma*-related EB, corresponding to the greater diversity of mycorrhizal OTUs. Desert samples showed the opposite, with higher abundance of *Burkholderia*-related EB. Overall, our results indicate co-occurrence of EB communities with their fungal hosts, and desert environments are likely reservoirs for opportunistic pathogens.

CS 3.6.1 - Deciphering the role of the key HR1 regulatory domain of protein kinase C (PkcA) in the fungal pathogen *Aspergillus fumigatus*

J.E.M. Campanella[1], M.C. Rocha[1,2], G.A. Sato[1], G. Yanes Filho[1], A.F. Cunha[1], I. Malavazi[1,3]
[1]Universidade Federal de São Carlos (UFSCar), Genética e Evolução, São Carlos, Brazil, [2]The Hebrew University of Jerusalem - School of Veterinary Medicine, The Robert H. Smith Faculty of Agriculture, Food and Environment, Rehovot, Israel, [3]National Institute of Science and Technology in Human Pathogenic Fungi, Sao Carlos, Brazil

Aspergillus fumigatus is a human pathogen responsible for severe infections, including invasive pulmonaryaspergillosis in immunocompromised individuals. In fungi, the Cell Wall Integrity (CWI) pathway regulates cell wall homeostasis and is activated by the apical kinase Protein Kinase C (PkcA). Unlike mammalian PKCs, fungal PKCs, such as PkcA, possess unique N-terminal extensions containing tandem HR1 (Homology Repeat) domains namely HR1A and HR1B subdomains. These domains are hypothesized to mediate interactions with Rho-GTPases and are essential for CWI pathway activity. Here, we investigate the role of the PkcA HR1A/HR1B domains in *A. fumigatus*. Mutants of PkcA lacking either HR1A or HR1B domains were generated and complemented using CRISPR-Cas9 technology. Deletion of the HR1A/B domains resulted in severe growth defects, reduced conidiation, and increased susceptibility to cell wall stressors and heat shock. Additionally, the double $pkcA^{\Delta HR1A/B}$ mutant was non-viable. These mutants exhibited altered subcellular localization patterns compared to the full-length PkcA::GFP, with $pkcA^{\Delta HR1A}$ losing its apical localization. HR1A and HR1B were essential for downstream activation of the CWI pathway through the transcription factor RlmA, with HR1B being dominant over HR1A. Recombinant expression of the HR1 domains and full-length PkcA enabled pull-down assays, which revealed that HR1A and HR1B act as effectors for Rho1 and Rho2 GTPases, but not for Rho4. Notably, only GTP-bound Rho1 binds to HR1A, while both GDP- and GTP-bound Rho1 can bind to HR1B. These findings demonstrate that the HR1 domains are pivotal for PkcA function and are critical for fungal development and, stress adaptation, and virulence.

CS 3.6.2 - QTL mapping of stress tolerance in the wheat pathogen Zymoseptoria tritici

J. Stapley[1], B. McDonald[1]
[1]ETH Zürich, Institute of Integrative Biology, Zürich, Switzerland

Climate change is expected to have important impacts on global agriculture, as it influences plant disease occurrence and severity. Changes in the environment can push organisms beyond their physiological limits and alter plant-pathogen interactions. Predicting how pathogens will respond to changing environments requires a detailed understanding of how pathogen growth is impacted by environmental stress, as well as knowledge of the molecular mechanisms underlying their response to stress. Using quantitative trait loci (QTL) mapping we have identified regions of the genome and candidate genes associated with multiple stressors in the wheat pathogen *Zymoseptoria tritici*. I will highlight the most exciting results to come from this QTL mapping approach. A better understanding of the genes involved in pathogens' responses to stress can help us better predict how pathogens will respond to climate change and other environmental stresses and may provide insights into the vulnerabilities of pathogens that could be exploited to find novel targets for control.

CS 3.6.3 - Post-translational modifications in response to hypoxia in the filamentous fungus *Aspergillus fumigatus*

L. Ivanova[1], T. Krüger[1], A. Bigalke[1], T. Heinekamp[1], M. Cyrulies[2], M. Agler-Rosenbaum[2,3], A.A. Brakhage[1,3], O. Kniemeyer[1]
[1]Leibniz Institute for Natural Product Research and Infection Biology, Molecular and Applied Microbiology, Jena, Germany, [2]Leibniz Institute for Natural Product Research and Infection Biology, Bio Pilot Plant, Jena, Germany, [3]Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany

The ability of the filamentous fungus *Aspergillus fumigatus* to adapt to hypoxia is an important virulence trait. The transcription factor SrbA is the central regulator of the fungal hypoxia response. However, little is known about the adaptation to hypoxia on the protein and posttranslational level. In order to get more insights, we performed quantitative proteomics, phospho- and thio redox-proteomics analyses by comparing fungal mycelium grown under either normoxic or hypoxic conditions. Protein were extracted from mycelium and analyzed after tryptic digest by LC-MS/MS. Phosphopeptides were enriched using a TiO_2/ZrO_2 solid phase extraction protocol, while the identification of oxidative thiol modification was determined by the OxICAT technology. We identified in total 5136 proteins, of which 318 proteins and 1674 phosphopeptides showed significantly different abundance upon hypoxia (fold change >4). In particular proteins involved in mitochondrion organization, amino acid metabolism, and lipid metabolic processes increased in abundance under hypoxia. The phosphoproteomic data indicated further that the mitotic cell cycle and autophagy processes were differentially regulated under hypoxic growth. Indeed, phosphopeptides derived from proteins of the Atg1 signaling complex, which is known to initiate autophagosome formation, showed drastic changes in phosphorylation under hypoxia. Redox proteomics revealed 44 cysteine-containing peptides with a decreased and 36 peptides with an increased level of thiol oxidation under hypoxia. A cysteine in the osmotic stress regulating mitogen-activated protein kinase SakA showed a most drastic level of thiol oxidation under hypoxia. Based on these findings, the observed regulatory mechanisms will be investigated further on the genetic level.

CS 3.6.4 - Zn stress in ectomycorrhizal symbiosis; a Laccaria bicolor point of view

M. Ottaway[1], *J. Swinnen*[1], *K. Verhaevert*[1], *J. Ruytinx*[1]
[1]Vrije Universiteit Brussel, Brussel, Belgium

The mutualistic symbiosis between ectomycorrhizal (ECM) fungi and host trees is found all throughout temperate and boreal forest ecosystems. While this relationship is important for optimal growth and functioning of both partners, knowledge on the sustainability of this mutualistic interaction in changing soil environments such as heavy metal pollution, nutrient excess/deficiency is largely lacking. In this study, we investigated the effect of both Zn excess and Zn deficiency on the formation of the symbiotic structures formed between *Laccaria bicolor* S238N and *Populus tremula x alba* 717-1B4. Specifically, how the formation of the hyphal mantle and hartig net are affected, as well as how the antioxidant response is regulated during symbiosis development when combined with these stress conditions. Microscopic analysis revealed a significant decrease in mantle thickness compared to control conditions, with the hartig net remaining unaffected under all conditions. Gene expression analysis of ROS scavenging genes, CAZymes and MiSSPs showed a differential regulation under Zn excess conditions compared to control during symbiosis development. Interestingly, MiSSP7 also showed differential regulation in free-living mycelium under Zn excess. Enzyme activity was also measured, but no significant changes could be detected in the symbiotic tissue when exposed to Zn stress. These results indicate that symbiosis marker gene expression is influenced not only by symbiosis development, but also by Zn stress. How developmental and stress response pathways in ECM fungi integrate needs further investigation and will lead to a better understanding of the sustainability of this pervasive mutualism.

CS 3.6.5 - A new principle for oxidative stress sensing in fungi based on heme oxygenases

L.O. Schuhmacher[1], *R. Fischer*[1]
[1]Karlsruhe Institute of Technology, Microbiology, Karlsruhe, Germany

Heme oxygenases, found in mammals, plants, bacteria, and fungi, are key enzymes in heme metabolism by catalyzing the oxidation of heme to biliverdin. Additionally, in mammals, they can function as stress sensors by localizing to the nucleus and interacting with transcription factors under specific stress conditions. Here, we investigated the functions of the heme oxygenases HoxA and HoxB from *Alternaria alternata*. Both HoxA and HoxB were previously found at mitochondria, where they interact with each other and with phytochrome (FphA). We proposed that they provide the linear biliverdin as chromophore for phytochrome. Interestingly, bimolecular fluorescence complementation revealed that HoxA and HoxB interact with each other not only at mitochondria, but also in the nucleus, suggesting a secondary function for the heme oxygenases. Furthermore, HoxB, but not HoxA, physically interacted with the stress regulated bZIP transcription factor AtfA as well as the blue-light receptor CryB in the nucleus. Mutation- and stress-assays with HoxB indicated its involvement in regulating ROS stress

responses, acting as negative regulator. Overall, the findings suggest that HoxA mainly contributes, through biliverdin synthesis, to red-light perception, while HoxB apparently acts as stress sensor, regulating ROS stress response and possibly blue light perception, therefore being involved in adaptation to environmental conditions. The stress sensing role of heme oxygenase appears to be conserved in *Aspergillus nidulans* and possibly other fungi. This stress-sensing principle may also be important in pathogenic fungi. Functional studies in the nematode-trapping fungus *Arthrobotrys flagrans* are on the way.

CS 3.6.6 - Initiation of endosymbiosis in *Rhizopus microsporus* indicates a shift from antagonism to commensalism

T. Gassler[1], *A. Holderbusch*[1], *G. Giger*[1], *A. Sintsova*[1], *M. Bortfeld-Miller*[1], *B. Dehapiot*[2], *S. Sunagawa*[1], *J.A. Vorholt*[1]
[1]ETH Zurich / Institute of Microbiology, Department of Biology, Zurich, Switzerland, [2]ETH Zurich / ScopeM, Zurich, Switzerland

Endosymbioses represent intricate and dynamic relationships between organisms that may involve pathogenic phases during their emergence. Here, we use fluidic force microscopy (FluidFM) to induce cell-in-cell interactions to directly probe the early stages of endosymbiosis. We introduced the opportunistic pathogen *Ralstonia pickettii* into a non-endosymbiotic strain of *Rhizopus microsporus*, simulating the unstable early phase of endosymbiosis. This approach allowed us to explore mechanisms that might overcome initial challenges in the stabilization of such an interaction. The intracellular presence of *R. pickettii* affected fitness and induced stress responses in the novel fungal host. Adaptations were observed at the phenotypic, genetic and transcriptional levels, indicating a shift from pathogenic antagonism to commensalism as the interaction progressed. Using high-throughput microscopy and custom-trained deep learning models, we tracked individual spores and quantified fungal growth and host responses. Our work offers insights into early processes of endosymbiosis, highlighting the role of mechanisms that mitigate pathogenicity and promote compatibility in stabilizing these interactions.

CS 3.6.7 - The overlooked functions of the elongation factor gamma in the control of protein translation under stress

M. Viloria[1], *R. Bchini*[1], *M. Morel-Rouhier*[1]
[1]University of Lorraine, UMR IAM Tree Microbe Interactions, Vandoeuvre les Nancy, France

Stress tolerance strategies improve organism survival in changing and sub-optimal environments. One of these strategies is the regulation of protein translation. Cells adapt to stress by altering the translatome to selectively express a subset of factors governing stress responses. This is in general accompanied by the downregulation of total protein synthesis, which contributes to the maintenance of cellular energy balance. Translation initiation was described as the rate-limiting step of translational control. However, balancing all four phases of translation (initiation, elongation, termination, and ribosome recycling) is important for maintaining proteostasis, with translation elongation being central to determining protein fate. The elongation complex (eEF1)

is composed of 3 subunits in fungi. These subunits are the guanidine nucleotide binding subunit eEF1A and the two eEF1Bα and eEF1Bγ that compose the nucleotide exchange factor eEF1B. The eEF1A/eEF1B complex promotes the exchange between GDP and GTP to regenerate active form of eEF1A allowing a proper translation process. Within this complex, the function of eEF1Bγ remains to be elucidated. eEF1Bγ is classified within the glutathione transferase (GST) superfamily. However, it is an atypical GST being composed of two domains: a GST domain at the N-terminal side and an elongation factor domain with unknown function at the C-terminal side. We focused our work on determining how eEF1Bγ regulates protein translation by determining how the eEF1Bγ/eEF1Bα association is controlled, and how the GST activity of eEF1Bγ participates in cell fitness under stress conditions.

CS 3.6.8 - UV induces translation in Fusarium species in a pKA and developmentally regulated manner

S. Covo[1], *Q. Hoang*[1], *S. Milo*[2]
[1]Hebrew University, Rehovot, Israel, [2]OpenU, Raanana, Israel

UV causes DNA damage and significantly delays DNA replication and transcription. One of the known effects of DNA damage on eukaryotic cells is growth arrest and consequently reduction in ribosome biogenesis. We discovered, in two Fusarium species, that UV induces ribosome biogenesis and translation capacity but only after the filaments are formed. The induction occurs at the transcription, association with the ribosomes and the proteome levels. The ribosome biogenesis induction contributes moderately but significantly to UV survival and UV repair. In addition to ribosome biogenesis, following UV, genes from other modules of the gene expression network from splicing to chaperones are differentially associated with the ribosomes. Hardly any UV repair genes are induced by the exposure to UV but DDB1 that is induced at the post transcription level. UV induced translation counteracts TOR signaling inhibition but is very much dependent on pKA. In conclusion, we describe a novel DNA damage response in filamentous fungi that we think is triggered by pKA activation when most of the nuclei in the filaments are dormant. We think that this UV response allows the filament to keep gene expression homeostasis when transcription is significantly impaired.

P1.101 - Proteome analysis of programmed cell death in *Aspergillus fumigatus* conidia

M. Händel[1], *K. Gonzales*[1], *M. Kawashima*[1], *T. Krüger*[1], *O. Kniemeyer*[1], *T. Heinekamp*[1], *A. Brakhage*[1]
[1]Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany

Conidia produced by the human-pathogenic fungus *Aspergillus fumigatus* are the main cause of invasive aspergillosis in immunocompromised patients. In the lung, alveolar macrophages phagocytose the inhaled conidia and process them intracellularly. However, the fungus interferes with this mechanism to avoid being killed. To study these host-pathogen interactions *in situ*, it is crucial to track dying conidia. Currently, the ability to visually distinguish between resting and dead conidia is very limited, as they show no easily detectable metabolic or morphological differences. By generation of a reporter strain that produces a cell death-associated protein fused with the green fluorescent protein, it would be possible to distinguish dead from living conidia by fluorescence microscopy. To identify a suitable reporter protein, we investigated the induction of programmed cell death in *A. fumigatus* conidia. First, we established an *in vitro* cell death assay by treating conidia with various cell-death inducing compounds. We found that H_2O_2 in a nutrient-rich medium has a greater ability to kill resting conidia than the antifungal drugs amphotericin B or voriconazole. By proteome analyses of treated resting and swollen conidia we identified several proteins which are differentially abundant during regulated cell death. Analysis of these proteins will provide insights into programmed cell death in *A. fumigatus* conidia. In addition, these data could be a suitable basis for the generation of a cell death reporter strain to study host-pathogen interactions.

P1.102 - Testing the role of the strawberry plant phyllosphere mycobiome in enhancing resistance to phytopathogenic fungi

J.S. Friedrich[1], *M. Dziomba*[1], *K. Mucha*[2,3], *J. Schulze*[4], *A. Fleißner*[1]
[1]TU Braunschweig, Institute of Genetics, Braunschweig, Germany, [2]TU Braunschweig, Braunschweig, Germany, [3]DSMZ, Braunschweig, Germany, [4]TU Braunschweig, Institute of Plant Biology, Braunschweig, Germany

Phytopathogenic fungi pose significant challenges to agriculture, contributing notably to crop losses. The primary control method employed is the use of broad-spectrum fungicides, which not only harm biodiversity but also lead to environmental pollution. Therefore, developing sustainable agricultural strategies is essential for eco-friendly farming.
Botrytis cinerea is a major plant pathogen affecting various crops, including strawberries. Recent studies indicate that the saprophytic fungus *Neurospora crassa* may influence *B. cinerea* development, diverting its spores from forming infection structures to creating non-infectious networks through germling fusion on plant surfaces. Given that *B. cinerea* and *N. crassa* inhabit distinct ecological niches, we are investigating whether similar developmental reprogramming

mechanisms exist within natural fungal communities containing *B. cinerea*. To explore this, we isolated and cultivated fungal species from the epiphytic phyllosphere mycobiome of strawberry plants, identifying a “core mycobiome” characterized by consistently present genera across sampling locations. We are characterizing the microscopic development of these fungi on plant surfaces through both single-species and co-cultivation with *B. cinerea*, aiming to identify isolates that influence *B. cinerea* in a similar manner to *N. crassa*. Furthermore, we established surface-sterile strawberry plants in sterile microcosms as an effective system for infection studies. We intend to re-establish the mycobiome in various compositions to examine its effects on the infectivity of *B. cinerea* and other isolated phytopathogens. Ultimately, this project aims to enhance our understanding of the phyllosphere mycobiome's role in the plant's defense against pathogenic fungi.

P1.103 - Immunoassay to detect urinary siderophore Triacetylfusarinine-C (TAFC) as a diagnostic biomarker of Invasive Aspergillosis

K. Walshe[1]
[1]Maynooth University, Maynooth, Ireland

The incidence of global fungal infection including Invasive Aspergillosis (IA) is expected to grow. Given the recent recognition that IA affects over 2 million people with COPD, the world’s population is rapidly aging, and the high incidence of death caused by Aspergillosis in patients with COVID-19 in ICU, non-invasive methods that enable rapid identification of infection will ideally avoid the need for disruption of care. The WHO has emphasized that Aspergillus fumigatus detection is key to facilitating faster and more accurate diagnosis of IA . However, even with the currently available rapid tests for detection of Aspergillus antigen, which require specimen pre-treatment, new methods for improved ease of detection with increased sensitivity and specificity are essential. Recent data has demonstrated that detection of biomarker Triacetylfusarinine C (TAFC), a virulence-associated metabolite produced by A. fumigatus, can be detected in the urine of infected patients with very high sensitivity and specificity using mass spectrometry. Until now, monoclonal IgG against A. fumigatus TAFC has proven difficult to produce. However, using a newly-generated recombinant monoclonal TAFC-specific IgG, we have developed a rapid, sensitive immunodiagnostic ELISA which demonstrates potential to detect TAFC at clinically-relevant levels directly from urine samples. The TAFC ELISA has high specificity, is reproducible and demonstrates excellent recovery of spiked TAFC in urine. Unlike other Aspergillus antigen assays, no sample pre-treatment is required. A proof-of-concept TAFC lateral flow device (TAFC LFT) has also been demonstrated.

P1.201 - A near-complete telomere-to-telomere genome assembly for *Batrachochytrium dendrobatidis* GPL JEL423 reveals a CBM18 gene family expansion and a M36 metalloprotease gene family contraction

N. Helmstetter[1], *J. Gregory*[1], *K. Harrison*[1], *J. Harrison*[1], *E. Ballou*[1], *R. Farrer*[1]
[1]University of Exeter, Biosciences, Exeter, United Kingdom

Batrachochytrium dendrobatidis (*Bd*) is responsible for mass extinctions and extirpations of amphibians, mainly driven by the Global Panzootic Lineage (*Bd*GPL). *Bd*GPL isolate JEL423 is a commonly used reference strain in studies exploring the evolution, epidemiology and pathogenicity of chytrid pathogens. These studies have been hampered by the fragmented, erroneous and incomplete *Bd* JEL423 genome assembly, which includes long stretches of ambiguous positions, and poorly resolved telomeric regions. Using Oxford Nanopore reads and Hi-C mapping, we generated a substantially improved, near telomere-to-telomere genome assembly and gene annotation for *Bd* JEL423. Our new assembly is 24.5 Mb in length, ~800 kb longer than the previously published assembly for this organism, comprising 18 nuclear scaffolds and 2 mitochondrial scaffolds and including an extra 839 kb of repetitive sequence. We discovered that the patterns of aneuploidy in *Bd* JEL423 have remained stable over approximately 5 years. We found that our updated assembly encodes fewer than half the number of M36 metalloprotease genes predicted in the previous assembly. In contrast, members of the crinkling and necrosis gene family were found in similar numbers to the previous assembly. We also identified a more extensive carbohydrate binding module 18 gene family than previously observed. We anticipate our findings, and the updated genome assembly will be a useful tool for further investigation of the genome evolution of the pathogenic chytrids.

P1.202 - Multi-omics analysis of a fungal cell factory producing recombinant enzyme controlled by a constitutive or inducible promoter

J.V. Bernardi[1,2], *E.P. Antoniel*[1], *M.F. Cavalheiro*[2], *L.O.S. Silva*[1], *M.M. Brandão*[2], *A. Damasio*[1]
[1]UNICAMP, Biochemistry and Tissue Biology, Campinas, Brazil, [2]UNICAMP, Center for Molecular Biology and Genetic Engineering, Campinas, Brazil

Aspergilli are filamentous fungi known for their high secretion capacity, making them effective cell factories for industrial enzyme production. However, some challenges can limit enzyme titers, so optimizing fungal strains is crucial to enhance enzyme production. This study compares two *Aspergillus nidulans* strains expressing α-L-arabinofuranosidase (AbfA) from *Aspergillus fumigatus*, controlled by either a constitutive (glyceraldehyde-3-phosphate dehydrogenase promoter, pgpdA, from *A. nidulans*) or an inducible (glucoamylase promoter, pglaA, from *Aspergillus niger*) promoter. The strain with the constitutive promoter showed 0.71-fold higher AbfA secretion and 4.11-fold increased enzyme activity. However, higher *abfA* mRNA expression (5.49-fold) was detected by qPCR in the strain with the inducible promoter. To further understand why the strain with higher mRNA levels secretes the lowest amount of enzyme, we performed RNA-seq and proteomic. Transcriptomic confirmed higher *abfA* levels in the pglaA::abfA strain (2.65-fold). Gene ontology enrichment revealed that oxidative stress was overrepresented under the control of the pglaA, while amino acid biosynthesis was highlighted for the pgpdA. Transcript levels of unfolded protein response genes were similar in both strains, suggesting that misfolded proteins may not limit AbfA secretion in the pglaA::abfA strain. Co-expression network identified genes whose expression regulation was directly associated with AbfA production, and their roles in fungal cell factories are under investigation. Proteomic data

showed differential expression of proteins involved in signaling, DNA packaging, and protein synthesis. AbfA was not found in the intracellular proteome, indicating effective secretion. The transcriptomic-proteomic correlations will contribute to unraveling potential bottlenecks that limit AbfA control by the inducible promoter.

P1.203 - Reference pangenomes improve 'omics analysis of fungi by capturing their genetic diversity: a demonstration from *Aspergillus fumigatus*

M. Perrier[1,2], *C. Puerner*[3], *A.A. Golicz*[4], *J.E. Stajich*[5], *R.A. Cramer*[3], *A.E. Barber*[1,2]
[1]Friedrich Schiller University, Institute of Microbiology, Jena, Germany, [2]Friedrich Schiller University, Cluster Balance of the Microverse, Jena, Germany, [3]Dartmouth College, Geisel School of Medicine, Department of Microbiology and Immunology, Hanover, United States, [4]Justus Liebig University, Department of Agrobioinformatics, IFZ Research Center for Biosystems, Land Use and Nutrition, Giessen, Germany, [5]University of California-Riverside, Department of Microbiology and Plant Pathology, Riverside, United States

Fungi harbour a tremendous amount of genomic diversity, including marked differences in gene content even within the same species. A prominent example is *Aspergillus fumigatus*, a ubiquitous environmental mould responsible for an estimated 1.5 million deaths annually. Only 69% of the total genes of the species are conserved in all isolates, with a large number showing presence-absence variation. Due to their absence in the reference strains, the role of these accessory genes in stress resistance, metabolism, and virulence remains unknown. To create a tool that captures species' diversity with the ultimate goal of understanding the function of these accessory genes, we used 26 near-chromosomal level genome assemblies to create a pangenome reference for *A. fumigatus*. This reference has a length of 38 Mbp, 30% longer than the current Af293 reference, and encodes 2,260 ORFs absent in Af293. This novel tool can be used for the unbiased but computationally straightforward analysis of genomic and transcriptomic data from diverse strains. As a demonstration that the graph pangenome better captures *A. fumigatus*' diversity, alignment of genomic and transcriptomic data resulted in notably more reads aligned than the linear reference. Ongoing work uses this new reference for the high-resolution quantification of the genomic adaptations that occur during chronic infection and to understand the role of the accessory genome in the virulence of *A. fumigatus* using a large transcriptomic dataset. This work highlights the value of reference pangenomes for improving our understanding of strain heterogeneity and how it contributes to diverse biological processes.

P1.204 - Taxogenomic analysis reveals the reticulate evolution of the genus *Trichoderma*

F. Cai[1], *A. Steindorff*[2], *F. Yi*[1], *M. Ding*[3], *R. de Vries*[4], *I.V. Grigoriev*[2], *I. Druzhinina*[5]
[1]Sun Yat-sen University, School of Ecology, Shenzhen, China, [2]Joint Genome Institute (JGI), Lawrence Berkeley National Laboratory, Berkeley, United States, [3]Nanjing Agricultural University, Jiangsu Provincial Key Lab for Solid Organic Waste Utilization, Nanjing, China, [4]Westerdijk Fungal Biodiversity Institute, Fungal Physiology, Utrecht, Netherlands, [5]Royal Botanic Gardens, Kew, Richmond, United Kingdom

The increasing importance of *Trichoderma* (Hypocreales, Ascomycota) for human well-being underscores the need for a solid taxonomy and deep understanding of its biology. Recent studies have challenged the hypothesis of *Trichoderma* primary association with plant roots or soil, instead highlighting its mycoparasitism and environmental opportunism. The parasitism of closely related fungi (adelphoparasitism) likely caused the massive transfer of plant cell wall degrading enzymes from plant-pathogenic fungi to ancestral *Trichoderma* lineages, along with some reciprocal transfers of other genes. The early molecular phylogeny has shown significant variations in evolutionary rates across different clades and identified taxonomic ambiguities, such as infrageneric "taxonomic clouds" or putative metaspecies (e.g. *T. harzianum* s.l.), where numerous phylospecies remained undefined due to the lack of genealogical concordance. The taxogenomic analysis of 66 *de novo Trichoderma* genome assemblies revealed four consistent phylogenetic clades. However, we also observed frequent gene tree incongruences, discordances between gene trees and the species tree, and inconsistent placement of individual lineages. Results from D Statistics and PhyloNet analyses were unanimous in indicating ancient gene flows within each clade (such as from an unsampled lineage or an extincted one to the ancestor of *T. effusum*) and putative hybrid speciation between certain *Longibrachiatum* species. Our findings suggest a complex history of introgression and reticulate evolution in some infrageneric groups of *Trichoderma* and the "conventional" speciation in the others. In this presentation, we'll propose a species concept accommodating the formation of metaspecies and discuss the importance of taxonomic stability in evaluating the safety of *Trichoderma*-based bioeffectors.

P1.205 - Evolutionary arms race between a yeast homing genetic element and its genomic target

M. Osborne[1], *K.H. Wolfe*[1]
[1]Conway Institute, University College Dublin., School of Medicine, Dublin, Ireland

We are investigating WHO elements, a new type of homing genetic element that was recently discovered by our lab in the yeast genus *Torulaspora*. WHO elements are related to, but different from, inteins and homing introns, two major classes of well known homing elements. WHO elements are about 4 kb long. They encode endonucleases that cut genomic DNA at one particular target site, in the glycolytic gene *FBA1*. The double-strand DNA break in *FBA1* is then repaired by integrating a WHO element. This process allows WHO elements to integrate into previously "empty" alleles of *FBA1*, causing them to spread through the population. WHO elements and their target site in *FBA1* are both highly variable in sequence, and they appear to be locked into an evolutionary arms race with each other. We hypothesise that in this arms race, WHO endonucleases are under selection to cleave as many alleles (sequence variants) of *FBA1* as possible, while *FBA1* alleles are under selection to avoid being cleaved. To test this hypothesis, we have determined the sensitivity and resistance of different *FBA1* alleles to cleavage by WHO endonucleases from different families. We are also mutation-scanning the target site of one WHO6 endonuclease in order to determine its precise sequence requirements for cleaving *FBA1*.

Our goal is to test the arms race hypothesis, and to
determine how these families of endonucleases and cleavage sites are coevolving.

P1.206 - Comprehensive genomic and proteomic analysis of a novel *Lindgomycetaceae* species

M. Pilz[1], *T. El-Elimat*[2], *H. Raja*[3], *F. Qoura*[4], *D. Awad*[1], *N. Oberlies*[3], *T. Brück*[1]
[1]Technical University of Munich, School of Natural Sciences, Department Chemistry, Werner Siemens-Chair of Synthetic Biotechnology, Garching, Germany, [2]Jordan University of Science and Technology, Department of Medicinal Chemistry and Pharmacognosy Faculty of Pharmacy, Irbid, Jordan, [3]The University of North Carolina at Greensboro, Department of Chemistry and Biochemistry, Greensboro, United States, [4]Fraunhofer-Institut für Grenzflächen- und Bioverfahrenstechnik IGB, Bio-, Elektro- und Chemokatalyse BioCat, Institutsteil Straubing, Garching, Germany

Saprobic fungi of the *Lindgomycetaceae* family are mostly found on submerged or decaying organic matter. The freshwater ascomycete, analysed in this study interacts with its environment by secretion of hydrolases, as well as diverse polyketides (PKS) and nonribosomal peptides (NRPS). Thus, the genome and proteome of the novel strain were explored in detail using next-generation sequencing and high-performance MS/MS analysis. Proteome analysis yielded insight into the expression profile of secreted and cellular proteins. Glycoside hydrolases were of special interest in this work due to their applicability as additives in washing detergents. Therefore, among secreted hydrolases, amylases and cellulases were further investigated. Amylase activity assays on starchy stains, as well as cellulase activity assays on fabric pilling yielded promising results. Additionally, the metabolic pathways concerning PKS and NRPS synthases were investigated, as polyketides and cyclodepsipeptides were detected within the samples of which the former exhibited activity in antibiotic assays. The PKS and NRPS synthases predicted via genome-based tools were clustered with reference sequences and compared with hits detected within proteomic analysis providing insight into the corresponding intracellular processes. The biosynthetic gene cluster analysis was based on conserved sequences of the predicted protein coding genes. Reference sequences from biosynthetic pathways concerning structures similar to the compounds of interest obtained from related organisms enabled the determination of plausible candidates. In further work, expression of the identified PKS and NRPS will be performed within a model organism, e. g. *Saccharomyces cerevisiae* or *Escherichia coli*, allowing the confirmation of target candidates via GC-MS and NMR.

P1.207 - Novel insights into the mode of action of the antifungal protein PeAfpA against *P. digitatum* revealed by high-throughput analysis

C. Ropero-Pérez[1], *M. Giner-Llorca*[1], *S. Garrigues*[1], *P. Manzanares*[1], *J.F. Marcos*[1]
[1]Instituto de Agroquímica y Tecnología de Alimentos (IATA), Consejo Superior de Investigaciones Científicas (CSIC), Biotechnology Department, Paterna (Valencia), Spain

Antifungal proteins (AFPs) from filamentous fungi are promising molecules to control fungal pathogens. However, their mechanisms of action remain largely unknown. The antifungal proteins PeAfpA and PdAfpB are encoded by the plant post-harvest pathogens *Penicillium expansum* and *Penicillium digitatum*, respectively. Both proteins show sequence divergence and belong to different phylogenetic classes, yet both are active against *P. digitatum*. Thus, the aim of this study is to elucidate the mode of action of PeAfpA against *P. digitatum* through high-throughput gene expression analysis and to compare it with the published transcriptomic data from PdAfpB.
We evaluated the gene expression profiling of *P. digitatum* with increasing concentrations of PeAfpA (0.1, 0.25, and 1 µg/mL). Only 218 DEGs were shared across all treatments, being 190 induced and 28 repressed. At the highest inhibitory concentration (1 µg/mL), 1,333 differentially expressed genes (DEGs) were identified (627 induced and 706 repressed). Comparison with the PdAfpB treatment profile revealed that 46% of the DEGs at 1 µg/mL of PeAfpA exhibited a common expression pattern, whilst 19% showed a reverse pattern. Additionally, gene ontology (GO) and KEGG enrichment analysis suggested that PeAfpA has a multi-target mode of action, affecting pathways related to cell wall biogenesis, lipid metabolism, apoptosis or stress. Based on these findings, we selected 17 genes to perform gene deletion analysis. Nevertheless, none of the mutants showed phenotypic alterations in axenic growth nor susceptibility changes to PeAfpA and PdAfpB treatment. Due to the potential multi-target roles of AFPs, future studies will focus on generating multiple deletions mutants.

P1.208 - Genome-wide structural variants uncover strain-specific genes linked to pathogenicity in *Colletotrichum graminicola*

S. Becerra[1], *B. Cuevas-Fernandez*[1], *P. García-Rodríguez*[1], *F. Rogério*[1], *S. Gutiérrez-Sánchez*[1], *T. Boufleur*[2], *R. Baroncelli*[3], *L. Faino*[4], *S. Sukno*[1], *M. Thon*[1]
[1]University of Salamanca, Salamanca, Spain, [2]University of Sao Paulo, Sao Paulo, Brazil, [3]University of Bologna, Bologna, Italy, [4]"La Sapienza" University of Rome, Rome, Italy

Structural variations (SVs) contribute to genomic plasticity, increasing genetic diversity and in adaptive evolution in plant pathogenic fungi. *Colletotrichum graminicola* is the fungus responsible for causing maize anthracnose, a significant disease with global implications. The study of this pathogen provides valuable insights into fungal pathogenicity and genome dynamics. To investigate the role of SVs in adaptations, we conducted a large-scale comparative genomic analysis by sequencing the genomes of five strains of *C. graminicola*. This analysis revealed that SVs are enriched in regions containing repetitive DNA elements and are close to secreted proteins and predicted effectors. A synteny analysis showed extensive collinearity across core chromosomes, although chromosomes 4 and 10 exhibited a higher incidence of structural variations, including translocations and inversions. Minichromosomes exhibited lower collinearity and a higher frequency of variations, reflecting distinct evolutionary trajectories. Segmental duplications in the reference strain span approximately 174 Kb; furthermore, these regions are enriched in repetitive elements and show evidence of repeat-induced point mutations but lack genes and effectors. Clustering of protein sequences identified 733 unique genes in the reference strain, including 121 located on minichromosomes. Of these, 457 strain-specific (SS) genes harbored high-impact variants that could affect gene function. Among the SS genes with

known functions, most were associated with pathogenicity. This study demonstrates that structural variations (SVs) are vital for the genomic adaptation of C. graminicola, enabling it to overcome host defenses and thrive in diverse environments. This enhances our understanding of plant-pathogen interactions and informs the development of anthracnose control strategies.

P1.209 - Zymocin-like killer toxin gene clusters in the nuclear genomes of filamentous fungi

P.G. Heneghan[1], *L.I. Salzberg*[1], *K.H. Wolfe*[1]
[1]Conway Institute, University College Dublin, School of Medicine, Belfield, Ireland

Zymocin-like killer toxins are anticodon nucleases secreted by some budding yeast species, which kill competitor yeasts by cleaving tRNA molecules. They are encoded by virus-like elements (VLEs), cytosolic linear DNA molecules that are also called killer plasmids. To date, toxins of this type have been found only in budding yeast species (Saccharomycotina). We discovered that the nuclear genomes of many filamentous fungi (Pezizomycotina) contain small clusters of genes for a zymocin-like ribonuclease (γ-toxin), a chitinase (toxin α/β-subunit), and in some cases an immunity protein. The γ-toxins from Fusarium oxysporum and Colletotrichum siamense abolished growth when expressed intracellularly in S. cerevisiae. Phylogenetic analysis of glycoside hydrolase 18 (GH18) domains showed that the chitinase genes in the gene clusters are members of the previously described C-II subgroup of Pezizomycotina chitinases. We propose that the Pezizomycotina gene clusters originated by integration of a yeast-like VLE into the nuclear genome, but this event must have been ancient because (1) phylogenetically, the Pezizomycotina C-II chitinases and the Saccharomycotina VLE-encoded toxin α/β subunit chitinases are sister clades with neither of them nested inside the other, and (2) many of the Pezizomycotina toxin cluster genes contain introns, whereas VLEs do not. One of the toxin gene clusters in Fusarium graminearum is a locus that has previously been shown to be under diversifying selection in North American populations of this plant pathogen. We also discovered that two genera of agaric mushrooms (Basidiomycota) have acquired toxin gene clusters by horizontal transfers from different Pezizomycotina donors.

P1.210 - Using multi-omics to establish how a change of diet affects anaerobic gut fungi and the wider gut microbiome of Jersey cows

S. Moñino Ramos[1], *T. Moses*[2], *M. Watson*[1], *J. van Munster*[1]
[1]Scotland's Rural College, Edinburgh, United Kingdom, [2]University of Edinburgh, EdinOmics, RRID:SCR_021838, Edinburgh, United Kingdom

The microbiome of the rumen, the first of four stomach chambers in ruminants such as sheep and cows, plays a critical role in sustainable farming, as microbial activity during plant feed digestion results in high methane emissions. Anaerobic gut fungi (AGF) are an essential members of the rumen microbiota; they possess an exemplary ability to digest the complex carbohydrates present in plant feed and support methanogens and other members via metabolic interactions. However, the roles and activity of AGF are not yet fully understood due to the complexity and limitations

in in-vivo studies. As a result, no strong links have been established between the microbiome composition, its activity, and the metabolites present in the rumen.
This study aims to use a time-resolved multi-omics approach to establish links between the bovine rumen metabolome and resident microbial communities, focusing on effects of a change of diet. Five rumen-fistulated Jersey cows from the same farm have been followed over a year where rumen fluid was collected monthly. We performed microbial profiling using bacterial 16S and fungal ITS amplicon sequencing and determined their metabolic profile via untargeted metabolomics studies, using zwitterionic-phase hydrophilic interaction chromatography (HILIC-Z) coupled to drift tube ion mobility-quadrupole time-of-flight (DTIMqTOF) mass spectrometry. These results will be integrated with the obtained volatile fatty acids (VFAs) profiles and pH measurements of the rumen fluid. With this knowledge, we aim to increase our understanding on how the microbiome, including AGF, contribute to individual metabolic activities in cattle, working towards improvement of sustainable farming practices.

P1.212 - A systematic screen for genetic factors underpinning transposon defense systems across the fungal kingdom

T. Badet[1], *D. Croll*[1]
[1]University of Neuchâtel, Neuchâtel, Switzerland

Fungal genome sizes vary more than 100-fold with repetitive sequences such as transposable elements (TE) being the primary driver for genome inflation. Genomic defenses evolved to counteract proliferation of TEs at the epigenetic or transcript level. In fungi, repeat-induced point mutation (RIP) target TE copies inserted in the genome by recognizing repeated sequences for targeted mutagenesis. The prevalence of RIP across the fungal kingdom is unknown as well as whether RIP mutations strictly follow the canonical C-to-T transition pattern. Here, we address these questions by designing a screen for mutational signatures specific to repetitive sequences across the fungal kingdom. Enrichment in mutational signatures at non-coding and repeated sequences was restricted to Ascomycota, consistent with a phylogenetically restricted occurrence of RIP-like genomic defenses. Then, we quantified mutational signatures to identify phylogenetic associations in gene functions. We identified a zing-finger protein as the strongest candidate underpinning a novel mechanism of genome defenses. Finally, we show that the loss of RIP components was at the origin of a 80 % (~30 Mb) genome size increase in the *Leotiomycetes*. We show that multifaceted drivers of TE defense systems have close ties to genome size evolution in the fungal kingdom elucidating how proximate mechanisms may impact genome evolution at deep phylogenetic scales.

P1.213 - Investigating the strain diversity in Cryptococcus neoformans using pangenomic analysis

C. Reichardt[1], *B. Billmyre*[2]
[1]University of Georgia, Microbiology, Athens, United States, [2]University of Georgia, Pharmaceutical and Biological Sciences, Athens, United States

Cryptococcus neoformans causes an estimated 160,000 deaths per year worldwide, primarily in immunocompromised patients. C. neoformans consists of 4 different molecular types: VNI, VNII, VNBI, and VNBII. While we understand sequence variation separating these groups, we have a limited understanding of the differences in gene content that differentiate the C. neoformans molecular types. We have assembled a pangenome using existing Illumina sequencing of 384 diverse C. neoformans isolates de novo. We curated a set C. neoformans genes that are either: core (present in >95% of genomes), accessory (present in >=95% and <1 genomes), or lineage-specific (present in 1 genome). We found a conservation of orthologs between molecular types, indicating potential for a speciation event. Additionally, we found most gene variance in C. neoformans is from accessory genes, not singleton. Our pangenome assembly of C. neoformans provides a novel angle to identify
1) novel antifungal targets and
2) unidentified mechanisms of drug resistance.
Coupled with large-scale transposon insertion sequencing in C. neoformans isolates, we can use the pangenome assembly to understand how gene content variation affects the development of antifungal drug resistance in vitro. We plan to perform Tn-seq in select isolates to identify variation in gene essentiality across C. neoformans as well as novel drug resistance/susceptibility genes.

P1.214 - Polyphasic approach revealed the genomic and phenotypic variation across the supreme plant biomass degradation genus *Trichoderma*

P. Chen[1,2], *N. Martinez Reyes*[2,3], *M. Peng*[2], *H. Senoglu*[2], *S.K. Reddy*[2,4], *B. Henrissat*[5,6], *A. Steindorff*[7], *I.V. Grigoriev*[7], *R. Berka*[8], *I.S. Druzhinina*[9], *R.P. de Vries*[2]
[1]Nanjing Agricultural University, College of Resources and Environmental Sciences, Nanjing, China, [2]Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands, [3]Universidad de León, León, Spain, [4]Wageningen University & Research, Wageningen, Netherlands, [5]Technical University of Denmark, Department of Biotechnology and Bioengineering, Lyngby, Denmark, [6]King Abdulaziz University, Jeddah, Saudi Arabia, [7]Joint Genome Institute, Berkeley, United States, [8]Archer Daniels Midland Company, Davis, United States, [9]Royal Botanic Gardens, Kew, London, United Kingdom

The genus *Trichoderma* (Hypocreales, Ascomycota), encompassing over 400 species, is ubiquitous and thrives in diverse environments, including dead wood, soil, litter, and even on fungi. *Trichoderma* spp. exhibit a broad spectrum of nutritional strategies, engaging in saprotrophic and biotrophic interactions with bacteria, fungi, plants, animals, and other organisms. *Trichoderma* has been intensively studied for over seven decades due to its extensive applications in cellulolytic enzyme production and biocontrol against plant pathogens, numerous genetic and genomic studies have been conducted on fungi within this genus, unveiling their genomic traits and evolutionary history.
This study specifically focuses on the genomic and phenotypic diversity of *Trichoderma* species. With the inclusion of *de novo* sequenced genomes and published ones, a comprehensive genome compendium of the genus was assembled. Our phylogenomic analysis identified at least three main clades/sections. Subsequently, we predicted sugar metabolism genes and secondary

metabolite genes, with the conserved sugar metabolism genes exhibiting a high correlation with the phylogenomic tree. Furthermore, a comparative analysis of the *Trichoderma* CAZome with those of other fungi revealed that, despite similar numbers of CAZyme families in *Trichoderma* genomes, only a quarter of these genes are conserved across all analyzed species. This finding suggests that recent gene deletions, likely driven by environmental pressures, have significantly shaped the current plant biomass degradation CAZyme profile within the *Trichoderma* genus. Our genotype-phenotype profiling contributes to a deeper understanding of the ecology, speciation, and biotechnological potential of *Trichoderma*.

P1.215 - Discovery of novel fungal carbon metabolic enzymes through transfer learning

M. Peng[1], *W. Wennekers*[1], *R.P. De Vries*[1]
[1]Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands

Developing fungal cell factories to convert plant biomass into value-added compounds is crucial for boosting the circular bioeconomy. This often requires engineering of their primary carbon metabolism (PCM) which frequently fails due to our incomplete understanding of the enzymes involved in these metabolic pathways. In this study, we aim to develop a transfer learning based approach to identify the missing metabolic genes by taking advantages of careful curation of high-quality enzymatic knowledge and maximal extraction of relevant features on both the enzymes and their corresponding substrates using pretrained models. The preliminary results revealed that our new approach successfully predicted several promising PCM candidates of *Aspergillus niger* that that can't be identified by commonly used sequence similarity searches. However, the current prediction model requires further optimization to improve its accuracy and generalizability, and additional biochemical experiments are required to further validate the prediction results. We expect this novel computational method will facilitate the prioritizing novel enzymes involved in fungal PCM to facilitate more effective metabolic engineering of fungal cell factories for a broad range of biotechnology applications.

P1.216 - 1000 fungal proteins project

J. Evans[1]
[1]Pacific Northwest National Laboratory, Environmental Molecular Sciences Laboratory, Richland, United States

The "1000 Fungal Proteins" project is an ambitious initiative aimed at associating structure and function with poorly characterized or previously uncharacterized proteins conserved across the fungal kingdom. The Environmental Molecular Sciences Laboratory (EMSL) is spearheading this effort by leveraging its extensive experimental and computational resources in structural biology to expedite the annotation of proteins whose functions remain elusive.
Central to this project is the integration of biochemical, structural biology, and cell biological methodologies to explore and characterize these proteins. This comprehensive approach will be facilitated by samples provided by EMSL users, which include target genes or protein sequences,

cell-free vectored target genes, purified target proteins, and fungal cells with high-copy target proteins in vivo. To systematically investigate these proteins, EMSL has established the 1000 Fungal Proteins Pipeline encompassing functional screening, structural annotation, and post-translational modification profiling. This presentation will provide an overview of the project, highlight recent results, and discuss how interested users can join the collaborative team.

P1.217 - Transposable elements are transcriptionally deregulated upon horizontal chromosome transfer in an entomopathogenic fungus

H. Griem-Krey[1], *M. Habig*[1]
[1]Kiel University, Fungal Evolutionary Genetics, Kiel, Germany

The horizontal transfer of entire chromosomes occurs in several fungal species, particularly involving accessory chromosomes. These horizontal chromosome transfers (HCTs) represent large genetic exchanges, often transferring hundreds of genes as well as non-genic elements such as transposable elements (TEs), which are typically enriched on accessory chromosomes, into a new genomic environment. However, how the activity of the transferred genes and TEs is influenced by the recipient genome is largely unknown. Moreover, the large number of transferred genes and TEs during HCT allows us to detect overall patterns of regulation that apply to horizontally transferred sequences in general and might also govern the fate of the more often observed individually horizontally transferred genes or TEs. We here examined transcriptional activity in two strains of the entomopathogenic fungus *Metarhizium robertsii*, between which an accessory chromosome (termed chrA) containing 364 genes and 474 TEs was experimentally transferred horizontally. While genes and TEs in the recipient genome exhibited small changes in their transcription, those located on the transferred chromosome underwent significant transcriptional deregulation in the new genomic background. Importantly, the TE repertoire of the recipient genome played a critical role in shaping the transcriptional activity of the TEs located on the horizontally transferred chromosome. We conclude that horizontal transfer leads to widespread transcriptional deregulation of transposable elements, likely governed by genomic-background-specific defences of the recipient genome. These genomic-background-specific defences might therefore help to explain the differences in the fate of horizontally transferred TEs observed in other fungal species.

P1.218 - Co-evolution of biological processes as revealed by whole genus genome sequencing of *Aspergillus*

R.P. de Vries[1], *I.V. Grigoriev*[2], *M.R. Andersen*[3], *S.E. Baker*[4]
[1]Westerdijk Fungal Biodiversity Institute, Fungal Physiology, Utrecht, Netherlands, [2]DOE Joint Genome Institute, Berkeley, United States, [3]Technical University of Denmark, Technical University of Denmark, Lyngby, Denmark, [4]Pacific Northwest National Laboratory, Environmental Molecular Sciences Division, Richland, United States

The rapid expansion of the set of fungal genomes has provided many new insights in the biology of these organisms and their diversity. *Aspergillus* is one of the best studied fungal genera, due to

its relevance as an opportunistic pathogen of humans and animals, a spoilage organism of many foods and its wide application in biotechnological processes. In recent years we have generated genome sequences for nearly 300 *Aspergillus* species, providing an unprecedented dataset of a fungal genus.
This dataset has now been used to generate an evolutionary roadmap of biological processes to identify at which level each of these processes has gone through major changes, focusing on the genus, subgenus, section and species level. Rather than studying the conservation of individual genes or gene groups, we analyzed the evolutionary pattern at the level of process covering the following topics: carbon utilization (CAZy-genes, primary carbon metabolism, sugar transporters), nitrogen utilization (nitrogen and amino acid metabolism), secondary metabolism, stress response, development and propagation (mating, sexual/asexual development, conidiation). Subsequently, the evolutionary patterns of these processes have been compared to reveal which processes co-evolve and which occur at different taxonomic levels. This has provided new insights into fungal evolution, which will also be a template for other whole genus genome projects, such as those of *Penicillium* and *Trichoderma*.

P1.219 - Population genomics of the yeast Kluyveromyces marxianus reveals no geographical stratification and incipient speciation

J.M. Moreno-Hernandez[1], J. Smets[2], L.F. García-Ortega[1], R.A. Ortiz-Merino[2], L. Morales[3], K. Verstrepen[2], A. DeLuna[4], E. Mancera[1]
[1]Cinvestav, Departamento de Ingeniería Genética, Unidad Irapuato, Irapuato, Mexico, [2]VIB—KU Leuven, Center for Microbiology, Leuven, Belgium, [3]UNAM, LIIGH, Juriquilla, Mexico, [4]Cinvestav, UGA, Irapuato, Mexico

Kluyveromyces marxianus is a non-conventional yeast known for its biotechnological potential due to its ability to ferment many carbon sources from industrial by-products. Despite its industrial relevance, the genetic diversity of *K. marxianus* remains underexplored. To breach the gap of knowledge we sequenced the genomes of 180 *K. marxianus* strains from diverse natural and human-related niches from around the world. Whole-genome sequence analysis revealed three distinct phylogenetic clades that do not correlate with the geographic origin of the strains. Instead, the type of environment that they inhabit may be driving population structure since the three clades are respectively formed by environmental, industrial (dairy) and agave fermentation isolates. In addition, phenotypic variation among the clades reflects niche-specific adaptations. At a genomic level, the clade from agave fermentations is considerably different when compared to the environmental and industrial clades. This, together with evidence of reproductive isolation, suggest that a process of incipient speciation may be occurring in this yeast. Our findings depict a population scenario different from that described for other yeasts, with implications on the way we understand geographical stratification and speciation of these microorganisms.

P1.220 - Integrating evolutionary genomics and experimental approaches to understand endophytism in entomopathogenic fungi

A. Kortsinoglou[1], *T. Butt*[2], *V. Kouvelis*[1]
[1]National and Kapodistrian University of Athens, Department of Biology, Athens, Greece,
[2]Swansea University, Department of Biosciences, Swansea, United Kingdom

Entomopathogenic fungi, known for infecting and killing insects, can also colonize plants as endophytes or rhizosphere associates, promoting plant growth and providing protection. This dual functionality makes them promising candidates for biocontrol products and biofertilizers. However, high-quality annotated genomes of these fungi are limited. This study combines evolutionary genomics with experimental approaches to explore the gene content and attributes that facilitate this dual lifestyle. The genomes of eight highly effective entomopathogenic and endophytic fungal strains from *Beauveria*, *Metarhizium*, and *Lecanicillium* genera were sequenced using Oxford Nanopore technology, resulting in high-quality assemblies. Comparative genomic analyses with other entomopathogenic fungi from the Order Hypocreales revealed variations in key gene families associated with endophytic traits, including those involved in host colonization, stress response, and nutrient acquisition. While all strains shared orthologous genes with other endophytic and entomopathogenic fungi, several species- or strain-specific singletons were identified, which are potentially linked to adaptive traits. The CAZyme (Carbohydrate-Active enZyme) repertoire, crucial for plant cell wall degradation and symbiosis, was highly conserved across species, underscoring its importance in fungal-plant interactions. In contrast, predicted secreted effector proteins and biosynthetic gene clusters showed greater variability, suggesting their role in adaptive dynamics of plant colonization and host specificity. Complementary plant inoculation experiments assessed phenotypic variation in endophytic colonization, linking genetic variations to observed behaviors. This integrative approach provides new insights into the mechanisms underpinning the dual ecological roles of entomopathogenic fungi, highlighting their potential for sustainable agriculture.

P1.221 - Towards a mechanistic understanding of fungus-growing ant coevolution

J. Robinson[1], *N. Gerardo*[1], *T. Read*[2]
[1]Emory University, Biology, Atlanta, United States, [2]Emory University, School of Medicine, Atlanta, United States

Fungal agriculture has arisen multiple times across domains of life. Of the more than 240 species of fungus-growing ant, those from the genus *Cyphomyrmex*, obligately cultivate *Leucocoprineae* fungi as their primary source of food (cultivar) and, like human agriculture, these cultivars are antagonized by pests with a paralleled coevolutionary history. Amongst these pests, *Sympodiorosea* acts a primary antagonist to *Cyphomyrmex* gardens whereby it exhibits chemotaxis toward cultivars *in vivo* and eventually degrades the cultivar. As cultivar genetic distance increases, *Sympodiorosea* success declines indicating potential lineage-specificity. However, both the extent of specialization and the underlying mechanism(s) that maintain the

associations between host and antagonist fungi remains poorly understood. In addition to comparative genomics of cultivars and *Sympodiorosea*, we leverage natural products chemistry to identify secretions from the cultivars that likely serve as selective boundaries between agricultural regimes. Furthermore, our analyses reveal that the pest, *Sympodiorosea*, experience purifying selective pressures which have led to constrained diversity in CAZymes, proteases, and lipases, likely reflecting the nutrient structure of the garden. Interestingly, biosynthetic gene cluster (BGC) diversity is less constrained within *Sympodiorosea* hinting at a potential mechanism of coevolution between host and antagonistic fungi. Together, our results reveal species-specific patterns of domestication and shed light on potential mechanisms that structure this highly successful, ancient agricultural system.

P1.222 - Coupling genome-wide positive selection analysis with transcriptomics to infer molecular bases of the *Rhizopus microsporus* – *Mycetohabitans* symbiosis

M. Branine[1], *E. Scales*[2], *S. Mondo*[3,4], *T. Pawlowska*[2]
[1]Cornell University, Department of Microbiology, Ithaca, United States, [2]Cornell University, School of Integrative Plant Science, Ithaca, United States, [3]U.S. Department of Energy Joint Genome Institute, Berkley, United States, [4]Colorado State University, Department of Agricultural Biology, Fort Collins, United States

The mucoromycete *Rhizopus microsporus* (*Rm*) and its bacterial endosymbiont *Mycetohabitans* spp. are an emerging model system for symbioses of early-diverging fungi and bacterial endosymbionts due to its experimental tractability and genomic resources. In this symbiosis, *Mycetohabitans* provides its host with secondary metabolite toxins, controls fungal asexual and sexual propagation, and alters fungal lipid metabolism. We hypothesize that the strong influence of *Mycetohabitans* on *Rm* host biology has significant consequences for the evolutionary trajectory of host fungi. Importantly, *Rm* isolates naturally free of endosymbionts (nonhosts) permit comparative analyses into if and how endosymbiotic bacteria influence the evolution of fungal hosts. To begin addressing this question, we implemented a genome-wide positive selection analysis in host and nonhost strains of *Rm* by calculating the non-synonymous to synonymous substitution rate ratio (dN/dS) of all single-copy orthologs. Under a branch model, we identified 28 genes putatively under positive selection in host *Rm* strains (dN/dS>1). To provide further evidence for the role of these genes in symbiosis, we leveraged transcriptomic data comparing two *Rm* hosts cured of their endosymbionts to their WT counterparts. While several genes from our positive selection analysis were differentially expressed (DE) in either host, only two were DE in both strains. One of these genes encodes a SRF-like protein with orthology to the transcription factor RlmA, a regulator of asexual differentiation in ascomycetes. Given the reproductive control exerted by *Mycetohabitans*, we hypothesize this transcription factor is playing a similar role in mucoromycete fungi, which we will test in future functional studies.

P1.223 - Comparative genomics of *Cryptococcus* and *Kwoniella* reveals karyotype dynamics and suggests evolutionary mechanisms of pathogenesis

M.A. Coelho[1], *M. David-Palma*[1], *T. Shea*[2], *K. Bowers*[2], *S. McGinley-Smith*[2], *A.W. Mohammad*[2], *A. Gnirke*[2], *A.M. Yurkov*[3], *M. Nowrousian*[4], *S. Sun*[1], *C.A. Cuomo*[2], *J. Heitman*[1]
[1]Duke University Medical Center, Department of Molecular Genetics and Microbiology, Durham, United States, [2]Broad Institute of MIT and Harvard, Cambridge, United States, [3]Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany, [4]Ruhr-Universität Bochum, Lehrstuhl für Molekulare und Zelluläre Botanik, Bochum, Germany

Understanding the genomic mechanisms driving fungal adaptation and pathogenicity is essential for revealing how certain species evolve into global human pathogens. In this study, we focused on the *Cryptococcus* genus (encompassing both human pathogenic and nonpathogenic species) and its related genus *Kwoniella* to investigate how genomic changes shape these processes. Chromosome-level assemblies generated for multiple species, representing nearly all known diversity within these genera, revealed distinct differences in genomic architecture. Despite comparable genome sizes (19.2–22.9 Mb), our analysis uncovered extensive variation in chromosome number and structure between the two genera. Most *Cryptococcus* species retain the ancestral 14 chromosomes, while most *Kwoniella* species exhibit fewer (11, 8, 5, or as few as 3), associated with giant chromosome formation (up to 18 Mb) through repeated fusion events marked by pericentric inversions and centromere loss. In contrast, *Cryptococcus* species with fewer than 14 chromosomes primarily show rearrangements involving the loss of repeat-rich centromeres and frequent translocations, including intercentromeric recombination facilitated by shared centromeric transposons. Gene content analysis revealed that pathogenesis-related genes are highly conserved, though pathogen-associated signatures of gene gain and loss were identified. For example, pathogenic *Cryptococcus* species have lost the zincophore Pra1 and its transporter Zrt1, retained in most nonpathogenic species. In *Candida albicans*, Pra1 recruits neutrophils to infection sites, so its loss in *Cryptococcus* may dampen neutrophil activation in early lung colonization. This study provides insights into the genomic features shaping both genera, offering a foundation for understanding centromere loss, chromosome fusions, and the emergence of pathogenic *Cryptococcus* species.

P1.224 - Unraveling the evolutionary history of genetically diverged lineages of *Colletotrichum graminicola*

F. Rogério[1], *S. De Mita*[2], *C. Van Oosterhout*[3], *B. Cuevas-Fernández*[1], *P. García-Rodríguez*[1], *S. Becerra*[1], *S. Gutiérrez-Sánchez*[1], *J. Dambolena*[4], *I. Buhiniček*[5], *S. Sukno*[1], *M. Thon*[1]
[1]University of Salamanca, Salamanca, Spain, [2]Univ Montpellier, Montpellier, France, [3]University of East Anglia, Norwich, United Kingdom, [4]Universidad Nacional de Córdoba, Córdoba, Argentina, [5]BC Institute for Breeding and Production of Field Crops, Dugo Selo, Croatia

Maize anthracnose, caused by the ascomycete fungus Colletotrichum graminicola, is an important crop disease worldwide. The disease can result in significant yield losses and is also an important model for genetic studies. The evolutionary history of crop pathogens is shaped by a complex interaction of natural and anthropogenic factors. We investigated the evolutionary origin of genetically diverged lineages of *C. graminicola*, using a collection of 212 field isolates from 17 countries including recently collected strains from Galicia, Spain. Genomic analyses supported the existence of three geographically isolated lineages, with a significant pattern of isolation by distance. We identified two distinct gene flow patterns, driven by short and long-distance dispersion, likely resulting from the natural spread of the pathogen and the exchange of contaminated seeds. Demographic modeling indicated that North America is an intermediate between Brazil, Europe and an ancestral, unsampled source population, hypothesized to be Mesoamerican. Our analyses revealed that the global genomic structure of C. graminicola is shaped by geographic differentiation driven by long-distance migration and a long history of recombination and introgression. We show historical relationships among these lineages, identifying a potential route for fungal spread, with the North American population emerging ancestrally, followed sequentially by the Brazilian and European populations. Our research indicates that the European lineage is more virulent, which has implications for the potential emergence of new outbreaks of maize anthracnose in Europe.

P1.225 - Meta-analysis of *Pleurotus ostreatus* transcriptomes: transcriptomic profiles and efforts in basidiomycete fungi

A.G. Pisabarro[1], *I. Jiménez*[1], *D. Rosales*[1], *E. Garde*[1], *J. Ruiz Umaña*[1], *G. Pérez*[1], *L. Ramírez*[1]
[1]Public University of Navarre (UPNA), Institute for Multidisciplinary Research in Applied Biology (IMAB), Pamplona, Spain

The energy cost of mRNA synthesis in fast-growing filamentous fungi can use up to 30% of the cell's ATP. In the basidiomycete *Pleurotus ostreatus*, this effort is spread across over 12,000 genes, supporting both maintenance and adaptation to environmental and developmental changes. Most transcriptomic studies identify differentially expressed genes between two conditions, often overlooking the overall distribution of gene expression, or *transcriptomic landscape*, of an organism.
Organismal systemic wisdom is required to efficiently allocate transcriptional effort and energy costs across different genes and gene groups.
We define ***transcriptional effort*** as the percentage of total mRNA synthesis devoted to a specific gene or group of genes. This study explores the transcriptomic profile of *P. ostreatus* and examines how transcriptional effort is allocated across different individuals and conditions. Key questions include: What is the transcriptomic profile of a growing filamentous fungus? Which genes are most expressed in a growing colony? Which genes remain consistently expressed across various growth conditions? What percentage of transcriptional effort do these genes consume? How is transcriptional effort divided among gene groups, such as those related to primary or secondary metabolism and information maintenance? Lastly, can a specific transcriptional effort profile correlate with different conditions, particularly under biotic or abiotic stress?

In summary, this article suggests a new approach to analyzing a large number of available transcriptomes, seeking insights into the adaptive and survival strategies of filamentous fungi.

P1.227 - Whole genome sequencing of Candida glabrata and Candida albicans for detection of resistance mutations against Echinocandins

F. Wegner[1], *P.S.D. Salik*[1], *S. Purushothaman*[1], *B. Schulthess*[1], *A. Egli*[1]
[1]Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland

Background: Invasive *Candida* infections (ICI) are a significant medical concern, particularly due to the rise of antifungal resistance (AFR). Echinocandins are first line treatment for ICI, targeting the glucan synthase necessary for cell wall synthesis, thereby inducing fungicidal activity. Classically, AFT assessment is culture dependent and slow requiring up to 72h from sample collection to AFR determination. In addition, EUCAST recommends, non-susceptible anidulafungin measurements to be referred to a reference laboratory for *fks1/2*sequencing and confirmation of minimal inhibitory concentrations (MIC). We aimed to explore a whole genome sequencing (WGS)-based approach and determine the performance to predict pheno- from genotypes.
Methods: We sequenced 110 clinical isolates of *Candida albicans* and *C. glabrata* comprising of susceptible and echinocandin resistant strains. Microdilution (Yeast one) was used to determine MICs and EUCAST was followed for categorical AFR interpretation. Combining our strains with publicly available genomic data available sources, we determined mutations conferring echinocandin resistance.
Results: In total, we had 81 genome-phenotype pairs. Anidulafungin, micafungin, and caspofungin resistance was present in 39%, 9%, and 14%, with median MICs of 0.125 (IQR=0), 0.125 (IQR=0.1), and 1 (IQR=1.25), respectively. Our results confirm the presence of known resistance mutations in the *fks2* genes in 44% of all *C. glabrata* resistant isolates, but none in *C. albicans.* Interestingly, *fks1/2* mutation failed to explain AFR in the majority of resistant isolates, highlighting novel AFR mechanisms.

P1.228 - Overestimation of proteome provides insights into transposable element landscape in *Podosphaera xanthii*, causal agent of cucurbit powdery mildew disease

A. Jimenez-Sanchez[1], *Á. Polonio*[1], *D. Fernández-Ortuño*[1], *A. Pérez-García*[1]
[1]University of Malaga, Department of Microbiology, Faculty of Sciences and Instituto de Hortofruticultura Subtropical y Mediterránea La Mayora (IHSM-UMA-CSIC), Malaga, Spain

Cucurbit powdery mildew, caused by *Podosphaera xanthii*, is one of the most destructive plant diseases of cucurbit crops worldwide. To date, there are discrepancies in the number of genes in the available genomic resources of the pathogen. We previously reported a predicted gene set of 16,030 in the *P. xanthii* isolate 2086, which was higher than that commonly described in other obligate biotrophic ascomycetes. The evaluation of the resulting proteome revealed approximately 7,000 consistent proteins and a surprising number of 8,940 unknown proteins. The

study of this "dark" proteome showed that it actually originated from divergent transposable elements (TEs). For its part, the *P. xanthii – Cucumis melo* dual RNA-seq analysis showed a significant activity of some of these TEs during infection. Refining genome annotation taking into consideration repetitive elements could be critical for future metabolic and evolutionary studies aimed at understanding pathogen-host interactions.

P1.229 - The lifestyle of mucoromycotina 'fine root endophytes' through the genomic lens

A. Wanke[1], *A. Williams*[2], *V.R. Morelos*[3], *A. Dallaire*[4,5], *S. Au*[5], *S. Pressel*[3], *K. Field*[2], *S. Schornack*[1]
[1]Sainsbury Laboratory Cambridge University, Cambridge, United Kingdom, [2]University of Sheffield, Sheffield, United Kingdom, [3]Natural History Museum, London, United Kingdom, [4]Royal Botanic Gardens Kew, Richmond, United Kingdom, [5]University of Cambridge, Cambridge, United Kingdom

'Fine Root Endophytes' from the Mucoromycotina clade (MFRE) play a crucial role in terrestrial nutrient cycling due to their dual saprotrophic and mycorrhizal traits. Over the last decade, microscopic and physiological studies have provided valuable insights into the mycorrhizal relationship between MFRE and plants, highlighting intracellular colonisation and nutrient-for-carbon exchange. However, the molecular mechanisms underlying the saprotrophic and mycorrhizal lifestyles of MFRE remain poorly understood. In this study, we employ genomic, transcriptomic and molecular genetic approaches on two MFRE isolates to explore symbiotic and saprotrophic strategies in this mycorrhizal lineage. We generated the first genome assemblies for MFRE and their bacterial endosymbionts, utilising both short-read (Illumina) and long-read (Nanopore) sequencing technologies. Through comparative genomic and transcriptomic analyses, we identified distinct signatures for transporters and secreted proteins (such as hydrolases and effector candidates) under *in vitro* and *in planta* conditions. Our findings highlight a unique molecular blueprint that distinguishes the saprotrophic and mycorrhizal lifestyles of MFRE from other mycorrhizal fungi.

P1.230 - Integrated study of fungal secondary metabolite and exoenzyme profiles, elucidating evolution of fungal interaction with competitors, substrate and host

L. Lange[1], *J.C. Frisvad*[2]
[1]Technical University of Denmark, Valby, Denmark, [2]Technical University of Denmark, Denmark/BioEngineering, Kongens Lyngby, Denmark

The secondary metabolite- and exoenzyme-profiles are studied separately, by researchers with different expertise, methods and objectives. In the current study we aim at making integrated secretome analysis, including both metabolites and exoenzymes. This approach is inspired by evolution, exerting selection pressure on the entirety of the interaction profile; composed of both biologically active metabolites and enzyme-proteins. This study aims at testing the hypothesis

that *Aspergillus* species, with strong degrading capacity, also have a strong profile of biologically active metabolites - e.g. giving added benefit as the fungus has a large amount of degraded substrate to defend against microbial foraging. In carrying out such integrated analysis, we take point of departure in *Aspergillus* genome-sequences, annotated to integrated F:F-observations (EC Function:Protein Family). Based on this the total number of CAZyme F:F-observations and the total number of unique observations (= function specificity diversity), can be summed up. Both scorings enable ranking, of enzyme profiles of all analyzed *Aspergillus* species, from highest to lowest. For integrated metabolite- and exoenzyme profiles, we selected four groups of *Aspergillus* species. Scoring highest, in enzyme degrading capacity (i), or in number of unique functions (ii); or scoring lowest in enzyme degrading capacity (iii), or lowest in number of unique functions (iv). Among these four groups of *Aspergillus* species, we selected species with secreted, well-characterized metabolite profiles, as analyzed by chromatography/mass spectrometry, together with exoenzyme profiles. The result from such integrated analysis of secondary metabolites and exoenzymes will provide an attempt to elucidate evolution of the *Aspergillus* interaction secretome.

P1.231 - Bioinformatics-driven identification of candidate pathogenicity-related genes in *Colletotrichum lupini* through comparative genomics and effector profiling

A. Menicucci[1], *C. Fiorenzani*[1], *S. Iacono*[1], *R. Baroncelli*[1,2]
[1]University of Bologna, Department of Agricultural and Food Sciences (DISTAL), Bologna, Italy, [2]University of Naples, Center for Studies on Bioinspired Agro-Enviromental Technology, Portici, Italy

Colletotrichum lupini is a host-specific fungal pathogen responsible for anthracnose in lupin, causing significant agricultural losses. Uncovering the genomic basis of its pathogenicity is essential for developing resistant cultivars and proper disease management strategies.
We employed a bioinformatics pipeline to identify species-specific genes potentially involved in virulence by analysing both lineage-specific regions (LSRs) and species-specific orthogroups (SSOGs) across multiple *Colletotrichum* species.
LSRs were identified by aligning raw sequencing data from closely related species to reference genomes, detecting zero-coverage genomic regions that may be involved in host specialization or horizontal gene transfer (HGT). In parallel, SSOGs were identified using *OrthoFinder*, revealing protein groups unique to each species, thus providing insights into phylogenomic relationships and proteins likely linked to pathogenicity.
Additionally, a combined bioinformatic workflow was utilized to analyse genes for their roles as candidate effectors, proteins likely involved in host interaction and immune evasion.
Furthermore, to gain a better understanding of the specialisation process, functional categories, often involved in fitness-related or virulence mechanism, such as carbohydrate-active enzymes (CAZymes), peptidases, transporters, and transcription factors (TFs) were annotated using the JGI's fungal genome database for three selected *Colletotrichum* genomes.
This comprehensive analysis identified several candidate genes, of which many within LSRs, SSOGs, and effector regions. These candidate genes are likely critical for host tissue degradation, immune suppression, and nutrient acquisition during infection. These findings provide key

insights into the molecular mechanisms of *C. lupini* pathogenicity and could be used to guide the design of novel biocontrol strategies to mitigate agricultural losses.

P1.232 - Exploring the complex secondary metabolism of the fungicolous genus *Cladobotryum* through comparative genomics

A.C Christinaki[1], *A.I Myridakis*[2], *D. Floudas*[2], *Z. Gonou-Zagou*[1], *V.N Kouvelis*[1]
[1]National and Kapodistrian University of Athens, Department of Biology, Athens, Greece, [2]Lund University, Department of Biology, Lund, Sweden

Fungi of the order Hypocreales engage in a wide array of biological interactions with various hosts. The fungicolous (or also known as mycophilic) species of the genus *Cladobotryum* are associated with other fungi. They colonize both wild and cultivated mushrooms, resulting in significant damage to edible mushroom production. However, there is limited knowledge of the genetic mechanisms driving fungicolous interactions. Understanding them could help develop mushroom protection strategies that improve cultivation efficiency through environmentally sustainable methods. These species exhibit a rich secondary metabolism, which seems to be strongly associated with their mode of life. Our previous work, integrating morphological, phylogenetic, and metabolomic data of 32 *Cladobotryum* strains, revealed high metabolic diversity within the genus. To deepen our understanding of this unique diversity, in the current study, the genomes of four *Cladobotryum* species were sequenced and analyzed, with an emphasis given on secondary metabolism related genes and genetic regions. Over 100 biosynthetic gene clusters (BGCs) responsible for secondary metabolite production were identified in each case, positioning *Cladobotryum* among the most BGC-rich genera of fungicolous fungi. Comparative genomic analysis with other Hypocreaceae species uncovers both conserved and unique BGCs, indicative of potential evolutionary processes, including BGC formation and losses and Horizontal Gene Transfer events, leading to the formation of novel metabolic products. These findings provide new insights on adaptive responses to environmental pressures and host interactions offering valuable insights for future studies in fungal ecology and agriculture.

P1.233 - Low complexity regions in fungi display functional groups and are depleted in positively charged amino-acids

K. Steczkiewicz[1], *A. Kossakowski*[1], *S. Janik*[2], *A. Muszewska*[1]
[1]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland, [2]University of Warsaw, Faculty of Mathematics, Informatics and Mechanics, Warsaw, Poland

Reports on the diversity and occurrence of low complexity regions (LCR) in Eukaryota are limited. Some studies have provided a more extensive characterisation of LCR proteins in prokaryotes. There is a growing body of knowledge about a plethora of biological functions attributable to LCRs. However, it is hard to determine to what extent observed phenomena apply to fungi since most studies of fungal LCRs were limited to model yeasts. To fill this gap, we performed a survey of LCRs in proteins across all Fungal Tree of Life branches. We show that

the abundance of LCRs and the abundance of proteins with LCRs are positively correlated with proteome size. We observed that most LCRs are present in proteins with protein domains but do not overlap with the domain region. LCRs are associated with many duplicated protein domains. The quantity of particular amino acids in LCRs deviates from the background frequency with a clear overrepresentation of amino acids with functional groups and a negative charge. Moreover, we discovered that each lineage of fungi favors distinct LCRs expansions. Early diverging lineages differ in LCRs abundance and composition pointing at a different evolutionary trajectory of each fungal group.

P1.234 - Genomic hints to Mortierellomycota resilience and adaptability

A. Muszewska[1], *D. Barua*[1], *M. Orłowska*[2], *B. Sokołowska*[1], *M. Ciach*[3], *S. Piłsyk*[1], *A. Okrasińska*[2], *J. Pawłowska*[2]
[1]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland, [2]University of Warsaw, Faculty of Biology, Biological and Chemical Research Centre, Warsaw, Poland, [3]University of Warsaw, Faculty of Mathematics, Informatics and Mechanics, Warsaw, Poland

Mortierellomycota encompasses ubiquitous soil- and plant-associated fungi, potentially the descendants of the first colonizers of land. Current ecological and biological knowledge of Mortierellomycota representatives is mostly related to their ability to produce valuable fatty acids. Mortierellomycota is related to both Mucoromycota and Glomeromycota, with which they share the predisposition to harbor endohyphal and associated bacteria. We performed genomic analyses for Mortierellomycota genomes deposited in GenBank database. Their genomes are compact with moderate amounts of genes and mobile elements. However, they display several unique traits, including potent metabolic capabilities, often reflected in gene duplications. They are also particularly adapted to aquatic environments and their lipid composition seems to be analogous to Blastocladiomycota. Mortierellomycota lost genes involved in ergosterol synthesis, and seem to have no diacylglycerol kinase. Instead, they have an expanded repertoire of lipid peroxidation, lipid degrading and sterol-binding proteins. Based on gene presence, they likely have sphingomyelin, like the Opisthokonta ancestors, animals and current Umbelopsidales. Mortierellomycota have the most complex fucose metabolism among fungi with two fucose synthesis pathways. Compared to Mucoromycotina, they possess several expansions of peptidases and oligopeptide transporters. They have duplicated genes enabling the usage of purines as secondary nitrogen sources in nitrogen-limiting conditions. Their proteomes are shaped by both vertical inheritance and horizontal gene transfer. Likewise Glomeromycota, they acquired NOD-like receptors, and like Basidiobolus, they acquired NRPS metabolic clusters from associated bacteria. Taken together, Mortierellomycota displays a list of unique properties among Fungi. Here, we summarize puzzling features we identified previously, together with new findings.

P1.235 - A genomic investigation into *Cryptococcus neoformans* hybrids

R. Anand[1], *R. Farrer*[1], *Q. Ma*[1], *D. P. Tamayo*[1]
[1]University of Exeter, MRC Centre of Medical Mycology, Exeter, United Kingdom

Fungi reproduce both sexually and asexually, occasionally resulting in hybridization. Fungal hybrids arise from interbreeding between genetically distinct populations or species. *Cryptococcus neoformans* typically reproduces mitotically, generating haploid daughter cells that inherit genetic material from one parent. Under stress, such as nutrient starvation, sexual reproduction is triggered, leading to meiotic recombination. *C. neoformans* hybrids have been identified that are the result from mating between VNIV and other lineages including VNI, VNII, VNB. We searched for signatures of hybrid genomes from 275 WGS samples of *C. gattii* and *C. neoformans* from our own collection and the NCBI SRA. We identified 67 putative hybrids, which coincided with diploidy and high genetic diversity. Phylogenetic analysis revealed three separate groups of hybrids, owing to different parents. We discovered differential proportions of parental alleles in hybrid, with a greater abundance of VNIV alleles owing to possible backcrossing or selection. Loss of heterozygosity and aneuploidy were evident across hybrid genomes. These analyses shed new light on the genomic consequences of hybridisation on the *C. neoformans* population.

P1.236 - Decoding *Basidiobolus*: a key member of the herpetofauna gut microbiome

L. Vargas Gastelum[1], *J.F. Tabima*[2], *K.C. Syring*[1], *D. Farthing*[1], *S. Mondo*[3], *I.V. Grigoriev*[3], *K.L. McPhail*[4], *D.M. Walker*[5], *J.E. Stajich*[6], *J.W. Spatafora*[1]
[1]Oregon State University, Botany and Plant Pathology, Corvallis, United States, [2]Clark University, Department of Biology, Worcester, United States, [3]Lawrence Berkeley National Lab, Department of Energy (DOE) Joint Genome Institute (JGI), Berkeley, United States, [4]University of Utah, Department of Medicinal Chemistry, College of Pharmacy, Salt Lake City, United States, [5]Middle Tennessee State University, Department of Biology, Murfreesboro, United States, [6]University of California, Riverside, Plant Pathology & Microbiology, Riverside, United States

Basidiobolus is a zygomycete fungus that possesses a complex life cycle. It produces multiple spore types adapted to different ecological niches. This fungus is best known for being associated with the gut microbiome of herpetofauna (amphibians and reptiles) species. Previous reports of two *Basidiobolus* species suggest relatively large genomes of ~100 MB, and unusual genome architecture compared to most eukaryotes (e.g. hundreds of chromosomes). Compared to other zygomycete fungi of Mucoromycota and Zoopagomycota, *Basidiobolus* genomes also possess a higher count of genes related to secondary or specialized metabolism. Many of these genes are hypothesized to be acquired by horizontal gene transfer (HGT) from bacteria. Previous studies suggest these HGT events have facilitated *Basidiobolus* adaptation to the herpetofauna gut environment. Here, we present a preliminary analysis of six new (Nanopore sequenced) and two reference (PacBio sequenced) *Basidiobolus* isolates obtained from different herpetofauna species.

The genomes presented a highly variable genome size, from 58 MB to ~100 MB, with hundreds of canonical telomeric sequences. *Basidiobolus* isolates that are closely related to the species *B. ranarum* presented the higher number of gene models and the greatest number of specialized metabolite gene clusters. An augmented pipeline for detecting HGT events enabled the identification of Betaproteobacteria as the predominant bacterial gene donor clade across all genomes. Our work focuses on establishing the baseline knowledge of *Basidiobolus*, highlighting the cryptic genome organization and the impact of HGT from co-occurring gut bacteria.

P1.237 - Pangenomics reveal genome compartmentalization and a putative mating-type locus on an accessory chromosome in a host-specific fungal insect pathogen

D.M. Parker[1,2], C.C.A. Nogueira[1], A. Wilson[1], K.N. Nielsen[3,1], L. Hestbjerg Hansen[1], T.K. Nielsen[1], H.H. de Fine Licht[1]
[1]University of Copenhagen, Department of Plant and Environmental Sciences, Frederiksberg, Denmark, [2]University of Copenhagen, Globe Institute, København, Denmark, [3]Technical University of Denmark, DTU Biosustain, Lyngby, Denmark

Fungal pathogen populations often harbor substantial functional diversity, which may be driven by presence/absence genomic polymorphisms and structural genetic variation. Here we present a pangenomic analysis of a fungal insect pathogen, *Metarhizium acridum*, using seven chromosome-scale genomes from four different continents. First, we discovered that approximately 25% of the *M. acridum* pangenome is comprised of lineage-specific genes (only present in one isolate) or shared lineage-specific genes (present in more than one isolate, but not all). These genes are enriched for functions in secondary metabolite production, nutrient transport, and chromosome organization. Second, we find evidence of functional compartmentalization of the genome, as the core genome of *M. acridum* is enriched in carbohydrate-active enzymes, while the lineage-specific components are enriched in effectors located in gene-sparse regions of the genome. Third, we identified the first naturally recovered accessory chromosome in *M. acridum*, which does not contain host-insect effector proteins but is enriched in functions related to sexual reproduction. Within the genus *Metarhizium*, *M. acridum* is one of few species considered to regularly reproduce sexually. The accessory chromosome contains putative copies of genes from both the *MAT1-1* and the *MAT1-2* idiomorphs. Our findings suggest that the presence of this accessory chromosome may facilitate sexual reproduction, possibly even primary homothallism, in the otherwise heterothallic *M. acridum*. Overall, our study provides insights into the functional genomic diversity of *M. acridum* and the structure of genomic compartments associated with host insect specialization and niche adaptation in fungal pathogens.

P1.238 - 3D model of Podospora anserina genome and integration of epigenomics and transcriptomics data

G. Lelandais[1], G. Royer[2], A. Gualdoni[2], F. Dumetz[3], N. Ponts[3], P. Grognet[1], F. Malagnac[1]
[1]Université Paris Saclay, CNRS I2BC, Orsay, France, [2]I2BC, Genome Biology, Gif sur Yvette, France, [3]INRAE, UR1264 Mycology and Food Safety (MycSA), Bordeaux, France

In eukaryotes, long molecules of DNA are packaged to fit inside the cell nucleus. This is done by wrapping DNA around histones to form nucleosomes, which are then stacked to form chromatin fibres. Histones undergo post-translational covalent modifications that affect the degree of genome compaction. In this context, elucidating the architecture of chromatin is of great interest. This is a challenging task because i) chromosomes belong to the mesoscale, i.e. a length scale larger than discrete molecular complexes, and ii) chromatin organisation is highly dynamic and variable from cell to cell. However, in recent years it has become easier to build 3D models of the whole genome, thanks to significant advances in experimental techniques such as HiC and the development of original computational tools to predict the 3D structures of genomes. Our team has succeeded in obtaining the first HiC data in the model species Podospora anserina and in constructing 3D models of its entire genome. Such an achievement opens the possibility to create original integrative views of epigenomics and transcriptomics datasets, ranging from molecular mechanisms of histone modifications to large-scale regulation of gene expression during critical developmental processes. We present here the first results we have obtained in this direction.

P1.239 - Whole genome sequencing of *Penicillium commune* spoilage mould from potato-cereal soft wrap production

A. Fagerlund[1], *C. Kummen*[1], *A.W. Åsli*[1], *C.K. Finne*[1]
[1]The Norwegian Food Research Institute Nofima, Department of Food Safety and Quality, Ås, Norway

Significant quantities of food are spoiled due to undesirable mould growth. Lompe and lefse, unleavened tortilla-like potato-cereal soft wraps, are popular commercial products in Norway but are periodically susceptible to substantial mould spoilage. Although the wraps undergo a baking step that inactivates most mould spores, the final products can become contaminated by spores from the air or production surfaces during transportation on conveyor belts for cooling before packing.
In previous work, *Penicillium commune* was identified as the main spoilage mould species for these wraps, and potential contamination sources in a production facility were revealed (C.K. Finne, Foods, 2023, 12:3238). However, identifying the spoilage mould to the species level using morphological characterization and sequencing of the ITS region was insufficient to resolve the root causes of contamination or understand the spread of *P. commune* spores in the facility.
In the current study, we subjected 76 *P. commune* isolates from the strain collection to whole genome sequencing (WGS) analysis. This enabled differentiation at the strain level and an assessment of whether spoilage was due to persistent sources of contamination in the facility or the continuous introduction of new strains, such as via raw materials. The isolates were collected on 19 different sampling days over a period of 16 months. Thirty-one isolates were from spoiled products, and 19 and 26 isolates, respectively, were from air and surfaces at different locations in the processing facility.

P1.240 - FungiDB: a bioinformatics resource for facilitating data exploration, analysis, and integration for fungal and oomycete species

E. Basenko[1], *O. Harb*[2], *D. Roos*[2], *A. Jones*[1]
[1]University of Liverpool, Institute of Systems, Molecular and Integrative Biology, Liverpool, United Kingdom, [2]University of Pennsylvania, Department of Biology, Philadelphia, United States

FungiDB, a component of the VEuPathDB project, provides a one-stop shop for omics data exploration and analysis for over 300 fungal and oomycete species, including organisms on the WHO fungal priority list. FungiDB integrates diverse data types and enables researchers to interrogate the data using various tools such as the search strategy system, a genome browser, and comprehensive gene pages. Integrated data types include genomic, transcriptomic, proteomic, metabolomic, population-level and phenotypic studies and more. Built-in orthology enables cross-species inferences and enhances the reusability of datasets across the integrated organisms. Key features of FungiDB include:
- Extensive information on genomes, including gene record pages and automated and expert-curated annotations.
- Tools for conducting comparative genomics, analyzing protein structures, exploring gene regulatory networks, and investigating pathogen-host interactions.
- Integration of publicly available datasets, enabling researchers to conduct in silico experiments and explore data enrichment analyses in the context of existing data.
- Ongoing curation and community-driven enhancements to genomes in the genome editor Apollo to ensure the capture of up-to-date, high-quality annotations by leveraging community expertise.
Future advancements are aimed at integrating AI-driven tools to enhance literature curation and metadata annotation and incorporating tools for new data types.

P1.241 - Analysis of the transcriptional regulatory network of lignocellulose degradation in Basidiomycota

N. Miklovics[1], *Z. Merényi*[1], *B. Hegedüs*[1], *R. Tengölics*[1], *L. Nagy*[1]
[1]Institute of Biochemistry, HUN-REN Biological Research Centre, Centre of Excellence of the European Union, Szeged, Hungary

Lignocellulose is a complex, recalcitrant plant biopolymer composed of lignin, cellulose and hemicellulose. Wood-decaying white rot fungi are able to degrade lignocellulose using a complex array of oxidative enzymes. However, how the corresponding genes are transcriptionally regulated is poorly understood. We aimed to elucidate the transcriptional patterns and the regulatory network controlling lignocellulose degradation in *Coprinopsis cinerea* and *Pleurotus ostreatus* based on RNA-seq analyses of time-series wood-decay degradation and complex carbon source utilization experiments. We identify transcription factor-encoding genes within the lignocellulose degradation pathways using co-expression analyses to identify key regulatory factors of the pathways. The RNA-seq data were correlated with measurements of lignin

degrading enzymatic activity and degradation efficiency. By investigating these regulatory pathways, this research aims to provide an improved understanding of lignocellulose degradation at the transcriptional level, which is important for both ecological processes and industrial applications.

P1.242 - Insights into the evolution of non-Dikarya (zoosporic & zygomycetous) fungi: a mitogenomic perspective

K.N. Podimata[1], *M. Andrikopoulos*[1], *S.R Ahrendt*[2], *T.Y James*[3], *J.E Stajich*[4], *V.N Kouvelis*[1]
[1]National and Kapodistrian University of Athens, Department of Biology, Athens, Greece, [2]DOE Joint Genome Institute, Berkeley, United States, [3]University of Michigan, Department of Ecology and Evolutionary Biology, Ann Arbor, United States, [4]University of California, Department of Microbiology and Plant Pathology, Riverside, United States

Fungi are highly adaptable organisms to nearly every habitat on Earth. The most well-known fungi belong to the phyla Basidiomycota and Ascomycota, comprising Dikarya, but fungi outside this subkingdom, i.e, non-Dikarya or also known as zoosporic and zygomycetous fungi, similarly play significant ecological roles, often interacting with plants, animals, and other microbes. Studying non-Dikarya is essential for understanding the transition from aquatic to terrestrial environments and their evolutionary history. While phylogenetic research has almost exclusively focused on nuclear datasets, mitochondrial (mt) markers (genes related to oxidative phosphorylation, along with rRNA genes) provide an alternative approach, due to their different evolutionary rate. This study presents a comparative phylogenetic and evolutionary analysis of 67 mt genomes from key non-Dikarya phyla: Cryptomycota, Aphelidiomycota, Monoblepharomycota, Chytridiomycota, Sanchytriomycota, Blastocladiomycota, Zoopagomycota, Kickxellomycota, Entomophthoromycota, Mortierellomycota, Mucoromycota and Glomeromycota. Manual annotation was required for most mtDNA due to limited publicly available characterization. Using a concatenated matrix of 14 mt genes related to oxidative phosphorylation, phylogenetic trees were constructed with Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods. The study additionally examines synteny, intron diversity, and intergenic regions. Ancestral intron insertion sites in *cox*1, *cox*3, *cob*, and *nad*5 genes were identified. Although the 14 genes were largely conserved as expected, gene order varied significantly, with syntenic units mostly found within the same genus. This diversity contributes to gene shuffling which is -in some cases- further supported by the presence of various G4 quadruplexes in the mt genomes. Therefore, valuable insights into the evolution of these phyla are drawn.

P1.243 - An improved method to identify *het* genes of *Aspergillus nidulans*

B. Auxier[1], *A.J. Debets*[1]
[1]Wageningen University & Research, Laboratory of Genetics, Wageningen, Netherlands

Hyphal fusion between two fungal individuals leads to rapid programmed cell death. In the model species *Neurospora crassa* and *Podospora anserina*, this process is known to be controlled by ~10 genes, termed *het* for heterokaryon incompatibility. However the identity of these genes, and their evolutionary origin, is unclear outside of these Sordariomycetes. Many *het* genes are related to genes involved in bacterial defence, and the selection maintaining the extreme *het* gene allelic diversity remains an enigma. To access the dense evolutionary sampling of the *Aspergillus* genus, we developed a method that can rapidly identify *het* genes. Using complementing nitrate auxotrophies, which can be easily induced using UV paired with chlorate selection, we can screen for rare outcrossed progeny that can form heterokaryons with their parents. Pools of compatible progeny can be combined and used for bulk segregant analysis. As a proof of principle, we have identified the *het* genes of *A. nidulans.* The eight *het* genes we identify include loci shared with *Aspergillus fumigatus* as well as undescribed loci. The speed of this method, which can identify *het* genes in weeks instead of the years previously necessary, allows for investigations on a scale not previously possible. We believe, this method can be applied to any species with an accessible sexual cycle and uninucleate conidia. Applying this method to targeted *Aspergillus* species, combined with the abundant genomic resources, we can pinpoint the origins of these genes.

P1.244 - A new high-quality genome for *Fomitopsis pinicola sensu stricto* for analysis of gene expression and secondary metabolite production

J. Österman-Udd[1], *T. Mali*[1], *M. Qurashi*[1], *E. Kiviniemi*[1], *T. Lundell*[1]
[1]University of Helsinki, Department of Microbiology, Helsinki, Finland

Fomitopsis pinicola (*Polyporales*, *Basidiomycota*) is a wood-decaying brown rot fungus that is widespread in European forests. For a long time, the reference genome for *F. pinicola* was that of the North American isolate FP-58527 SS1, which was recently transferred to a new *Fomitopsis* species named *F. schrenkii*. Isolates of *F. pinicola sensu stricto* are found only in Eurasia, while the well-studied North American isolates belong to other species in the *F. pinicola* species complex: *F. mounceae*, *F. schrenkii* and *F. ochracea*. More information on the European isolates is thus needed to gain up-to-date information on *F. pinicola sensu stricto*.
Currently there is only one genome sequence publicly available for *F. pinicola*, represented by the draft genome of the Swedish isolate GR9-4. We have sequenced the genome of a Finnish isolate, FBCC 1181, which has been included in studies on wood decay and fungal interactions in our team. Both the nuclear and mitochondrial genome of *F. pinicola* FBCC 1181 have been assembled and annotated. The circular mitochondrial genome sequence is completely assembled, and the contig-level haploid nuclear genome assembly includes seven complete chromosomes. This high-quality genome allows for comparative genome analyses with other *Basidiomycota* species, but also studies applying RNA-Seq and analyses of metabolites produced under different growth conditions, with varying substrates and different levels of interaction with other microorganisms. This work will provide important information on *F. pinicola sensu stricto*, one of the most prevalent brown rot fungi in the Nordics and in Europe.

P1.245 - Anaerobic fungi as treasure trove for biomass degradation machinery for exploitation in renewables-based biotechnology

S. Shen[1], J. Matthews[1], M. Bowden[2], M. Blow[3], K. LaButti[3], V. Ng[3], C. Nicora[2], S. Leichty[2], S. Purvine[2], I. Grigoriev[3], J. van Munster[1]
[1]SRUC, Department of Plant and Soil Sciences, Edinburgh, United Kingdom, [2]Pacific Northwest National Laboratory, Richland, United States, [3]U.S Department of Energy Joint Genome Institute, Berkeley, United States

Lignocellulosic biomass is a promising feedstock for renewables-based biotechnology, but complete degradation of this material remains challenging. We investigate anaerobic gut fungi (AGF, phylum *Neocallimastigomycota*) to provide potential solutions. These fungi have distinct degradation mechanisms compared to the aerobic fungi usually employed as enzyme producers in biotechnology: they employ plant-biomass penetrating hyphae and leverage an arsenal of enzyme complexes that are unique among fungi. Despite lacking oxygen dependent lignin-degrading machinery or LPMO's, these AGF are highly effective in degrading raw lignocellulose.
Here we demonstrate how the degradative activity of three AGF isolates from genera *Neocallimastix, Caecomyces and Piromyces*, has distinct effects on plant biomass composition and architecture, via integration of distinct lignocellulose characterization techniques. Growth experiments indicated the fungi also had distinct capacities for metabolism of simple sugars that are derived from lignocellulose. These findings suggest that each species has unique roles and degradation capabilities. To further explore this, we are now using RNA-seq and untargeted proteomics to compare how our *Neocallimastix* and *Caecomyces* isolates leverage their degradative machinery during wheat straw fermentation.

P1.246 - The life-cycle switch in the Scots pine blister rust fungus, *Cronartium pini*, is associated with differences in mating type loci

P. Gomez-Zapata[1], M. Brandström Durling[1], M. Seidl[2], Å. Olson[1]
[1]Swedish University of Agricutural Sciences, Forest Mycology and Plant Pathology, Uppsala, Sweden, [2]Utrecht University, Theoretical Biology & Bioinformatics, Utrecht, Netherlands

Cronartium pini is a rust fungus that infects Scots pine across Asia and Europe, causing significant yield losses in Swedish and Finnish forests over the past decade. This pathogen has two life cycle forms: a heteroecious (complex) form and an autoecious (simple) form—each with potentially distinct impacts on reproduction and virulence. Given that the heteroecious form reproduces sexually while the autoecious form is largely clonal, we hypothesized that this life cycle shift is linked to variation in mating type loci. Using population genomic analyses on a collection of *C. pini* strains from Scandinavia, we examined the genetic diversity in mating genes. Our findings revealed multiallelic genes across all mating type loci in the heteroecious form. In the autoecious form, which is thought to be primarily clonal, we observed evidence of self-compatibility due to mating gene duplication in certain strains. This discovery challenges traditional views on reproduction in rust fungi and suggests mechanisms through which the autoecious form might retain genetic diversity. These insights advance our understanding of

mating systems in rust fungi, establishing *C. pini* as a valuable model for exploring life cycle-driven genetic diversity. Furthermore, the observed mating gene variation between forms has broader implications for studying pathogen evolution and virulence in forest ecosystems.

P1.247 - A pangenomic approach to understand genome evolution in *Cercospora*

J. Richards[1], *P. dos Santos*[1], *J. Searight*[1], *V. Doyle*[1]
[1]Louisiana State University Agricultural Center, Plant Pathology and Crop Physiology, Baton Rouge, United States

Understanding how genomes have changed over time can provide insight into ecological adaptation and diversification. Despite its threat to agricultural production across many crops, genome evolution within the fungal genus *Cercospora* is relatively unexplored due to the lack of genomic resources that adequately represent the breadth of diversity in the genus. We used a pangenomic approach to characterize gene family expansions/contractions, effector gene content, and repetitive element repertoires to understand genome evolution in *Cercospora*. Highly contiguous annotated assemblies were generated for 9 species and analyzed alongside 7 previously published genomes. Ortholog clustering revealed 14,173 orthogroups, of which, 68.2% were classified as accessory orthogroups. We also found 717 effector gene families and 532 species-specific singleton effector genes. Species-specific effector genes ranged from 11 to 79 per species. Additionally, lineage-specific expansions/contractions of virulence-related gene families were identified. These results indicate that the genus *Cercospora* has a highly diverse and variable effector/virulence gene repertoire and, in some cases, may reflect host specialization through co-evolutionary adaptation. Repetitive element content was also highly variable among species. Proportions of repeats ranged from 1.46% to 34.84% with four species having substantially elevated repeat content (>23%). Genome size in *Cercospora* has decreased over time and is correlated with overall repeat content, suggesting that differences in genome defense mechanisms among species may exist. Our preliminary results have shown that genome structure is highly diverse in *Cercospora* and future work will infer ancestral genome configurations to provide further insight into the mechanisms of genome diversification.

P1.248 - Transcriptional insights into the lignocellulose degradation mechanisms of white and brown rot fungi

I. Jiménez[1,2], *M. Alfaro*[1,2], *E. Garde*[1,2], *A. Fernández-Morales*[1,2], *J. Ruiz*[1,2], *G. Pérez*[1,2], *A. Lipzen*[3], *K. Lail*[3], *D. Bauer*[3], *K. Barry*[3], *I.V. Grigoriev*[3,4], *L. Ramírez*[1,2], *A.G. Pisabarro*[1,2]
[1]Public University of Navarre, Pamplona, Spain, [2]Institute for Multidisciplinary Research in Applied Biology, Pamplona, Spain, [3]DOE Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, United States, [4]University of California Berkeley, Department of Plant and Microbial Biology, Berkeley, United States

Wood-degrading fungi play a vital role in ecosystems by breaking down lignocellulose, the complex structure of plant cell walls. Among these fungi, white rot fungi (WRF) and brown rot

fungi (BRF) have evolved distinct strategies for lignocellulose degradation. WRF utilize oxidative enzymes to decompose both lignin and cellulose, allowing for complete breakdown, while BRF primarily target cellulose through a low-oxidation Fenton reaction, largely preserving lignin.
To investigate the genetic adaptations of these fungi to different carbon sources, we analyzed the transcriptomes of three WRF species—*Phanerochaete chrysosporium*, *Heterobasidion irregulare* and *Pleurotus ostreatus*—and two BRF species—*Fomitopsis schrenkii* and *Rhodonia placenta*—cultured on glucose and poplar wood media. Our findings revealed distinct gene expression patterns between the groups. WRF showed significant upregulation of genes related to carbohydrate metabolism and transport, highlighting their extensive enzymatic repertoire, including cellulases and lignin-degrading enzymes from families such as AA3_1, AA9, GH6, and GH7.
Notably, GH16 was the only enzyme upregulated across all five species, indicating its unique role in lignocellulose degradation. In BRF, GH16 exhibited higher transcription levels than in WRF, suggesting an important adaptation to their lignocellulose degradation strategy, in which cellulose is mainly degraded by Fenton reaction. Furthermore, the absence of GH6 and GH7 in BRF genomes implies that GH16 may have evolved earlier than these specialized cellulases. Overall, this research highlights the distinct evolutionary paths of WRF and BRF, providing valuable insights into their lignocellulose degradation mechanisms and paving the way for optimizing biofuel production.

P1.249 - Discoveries of genomic treasures in deep-sea fungi

Y. Nagano[1], *K. Mizuno*[1], *A. Doan*[2], *I. Gregoriv*[3], *E. Record*[2], *F. Martin*[4], *S. Miyauchi*[5]
[1]Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Marine Biodiversity and Environmental Assessment Research Center, Research Institute for Global Change (RIGC), Yokoshuka, Japan, [2]INRAE, Aix Marseille Univ, UMR1163, Biodiversité et Biotechnologie Fongiques, Marseille, France, [3]US Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, United States, [4]Université de Lorraine, INRAE-Grand Est-Nancy, UMR Interactions Arbres/Microorganismes, Nancy, France, [5]Okinawa Institute of Science and Technology, Evolutionary and Synthetic Biology unit, Okinawa, Japan

Deep in the ocean where sunlight does not reach, fungi live under extremes of temperature, salinity, hydrostatic pressure, and in the presence of toxic organic compounds. Such fungi have rare adaptations to their harsh environment. However, deep-sea fungi have remained unexplored. Here we study genomes of deep-sea fungi and identify genomic factors enabling the fungi to survive in the extreme environment. We sequence genomes of various marine fungi and compare them with a range of aquatic and terrestrial fungi. We clarify how the fungi have adapted to the deep sea. Our discoveries give insights into fungal machineries converting nutrition from unusual sources in the deep ocean, and advance understandings of microbial nutrient cycles in marine ecosystems.

P1.250 - Fungal genomes in the Carbohydrate-Active enZYme database

E. Drula[1], *V. Lombard*[2], *M. Boulinguiez*[3], *P. M Coutinho*[3], *M.-L. Garron*[3], *I.V. Grigoriev*[4], *N. Terrapon*[3], *B. Henrissat*[5]
[1]INRAE, Fungal Biodiversity and Biotechnology, Marseille, France, [2]CNRS, Architecture et Fonction des Macromolécules Biologiques, Marseille, France, [3]Aix Marseille University, Architecture et Fonction des Macromolécules Biologiques, Marseille, France, [4]Lawrence Berkeley National Laboratory, DOE Joint Genome Institute, California, United States, [5]Technical University of Denmark, Department of Biotechnology and Biomedicine, Copenhagen, Denmark

Over thirty years have passed since the emergence of the classification of carbohydrate-active enzymes (CAZymes) into sequence-based families, which underpins the CAZy database (www.cazy.org) [1]. The database, which is manually curated, continuously updates the families of enzymes that catalyze the cleavage and assembly of glycans and glycoconjugates. Historically and for maintenance reasons, CAZy was only updated using sequences from the daily releases of Genbank [2]. However, Genbank releases induce a taxonomic bias due to the incompleteness of many eukaryotic genomes, including fungal ones. Thanks to our long-term collaboration with the JGI, we recently released our annotations of published MycoCosm [3] and PhycoCosm [4] projects, amounting to 1141 genomes of fungal, algal and related species. These annotations are continuously updated, meaning that when new CAZyme families are discovered, the corresponding members are identified and integrated into previously annotated genomes.
A few years ago, our database started collecting and presenting the origin of the functional information (PubMed or DOI links) for CAZymes that have been experimentally characterized and published in the literature. The extracted information is disclosing the substrate diversity in each family which could be difficult to apprehend in its current form (EC numbers). To better harness the functional details of CAZymes and to circumvent limitations of the EC number system, we recently developed a system that describes mechanisms, glycosidic bond orientations, subsites and inter-residue connectivity. This new system allows complex searches in the CAZy database to uncover the evolution of substrate specificity and mechanisms of CAZymes across families [5].

P1.251 - Four microplastic-associated marine basidiomycete yeasts: genomic insights into resilience, adaptation, and innovation

F. Venice[1], *M. Florio Furno*[1], *F. Spina*[1], *G.C. Varese*[1]
[1]University of Turin (UNITO), Department of Life Sciences And Systems Biology, Turin, Italy

Microplastics (MPs) pollution is a critical environmental issue affecting ecosystems globally, with significant implications for their inhabitants. We examined the genomes of four Basidiomycete yeasts sourced from sediment MPs in the Livorno region of Italy. The genomes of *Cystobasidium slooffiae* and *Sakaguchia dacryoidea* (Cystobasidiomycetes), *Vishniacozyma carnescens* (Tremellomycetes), and *Kondoa aeria* (Agaricostilbomycetes) are similar in size, approximately 50 Mbp, and average around 10,000 genes each. These fungi are known to

produce bioactive compounds with properties that are antiviral and osteogenic; their genomes contain a notably high number of gene clusters associated with terpene-like molecule production and export, but a Mass Spectrometery-driven discovery is also ongoing. All four fungi demonstrate some capability to depolymerize plastics, evidenced by the presence of potential plastic-degrading enzymes like esterases and laccases, as well as hydrophobins that may assist in adhering to MPs. Their ability to degrade plastics is also supported by horizontally acquired genes, including a bacterial-derived plastic depolymerase identified in *C. slooffiae* and its close relatives. Furthermore, given their presence in terrestrial environments, studying their genomes may provide insight into the evolutionary adaptations that allowed fungi to thrive in marine settings, such as shifts in tRNA-adaptation indices for specific class of genes. We propose these four strains as potential candidates for biotechnological and biomedical innovations, as well as important evolutionary models highlighting the adaptability of fungi.

P1.252 - Assessment of dsRNA uptake in Stemphylium vesicarium for RNAi - based control strategy

E. Baraldi[1], *M Karas*[1], *F Negrini*[1], *X Chen*[1], *C Minnelli*[2], *G Mobbili*[2], *A Ricci*[3], *S Sabbadini*[3], *B Mezzetti*[3]
[1]University of Bologna, Department of Agricultural and Food Sciences, Bologna, Italy, [2]Polytechnic University of Marche, Department of Life and Environmental Sciences (DISVA), Ancona, Italy, [3]Polytechnic University of Marche, Department of Agricultural, Food, and Environmental Sciences, Ancona, Italy

Since almost ten years ago, *Stemphylium vesicarium* (Sv) the fungal pathogen of brown spot of pears is causing dramatic losses in the pear production in Italy where the cultivation of 'Abate' pear represents a distinctive agricultural hallmark with great economic and social impact.
SIGS (Spray Induced Gene Silencing) is an innovative control strategy based on the cellular mechanism of RNA interference and for its efficiency relies on the pathogen capability of uptaking dsRNA molecules which are supposed to be administered in field. Several reports indicate that SIGS is working against a large range of different pests, including fungi, viruses and insects.
In this study we used fluorescence-labelled dNTPs to evaluate dsRNA uptake as naked molecules and in formulation with nanoparticles to facilitate dsRNA delivery. Fluorescence microscopy and in vitro Sv growth assays were used to measure dsRNA absorbance by the pathogen. We compared several naked dsRNA delivery methods and formulations-mediated ones to quantify the efficiency of dsRNA uptake using different molecules-formulant combinations, mix-ratios, application methods and timings in hundreds of in infection tests. Our data show that, for some phytopathogenic fungi, dsRNA uptake process can indeed be the crucial bottleneck to overcome in order to achieve efficiency in RNAi- control strategies and emphasize the need to develop innovative delivery methods to overcome this aspect. Further studies should address this issue for pathogens that appear reluctant and not sensitive to RNAi applications.
This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

P1.253 - Conidia not identical as you may think

S. Covo[1], *D. Mukherjee*[1]
[1]Hebrew University, Rehovot, Israel

Microconidia are oval mostly G1 asexual spores of *Fusarium oxysporum* that start the fungus disease cycle. We found that conidia isolated from solid media are smaller and shifted more towards G1 population than conidia isolated from liquid culture. In agreement. liquid based conidia are more metabolically active and germinate faster. Interestingly, liquid based conidia repair UV damage faster, survive UV damage better with no increase in the rate of mutagenesis. We then asked are all the spores of liquid-based conidia respond the same way to UV? Surprisingly, 5-10% of all spores do not germinate in response to UV even after 24 hours, some don't germinate even after 48 hours. We were able to isolate this arrested population based on its size and characterize it. This arrested population shows decreased plating efficiency and increased mutagenesis. Surprisingly even the mutation spectrum of this population is different from the entire irradiated conidia. Unlike our expectation, the amount of DNA damage in this population is relatively low and just slightly higher than the filaments that are able to recover from the irradiation. To sum up we will present how metabolic and genetic components affect mechanisms of mutagenesis that drive the evolution of a fungal plant pathogen.

P1.254 - Phylogenomics and adaptive evolution of the *Colletotrichum gloeosporioides* species complex

F. Liu[1,2], *Z. Ma*[1,2], *L. Cai*[1,2]
[1]State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China, [2]University of Chinese Academy of Sciences, Beijing, China

The *Colletotrichum gloeosporioides* species complex (CGSC) is one of the most devastating fungal phytopathogens, which is composed of three main clades: Kahawae, Musae, and Theobromicola. Despite the diversity of CGSC, there is limited understanding on their evolutionary mechanisms. By analysing 49 genomes, we found that the expansion of transposable elements, especially long terminal repeat retrotransposons, facilitates the expansion of genome size and genetic variation. Further analyses suggested that an intra-chromosomal inversion may have been the driving force behind the divergence of Kahawae clade from its ancestor. Within the Kahawae clade, the narrow-hosted quarantine species *C. kahawae* has undergone extensive chromosomal rearrangements mediated by repetitive sequences, generating highly dynamic lineage-specific genomic regions compared to the closely related broad-hosted species *C. cigarro*. The results of this study highlight the role of chromosomal rearrangements in promoting genetic diversification and host adaptation, and provide new perspectives for understanding the evolution of phytopathogenic fungi.

P1.255 - Comparative genomics reveals sources of genetic variability in the asexual fungal plant pathogen *Colletotrichum lupini*

J. Alkemade[1,2], *P Hohmann*[3], *M.M. Messmer*[4], *T.G. Barraclough*[1,2]
[1]University of Oxford, Department of Biology, Oxford, United Kingdom, [2]Magdalen College, Calleva Research Centre for Evolution and Human Science, Oxford, United Kingdom, [3]Universitat de Barcelona, Department of Biology, Healthcare and the Environment, Faculty of Pharmacy and Food Sciences, Barcelona, Spain, [4]Research Institute of Organic Agriculture (FiBL), Department of Crop Sciences, Frick, Switzerland

Fungal plant pathogens are a major cause of global crop losses, with many exhibiting compartmentalized genomes comprising core and accessory regions that facilitate rapid adaptation. The host-specific fungus *Colletotrichum lupini*significantly affects lupin (*Lupinus* spp.) cultivation and belongs to clade 1 of the *C. acutatum* species complex. This pathogen consists of four genetically uniform clonal lineages (I–IV), yet notable variation in virulence and morphology exists within these lineages. To uncover potential sources of genetic variability in this asexual species, we analyzed the genomes of 16 *C. lupini* strains alongside 17 related *Colletotrichum* species. Phylogenomic analysis reaffirmed the presence of four distinct lineages but revealed that lineage II could be further divided into two subgroups, II-A and II-B, based on differences in genome size, gene content, transposable elements (TEs), and deletions. Variation in TE content strongly correlated with genome size, implicating TEs in driving genome expansion. Pangenome analysis highlighted a highly dynamic accessory genome, including a mini-chromosome present in lineages II, III, and IV, but absent in lineage I. Accessory genes and putative effectors were often clustered near TEs, and their presence/absence patterns were lineage-specific, indicating a role in shaping host specificity. Interestingly, no effectors were identified on the TE-rich mini-chromosome. These findings reveal mechanisms of genetic diversification in this asexual fungal pathogen and provide insights that could inform strategies for future disease management.

P1.256 - Characterization and transcriptome analysis of a CRISPR/Cas9 generated *Botrytis cinerea*mutant possessing the MDR-related V575G mutation in *Mrr1* gene

G. Sofianos[1], *A. Eleftheriadou*[1], *M. Hahn*[2], *G. Karaoglanidis*[1]
[1]Aristotle University of Thessaloniki, School of Agriculture, Lab of Phytopathology, Thessaloniki, Greece, [2]University of Kaiserslautern, Department of Biology, Kaiserslautern, Germany

Botrytis cinerea is a major plant pathogen causing gray mold across many hosts. While chemical control is common, fungicide resistance is a frequent issue. Resistance is mediated either by target site alterations or enhanced activity of efflux transporters that leads to Multidrug resistance (MDR). The genetic background of isolates possessing MDR-related mutations varies, thus the need to study MDR mutants is urgent. In this study, we created *B. cinerea* strain B05.10 mutants possessing the newly found mutation V575G in the transcription factor *Mrr1*, by using a highly

efficient CRISPR/Cas-based protocol. The transformed strains were used in bioassays to test the range and level of fungicide sensitivity. It was shown that this mutation yielded significantly lower sensitivity to almost every fungicide tested. To further uncover genes potentially affected, directly or indirectly, by the V575G mutation in the transcription factor, we performed RNA sequencing with the control strain B05.10 and the mutant. The results showed a large number of DEGs between the two strains. Many DEGs were linked to MFS and ABC transporters, indicating a role of this mutation in their regulation and resulting MDR phenotypes. In addition, comparison of pathogenicity on several hosts between V575G mutants, KO mutants, and the B05.10 strain, revealed lower virulence of the V575G mutants. Ultimately, MDR in the field may be more impactful than previously realized. The research project was supported by H.F.R.I. under the "2nd Call for H.F.R.I. Research Projects to support Faculty Members & Researchers" (Project Number: 2959).

P1.257 - Pangenome analysis of *Candida parapsilosis*: gene fusion and stop-gain variation

A.P. Ryan[1], *J. Scully*[1], *S.A. Bergin*[1], *C. Hession*[1], *K.H. Wolfe*[2], *G. Butler*[1]
[1]University College Dublin, School of Biomolecular and Biomedical Science, Dublin, Ireland,
[2]University College Dublin, School of Medicine, Dublin, Ireland

Pangenomes are a means of characterising genomes by determining the gene repertoire in a population, rather than in a reference isolate. Here we define and analyse the pangenome of *Candida parapsilosis* from a population of 372 isolates. Gene sequences were clustered based on orthology, and gene absences were confirmed using stringent filtering supported by gene order. The pangenome consists of 5859 genes, of which 5791 were considered core. Core genes were defined as those resolved to single orthologous clusters present in ≥371 isolates (5635 genes), those unresolved by clustering but were present in ≥1-copy by coverage analysis (152 genes), and paired allele clusters resulting from premature stop-codons (4 genes). One allele pair is the allantoin permease *DAL4*: one allele is truncated by a premature stop codon, whereas the second encodes a full-length protein. CRISPR-Cas9 gene editing showed that the full-length allele is associated with improved use of allantoin as a sole nitrogen source. Accessory genes were defined as those absent in >1 isolate and consists of sixty-eight genes. These includes thirty-eight previously annotated genes, twenty-seven novel paralogues and three uncharacterised open-reading-frames. Gene ontology shows that transmembrane transporters are enriched in the accessory genome, predominantly of Major Facilitator Superfamily (MFS) proteins. Approximately one-third of the accessory genome is associated with tandem duplication and subsequent gene fusion between homologous MFS genes. Contraction of the inorganic sulphur transporter *SOA1* from six to three non-identical copies was observed, with six copies representing the ancestral state and subsequent gene fusions resulting in contractions.

P1.258 - Incongruence and structural variation shape rapid mitogenome evolution in a 2000-isolate panel of the wheat pathogen Zymoseptoria tritici

I. Skakov[1], *A. Feurtey*[2,1], *G. Puccetti*[1], *D. Croll*[1]
[1]University of Neuchatel, Institute of Biology, Neuchâtel, Switzerland, [2]ETH, Plant Pathology, D-USYS, Zurich, Switzerland

Mitochondria play crucial roles in fungal cells serving as hubs for energy conversion. Despite the conservation of mitochondrial functions across the fungal tree of life, mitogenomes exhibit astonishing incidences of genome expansions and rearrangements among species. Genome expansions are best explained by selfish element proliferation, however, how such variation arises in its earliest stages (i.e. within species) remains largely unknown. Here, we address this question by establishing the largest intra-species survey of mitochondrial genomes focused on the major wheat pathogen Zymoseptoria tritici. We found that mitochondrial and nuclear population structures assessed for 2120 isolates were incongruent across the global distribution range. We systematically assembled mitochondrial genomes and validated major structural variants using long-read sequencing. We found that 97% of all mitochondria belong to five primary haplotypes distinguished mainly by two large insertions/deletions underpinning variation in mitogenomes by 18%. Selfish elements were absent from the mitochondria suggesting efficient purifying selection with the notable exception of a rare Eastern Europe haplotype encoding a GIY-YIG homing endonuclease. Assembling mitogenomes from closely related Zymoseptoria species revealed that the dynamics observed within the species expand to substantial interspecies variability in mitogenomes (44-60 kb) and essential gene content variation. The GIY-YIG homing endonuclease shows a sparse and patchy distribution in ascomycetes with highly similar copies found in distant groups of fungi raising the possibility of horizontal transfer. In conjunction, our study shows how the extensive variability of mitogenomes has its origins within species. This facilitates mechanistic studies on how selfish element activity drives genome evolution.

P1.259 - Exploring population genomics in *Malassezia*: evidence for sexual reproduction

M. David Palma[1], *M. A. Coelho*[1], *L. Díaz*[2], *F.J. Cabañes*[2], *J. Heitman*[1]
[1]Duke University Medical Center, Department of Molecular Genetics and Microbiology, Durham, United States, [2]Universitat Autonoma de Barcelona, Veterinary Mycology Group, Department of Animal Health and Anatomy, Bellaterra, Spain

Malassezia species are lipid-dependent yeasts inhabiting the skin of warm-blooded hosts, acting as both commensal and opportunistic pathogens. Although emerging evidence suggests a potential for sexual reproduction, a complete sexual cycle has yet to be confirmed. In this study, we explored the genomic diversity and population structure of *M. pachydermatis*, *M. sympodialis*, and *M. vespertilionis* isolates from various locations and hosts.
For *M. pachydermatis*, phylogenetic analyses based on single nucleotide variants revealed three distinct populations, independent of host, health status, or geography. Phylogenetic network

analysis indicated possible reticulate events (e.g., recombination or hybridization) within populations, but limited between them. Several strains were identified as heterozygous diploid or aneuploid, exhibiting genetic contributions from two distinct populations, including the pairing of *P/R* and *HD* mating-type alleles from different parental lineages. In contrast, *M. sympodialis* showed no clear population structure, consistent with network analysis suggesting recombination. *M. vespertilionis* exhibited population differentiation related to geography, with reticulate evolution detected in the California population, where two distinct *P/R* mating-type alleles were identified.
Sequence analysis of the *HD* loci across species revealed multiple DNA-level alleles but limited protein variation, suggesting a unique mechanism of mating-type determination. Co-culture experiments between compatible strains showed no morphological signs of sexual reproduction, indicating that further investigation into *HD* allele compatibility is needed to clarify the mechanisms of mating-type determination in *Malassezia*.
This study expands our understanding of the genomic diversity and reproductive potential of *Malassezia*, contributing to knowledge of its role in the human and animal mycobiome.

P1.260 - Coordinated adaptation of gene families has shaped the genome of dimorphic fungi

G. Tahiri Zainane[1], *C. Lax*[1], *T. Gabaldón*[2], *V. Garre*[1], *F.E. Nicolás Molina*[1]
[1]University of Murcia, Genetics and Microbiology, Murcia, Spain, [2]Barcelona Supercomputing Center, Life Sciences - Comparative Genomics, Barcelona, Spain

Dimorphism is a critical virulence factor in fungi, enabling pathogens to transition between yeast and the invasive mycelium form, which helps them evade the host immune response. Through comprehensive transcriptomic analysis of the yeast and mycelial forms of the dimorphic fungus *Mucor lusitanicus*, we identified that approximately 30% of its protein families are dimorphic, characterized by paralog genes that are expressed in yeast and those specifically expressed in mycelium. These dimorphic families are randomly distributed along genome and are involved in multiple biological processes.
Notably, a thorough genomic analysis revealed that *M. lusitanicus* utilizes head-to-head (H2H) genes to coordinately regulate genes from dimorphic families associated with related functions. These conserved genetic structures across the Mucorales control around 78% of the H2H genes co-expressed within the same morphology. This indicates that these genetic structures could serve as predictors of the morphology in which a particular paralog is expressed, as orthologs expressed in mycelium cluster together in phylogenetic analyses.
Moreover, comparative genomic analysis between dimorphic and non-dimorphic fungi showed that dimorphic fungi have an expanded set of genes within dimorphic families, whereas non-dimorphic fungi possess an expanded array of genes predominantly expressed in the mycelial form.
Altogether, the integration of transcriptomic, genomic, and phylogenetic analyses enables the development of a predictive model to distinguish between dimorphic and non-dimorphic fungi. This model enhances our understanding of their evolutionary mechanisms and potential strategies for evading the immune response.

P1.301 - Tracking hyphal fusion in highly diverse *Aspergillus fumigatus* populations to identify the formation of multi-drug resistance heterokaryon compatibility groups

M. Bottery[1], *E. Tancredi*[1], *A. Sarkar*[2], *S. Gupta*[3], *N. Osherov*[2], *M. Bromley*[1]
[1]University of Manchester, Division of Evolution, Infection and Genomics, Manchester, United Kingdom, [2]Tel Aviv University, Tel Aviv, Israel, [3]Trinity College Dublin, Dublin, Ireland

Aspergillus fumigatus is a significant human pathogen capable of causing severe pulmonary infections. Heterokaryon formation, achieved through hyphal fusion, promotes the horizontal transfer of ecologically important traits among related individuals. This is particularly important within long-term chronic infections where local genetic diversity, generated through mutation, can be disseminated within mycelial networks. Stressors such as antifungal treatment can induce hyphal fusion, potentially stimulating the accumulation of multiple resistance alleles within single individuals. However, hyphal fusion is tightly regulated to limit non-self-fusion through a multigenic incompatibility system controlled by heterokaryon incompatibility (*het*) loci, which likely evolved to prevent the spread of selfish genetic elements such as viruses and transposable elements. To investigate hyphal fusion dynamics and identify novel *het* loci, we developed a high-throughput method to track hyphal fusion events between *A. fumigatus* isolates with distinct resistance profiles within large, diverse populations. Our results demonstrate that fusion preferentially occurs between isogenic partners but can also take place with genetically distinct but phylogenetically closely related individuals, suggesting the existence of fusion compatibility groups. By screening genetic loci for high divergence between groups and low divergence within groups, we identified putative novel *het* loci. These results highlight that cell fusion is not restricted to isogenic strains and can promote the sharing of genetically distinct nuclei harbouring different resistance alleles between individuals. This mechanism could contribute to the rapid spread of multiple resistances within *A. fumigatus* populations.

P1.302 - Genomic patterns of adaptation to antifungal drugs in the Candida parapilosis complex

Á. Redondo-Río[1,2], *J.C. Nunez-Rodriguez*[1,2], *V. Biriukov*[1,2], *V. del Olmo*[1,2], *M. Parmar*[1,2], *T. Gabaldón*[1,2]
[1]Barcelona Supercomputing Centre, Life Sciences, Barcelona, Spain, [2]Institut de Recerca Biomèdica de Barcelona, Comparative Genomics, Barcelona, Spain

Pathogens within the C. parapsilosis complex have been observed to adapt and develop resistance to antifungal drugs in clinical settings. While the mechanisms behind these adaptations are generally well-understood in other Candida species, the C. parapsilosis complex has a very poor understanding of resistance, especially in hybrid species. Notably, the high prevalence of hybrid isolates within this complex suggests an evolutionary advantage that may aid in adaptation to treatment, driven by their highly heterozygous, unstable genomes and frequent loss of heterozygosity (LOH) events.
To investigate the mechanisms of adaptation in these hybrid species, we evolved isolates from C.

parapsilosis sensu stricto, C. orthopsilosis and C. metapsilosis, incorporating both hybrid and non-hybrid strains from clinical and environmental sources. These isolates were exposed to gradually increasing concentrations of two commonly used antifungal agents, fluconazole and anidulafungin. This process generated a robust collection of over 200 drug-resistant strains, with WGS data from more than 100 of them to pinpoint the genomic changes responsible for their adaptation.
Here we present the key findings from these evolution experiments, focusing on the distinct and shared mechanisms of adaptation in hybrid and non-hybrid species. We examine the roles of small genetic variants, copy number variations, aneuploidies, and LOH events, offering a comprehensive look at both conventional and alternative pathways to drug tolerance. This study offers a comprehensive examination of drug resistance mechanisms, being the most extensive and wide study of its kind to date in this field.

P1.303 - Unraveling Fitness Trade-offs in Multidrug-Resistant Fungi: A Path to Optimized Treatments

H. Carolus[1], D. Sofras[1], G. Boccarella[2,3], V. Biriukov[4,5], S. Jacobs[1], C. Lobo Romero[1], R. Vergauwen[1], J. Vreys[1], S. Pierson[1], L. Goossens[1], A. Chen[1], I. Vantyghem[1], T. Verbeeck[1], S. Wijnants[1], P. Vandecruys[1], N.C. Cauldron[6], B.B. Xavier[7,8], P. Sephton-Clark[9], L. Vinken[10], J.P. Herrera Avila[7], R. Naesens[7,11], J.F. Meis[12,13], H. Steenackers[14], K. Lagrou[10], C.A. Cuomo[6,9], J.M. Rybak[15], P. van den Berg[2,3], J. Berman[16], T. Gabaldón[4,5,17,18], P. Van Dijck[1,19]
[1]KU Leuven, Laboratory of Molecular Cell Biology, Department of Biology, Leuven, Belgium, [2]KU Leuven, Evolutionary Modelling Group, Department of Biology, Leuven, Belgium, [3]KU Leuven, Evolutionary Modelling Group, Department of Microbial and Molecular Systems, Leuven, Belgium, [4]Barcelona Supercomputing Centre (BSC-CNS), Barcelona, Spain, [5]Institute for Research in Biomedicine (IRB), Barcelona Institute of Science and Technology, Barcelona, Spain, [6]Brown University, Molecular Microbiology and Immunology Department, Rhode Island, United States, [7]Ziekenhuis Netwerk Antwerpen, Infectious Diseases Department, Antwerp, Belgium, [8]University of Groningen, Department of Medical Microbiology and Infection Prevention, Groningen, Netherlands, [9]Broad Institute of MIT and Harvard, Cambridge Massachusetts, United States, [10]KU Leuven, Department of Microbiology, Immunology and Transplantation, Leuven, Belgium, [11]Ziekenhuis Aan de Stroom Antwerp, Laboratory of Clinical Microbiology, Antwerp, Belgium, [12]Radboudumc/CWZ, Centre of Expertise in Mycology, Nijmegen, Netherlands, [13]University of Cologne, Institute of Translational Research, CECAD, Cologne, Germany, [14]KU Leuven, Centre for Microbial and Plant Genetics, Department of Microbial and Molecular Systems,, Leuven, Belgium, [15]St. Jude Children's Research Hospital, Memphis Tennessee, United States, [16]Tel Aviv University, Shmunis School of Biomedical and Cancer Research, Tel Aviv, Israel, [17]Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain, [18]Instituto de Salud Carlos III, CIBER de Enfermedades Infecciosas, Madrid, Spain, [19]KU Leuven, KU Leuven One Health Institute, Leuven, Belgium

The increased prevalence of antifungal drug resistance and emergence of multidrug-resistant species such as *Candida auris* and *Candida glabrata* represent a global public health threat. Amphotericin B (AMB) has a critical role in treatment of invasive resistant infections, but resistance is increasingly reported. We investigated the mechanisms underlying acquired AMB

resistance and their associated fitness trade-offs in *Candida auris*. Using *in vitro* and *in vivo* experimental evolution, whole genome sequencing, CRISPR-Cas9 gene editing and sterol profiling, we identified several novel mechanism of acquired AMB resistance, all resulting in altered membrane sterol compositions and reduced cellular fitness. These fitness trade-offs, while limiting general growth potential, did not uniformly hinder the pathogen's ability to cause infections, highlighting the complex interaction between fitness and resistance. In addition, we discovered how specific fitness trade-offs can be mitigated by compensatory mutations, a mechanism that potentially drove resistance in a clinical infection.
One of the common trade-offs of AMB resistance was collateral sensitivity (CS) to echinocandins. Mathematical modeling, competition experiments and *in vitro* experimental evolution demonstrated that CS-based drug cycling regimens can reduce resistance development and eliminate existant resistant subpopulations. This represents a promising strategy for preventing resistance or overcoming resistance while mitigating the risks of treatment failure. Building on these insights, we also examined the potential of repurposed drugs as collateral-sensitivity-based treatments. In a case study of a complicated hyper-multidrug resistant *C. glabrata* infection, we detected collateral sensitivity to the antibiotic nitroxoline, showcasing the value of repurposing agents and collateral sensitivity in cases with no therapeutic prospects. Combined, this work provides a comprehensive new view on the complex relationship between antifungal drug resistance and fitness and the value of studying this interaction. We show that by understanding and exploiting fitness trade-offs such as collateral sensitivity, new avenues for treatment optimization can open, such as the use of dynamic drug regimens and repurposed agents to counter multidrug-resistant fungal pathogens.

Related publications:
https://www.nature.com/articles/s41564-024-01811-w
https://www.nature.com/articles/s41564-024-01854-z

P1.304 - Boosting *Aspergillus* antifungal research combining multiplex CRISPR/Cas9 with counter-selection

L.E. Sastré-Velásquez[1], *N. Schiefermeier-Mach*[2], *A. Kühbacher*[1], *B. Mertens*[1], *C. Baldin*[3], *L. Lechner*[2], *G. Diallinas*[4], *F. Gsaller*[1]
[1]Medical University of Innsbruck, Institute of Molecular Biology, Innsbruck, Austria, [2]Health University of Applied Sciences Tyrol/FH Gesundheit Tirol, Research and Innovation Unit, Innsbruck, Austria, [3]Leopold-Franzens University of Innsbruck, Institute of Microbiology, Innsbruck, Austria, [4]National and Kapodistrian University of Athens, Department of Biology, Athens, Greece

Aspergillus fumigatus, the main causative agents of invasive aspergillosis, represents one of the deadliest fungal species worldwide and accounts for hundreds of thousands of deaths each year. In addition to a shortage in the antifungal armory comprising only three drug classes, resistance to the major class employed in the clinical setting, the azoles, is steadily increasing. A comprehensive understanding of the molecular mechanisms driving azole resistance is essential for developing new strategies to combat this problem.
The recent discovery of multiple, endogenous counter-selectable markers enabled site-directed

insertion of numerous additional expression cassettes into the genome of *A. fumigatus*, which facilitated research applications that required multigene integration. The shortcoming, gene cassettes had to be transformed sequentially.
In this study, we overcame this obstacle by combining the use of CRISPR/Cas9 with the mentioned markers, successfully integrating multiple expression cassettes in a single transformation event. Exploiting this new technique, we generated mutants carrying different resistance alleles and strain-specific fluorescent protein tags to simultaneously analyze four strains during azole treatment employing multicolor fluorescence microscopy.
We anticipate that the presented method will bolster a wide range of research applications that require facile and rapid equipment of strains with multiple expression cassettes and will therefore open new avenues in antifungal research.

P1.305 - Synthetic genetic array screening can identify novel therapeutic targets and inhibitors which combat azole resistance in *Candida glabrata*

C.C. Williams[1], *J. Usher*[1]
[1]University of Exeter, MRC Centre for Medical Mycology, Exeter, United Kingdom

Candida glabrata (*Nakaseomyces glabratus*) is the second most common cause of invasive *Candida* infections, with an attributed mortality rate of ~40%. It exhibits intrinsic low-level azole resistance and rapidly acquires high-level resistance, often becoming multi-drug resistant, posing a significant treatment challenge. Its azole resistance is primarily due to gain-of-function mutations in *CgPDR1*, leading to hyperactivity of the CgPdr1p transcription factor and upregulation of efflux pumps, reducing intracellular azole levels.
We used synthetic genetic array (SGA) screening in *Saccharomyces cerevisiae* to explore genetic interactions underlying azole resistance in *C. glabrata*. SGA generates double mutants expressing a $CgPDR1^{+}$ allele and a gene deletion, with the combination potentially causing synthetic sick or lethal interactions. Our screens with wild-type *CgPDR1* and a clinically derived $CgPDR1^{R592S}$ allele highlighted the importance of chromatin remodelling and transcription pathways in resistance, identifying *CgGCN5* of the SAGA complex as a therapeutic target. *In silico* screening revealed Methotrexate, an FDA-approved drug, as a potential inhibitor of *CgGCN5*. *In vitro* testing demonstrated synergy between Methotrexate and Fluconazole against *C. glabrata* and other *Candida* species. These findings highlight the utility of SGA in discovering therapeutic targets and suggest Methotrexate as a promising candidate for combination therapy with Fluconazole.

P1.306 - Starship transposons spread mercury resistance genes between fungal species

A.S. Urquhart[1], *A. Vogan*[2]
[1]University of Melbourne, School of BioSciences, Melbourne, Australia, [2]Uppsala University Systematic Biology, Uppsala, Sweden

The spread of antibiotic resistance genes in bacteria poses a significant threat to human health. These genes are carried between bacteria on mobile genetic elements (MGEs) including integrative and conjugative elements, and plasmids. However, clinically relevant antibiotics are not the only antimicrobial compounds that these elements are known to provide resistance to. A second well-studied example is resistance to mercury. No parallel mechanisms of horizontal gene transfer (HGT) have been established in fungi. Recently, a candidate for such mechanisms in fungi has emerged: *Starship* transposons. *Starships* are giant transposable elements (typically 100 kb) which can be mobilized within and between genomes. To explore the role that *Starships* play in fungal adaptation we investigated the link between mercury resistance, a trait known to be mobilized in the prokaryotic world, and *Starships* to see if *Starships* might be playing a parallel role in fungi. In this work we found a remarkable association between *Starship* elements and mercury resistance genes and can see that these elements have mediated multiple horizontal gene transfers of these genes between fungal species following an initial HGT of these traits from bacteria. Additionally, we have identified novel genes involved in mercury resistance in fungi. The results strengthen our hypothesis that *Starships* are fungal analogs of bacterial MGEs. Furthermore, they suggest that research into the roles of *Starships* in the evolution of resistance to other antimicrobial compounds including medically relevant antifungals is urgent.

P1.307 - Targeting Vulvo-Vaginal Candidiasis (VVC): Glycomimetic-Mediated Virulence Inhibition and Resistance Reversal

H. David[1], *A.P. Solomon*[1]
[1]SASTRA Deemed University, School of Chemical and Biotechnology, Thanjavur, India

The consequential clinical challenge posed by biofilm-associated Vulvo-Vaginal Candidiasis (VVC) primarily arises due to the diminished efficacy of existing antifungals against *Candida albicans*. However, it is estimated that at least 75% of women suffer from VVC infections, and in 40% of them, infection recurs. Increased pathogenic virulence, multidrug resistance, and the poor penetration of antimicrobials into biofilm pose a serious challenge in treatment strategies. Novel treatment strategies targeting virulence can potentially solve these existing challenges. Biofilm formation by adhesion and tissue invasion of the fungus occurs because of the change of morphological type of *C. albicans* from yeast to hyphal form. The cell wall of the fungus consists mainly of mannoproteins, which have very crucial roles in fungal-host interactions, making this structure an attractive target for new antifungal strategy development. In this context, the processing of mannoproteins by α-glucosidase was identified as a critical target. Screening 114 FDA-approved glycomimetic drugs for their potential role identified acarbose as the top hit (G Score: -11.427). At nanomolar concentrations of acarbose (90nM-200nM), it completely inhibited biofilm formation, virulence, and morphological switching. Transcriptomic (RNAseq) analysis further ascertained its action on α-glucosidase. Its additive combination with fluconazole synergistically potentiates the antifungal efficacy at a sub-breakpoint concentration. A mucoadhesive gel incorporating acarbose and antifungal drugs was also prepared with a polymer combination of poloxamer, HPMC, and chitosan. The dual drug mucoadhesive gel will offer better therapeutic treatment due to the localized and sustained release of the drug at the point of infection to treat vaginal candidiasis.

P1.308 - Genetic basis and evolution of resistance to a polyene preservative natamycin

A. Grum-Grzhimaylo[1], *R. van den Bos*[2], *J. Houbraken*[1], *E. Breukink*[2], *J. Dijksterhuis*[1]
[1]Westerdijk Fungal Biodiversity Institute, Food and Indoor Mycology, Utrecht, Netherlands, [2]Utrecht University, Membrane Biochemistry and Biophysics, Utrecht, Netherlands

The polyene antifungal natamycin is used for the protection of cheese and sausages against fungal growth, posing risk for resistance evolution. Resistance to polyenes is considered rare compared to other antifungal compounds. Here, we discover high-level resistance conferred by a natamycin-degrading enzyme, which we call natamycinase, in the cheese-spoiling fungus *Penicillium discolor*. The gene is present within a large transposable element carrying many other genes of unknown function. In resistant genomes, we found multiple identical gene copies coding for natamycinase, also likely spread by transposons. Further genomic analyses and phylogenetic reconstructions of resistant and sensitive strains demonstrated that the acquisition of resistance likely happened once and clonally spread across the globe. The clonal mode of propagation and the inability to form anastomoses between resistant and closely related sensitive *P. discolor* strains suggests a low risk of horizontal spread of natamycin resistance within species. Homologs of the natamycinase gene are present in unrelated fungal species (mainly aspergilli), also conferring resistance to natamycin. As some of those fungi can cause human infections, natamycin would not be the preferred antifungal for treatment.

P1.309 - Tracking antifungal resistance in A. fumigatus from compost samples in Norway

M.M. Owczarek-Kościelniak[1], *M. Mølmann Kåråsen*[1], *M.T. Noer*[1,2], *I. Skaar*[1]
[1]Norwegian Veterinary Institute, Chemistry and Toxinology Research Group, Ås, Norway, [2]Oslo University Hospital, Department of Microbiology, Oslo, Norway

Fungi from the genera *Aspergillus* are ubiquitous and easily spreading organisms. Particularly *A. fumigatus* poses significant risks to immunocompromised individuals and other at-risk groups. Recent studies and reports point out *A. fumigatus* as an emerging pathogen with an increasing occurrence of azole-resistant strains. Our study employed citizen science and additionally involved several composting facilities to gather compost samples from across Norway in order to enhance our understanding of environmental *A. fumigatus* populations. Over 100 citizen scientists participated, reflecting strong community engagement in this public health initiative. Using selective cultivation, we screened the samples for potentially resistant strains. Through Minimum Inhibitory Concentration (MIC) and broth microdilution method analyses, the susceptibility of the strains was evaluated, allowing for a comprehensive understanding of antifungal resistance patterns. Based on morphological characteristics and calmodulin/β-tubulin sequencing, the taxonomical identity of the strains was verified, ensuring accurate species identification.
Sequencing of the *cyp51A* gene and its promoter region revealed distinct mutation profiles across the samples. Our findings have shown that resistant isolates were present in approx. 24% of the

collected samples. Sequencing of *cyp51A* uncovered the TR34/L98H, TR46/Y121F/T289A, and TR46/Y121F/T289A/S363P/I364V/G448S mutations, occurring individually or in combinations, with highly variable MIC values among isolates, ranging to > 32 mg/L. Obtained results underscore the importance of monitoring environmental *A. fumigatus* for antifungal resistance and its implications for public health, highlighting the need for proactive surveillance strategies.

P1.310 - Multi-drug and -fungicide resistance in the pathogen *Aspergillus fumigatus*

M. Momany[1], B.N Celia-Sanchez[1], B Mangum[1,2], L.F Gómez Londoño[2], C Wang[2], B Shuman[1], M.T Brewer[2]
[1]University of Georgia, Fungal Biology Group & Plant Biology Dept., Athens, United States, [2]University of Georgia, Fungal Biology Group & Plant Pathology Dept., Athens, United States

Fungi are important pathogens of plants and people. Fungi destroy 125 million tons of food crops annually, about 20% of the global food crop total. Invasive fungal infections kill 2.5 million people each year, with the pathogen *Aspergillus fumigatus* responsible for 1.8 million of those deaths. Azole compounds account for 25% of fungicides used on pathogens of crop plants and are also the first-line treatment used to combat *A. fumigatus* infections of people.
A. fumigatus resistance to azole antifungal drugs, a recognized problem in Europe and Asia for twenty years, has recently been gaining attention in the U.S. We isolated azole-resistant *A. fumigatus* from soil, plant debris, and compost in agricultural environments in seven U.S. states. We tested isolates for sensitivity to azole fungicides and antifungals along with other fungicides used only on crop plants. We found multi-drug and -fungicide resistant *A. fumigatus* is widespread in U.S. agricultural environments. Analysis including publicly-available sequence data showed that the U.S. isolates belong to three defined world-wide clades, with multi-drug and -fungicide resistant isolates largely in a single clade. Our analysis also showed high levels of recombination among isolates. Strikingly, clinical isolates of pan-azole resistant *A. fumigatus* also contained alleles conferring resistance to fungicides that have only been used on crop plants, showing that these clinical isolates have a clear agricultural history.

P1.311 - Antifungal tolerant *Candida glabrata* experience chromosomal abnormalities, upregulation of efflux pumps and decreased virulence

C. Chedraoui[1], N. Fattouh[1,2], S. El Hachem[1], M. Younes[1], R.A. Khalaf[1]
[1]Lebanese American University, Department of Biological Sciences, Byblos, Lebanon, [2]Saint George University of Beirut, Department of Biology, Beirut, Lebanon

Candida glabrata is an opportunistic, pathogenic fungus that is increasingly isolated from hospitalized patients. The incidence of drug tolerance, heteroresistance, and resistance is on the rise due to an overuse of antifungal drugs. The aim of this study was to expose a sensitive *C. glabrata* strain to sequentially increasing concentrations of two antifungal drugs, fluconazole, an azole that targets ergosterol biosynthesis, or caspofungin, an echinocandin that targets cell wall

glucan synthesis. Analysis of the drug exposed isolates showed development of antifungal tolerance, chromosomal abnormalities, decreased adhesion on both epithelial cells, and agar surfaces, attenuated virulence, and an increase in efflux pump activity. Furthermore, Illumina whole genome sequencing of all isolates exposed to different concentrations of fluconazole or caspofungin was performed to determine mutations in key genes that could correlate with the observed phenotypes. Mutations were found in genes implicated in adhesion, such as in the *AWP*, *PWP*, and *EPA* family of genes, resulting in the above-mentioned decreased adhesion. Isolates exposed to higher drug concentrations displayed more mutations than those at lower concentrations. In conclusion, this is the first study to attempt induction of resistance and tolerance in *C. glabrata* followed by genotypic and phenotypic analysis of isolates to determine mechanisms of drug resistance.

P1.312 - Characterization of organotin resistance in the sugar beet pathogen *Cercospora beticola*

L. Martinez[1], *V. Rivera*[1], *G. Secor*[1], *M. Bolton*[2], *N. Wyatt*[2]
[1]North Dakota State University, Plant Pathology, Fargo, United States, [2]U.S. Dept. Agriculture, Fargo, United States

Cercospora beticola (*Cb*) is the most destructive pathogen of sugar beet. Organotin (OTs) fungicides are broad-spectrum fungicides acting on the early stages of infection and are commonly used as protectants against *Cb*. OT sensitivity surveys have identified high prevalence of resistance in the United States. However, the molecular mechanisms underlying OT resistance in *Cb* are completely unknown. To identify genetic associations corresponding to OT resistance, we isolated, phenotyped, and whole genome sequenced *Cb* populations sampled in the USA across different years. We leveraged population genomic analysis and association mapping comparing OT-resistant and -sensitive *Cb* populations to identify genomic regions associated with OT resistance. One of the identified associations was within the coding region of an omega class glutathione S-transferase (GST), ubiquitous enzymes commonly involved in cellular detoxification. Two alleles of the GST gene were identified and frequencies varied across years. Preliminary assays using a known GST inhibitor, diethyl malate (DEM), in combination with OTs revealed a synergistic effect, suggesting the inhibition of this GST affects the OT resistance mechanism. Further experiments to knock-out the GST gene and examining its expression under OT conditions are underway to functionally validate the GST involvement in OT resistance.

P1.313 - Exploring azole resistance mechanisms in mucor iusitanicus: the impact of genetic mutations and efflux transporters

M. Lackner[1], *M.V. Keniya*[2], *C. Lax*[3], *J. James*[1], *S. Töpfer*[1], *L. Cesarini*[4], *L.-M. Zenz*[1], *C. Müller*[5], *F.E. Nicolas Molina*[3], *U. Binder*[1], *F. Bracher*[6], *B. Monk*[2], *E. Lamping*[2]
[1]Medical University of Innsbruck, Institut for Hygiene and Medical Microbiology, Innsbruck, Austria, [2]Sir John Walsh Research Institute, Faculty of Denistry, University of Otago, Dunedin, New Zealand, [3]Departamento de Genetic y Microbiologia, Faculty de Biologia, Universidad de Murcia, Murcia, Spain, [4]Department of Chemestry, Biology and Biotechnology, Univerita degli Studi di Perugia, Perugia, Italy, [5]Department of Pharmacy, Center for Drug Research, Ludwig

Maximilian University Munich, Munich, Germany, [6]Department of Pharmacy, Center for Drug Research, Ludwig Maximilians University Munich, Munich, Germany

Mucormycetes, classified by WHO as high-risk fungal pathogens, cause difficult to treat invasive infections due to rapid disease progression and limited therapeutic options. We show two conserved amino acid substitutions in the *M. lucitanicus* sterol 14a-demethylase F5 paralogue (MlSDM-F5) significantly impair the binding of short- and mid-length tailed azoles. This was demonstrated by high minimal inhibitory concentrations (MICs) for voriconazole and isavuconazole, while posaconazole retained efficacy. We also analyzed eight pleiotropic drug resistance (PDR) ATP-binding cassette (ABC) transporters, which cluster in two groups: A (Pdr1, Pdr6-8) and B (Pdr2-5). Transcriptome analysis of the azole-resistant clinical isolate CBS277.49 revealed significant upregulation of *PDR1* and *PDR6*, alongside downregulation of *PDR7* and *PDR8* after azole exposure.
Heterologous expression in *Saccharomyces cerevisiae* of the novel plasmid pABC3XL, containing Pdr1, Pdr6, and Pdr7, conferred increased resistance to short- and mid-length tailed azoles. Structured illumination microscopy confirmed the expected plasma membrane localization of these transporters.
Our study reveals two critical mechanisms contributing to azole resistance in *Mucor lusitanicus*: azole target site substitutions and the activity of PDR transporters. Understanding of these mechanisms is needed for developing more effective therapeutic strategies. Circumventing the effects of both azole target site substitutions and PDR activity may provide new avenues to improve treatment of *M. lusitanicus* infections.

P1.314 - Unlocking the fungicidal activity of triazoles against Candida auris

L. Doorley[1], V. Meza-Perez[1], S. Jones[1], J. Parker[2], S. Kelly[3], J. Rybak[1]
[1]St. Jude Children's Research Hospital, Pharmacy and Pharmaceutical Sciences, Memphis, United States, [2]Cardiff University, Cardiff, United Kingdom, [3]Swansea University, Swansea, United Kingdom

Treatment of *Candida auris* infections is significantly limited by antifungal resistance. To identify molecular vulnerabilities which can be exploited to develop novel therapeutics, we used our *C. auris* gene-editing system to disrupt zinc cluster transcription factor (ZCF) genes in a virulent clinical outbreak-derived *C. auris* isolate exhibiting resistance to all major antifungal classes and then evaluated for changes in antifungal susceptibility. This revealed one ZCF gene, B9J08_02229, which when disrupted not only increased susceptibility to all clinically available triazoles, but also transformed the normally fungistatic triazole activity to a rapid fungicidal activity (>3 $\log_{10}$ colony forming unit reduction) in time kill analysis. Susceptibility to echinocandins and amphotericin B was not improved. Importantly, restoring the wildtype B9J08_02229 allele, restored triazole resistance and abolished triazole fungicidal activity. Furthermore, disruption of B9J08_02229 in triazole-resistant *C. auris* isolates from additional clades also transformed triazole activity to fungicidal. Consistent with previous studies, loss of the predicted B9J08_02229 ortholog in *Candida albicans* (*UPC2*) did not result in triazole

fungicidal activity in time kill analysis, suggesting a potentially *C. auris*-unique vulnerability. RNA-seq analysis revealed B9J08_02229 impacts regulation of small molecule transporters and ergosterol biosynthesis. Sterol analysis revealed significantly less ergosterol in strains lacking B9J08_02229. The sterol biosynthesis inhibitors terbinafine, fenpropimorph, and fluvastatin demonstrated greater activity against strains lacking B9J08_02229, but did not exhibit fungicidal activity in time kill analyses. Future research is needed to identify potentially exploitable targets in the B9J08_02229 regulon which can reclaim the use of triazoles for the treatment of *C. auris* infections.

P1.315 - Mechanistic insights into combinatorial stress adaptation in *Candida glabrata*: implications for antifungal resistance and pathogenicity

J. Usher[1]
[1]University of Exeter, MRC Centre for Medical Mycology, Exeter, United Kingdom

Candida glabrata has emerged as a major human pathogen, responsible for a significant proportion of mucosal infections. Its high tolerance to commonly administered antifungals and host-induced stressors allows it to persist and thrive during infection. My research focuses on understanding the molecular mechanisms behind *C. glabrata's* remarkable resistance to combinatorial stress (CS) — the simultaneous exposure to oxidative, cationic stress and antifungal drugs. Unlike individual stress responses, the CS adaptation is distinct and not simply additive, with critical implications for virulence and immune evasion.
Using forward genetics and high-throughput analysis, I have identified key genes involved in *C. glabrata*'s CS resistance, particularly those related to cell wall biosynthesis, metal ion homeostasis, and stress responses. My work aims to (1) identify mutations conferring CS resistance, (2) characterize gene regulation during CS adaptation, and (3) determine the role of CS-related genes in infection and immune evasion.
This research will yield novel insights into *C. glabrata* pathogenicity and potentially reveal therapeutic targets for antifungal drug development. In addition, it may uncover biomarkers for improved diagnosis of *C. glabrata* infections. These findings are pivotal in addressing the growing threat posed by this highly resistant fungal pathogen.

P1.316 - Insights into the chitin metabolism of pathogenic oomycetes: a promising target for fungicide development and biocontrol

L. Kappel[1], *S.F. Ullah*[1], *V. Srivastava*[1]
[1]KTH Royal Institute of Technology, Division of Glycoscience, Department of Chemistry, Stockholm, Sweden

Oomycetes are among the most devastating eukaryotic pathogens in crop production and aquaculture. Due to increasing resistance, the number of effective fungicide classes is narrowing, making the development of alternative solutions a high priority. The cell wall of oomycetes plays a vital role in maintaining cellular integrity, growth, and virulence. In fungi, cell wall chitin is

one of the promising targets for fungicide development. Unlike in fungi, the importance of chitin to oomycete infection has not been investigated in detail. Although chitin is present in considerably lower amounts in the oomycete cell walls than in fungi, evidence suggests it is an important pathogenicity determinant for oomycetes. Similar to fungi oomycetes harbour chitin synthases and chitinases, albeit often in lower numbers. However, specialized extracellular chitinolytic enzymes may assist oomycetes in evading the attack of mycoparasitic species used in biocontrol. Here we explore the role of chitin metabolism in oomycetes in relation to their competitive behaviour and virulence. We provide new insights into how this polysaccharide and the associated chitin metabolic machinery may serve as potent targets for the development of new fungicides and effective biocontrol measures.

P1.317 - Mathematical modeling of fungal growth provides insights into antifungal drug resistance

D. Cánovas[1]
[1]University of Sevilla, Dept of Genetics, Sevilla, Spain

Microbial growth analysis is a crucial tool for microbiologists, providing insights into various strains under different conditions. However, these analyses typically focus on qualitative assessments of quantitative data. To enhance the analysis of fungal growth, I tested five commonly used mathematical growth models using real experimental datasets. One model stood out as the most cost-effective for fitting the data.
To validate the model, I used growth datasets of *Aspergillus nidulans* grown under varying nitrogen concentrations and inoculum sizes. The model and a new parameterization identified three and four parameters, respectively, defining the growth curve. Changes in nitrogen concentration affected both maximum growth and growth rate, while inoculum size inversely correlated with the inflection time point. The model was also useful for studying growth in other ascomycete fungal species, such as *Fusarium* and *Neurospora*. After validation, the model was applied to study the growth of *A. fumigatus* in response to antifungal drugs. Voriconazole at subinhibitory concentrations mainly reduced the growth rate without affecting other parameters. Accordingly, the Δ*nctA* and Δ*nctB* mutants, which display increased azole resistance, showed higher growth rates but no changes in other growth parameters in the presence of voriconazole. Environmental isolates of *A. fumigatus* resistant to azoles due to mutations in the *cyp51A* gene were also tested, and their growth parameters revealed different fitness costs under various growth conditions. This method enables automated characterization of numerous samples simultaneously, offering significant potential for high-throughput antibiotic drug screenings.

P1.318 - Unveiling the genetic background of azole-resistant and susceptible *Aspergillus fumigatus* environmental isolates of the Basque Country

S. Cendon-Sanchez[1], *L. Fabre*[2], *E. Pelegri-Martinez*[1], *U. Perez-Cuesta*[3], *X. Guruceaga*[4], *A.E. Barber*[2], *A. Ramirez-Garcia*[1], *A. Abad-Diaz-de-Cerio*[1], *A. Rementeria*[1]
[1]University of the Basque Country (UPV/EHU), Department of Immunology, Microbiology and Parasitology, Bilbao, Spain, [2]Institute of Microbiology, Friedrich Schiller University Jena (FSU),

Jena, Germany, [3]University of Tennessee Health Science Center (UTHSC), Department of Clinical Pharmacy and Translational Science, Memphis, United States, [4]Institute for Multidisciplinary Research in Applied Biology (IMAB), Public University of Navarre (UPNA), Pamplona, Spain

Aspergillus fumigatus is an opportunistic pathogen, whose infections are treated mainly with azoles. Nevertheless, in the recent years, the fungus has developed resistance mechanisms to the available drugs. This is one of the reasons why WHO included *A. fumigatus* in the group of critical priority fungal pathogens in 2022. The origin of these resistances is both clinical, due to prolonged treatments of chronic patients with medical azoles, and environmental, due to extensive use of azole fungicides in agriculture. In this work, six environmental *A. fumigatus* strains were sequenced (n=2 susceptible; n=4 resistant). Regardless of their origin and susceptibility, phylogeny results showed that the isolates from the Basque Country shared numerous variants compared to the reference genome Af293. The four resistant isolates carried the previously described mutation TR_{34}/L98H in *cyp51A*, the azoles target. Variant calling analysis allowed the identification of 13 genes with possible involvement in triazole resistance based on their function: four play a role in the ergosterol biosynthesis, the pathway targeted by the azoles, and nine encode for multidrug efflux pumps. To assess the impact of those variants, the expression of some genes was analyzed by RT-qPCR in absence and presence of voriconazole for the six abovementioned strains as well as for the Af293 strain.
This study was funded by project IT1657-22 of the Basque Government. SCS and EPM have received predoctoral grants from the Basque Government. SCS also received the FEMS RTG.

P1.319 - In-vitro evolution of known pathogenic Candida species to understand genomic adaptations

M. Parmar[1], *V. Biriukov*[1,2], *T. Gabaldon*[1,3]
[1]Institute of Research in Biomedicine, Mechanism of Disease, Barcelona, Spain, [2]Institute of Research in Biomedicine, Barcelona, Spain, [3]Barcelona Supercomputing Centre, Barcelona, Spain

Each year, about 2.5 million deaths globally are directly associated with fungal infections. In 2022, the World Health Organization (WHO) issued the first fungal pathogen priority list, including five species causing candidiasis namely Candida albicans, Candida auris, Candida parapsilosis, Candida glabrata (Nakaseomyces glabratus) and Candida tropicalis. A major concern for these pathogens is the increased rates of acquired resistance against antifungals that are used to treat these infections in clinics. Although mutations in certain genes have been associated with lower levels of susceptibility to different drugs, we still have a poor understanding of the genomic changes underlying drug adaptation.
Our work is aimed at understanding mutational adaptations of Candida species against the major classes of commercially used antifungals, Fluconazole, Anidulafungin and Amphotericin B. We used an experimental protocol which utilizes the in-vitro evolution approach of phylogenetically distinct Candida species; C.albicans, C. auris, C. glabrata, C. tropicalis and C. parapsilosis. We evolve all strains from a lower to high value of their MIC. Independently evolved populations are

subjected to next generation sequencing for genomic analysis. The genomic data allows us to map mutations that occurred as a consequence of drug exposure. Additionally, we tested these evolved strains in other drugs to identify cases of collateral sensitivity and cross-resistance. Combining the knowledge from our work with available literature would allow us to come up with alternative strategies to better tackle fungal infections.

P1.320 - Cytochrome bc1 quinone inside inhibitor fungicide suppresses fungal sensitivity to widely-used azole-based antifungals

S.M. Pociunaite[1], *S.A. White*[1], *A. Pidoux*[1], *R. Allshire*[1]
[1]Institute of Cell Biology, University of Edinburgh, Edinburgh, United Kingdom

Azole-based antifungal compounds that inhibit lanosterol 14a-demethylase(known as DMIs: demethylase inhibitors) encoded by fungal *erg11/cyp51* genes are widely-used to combat human and crop plant fungal pathogens. DMIs such as fluconazole(FLC) are frequently used alone to ameliorate human Cryptococcus and other fungal infections. Furthermore, cereal crops are treated with various antifungals, including DMIs, several times per growing season. To manage infections by *Zymoseptoria tritici*(causes Septoria Leaf Blotch) in winter wheat, crop fields are treated with fungicide combinations containing DMIs, and Succinate Dehydrogenase Inhibitors(SDHIs), or Cytochrome bc1 inhibitors(Quinone outside/inside Inhibitors; QoI/QiIs) multiple times a year. Controlling fungal diseases in cereal crops is imperative to ensure global food security. However, mitochondrial dysfunction is known to confer drug resistance in *Schizosaccharomyces pombe* and other fungal systems. We hypothesised that the combined use of fungicides, such QiIs, that interfere with mitochondrial electron transport chain function, might inadvertently suppress the effectiveness of DMIs. Here we demonstrate that exposure of fission yeast to the QiI Fenpicoxamid-phenol(FNP^{QiI}), recently introduced to control *Z. tritici*, renders them resistant to DMIs. Even transient FNP^{QiI} exposure permits subsequent growth in the presence Azole-based antifungals. Importantly, prior FNP^{QiI} exposure also suppressed the effectiveness of Azole-based antifungals in preventing *Z. tritici* growth. RNA-seq analyses shows that low level exposure to QiI in fission yeast induces oxidative stress pathways and consequently efflux pumps reducing sensitivity to DMIs. Thus, the use of antifungals that interfere with ETC function are likely counterproductive, under certain conditions, with respect to controlling the emergence of resistance to other antifungal agents.

P1.321 - Harnessing green-synthesized nanoparticles to combat fungal diseases in citrus: a sustainable approach to fungicide resistance

A. Malandrakis[1,2], *C. Chrysikopoulos*[3], *N. Kavroulakis*[2], *K Varikou*[2]
[1]Technical University of Crete, School of Chemical and Environmental Engineering, Chania, Greece, [2]ELGO-DIMITRA, Institute of Olive Tree, Subtropical Plants and Viticulture, Chania, Greece, [3]Khalifa University of Science and Technology, Department of Civil Infrastructure and Environmental Engineering, Abu Dhabi, United Arab Emirates

Pre- and post-harvest fruit rots caused by *B. cinerea* and *Penicillium* spp. represent significant both economic and health hazard threats due to the latter's ability to produce a variety of mycotoxins such as patulin and citrinin. Currently, synthetic fungicides are the most effective means of controlling these diseases and preventing mycotoxin contamination. However, the effectiveness of chemical control is being undermined by the development of resistance to many fungicides and stringent EU regulations, which have led to the withdrawal of numerous active ingredients. To address these challenges, green-synthesized nanoparticles (GNPs) have emerged as promising eco-friendly alternatives. In the present study, an eco-friendly approach was followed to synthesize metallic GNPs using bacterial and plant metabolites as reducing and capping agents. Their effectiveness was evaluated against sensitive and fungicide-resistant *B. cinerea* and *Penicillium* sp. isolates *in vitro* and *in planta*. Results revealed that GNPs significantly inhibited mycelial growth and reduced disease symptoms more effectively than a reference $Cu(OH)_2$ containing fungicide. A profound synergistic effect was observed when GNPs were combined with conventional fungicides registered against these pathogens. In summary, GNPs have shown significant potential in managing sensitive and fungicide-resistant fungal strains, making them a promising strategy for minimizing the environmental impact of chemicals used to combat citrus diseases.

This study was co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Rural Development Program (RDP/ΠΑΑ) 2014 – 2020, under the call "Cooperation for environmental projects, environmental practices and actions for climate change" (project code: M16SYN2-00354), and the European Union- Next Generation EU, Greece 2.0 National Recovery and Resilience plan (project code: TAEDR-0535675).

P1.322 - Structure-function characterization of antifungal peptides and development of high-level production chassis strains

L. Heber[1], *C. Cairns*[1], *S. Muralikumar*[2], *M.A. Mroginski*[2], *V. Meyer*[1], *S. Jung*[1]
[1]Technische Universität Berlin, Chair of Applied and Molecular Microbiology, Berlin, Germany,
[2]Technische Universität Berlin, Chair of Modeling of Biomolecular Systems, Berlin, Germany

Fungal infections claim more human casualties than both, tuberculosis and malaria. Cysteine-stabilized antifungal peptides (AFPs) from filamentous fungi contain several promising future lead drugs. These AFPs form a peptide family of more than 150 members. Extensively studied members of this family are AFP from *Aspergillus giganteus*, PAF from *Penicillium chrysogenum* and AnAFP from *A. niger*. These peptides act as antifungals, exert a specific target spectrum and are considered promising molecules for use in medical or agricultural applications to combat human- and plant-pathogenic fungi. AFP was found to exclusively act on filamentous fungi without affecting the growth of bacteria, yeast, mammalian or plant cells. Although similar in amino-acid sequence, 3D structure and amphipathic character, this ribosomally synthesized peptide differs considerably in its antifungal mode of action, specificity, and efficacy compared with AnAFP and PAF.
To investigate the underlaying peptide moieties that are responsible for the differences in specificity we apply Molecular Dynamics simulations and peptide epitope reshuffling. The peptides' 3D structures, antifungal activities, and simulated membrane interactions deliver

insights to further elucidate their antifungal mode of action.
In parallel, we are interested in the regulation of the biological synthesis of AFPs to increase their production in filamentous fungi by design of high-level production chassis strains. Thereby, we consider two aspects: i) transcriptional regulation and ii) post-translational processing.

P1.323 - Azole tolerance and resistance in *Candida parapsilosis*

C. Hession[1], *A. Ryan*[1], *S. Bergin*[1], *E. Zuniga*[1], *J. Scully*[1], *G. Butler*[1]
[1]University College Dublin, School of Biomolecular and Biomedical Sciences, Dublin, Ireland

Candida parapsilosis is a common opportunistic fungal pathogen. Since 1990, there has been a notable surge in the incidence of *C. parapsilosis* isolations, making it the second or third most frequently isolated Candida strain globally. The emergence of fluconazole resistant isolates has become more prevalent, resulting in treatment failures. This failure might be attributed, at least in part, to a phenomenon known as "drug tolerance," wherein a subset of cells can grow slowly at drug concentrations above the minimum inhibitory concentration.
This project aims to characterize both the phenotypic and genotypic variations among *C. parapsilosis* strains, with a specific focus on identifying traits associated with fluconazole tolerance and resistance. Utilizing Illumina technology, the genomes of 386 *C. parapsilosis* strains were sequenced. Fluconazole tolerance and resistance were assessed through disk diffusion assays. Among the strains examined, 25.9% demonstrated fluconazole tolerance, while 2.6% exhibited resistance. Resistance is associated with Y132F variants in *ERG11*, as well as changes in copy number of drug transporters. There is a strong phylogenetic signal among strains exhibiting increased tolerance to fluconazole. Further analyses and tests are ongoing to pinpoint variants associated with this tolerance.
There remains a gap in our understanding of tolerance in *C. parapsilosis*. The mechanisms underlying tolerance may differ from other Candida species. Notably, fluphenazine, a common antipsychotic, has demonstrated synergy with fluconazole in reducing tolerance in *Candida albicans*. However, in *C. parapsilosis*, fluphenazine does not reduce tolerance in the same fashion. It acts antagonistically with fluconazole, rendering susceptible isolates resistant to the drug.

P1.324 - The role of membrane transporters in *Aspergillus fumigatus* drug resistance

L. Chidi-Onuorah[1], *T. Furukawa*[2], *S. Gago*[1], *M. Bromley*[1], *P. Bowyer*[1]
[1]The University of Manchester, Manchester, United Kingdom, [2]Teesside University, Middlesbrough, United Kingdom

Drug efflux or influx proteins are often important mechanisms of antifungal resistance. Previous studies have focused only on single or a few transporter genes and so it is difficult to say which are the most important efflux or influx transporters. To establish the most important transporters involved in *Aspergillus fumigatus* drug resistance, the whole set of Adenosine triphosphate Binding Cassette (ABC) transporters were identified by a 1-2-1 reciprocal BLAST method and

grouped into families based on Maximum Likelihood phylogeny. The RNA expression patterns of the transporters were determined using RNA-seq in the absence and presence of itraconazole (0.125, 0.25, 0.5, 1, 2 mg/L). All of these genes were knocked out then an *in vitro* competitive fitness assay (Bar-seq method) was performed at the IC_{50} values for itraconazole (0.02 mg/L), simvastatin (0.07 mg/L), terbinafine (0.05 mg/L), voriconazole (0.02 mg/L), carbendazim (2 mg/L). Olorofim and Amphotericin B were tested at 0.000625 mg/L and 0.0625 mg/L respectively. Several ABC transporter genes showed significantly higher or lower transcription in the presence of azoles with AFUB_053630 displaying >3 log2 fold induction. RNA-seq analysis corresponds with the ABC transporter gene phylogeny. Bar-seq analysis revealed many knockout genes made the strains either more or less fit to the drug conditions in comparison to the parental strain. Many more genes could be responsible for *Aspergillus fumigatus* drug resistance either by efflux or influx and will be ascertained with further analysis.

P1.325 - Combination therapy suppresses the emergence of resistance in Aspergillus fumigatus

J. Tindale[1], *A. Anna*[1], *C. Valero*[1], *M. Bromley*[1], *M. Bottery*[1]
[1]University of Manchester, Manchester Fungal Infection Group, Division of Evolution, Infection and Genomics, Manchester, United Kingdom

The fungal pathogen *Aspergillus fumigatus* is responsible for invasive and chronic diseases that causes an estimated 300,000 deaths each year. Therapeutic options for treating aspergillosis are limited to only a few classes of antifungal drugs. Treatment of chronic disease is characterised by prolonged azole therapy, often resulting the evolution of azole-resistant strains. As azoles are the first-line antifungal for aspergillosis and the only orally available class, new treatment strategies are crucial to prevent resistance. Combination therapy is one promising solution that has already demonstrated clinical effectiveness through reducing fungal burden and improving patient outcomes. The application of this strategy in the context of suppressing the emergence of resistance is yet to be explored. Using a fluctuation assay, we demonstrated that the rate of spontaneous resistance was significantly lower in response to combination treatment of paired antifungals compared to the respective drugs individually. We show using combinations of two antifungals either from the same (voriconazole and itraconazole) or different (itraconazole plus the novel orally bioavailable antifungal olorofim) classes, there is a lower probability of acquiring mutations that confer resistance to both drugs simultaneously. We conducted whole genome sequencing on resistant isolates to uncover mechanisms of cross-resistance to compounds from both the same and different antifungal classes. Combination therapy through the use of multiple antifungal drugs may provide a promising strategy to reduce the risk of antifungal resistance emerging during the treatment of aspergillosis.

P1.326 - Molecular identification and fungicide resistance profiles of *Colletotrichum* spp. strains associated with Olive Anthracnose disease in Greece

A.G. Papageorgiou[1], *S. Bitsika*[1], *D.I. Tsitsigiannis*[1]
[1]Agricultural University of Athens, Department of Crop Science, Athens, Greece

Olive anthracnose disease, caused by fungi belonging to *Colletotrichum* species, stands as a major global threat to olive drupes, leading to significant quantitative and qualitative deterioration of harvested products. This study aims to identify the *Colletotrichum* species associated with olive anthracnose disease in Greece and investigate the sensitivity of several *Colletotrichum* spp. strains to various chemical plant protection products (PPPs). As part of the above objectives, 110 *Colletotrichum* spp. strains were isolated from symptomatic olive drupes and leaves from different olive-producing areas of the country, while a multilocus analysis of ITS, TUB2, and GAPDH genes was conducted for their molecular identification. Selected strains were *in vitro* evaluated for their sensitivity/resistance to ten different fungicides, assessing the inhibitory effect on mycelial growth and conidial germination. Subsequently, detached olive drupes of Greek olive cultivars *cv*. Kalamon and *cv*. Koroneiki were artificially inoculated with five *Colletotrichum* spp. isolates and six different PPPs approved for olive cultivation in Greece were evaluated focusing on disease severity reduction and inhibition of fungal conidia production of each strain. The analysis found that the *C. acutatum* species complex is mainly responsible for the disease in Greece, with some of the isolates showing strong resistance profiles to several approved PPPs.

P1.327 - Development of a novel siderophore-based antifungal for treatment of *A. fumigatus*

C. Murphy[1], *S. Doyle*[1], *K. Walshe*[1]
[1]Maynooth University, Biology, Maynooth, Ireland

As prevalence of antifungal resistance worldwide increases and the effectiveness of current antifungal regimens decrease, there is a greater need for novel antifungals that do not share the same targets as approved antifungal drugs. As such, one avenue for antifungal development is the repurposing of already approved chemotherapy drugs and one such candidate is methotrexate (MTX) which inhibits the folate pathway which is present in all domains of life. However, MTX is used as a chemotherapy treatment for rheumatoid arthritis and suppresses the immune system, thereby mediating infection in patients and therefore, to reduce the risk of infection, MTX must be conjugated to a suitable carrier. Herein, MTX is conjugated to diacetylfusarinine C (DAFC), an analogue of the fungal siderophore triacetyl fusarinine C (TAFC) which is crucial to the virulence of the human pulmonary pathogen *Aspergillus fumigatus*. The DAFC-MTX conjugate is then labelled with iron and gallium to aid its uptake into *A. fumigatus* and *In vitro* assays demonstrate that the both the FeDAFC-MTX and the GaDAFC-MTX conjugate inhibit the growth of *A. fumigatus* in iron-deplete conditions and GaDAFC-MTX exhibits similar antifungal activity against *A. fumigatus* to MTX by itself. Analysis of liquid cultures shows that both MTX and GaDAFC-MTX inhibit the production of the siderophore TAFC, but the GaDAFC-MTX conjugate by itself inhibits overall siderophore production in *A. fumigatus* demonstrating that not only can *A. fumigatus* growth can be inhibited, but also the virulence of *A. fumigatus* be attenuated which may improve patient outcome.

P1.328 - Resistance towards biological control? Screening of fungal isolates from a long-term field trial

E. Kuper[1], *J. Stenberg*[1]
[1]Swedish University of Agricultural Sciences, Lomma, Sweden

Intensive use of synthetic fungicides in horticultural production has favoured the rapid evolution of resistant fungal strains. The phytopathogenic fungus *Botrytis cinerea* developed resistances to a wide range of synthetic compounds, in some cases by mutation of a single gene (Leroch et al., 2013). Biological control – by purposely applying living antagonists to the production system – is expected to provide a more durable way of disease control due to its more complex mode of action. However, recent studies demonstrate that phytoapthogens can evolve tolerances to microbial biological control when selection pressure is maintained over multiple generations (Clough et al., 2024).
Here we present the starting point of a long-term field trial in a strawberry production system to assess the response of the natural *B. cinerea* population to reoccurring treatment with a synthetic fungicide (Switch®) or a biological control fungus (*Aureobasidium pullulans).* Thus far we collected *B. cinerea* strains from strawberry plants with a history of up to 3 years of chemical or biological treatment. In addition we developed *in-vitro* screening methods to evaluate tolerances towards chemical and biological control products and are currently applying those methods to our collected field strains. Once we identify strains with a tolerant phenotype we will proceed with genetic characterizations by analysing the presence of known resistance-conferring mutations. This project opens the conversation about the risk of resistance development towards biological control and provides methodology applicable to screen for antimicrobial resistance in different pathosystems.

P1.329 - Acquired azole resistance in black Aspergilli in the Netherlands

M. Punt[1], *A. de Jong*[1], *J. Zhang*[2], *P. Verweij*[2]
[1]National Institute for Public Health and the Environment, Centre for Infectious Diseases, Bilthoven, Netherlands, [2]RadboudUMC, Medical Microbiology, Nijmegen, Netherlands

Aspergillus section *Nigri* isolates are commonly found in patients and increasingly resistant to azoles. Yet, the antifungal susceptibility of these species is not well known. Recent work on azole-resistant *A. fumigatus* provided strong evidence that patients are infected by isolates that acquired their resistance to azoles in the environment. Whether a similar route of acquired resistance is likely for the black *Aspergilli* has not yet been studied. In this study, the MICs of black *aspergilli* obtained from Dutch hospitals and environmental sources were determined using EUCAST and Etest methods. As the cryptic nature of species belonging to section *Nigri* complicates correct identification, whole-genome sequencing was used to determine species identity and to study population genomics as well as specific azole-resistance loci, such as *Cyp51*. Together, these data provided insight into the molecular epidemiology of *Aspergillus*

section *Nigri* in the Netherlands and allows for further comparative genomic studies in search for resistance mechanisms in clinically relevant black *Aspergilli*.

P1.330 - Identification of novel genetic determinants of fluconazole resistance in *Candida auris*

T.L. Peters[1], *N. Cauldron*[2], *C.A. Cuomo*[2], *P.D. Rogers*[1]
[1]St. Jude Children's Research Hospital, Pharmacy and Pharmaceutical Sciences, Memphis, United States, [2]Brown University, Molecular Microbiology and Immunology, Providence, United States

Candida auris is an emerging fungal pathogen associated with outbreaks in healthcare settings with up to 90% of clinical isolates resistant to fluconazole. While azole resistance has been most widely attributed to mutations in the *ERG11* gene encoding the azole target sterol demethylase, mutations in the gene encoding the zinc-cluster transcription factor Tac1b, and increased expression of the gene encoding the efflux pump Cdr1, these do not account for azole resistance observed in all clinical isolates. To identify novel mechanisms of fluconazole resistance, we evolved fluconazole resistance in two separate *C. auris* strains in which the *CDR1* gene was disrupted. Every 3 days, we increased the drug concentration and sampled the population. For each sample collected, we determined the minimum inhibitory concentration (MIC) and found that 79 of the 96 samples had a 2- to 64-fold increase in MIC above the parental strain as well as their DMSO-evolved control strain counterparts. We then sequenced the genomes of all strains with an elevated MIC and identified mutations associated with increased resistance. While no mutations were observed in *ERG11* or *TAC1B*, we discovered mutations in 33 genes, 29 of which have similar mutations found among a collection of resistant clinical isolates. The genes encoding the bZIP transcriptional regulator Cap1, zinc-cluster transcription factor Mrr1a, and ubiquitin-conjugating enzymes were among those most frequently observed and of particular interest for follow-up analysis. These findings reveal potential *CDR1*-independent resistance determinants in *C. auris* that may be operative in resistant clinical isolates.

P1.331 - The genetic basis for fluconazole resistance in Clade III *Candida auris* clinical isolates

K. Barker[1], *Q. Zhang*[1], *T. Peters*[1], *J. Rybak*[1], *C. Cuomo*[2,3], *D. Rogers*[1]
[1]St. Jude Children's Research Hospital, Pharmacy & Pharmaceutical Sciences, Memphis, United States, [2]Brown University, Molecular Microbiology and Immunology, Providence, United States, [3]Broad Institute, Cambridge, United States

Candida auris is categorized as a fungal priority pathogen by the World Health Organization, primarily due to its ease of transmissibility and its decreased susceptibility to antifungals. Over 90% of *C. auris* clinical isolates are resistant to fluconazole (FLC). Studies to elucidate molecular resistance determinants in *C. auris* have examined orthologs of known fluconazole resistance factors from the related yeast *C. albicans*, including the fluconazole drug target sterol demethylase (Erg11) and the Mdr1 Major Facilitator Superfamily transporter controlled by the

zinc-cluster transcription factor Mrr1. Most *C. auris* Clade III isolates harbor an Erg11 VF125AL amino acid substitution and Mrr1A N647T substitution. We examined the contributions of these mutations to FLC minimum inhibitory concentration (MIC). Correction by CRISPR-Cas9 gene editing of the N647T Mrr1A substitution to the wild-type sequence, or *MDR1* disruption, in the Clade III clinical isolates AR0384 (FLC MIC= 128 mg/L) and AR1102 (FLC MIC= 256 mg/L) resulted in only a one-dilution decrease in FLC MIC. However, correction of the VF125AL Erg11 substitution in AR0384 and AR1102 and their Mrr1A-corrected or *MDR1*-disrupted strains resulted in FLC MICs of 4-8 mg/L. Introduction of the Mrr1A N647T substitution alone in a Clade I strain (FLC MIC= 2mg/L) resulted in no change in FLC MIC. We conclude that this Erg11 substitution is a major contributor to FLC resistance in Clade III isolates with a modest contribution made by the N647T Mrr1A substitution. Furthermore, the residual elevated FLC MIC in these strains indicate that additional resistance determinants remain to be identified.

P1.332 - Characterisation of *Mucor lusitanicus* PDR transporters with a focus on Cluster A transporters that confer multidrug resistance

S. Töpfer[1], J. James[1], E. Lamping[2], J. Loacker-Schoch[1], K. Rosam[1], L.-M. Zenz[1], O. Majer[3], M. Lackner[1]
[1]Medical University of Innsbruck, Innsbruck, Austria, [2]University of Otago, Dunedin, New Zealand, [3]Max Planck Institute for Infection Biology, Berlin, Germany

Mucorales are ranked among the high-priority group of the fungal pathogens' priority list published for the first time by the World Health Organization in October 2022. Invasive Mucorales infections are extremely difficult to treat due to the limited treatment options and the fast disease progression. No doubt, novel antifungals are urgently needed. The mechanisms of azole resistance in Mucorales species remain poorly understood and the possible contribution of efflux pumps to the azole resistance phenotypes observed for many Mucorales species has largely been ignored to date.
This study aimed to characterize the possible contribution of eight pleiotropic drug resistance (PDR) transporters in the azole resistance phenotype of *Mucor lusitanicus*. The goal was to identify drug efflux pumps as potentially novel drug targets and to extend our understanding of drug resistance in Mucorales species.
The eight *M. lusitanicus* PDR transporters separate into two evolutionarily distinct clusters, Cluster A (*pdr1*, *pdr6-8*) and Cluster B (*pdr2-5*). Transcriptome analysis of *M. lusitanicus* revealed strong upregulation of *pdr1* and *pdr6* in response to azole exposure. Characterization of the PDR transporters overexpressed in the *Saccharomyces cerevisiae* model host, ADΔΔ, revealed all Cluster A PDR transporters as candidate efflux pumps of short- and mid-length tailed azoles while Pdr8 caused a pan-azole resistance phenotype.
In conclusion, due to their strong phenotype and upregulation in response to azoles, Pdr1 and Pdr6 are regarded as major players in azole resistance and should be considered along with the other Cluster A transporters for the evaluation of novel efflux pump inhibitors.

P1.333 - Characterization of the *Sordaria macrospora* PDR16 protein: a lipid transfer protein putatively regulating the lipid composition of membranes

S. Pöggeler[1], *M. Nater*[1], *D. Stastny*[2], *A. Werner*[3], *S. Erb*[1], *G. Stahlhut*[1], *P. Griac*[2]
[1]Georg-August University Göttingen, Genetics of Eukaryotic Microorganisms, Göttingen, Germany, [2]Centre of Biosciences SAS, Institute of Animal Biochemistry and Genetics, Bratislava, Slovakia, [3]Georg-August University Göttingen, Molecular Structural Biology, Göttingen, Germany

Pulldown experiments with the Striatin interacting phosphatase and kinase (STRIPAK) component SCI1 identified an orthologue of the yeast PDR16 phosphatidylinositol (PtdIns) transfer protein as a putative target of the STRIPAK complex in *S. macrospora*.
The *S. cerevisiae* homolog of the *S. macrospora* PDR16 protein is predominantly associated with lipid droplets or is localized either to the plasma membrane (PM) or the PM-associated ER. The role of the Pdr16 protein in yeast metabolism is not fully understood. However, the absence of Pdr16p leads to increased susceptibility of yeast cells to azole antifungals, indicating its role in sterol biosynthesis. By regulating the phospholipid/sterol composition of plasma- and endo-membranes, *S. macrospora* PDR16 may be involved in membrane recruiting of the STRIPAK signaling complex required for hyphal fusion.
Deletion of *Smpdr16* in *S. macrospora* resulted in no obvious changes in growth, fruiting-body morphology or sexual reproduction under regular conditions. Only when grown at decreased temperatures the ΔSmpdr16 mutant displayed a reduced growth rate in comparison to the wt. SmPDR16 is localized at septa and endo-membranes, but localization at the plasma membrane cannot be ruled out.
To examine the functional conservation of PDR16 proteins, we heterologously expressed the *S. macrospora pdr16* cDNA in a yeast *Δpdr16* mutant and verified that the *S. macrospora* gene can substitute for the absence of the yeast protein. To address the lipid/sterol binding capacity of PDR16, we will overexpress SmPDR16 in *E. coli* followed by purification to perform a lipid binding assay in HL60 cells.

P1.334 - Rapamycin sensitivity is induced by lithium in *Aspergillus fumigatus*: structural, binding, and phenotypic effects of lithium induced rapamycin binding to FKBP12

B.G Bobay[1], *D.C. Cole*[2], *W.J Steinbach*[3], *P.R Juvvadi*[3]
[1]Duke University Medical Center, Department of Radiology, Durham, United States, [2]North Carolina State University, Department of Biological Sciences, Raleigh, United States, [3]University of Arkansas for Medical Sciences, Department of Pediatrics, Little Rock, United States

Antifungal resistance in fungal pathogens is a multifaceted clinical challenge making combination therapy a valuable alternative to monotherapy. The target of rapamycin (TOR), also known as FKBP-rapamycin associated protein (FRAP), is a conserved serine/threonine kinase from yeast to humans. It is a multi-functional kinase regulating key aspects of fungal growth and

pathogenicity making it an attractive target for antifungal development and combinatorial regimens with current clinical antifungals. The inhibition of TOR kinase occurs via rapamycin binding to the immunophilin FKBP12, and the FKBP12-rapamycin complex binding to TOR. Despite the essential nature of TOR kinase in *Aspergillus fumigatus*, previous studies have shown that rapamycin (sirolimus; everolimus) or rapamycin analogs (INK128; AZD8055) exhibit poor antifungal activity alone but are synergistic with azoles. To distinguish between the fungal versus mammalian FKBP12 counterparts mediating rapamycin inhibition, we compared the *A. fumigatus* strain expressing native FKBP12 (Af-FKBP12) and human FKBP12 (H-FKBP12). We show that LiCl enhanced rapamycin antifungal activity and was synergistic with the calcineurin inhibitor FK506 in the Af-FKBP12 expression strain. However, the H-FKBP12 expression strain showed sensitivity to rapamycin in the absence of LiCl. Molecular dynamic (MD) simulations were performed to more accurately characterize FKBP12's solution structure bound to rapamycin and/or (m)Tor (FRAP) in the presence and absence of Li^{1+}. Our MD simulations provided plausible reasons for the observed phenotype of Li^{1+} increasing rapamycin sensitivity in Af-FKPB12 with no observable effect in the h-FKPB12 system. FKBP12-rapamycin interactions distinguishing the fungal versus the human system might be useful in designing fungal-specific rapamycin analogs.

P1.335 - From Crops to Clinic – The Dual Use of Azoles Driving Antifungal Resistance in *Candida* species

J. Allison[1], *E. Hyland*[1], *S. Cameron*[1]
[1]Queen's University Belfast, School of Biological Sciences, Belfast, United Kingdom

Invasive fungal infections are posing a significant One health threat. In clinical settings fungal disease amounts for 3.8 million deaths annually, with *Candida* species accounts for 70% of deaths. While in agricultural, fungal disease results in annual ~40% harvest loss posing a global food security risk. Antifungal resistance is limited our supply of effective treatments, with ergosterol biosynthesis inhibitors (azoles) being the primary drug class in both clinical and agricultural settings. Azoles function to alter to cell membrane, inhibiting fungal growth by targeting the cytochrome P450-dependent enzyme 14-α-demethylase *CYP51* and *ERG11*. *Candida* species have acquired several resistance mechanisms against azole antifungals, including overexpression and mutations of the drug target genes, and the upregulation of drug efflux pumps. Concerningly, azoles have identical modes of action in both clinical and agricultural settings, and in turn, similar resistance mechanisms leading to the evolution of cross-resistant fungal pathogens, and potentially driving clinical antifungal resistance. However, we still lack the understanding of the mechanisms underlying cross-resistant in *Candida* species human pathogens. Using a combination of culture-based, metagenomic and phylogenomic assays, this research aims to phenotype cross-resistant *Candida* species and identify the resistance mechanisms governing cross-resistance. We will also focus on the environmental origins of *Candida* species, and the phylogenetic relationship between clinically and environmentally isolates *Candida.*

P1.336 - Phylogenetic analysis of members of the Antifungal Protein and Bubble Protein family

C. Cairns[1], *L. Heber*[1], *V. Meyer*[1], *S. Jung*[1]
[1]Institute of Biotechnology, Technische Universität Berlin,, Applied and Molecular Microbiology, Berlin, Germany

The rising number of infections due to microbial infection including invasive fungal infection will exceed the current number of cancer death by 2050. This is further exacerbated by vast annual destruction of crop yields by fungi and the spread of antibiotic-resistant strains. Therefore, the discovery of new antifungal drug reservoirs is critical.

The Antifungal Protein (AFP) and Bubble Protein (BP) families contain peptides produced by filamentous fungi. Several members of these families have been shown to exert a wide spectrum of efficacy against filamentous fungi, yeast, bacteria, and viruses, yet did not show animal or plant cytotoxicity. Hence, these peptides are a vital source for novel antifungal drugs.

Evolutionary analysis was performed to gain a deeper understanding of the AFP and BP families. Iterated PSI-BLAST was used to identify further members of both families. This increased the total number of peptides to 167 and 86 for the AFP and BP family, respectively. Subsequently, MAFFT multiple sequence alignment was used and two phylogenetic trees for the peptides and their producing organisms were constructed using the maximum-likelihood approach. Results show (i) all members of the AFP and BP families are produced by filamentous fungi, (ii) originate most likely in *Aspergillus* spp., (iii) are not associated with virulence, and (iv) support their assignment as secondary metabolites.

This study aided an enhanced understanding of the structure-function relationship of antifungal peptides from filamentous fungi, which can be harnessed as templates for the development of novel antifungal drugs.

P1.337 - High recombination rates in *Aspergillus fumigatus* allows for bulk quantitative trait locus (QTL) mapping of known and novel azole resistance and fitness traits

C. Valero[1], *M. Bottery*[2], *B. Auxier*[3], *F. Stanford*[4], *P.S Dyer*[4], *M. Bromley*[1]
[1]The University of Manchester, Manchester Fungal Infection Group, Manchester, United Kingdom, [2]The University of Manchester, Manchester Fungal Ingection Group, Manchester, United Kingdom, [3]Wageningen University, Wageningen, Netherlands, [4]University of Nottingham, Nottingham, United Kingdom

Aspergillus fumigatus is an environmental fungus that can cause life-threatening or debilitating lung diseases. The number of *A. fumigatus* human infections resistant to the first-line treatment azole drugs have increased over last years which has been linked to the widespread use of azoles fungicides in agriculture. Azole resistance is primarily caused by variants in the gene coding the

azole target Cyp51A, however alternative and complementary non-target variants are increasingly recognized as the cause for antifungal resistance which are potentially coupled with variants associated with increased fitness. Recently, it has been demonstrated that *A. fumigatus* harbours the highest known rate of meiotic crossovers during sexual reproduction generating a highly recombinant progeny which allows for fine mapping of traits of interest. Here, we have developed a high-throughput bulk QTL mapping approach to identify variants causing azole resistance in *A. fumigatus*. An azole sensitive strain was crossed with an environmental strain with known mechanism of azole resistance (*cyp51A*$^{TR34/L98H}$) and pooled F1 progeny was exposed to voriconazole (0.5µg/ml). Using a custom QTL bioinformatic pipeline we were able to identify not only the variant conferring azole resistance (*cyp51A*$^{TR34/L98H}$) but also complementary variants contributing to general fitness. This technique offers a great potential for identifying the underlying mechanism of complex polygenic traits such as antifungal resistance and fitness.

P1.338 - Multi-drug resistant *Aspergillus fumigatus* are more fit at sub-inhibitory concentrations of DHODH inhibitors

N. van Rhijn[1], *J. Rhodes*[2], *C. Valero Fernandez*[1], *M. Bottery*[1], *M. Fisher*[3], *M. Bromley*[1]
[1]University of Manchester, Manchester Fungal Infection Group, Manchester, United Kingdom, [2]Radboud UMC, Nijmegen, Netherlands, [3]Imperial College London, London, United Kingdom

Pesticides, including fungicides, are extensively used in agricultural practice to protect plants from unwanted growth of weeds, plant pathogens and other pests. Dual use of antifungals in the environment and in the clinic, with similar mode of actions, has been shown to drive the development of resistance. Although not a plant pathogen, *A. fumigatus* is ubiquitous in the environment and therefore exposed to agricultural fungicides. Extensive use of triazoles in the environment has led to high rates of resistance found in clinical *A. fumigatus* isolates. These resistant isolates are not only triazole resistant, but we also show that multi-drug resistant *A. fumigatus* to several fungicides are common. It is critical that the use of novel antifungals and fungicides remains effective. Olorofim is a novel antifungal for clinical use, targeting the essential protein DHODH, for which resistance is rare. Recently, several agricultural DHODH inhibitors, including ipflufenoquin , have gone through the approval process. We show here through Bar-seq experiments, in which we compete 180 genetically barcoded environmental and clinical isolates of *A. fumigatus*, that we can identify azole and multi-drug resistant signatures. Furthermore, we show that multi-drug resistant strains are more fit at sub-inhibitory concentration of ipflufenoquin and olorofim. On solid plate transfer experiments, we show that after one passage, multi-drug resistant strains take up the majority of the spore producing population. Our results highlight the potential dangers of using DHODH inhibitors in agriculture and selecting for multi-drug resistant strains upon sub-inhibitor concentrations of DHODH inhibitors.

P1.339 - First observation of genetically acquired echinocandin tolerance in *Candida auris*: from treatment failure *in vivo* to mechanism of action

D. Sofras[1], A. Zile[1], S. Jacobs[1], A. Mottola[2,3], V. Biriukov[4,5], L. Walker[6], C. Lobo Romero[1], Z. Wang[3], D. Ludwig[3], T. Gabaldón[4,5], M. Ralser[3], J. Berman[2], C. Munro[6], P. Van Dijck[1], H. Carolus[1]
[1]KU Leuven, Department of Biology, Leuven, Belgium, [2]Tel Aviv University, Shmunis School of Biomedical and Cancer Research, Tel Aviv, Israel, [3]Charité Universitätsmedizin, Department of Biochemistry, Berlin, Germany, [4]Barcelona Supercomputing Centre (BSC-CNS), Barcelona, Spain, [5]Institute for Research in Biomedicine (IRB), Barcelona Institute of Science and Technology, Barcelona, Spain, [6]University of Aberdeen, School of Medicine, Medical Sciences and Nutrition, Aberdeen, United Kingdom

Candida auris is a rapidly emerging fungal pathogen and a significant challenge to global healthcare, partly due to its resistance to antifungal treatments. *In vitro* experimental evolution of 180 independent lineages exposed to three different echinocandins, revealed that cells commonly acquire echinocandin tolerance besides resistance. Despite a reduction in minimum inhibitory concentration (MIC) values-suggesting decreased resistance- the tolerant strains displayed increased growth within the ellipse of drug diffusion strip assays after prolonged incubation, indicating tolerance. Genome sequencing showed a novel mechanism of acquired echinocandin tolerance involving disruptions in the HOG MAPK pathway. We confirmed this by deleting *HOG1* and other effectors using CRISPR-Cas9 in three *C. auris* clades. Additionally, we observed an echinocandin-hypersensitive phenotype in the *mkc1Δ* mutant, lacking a putative downstream effector of Hog1. In an immunocompromised murine systemic infection model treated with micafungin, we found that the *hog1Δ* strain caused significantly higher fungal burden than the *mkc1Δ* strain, despite their identical MICs, showing that tolerance can jeopardize treatment. Genome mining revealed HOG pathway mutations in clinical isolates of *Candida* species, suggesting that this mechanism of acquired tolerance might influence clinical treatment success. To investigate the mechanism of action, we performed cell wall component and thickness measurements in the presence and absence of caspofungin. Comparative proteomics revealed changes in biological processes in both gain-of-tolerance and loss-of-tolerance strains compared to the wild type. In conclusion, our data suggest that echinocandin tolerance can escape detection by current clinical susceptibility tests and may play a critical role in treatment failure.

P1.340 - Transcriptomic response of *Madurella mycetomatis* exposed to azoles

I. Abugessaisa[1], M. Konings[2], T. Baltussen[2], R.-i. Manabe[1], M. Tagami[1], A. Hasegawa[1], K. Eadie[2], Y. Okazaki[1], T. Kasukawa[1], W. van de Sande[2]
[1]RIKEN Center for Integrative Medical Sciences, Yokohama City, Japan, [2]Erasmusmc, Medical Microbiology and Infectious Diseases, Rotterdam, Netherlands

Mycetoma is a neglected tropical disease with high morbidity. The disease is characterized by large lesions in the subcutaneous tissue in which grains are present. The causative agent can be bacterial (actinomycetoma) or fungal (eumycetoma). Fungal mycetoma is most often caused by *Madurella mycetomatis*. Eumycetoma is treated for 6 months with an azole and then the lesion is removed by surgery. To determine how the azoles influence *M. mycetomatis*, we analyzed the transcriptomic response of *M. mycetomatis* exposed to itraconazole and ravuconazole. A time-series RNA-seq was performed using time points 4h, 24h and 72h after adding the azole. Untreated and treated samples were compared with each other as well as consecutive treated samples. *M mycetomatis* showed a slow transcriptomic response to azole treatment with differentially expressed genes identified only after 72h when treated and untreated samples were compared. Gene ontology analysis identified sterol biosynthesis and efflux transporters such as ABC and MFS transporters enriched among the differentially expressed genes. Other biological processes enriched during itraconazole and ravuconazole treatment were translation, peptide biosynthetic process, amide biosynthetic process, cellular protein metabolic process and cellular nitrogen compound biosynthetic process among others. In this study, we described the *M. mycetomatis* transcriptome after exposure to azole compounds and found a sluggish response compared with other fungal pathogens such as *Aspergillus fumigatus*. The slow azole response might be a result of the slow growth of *M. mycetomatis*. Additionally, ergosterol biosynthesis genes as well as membrane efflux transporters were differentially regulated in *M. mycetomatis*.

P1.401 - A mutation in the 3´-downstream of Pleurotus ostreatus roc1 is responsible for high production of cellulose-degrading enzymes

T. Nakazawa[1], *Y. Honda*[1]
[1]Kyoto University, Kyoto, Japan

White-rot fungi degrade all the three major wood components, cellulose, hemicellulose, and lignin, by secreting numerous enzymes; however, the regulation mechanisms remain largely unknown. Here, we conducted forward genetics to identify a gene that plays a crucial role in the regulation of cellulose-degrading enzyme-encoding genes in the white-rot fungus *Pleurotus ostreatus*. Strain AZp1 with the enhanced ability to release blue dye from AZCL-H-Cellulose on YMG agar plate [0.4%(w/v) yeast extract, 1%(w/v) Malto extract, 0.4% (w/v) glucose, and 2% (w/v) agar] was isolated after UV irradiation to protoplasts from monokaryotic wild-type PC9. Extracellular activities related to cellulose degradation were 10–100-fold higher in AZp1 than in PC9 grown on beech wood sawdust supplemented with 1.3%(w/w) wheat bran. When AZp1 was mated with PC15, the resulting dikaryon did not exhibit the mutant phenotype, suggesting it was recessive. Linkage analysis using conventional PCR (Nakazawa *et al*. 2017 Environ. Microbiol.) identified a marker for which recombination rate was 0 among 11 F_1 progeny from AZp1×PC15. Whole-genome resequencing identified a single nucleotide mutation close to this completely linked marker, which locates approximately 300-bp downstream from the transcriptional termination site of a putative agaricomycetes-specific Zn_2Cys_6-type transcription factor-encoding gene homologous to *Schizophyllum commune roc1* (Marian *et al*. 2022 mBio). The mutant phenotype of AZp1 was complemented in 14 out of 27 carboxin-resistant transformants when *P. ostreatus roc1* from PC9 was introduced along with pPTM1. These results indicated that a mutation in the 3´-downstream of *roc1* is the responsible for the mutant phenotype of AZp1.

P1.402 - Roles for heterotrimeric G-proteins and adenylyl cyclase in transcriptional control of cellulase gene expression in Neurospora crassa

K. Borkovich[1], *Y. Oza*[1], *L. Collier*[1], *M. Quinn*[1], *A. Carrillo*[1]
[1]University of California, Riverside, Microbiology and Plant Pathology, Riverside, United States

Our previous work demonstrated roles for several heterotrimeric G protein subunits and adenylyl cyclase in the complete conversion of Avicel (cellulose) to glucose (Avicelase activity) in *Neurospora crassa*. We obtained evidence for transcriptional regulation of 5-6 cellulase genes in some mutants using qRT-PCR. Both the Avicelase activity and cellulase gene expression defects were rescued by exogenous cAMP in several mutants. In this study, we performed mRNAseq to identify global patterns of gene expression in wild type and three mutants–the Gα mutants Δ*gna-1* and Δ*gna-3* and the adenylyl cyclase mutant Δ*cr-1*–after overnight growth on glucose followed by transfer to glucose or cellulose. We identified more than 2000 genes that were upregulated in the wild-type strain on cellulose as compared to glucose. Predicted cellulases were among the top up-regulated genes in this group in wild type. Expression of most cellulases and several transcription factors previously implicated in regulation of cellulase gene expression were downregulated in the three mutants, with Δ*cr-1* displaying the greatest defects. Overexpression of the transcription factor *clr-2* restored Avicelase activity in the mutants. Our results demonstrate that heterotrimeric G protein and cAMP signaling strongly impact transcriptional control of cellulase activity, through regulation of the downstream CLR-2 transcription factor in *N. crassa*.

P1.403 - Organic acid utilization by *Aspergillus niger*

R. de Vries[1], *A. Manassero*[1], *M. Peng*[1]
[1]Westerdijk Fungal Biodiversity Institute, Fungal Physiology, Utrecht, Netherlands

Fungi produce substantial quantities of organic acids under specific stress conditions, leading to their accumulation in the soil. While it is widely presumed that fungi utilize many of these organic acids as a carbon source, particularly by entering the tricarboxylic acid (TCA) pathways, there is a scarcity of experimental evidence supporting this assumption in literature. Employing growth profiling, we conducted an analysis to assess the ability of the *Aspergillus niger* to grow on nine different organic acids as the sole carbon source. The fungus was inoculated on solid minimal medium (MM) plates with the addition of organic acids and it was derived from both mycelium and spores, under two distinct conditions: directly on the organic acid and in the presence of glucose starvation. In each instance, the result was compared to a positive control (glucose) and a negative control (no carbon source). The findings revealed challenges in the utilization of most organic acids as a sole carbon source, both from mycelium and spores, with and without glucose starvation. The phenotypic data was complemented with transcriptomic analysis of transfer cultures to these organic acids to reveal changes in gene expression profiles when the organic acids are the only carbon source present.

P1.404 - Need for sweet: exploring the how and why of erythritol biosynthesis in fungi

F. Zlati[1], *E. Bredeweg*[2], *S. Baker*[2], *N. Wierckx*[1]
[1]Forschungszentrum Jülich, Institute of Bio- and Geosciences (IBG-1), Jülich, Germany, [2]Pacific Northwest National Laboratory (PNNL), Environmental Molecular Sciences Laboratory (EMSL), Richland, United States

Ustilaginaceae fungi naturally produce a variety of value-added chemicals, including organic acids, glycolipids, and polyols. While primarily known for their plant pathogenicity, these fungi are gaining biotechnological interest due to their diverse product spectra. *Ustilago maydis* serves as an established model organism with available genetic tools transferable to other family members. Here, a library of 123 wild-type strains from the genera *Pseudozyma, Ustilago, Macalpinomyces, Sporisorium,* and *Anthracocystis* was screened for natural erythritol production. Erythritol, a four-carbon polyol primarily used as a sugar substitute, is 60–80% as sweet as sucrose and is considered safe, zero-calorie, and noncariogenic. Its popularity is growing in response to increasing awareness of the harmful effects of sugar consumption.
Erythritol is produced as a compatible solute by osmotolerant fungi under osmotic stress. The screening, conducted under high osmotic pressure to induce erythritol accumulation, revealed that nearly all tested Ustilaginaceae produced varying concentrations of erythritol, achieving titers over 150 g/L and yields of approximately 0.5 g/g, outperforming the reference strain *Moniliella pollinis*. Building on these findings, our efforts are now focused on characterizing the erythritol production pathway in Ustilaginaceae through comparative transcriptomic and proteomic analyses, as well as existing knowledge from other fungi, in particular *Yarrowia lipolytica*. This approach enables the exploration of the mechanisms behind erythritol production in both *Yarrowia* and Ustilaginaceae. A clear understanding of the erythritol biosynthetic pathway is crucial for strain engineering and improved production, as erythritol biosynthesis in fungi is still full of mysteries despite its large-scale industrial production.

P1.405 - Phenotypic and transcriptomic analysis of bZIP transciption factors in Aspergillus nidulans

B. Kocsis[1,2], *M.-K. Lee*[3], *K. Antal*[4], *J.-H. Yu*[5,6], *T. Emri*[1,2], *T. Nagy*[7], *L. Daróczi*[8], *G. Batta*[9], *I. Pócsi*[1,2], *É. Leiter*[1,2]
[1]University of Debrecen, Department of Molecular Biotechnology and Microbiology, Debrecen, Hungary, [2]HUN-REN-UD Fungal Stress Biology Research Group, Debrecen, Hungary, [3]Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Ipsingil, Jeongeup, Jeonbuk, Korea, Republic of, [4]Eszterházy Károly Catholic University, Department of Zoology, Eger, Hungary, [5]The University of Wisconsin-Madison, Department of Bacteriology, Wisconsin-Madison, United States, [6]Konkuk University, Department of Systems Biotechnology, Seoul, Korea, Republic of, [7]University of Debrecen, Department of Applied Chemistry, Debrecen, Hungary, [8]University Of Debrecen, Department of Solid State Physics, Debrecen, Hungary, [9]University of Debrecen, Department of Genetics and Applied Microbiology, Debrecen, Hungary

The bZIP transcription factors (TFs) are crucial elements of the secondary metabolism, sexual development and stress response pathways in *Aspergilli*.
A comprehensive study including phenotypic and transcriptomic characterization of AtfA and AtfB TFs was performed on gene deletion and overexpression mutants in *Aspergillus nidulans*.
The role of AtfA in the protection against oxidative stress was confirmed because the *ΔatfA* mutant was sensitive in the presence of diamide, *t*BOOH as well as menadione. This mutant showed a moderately tolerant phenotype against cell wall integrity stress. Meanwhile AtfB plays an important role in the sensitivity to heavy metal and heat stress.
Both AtfA and AtfB affect the sexual and asexual reproduction in *A. nidulans*. In addition, AtfB coordinates the size of the conidiospores, since *atfB*OE was characterized by significantly larger spore size.
Examining the secondary metabolism of the mutants, in the *ΔatfA* strain no sterigmatocystin production was detected.
The link between sexual reproduction and secondary metabolism was confirmed since in the *ΔatfA* strain the disturbance in sexual reproduction was accompanied by the complete inhibition of sterigmatocystin synthesis.
According to the transcriptomic data neither MSB treatment nor *atfB* deletion caused changes in the gene expression of *atfA*, the MSB treatment in the mycelium samples and deletion of *atfA* in the mycelium and conidia samples induced the repression of *atfB* gene and the transcriptomes of both conidia and mycelia were significantly modified by the MSB treatment. Our transcriptomic study underlined that both AtfA and AtfB have a significant regulatory role in the conidiospores.

P1.406 - Functional and physiological characterization of polyol transporters of *Aspergillus niger*

C. Lyra[1,2], *L. Nummela*[1,2], *R. Mewalal*[3], *I. Blaby*[3], *H.N. Maina*[2], *J.T. Pronk*[4], *R. Mans*[4], *I.V. Grigoriev*[3], *M.R. Mäkelä*[1,2]
[1]Aalto University, Espoo, Finland, [2]University of Helsinki, Helsinki, Finland, [3]Lawrence Berkeley National Laboratory, Berkeley, CA, United States, [4]Delft University of Technology, Delft, Netherlands

Aspergillus niger is a crucial contributor to sustainable solutions within the bio-based economy. It converts plant biomass monosaccharides, such as D-galactose and D-xylose, to polyol intermediates through the oxido-reductive and pentose catabolic pathways[a]. Despite extensive *in silico* identification of polyol transporters in *A. niger* and other fungi, most of these proteins remain uncharacterized. To date, only L-arabitol-transporting LatA has been characterized in *A. niger*[b].
We selected ~30 Major Facilitator Superfamily[c] proteins from three phylogenetic groups[d] for characterization in *A. niger* and a *Saccharomyces cerevisiae* platform strain[e]. Since *S. cerevisiae* lacks efficient pathways to metabolize xylitol, sorbitol and mannitol, we engineered the platform strain with the genes required to metabolize these polyols using CRISPR/Cas9-technology. We used the same technology to generate *A. niger* polyol transporter deletion mutants.
Functional characterization showed that part of the recombinant strains expressing *A. niger* polyol transporter genes grow on media containing 0.05% and 0.5% xylitol and sorbitol, and 0.05% mannitol as carbon source. The improvement of *S. cerevisiae* strains for enhanced

metabolism and the generation of some of the desired *A. niger* polyol transporter deletion mutants for physiological characterization are still in process.
Classification of sugar transport connecting the exogenous and endogenous processes in *A. niger* could highly enhance our understanding of plant biomass conversion.
[a]Chroumpi *et al*. *Microb. Biotechnol.* 159:1–10 (2022)
[b]Meng *et al*. *Biomolecules* 13:1–11 (2023)
[c]Saier *et al. Nucleic Acids Res.* 49(D1):D461-7 (2021)
[d]Xu *et al. Biores Technol.* 391:1–7 (2024)
[e]de Valk *et al. Biotechnol. Biofuels* 15:1–16 (2022)

P1.407 - Starch metabolism ability is variable between genera of anaerobic gut fungi: effective degrader *Neocallimastix frontalis* employs multiple degradative enzymes

L. Edge[1], *M. Yang*[1], *T. Hong*[1], *J.M van Munster*[1]
[1]Scotland's Rural College, Plant and Soil Science, Edinburgh, United Kingdom

Anaerobic Gut Fungi (AGF, Phylum *Neocallimastigomycota*) are primary degraders of plant cell wall materials within the specialised digestive system of ruminant herbivores, including agriculturally important animals such as cattle. This important role in ruminant digestion makes them a promising target for microbiome modulation.
High starch-containing grains are an important component in cattle diets due to their high calorific content. However, in animals fed these high-starch diets, some AGF increase in abundance while others are impaired. The reasons for this difference, and the roles AGF play in starch metabolism, are largely unknown.
Here we uncovered how distinct metabolic and degradative activities underpin differences in starch degradation abilities in fungi from the genera *Neocallimastix*, *Caecomyces* and *Piromyces*. Fungal growth was measured alongside the rate of starch degradation and product formation in *in vitro* cultures. The amylolytic machinery of *N. frontalis* was biochemically characterised.
We confirmed that starch is completely degraded to glucose extracellularly, by analysing product formation after fungal inactivation via hygromycin. Amylases were purified via two chromatography steps, their activities characterised using colourimetric assays, and their end-products identified using thin layer chromatography.
Three distinct enzymes were identified: one glucose producing exo-amylase and two endo-amylases; one which produces an oligosaccharide with 5 degrees of polymerisation (DP); the second which produces oligosaccharides of DP8 and longer. We have also confirmed the presence of a biomass-bound α-glucosidase.
Together these results give insight in the different metabolic and degradative abilities of AGF, which may enable better prediction of their activities and importance *in vivo.*

P1.408 - Adsorption of cutinase CutL1 to the Langmuir membrane of hydrophobin RolA derived from the fungus Aspergillus oryzae

S. Iio[1], N. Takahashi[1], Y. Terauchi[2], T. Tanaka[3], A. Yoshimi[4,5], M. Mitsuishi[6], H. Yabu[7], F. Hasegawa[8], K. Abe[1]
[1]Tohoku University, Graduate School of Agricultural Science, Sendai, Japan, [2]Yamaguchi University, RCTMR, Ube, Japan, [3]Osaka University, Graduate School of Engineering, Suita, Japan, [4]Kyoto University, Graduate School of Global Environmental Studies, Kyoto, Japan, [5]Kyoto University, Graduate School of Agriculture, Kyoto, Japan, [6]Tohoku University, Graduate School of Engineering, Sendai, Japan, [7]Tohoku University, WPI-AIMR, Sendai, Japan, [8]National Institute of Technology and Evaluation, Tokyo, Japan

Filamentous fungi play an important role in degradation of solid polymers such as proteins and polysaccharides in nature. When a filamentous fungus *Aspergillus oryzae* is cultured using the biodegradable plastic polybutylene succinate-*co*-adipate (PBSA) as the sole carbon source, the fungus co-expresses a hydrophobin RolA and polyesterase CutL1. RolA attached to the surface of PBSA recruits CutL1 via ionic interactions, then promoting the degradation of PBSA. When RolA is adsorbed to a hydrophobic substrate, RolA molecules form an amorphous membrane and then self-assemble to form rod-shaped multimeric structures called rodlet. However, it remains unclear whether the recruitment of CutL1 by RolA takes place on the amorphous or rodlet membrane. In this study, we used the Langmuir membrane to clarify which state of RolA, amorphous or rodlet, interacts with CutL1. RolA solution was spread on the buffer surface in a Langmuir trough and compressed from both sides until reaching the target surface pressure. Then, the surface area of the RolA monolayer was fixed and CutL1 was injected into the buffer. The change in surface pressure was further measured over time. As a result, the surface pressure increased significantly only when amorphous RolA was used. In an experiment using RolA HKK46S, a mutant in which triple positively charged residues were substituted to serine, CutL1 addition scarcely increased the surface pressure of RolA HKK46S amorphous membrane. These results suggest that CutL1 interacts with amorphous RolA via electrostatic interactions, not with RolA rodlet. We discuss the CutL1 recruitment by the form of RolA membranes.

P1.409 - Exploring the role of *Candida albicans* Rap1 in iron homeostasis, azole resistance and virulence

W.L. Huang[1], M.C. Yang[1], C.Y. Lan[1,2]
[1]Institute of Molecular and Cellular Biology, National Tsing Hua University, Hsinchu, Taiwan, China , [2]School of Medicine, National Tsing Hua University, Hsinchu, Taiwan, China

C. albicans is one of the critical priority fungal pathogens listed by the WHO. Rap1 is a conserved DNA-binding protein found in yeast, including *C. albicans*, and in mammals, where it is involved in telomere maintenance. In this study, we aimed to explore the non-telomeric functions of Rap1 in *C. albicans*. RNA sequencing analysis was performed to identify differentially expressed genes (DEGs) in the *RAP1*-deleted mutant compared to the wild-type strain under different iron conditions. These DEGs included those related to iron homeostasis,

drug transport, and ergosterol biosynthesis. Further analysis revealed that the *RAP1*-deleted mutant exhibited altered iron acquisition abilities and associated gene expression. Additionally, deletion of *RAP1* changed cellular susceptibility to fluconazole, especially under low iron condition. Finally, using a mouse model of disseminated infection, we demonstrated that Rap1 is correlated with *C. albicans* virulence. Together, this study revealed novel functions of Rap1 in *C. albicans*, and highlighted complex regulatory mechanisms in fungal pathogens.

P1.410 - Identification of the key enzymatic genes of *Penicillium camemberti* responsible for the development and ripening of mould-ripened cheeses

A. Kuria[1], *R. Layfield*[2], *M. Brock*[1], *P. Dyer*[1]
[1]University of Nottingham, Fungal Biology Group, School of Life Sciences, Nottingham, United Kingdom, [2]University of Nottingham, Protein Biochemistry Group, School of Life Sciences, Nottingham, United Kingdom

The fungus *Penicillium camemberti* is used in production of outer mould-ripened cheeses such as Camembert and Brie. However, despite its economic significance, *P. camemberti* has not been widely studied and its mechanism of action with regards to cheese production and maturation has not been elaborated. One of the challenges of mould-ripened cheeses is that these contain live fungi from the point of production all the way to the supply chain, which can limit shelf-life due to ongoing fungal activity and possible over-ripening and food spoilage. This study aims to bridge this knowledge gap by identifying and characterizing at a molecular genetic level the extracellular enzymes that are key for flavor, taste, and appearance of these cheeses, which would help identify potential areas for strain improvement. A multi-faceted approach has been used to identify candidate lipolytic and proteolytic genes of *P. camemberti* involved in cheese maturation. Firstly, bioinformatic analysis based on the genome of the FM013 strain identified 20 and 49 candidate extracellular lipolytic and proteolytic enzymes, respectively. Secondly, a combination of gene expression techniques, initially involving end-point RT-PCR, and a proteomics approach were used to further identify key enzymes. For lipolytic enzymes, five genes appeared to be primarily responsible for lipolysis and work is ongoing to further characterize these genes via deletion with a newly developed CRISPR-Cas9 system. A similar approach has been adopted for proteolytic enzymes, with qRT-PCR work ongoing to select key candidate genes from a list of 17 genes already identified using end-point RT-PCR and proteomics techniques.

P1.411 - Ace3 of the white-rot fungus *Dichomitus squalens* regulates cellulose degradation and utilization through cellobiose induction

V.M. Gonzalez Ramos[1,2], *H. Böke*[1,2], *L. Nummela*[1,2], *C. Lyra*[1,2], *R. Mewalal*[3], *I. Blaby*[3], *J.T. Pronk*[4], *R. Mans*[4], *I.V. Grigoriev*[3,5], *H.N. Maina*[6], *J.E. Kowalczyk*[2,7], *M.R. Mäkelä*[1,2]
[1]Aalto University, Department of Bioproducts and Biosystems, Helsinki, Finland, [2]University of Helsinki, Department of Microbiology, Helsinki, Finland, [3]Lawrence Berkeley National Laboratory, US Department of Energy Joint Genome Institute, Berkley, United States, [4]Delft University of Technology, Department of Biotechnology, Delft, Netherlands, [5]University of

California Berkeley, Department of Plant and Microbial Biology, Berkeley, United States, [6]University of Helsinki, Department of Food and Nutrition, Helsinki, Finland, [7]Catalyze Group, Amsterdam, Netherlands

The basidiomycete white-rot fungus *Dichomitus squalens* is an efficient wood-degrading species with a flexible physiology and finely tuned molecular response to different types of plant biomass[a]. Previously, we revealed that a candidate transcription factor *Ds*Ace3, an orthologue of the *Trichoderma reesei* Ace3, co-regulates predicted intra- and extracellular carbohydrate-active enzyme and sugar transporter encoding genes. In this study, we investigate the role of *Ds*Ace3 in cellulose degradation and utilization through cellobiose induction. Disruption of the *ace3* gene in *D. squalens* resulted in a substantial reduction in growth and caused morphological changes when the mutant strain was cultivated on cellulose. The *Dsace3* mutant showed little to no activity of key hydrolytic cellulases, i.e. β-1,4-glucosidases, cellobiohydrolases, and endoglucanases, together with reduced production of extracellular enzymes. Transcriptomic analysis of the mutant cultivated on cellobiose and cellulose confirmed the downregulation of cellulase-encoding genes, together with increased expression of genes related to sugar metabolic pathways, such as the oxidoreductive, Leloir, and pentose phosphate pathways. Preliminary data suggest that *Ds*Ace3 also affects sugar uptake, as several putative cellodextrin transporter encoding genes were downregulated in the *ace3* mutant. These findings highlight *Ds*Ace3 as a critical regulator of cellulase expression and cellulose utilization in *D. squalens*, advancing our understanding of lignocellulose degradation in white-rot fungi.

[a]Gonzalez, V. M. et al. (2014) *Curr Res Biotechnol*. 7,100198

P1.412 - Diversity of iron uptake systems and their roles in the skin fungus *Malassezia*

B. Minseok[1], *C. Sohyeong*[1], *C. Young-Joon*[2,3], *J. Won Hee*[1]
[1]Chung-Ang University, Systems Biotechnology, Anseong, Korea, Republic of, [2]Kangwon National University, Molecular Bioscience, Chuncheon, Korea, Republic of, [3]Kangwon National University, Multidimensional Genomics Research Center, Chuncheon, Korea, Republic of

Iron is an essential element for most organisms, serving as a cofactor for enzymes involved in various cellular processes. It also plays a critical role in the interaction between pathogenic microorganisms and their hosts. Many pathogens, including fungi, possess iron uptake systems that enable them to acquire iron from host environments. In this study, we aimed to identify and characterize the iron uptake systems in *Malassezia*, a fungal genus predominantly found on human skin and implicated in skin conditions such as seborrheic dermatitis and dandruff. Our genome mining analyses revealed the presence of siderophore synthesis and transport pathways in major *Malassezia* species found on human skin. In contrast, the high-affinity reductive iron uptake pathway, which is required for the utilization of ferric iron, was found to be species-dependent. These differences were reflected in the varying growth capabilities of different *Malassezia* species when exposed to different iron sources. We also investigated the transcriptome profiles of *M. restricta* and *M. sympodialis* and found that genes involved in iron

transport and metabolism, including siderophore synthesis and transport, were differentially expressed in response to iron availability. Strains lacking iron transport genes are currently being constructed, and their phenotypic and physiological characteristics will be studied. Overall, our study highlights the functional diversity within the *Malassezia* genus and provides insights into how these fungi adapt to iron-depleted environments, such as the human skin surface.

P1.413 - Role of the sugar transporter Tr44175 in cellulase induction in *Trichoderma reesei*

L.M. Soares Pereira[1], *D. Batista Maués*[1], *R. do Nascimento Silva*[1]
[1]University of São Paulo, Department of Biochemistry and Immunology, Ribeirão Preto, Brazil

Sugar transporters are essential for cellulase production in *Trichoderma reesei*. However, the role of most sugar transporters encoded in its genome remains unknown. In this study, the role of the cellobiose and sophorose transporter *Tr44175* in the *T. reesei* TU-6 strain was investigated. Deletion of the gene encoding Tr44175 in *T. reesei* reduced growth on cellobiose, L-arabinose, and lactose in solid media, as well as on glucose and carboxymethyl-cellulose in submerged media. However, it did not affect growth on sophorose in the Biolog assay. The role of *Tr44175* in the presence of cellulose, sophorose, and cellobiose was also examined, and the results showed that deletion of *Tr44175* affected cellulase gene transcription distinctly depending on the carbon source. Expression of the cellobiohydrolase *cel7a*, endoglucanase *cel7b*, and β-glucosidase *cel3a* was abolished in the mutant strain on cellulose at 12 hours of culture and drastically reduced on sophorose at the same time point. However, *cel7a* expression on cellobiose was higher in the mutant compared to the parental strain at 12 hours. Specific activities of CMCase, β-glucosidase, and β-xylosidase in cellulose culture were higher in the Δ*44175* mutant than in the parental strain at 48 hours, while β-glucosidase activity was lower in the Δ*44175* strain at 72 hours. These findings suggest that the sugar transporter *Tr44175* plays a key role in cellulase induction in the early stages of cultivation on cellulose, sophorose, and cellobiose and for *T. reesei* growth on various carbon sources.

P1.414 - The PacC transcription factor responds in a carbon source specific manner in *Aspergillus niger* and *Aspergillus nidulans*

A. Müller[1], *N. Martinez Reyes*[1], *R.P. de Vries*[1]
[1]Westerdijk Fungal Biodiversity Institute, Fungal Physiology, Utrecht, Netherlands

The fungal transcriptional regulator PacC plays an important role in adapting to fluctuating ambient pH, particularly under alkaline conditions. In neutral or alkaline environments, PacC undergoes proteolytic activation, resulting in its transcriptional regulation of various pH-responsive genes, thus enabling the fungus to modulate processes such as nutrient acquisition, ion transport, and secondary metabolism. The deletion of the *pacC* gene impairs the organism's ability to adapt to alkaline conditions, leading to downregulation of alkaline-expressed genes and an overall reduction in fungal fitness. Conversely, constitutive activation of *pacC* ($PacC_C$) mimics alkaline conditions, even in acidic environments, resulting in the inappropriate expression

of alkaline genes and repression of acid-expressed genes.
This study compares the carbon source dependency of the PacC mediated response in *Aspergillus niger* and *Aspergillus nidulans*. The phenotypes of deletion and constitutive activation PacC mutants of both species were evaluated in the presence of different carbon sources and at different pH and the genes influenced under these conditions were identified.
Initial results suggest a strong carbon source specific effect on the function of PacC and significant differences between these two *Aspergillus* species.

P1.415 - Functional characterization of the saccharopine dehydrogenase LYS1 from filamentous fungi relevant for solid-state plant food fermentation

T. Vellozo-Echevarría[1], *K. Barrett*[1], *M. Vuillemin*[1], *A. S. Meyer*[1]
[1]Technical University of Denmark, Bioengineering, Kgs. Lyngby, Denmark

The escalating global population is challenging the food industry. While plant-based foods offer advantages in terms of reduced emissions and water usage, they often lack sufficient levels of certain essential amino acids, notably lysine. Lysine is an essential amino acid and is critical for human health as it plays a vital role in protein synthesis. Solid-state fungal fermentation of plant materials has emerged as a promising approach to enhance the amino acid profile and digestibility of plant-based foods, as demonstrated in traditional foods like tempeh. In fungi, lysine biosynthesis proceeds through the unique α-aminoadipate pathway, the details of which are still unexplored in many species of filamentous fungi of interest in the food industry. This study focuses on LYS1, the enzyme catalyzing the final step in the synthesis of lysine through the α-aminoadipate pathway, and comprises the identification, expression and characterization of the activity of LYS1 from *Aspergillus niger*, *A. nidulans*, *Penicillium roqueforti*, *Rhizopus microsporus*, *R. arrhizus*, and *R. stolonifer* – of which the *Rhizopus* spp. are used in tempeh production. Benchmarking was conducted by comparison to the previously characterized LYS1 from baker's yeast, *Saccharomyces cerevisiae*. The assessment of the kinetic parameters for the enzymes has shown shared characteristics inside the Ascomycetes enzymes and differences when compared with the Mucoromycetes. Interestingly, variations in enzymatic properties were also observed between closely related species within the same genera. These findings have important implications regarding the optimization of the selection of species for solid-state fermentation of plant-based foods.

P1.416 - Re-evaluating the fungal D-glucuronic acid pathway

N. Rahnama[1], *A. Manassero*[1], *P. Chen*[1], *A. Mueller*[1], *R.P. de Vries*[1]
[1]Westerdijk Fungal Biodiversity Institute & Fungal Molecular Physiology, Utrecht, Netherlands

D-glucuronic acid is a common compound in nature that is found as a side-group on the plant cell wall polysaccharide xylan, as well as a component of many gums, such as Arabic and xanthan gum. Its pathway has been well studied in bacteria, but to a much lesser extent in fungi. In recent years, the D-glucuronic acid pathway has been studied in *Aspergillus niger* and several genes

encoding the enzymes catalyzing the metabolic steps have been identified. However, deletion mutant strains for these genes showed mostly reduced rather than no growth phenotypes, suggesting that additional enzymes could be involved in this pathway. A similar situation has recently been described for the *A. niger* pentose catabolic pathway, showing that some metabolic steps are catalyzed by at least three different enzymes.
In this study, we aimed to revisit the D-glucuronic acid pathway and identify the full range of genes involved in the pathway in *A. niger*. RNA-seq analysis of *A. niger* wild type strain cultivated on D-glucuronic acid revealed several additional candidate genes for most steps of the pathway. Construction of strains with single and multiple gene deletions were performed to demonstrate their in vivo functionality in the pathway. The resulting strains were compared with respect to phenotypes on D-glucuronic acid and other carbon sources to determine the full scope of genes involved in the pathway, as well as the possible role of these genes in other pathways.

P1.417 - Exploring variations and similarities between *Serpula lacrymans* isolates

K. Russ[1], *F. Peters*[2], *M. Kirchmair*[1], *S. Zeilinger-Migsich*[1], *S. Neuhauser*[1]
[1]Universität Innsbuck, Microbiology, Innsbruck, Austria, [2]Leibnitz Universität Hannover, Institute of Vocational Sciences in the Building Trade, Hannover, Germany

One of the most potent brown rot fungi is *Serpula lacrymans*, which colonises houses in temperate and boreal regions around the world. People fear *S. lacrymans* because removing it is very expensive and laborious, and it must be done according to official guidelines.
S. lacrymans spreads quickly in houses by vegetative mycelium and by the formation of mycelial cords, which are responsible for transporting nutrients. Once the fungus is established on wood in houses, it begins to break down cellulose and hemicellulose.
We have eleven *Serpula lacrymans* isolates from Austria, belonging to the three genotypes known in Europe. With these isolates, experiments to explore the similarities and distinctions among the strains and the three genotypes were conducted. Isolates showed strong variation in growth rate, which could not be linked to the genotype. We carried out degradation tests on different biopolymers (cellulose, pectin, starch and xylan) and a wood degradation experiment using pieces of *Picea abies* wood with the different isolates. In these experiments, we were not able to find any similarities between the individual fungi of the same genotype, hence differences in behaviour/physiology appear to be strain specific.
Because fungi, such as *S. lacrymans*, are known for their ability to produce a wide range of secondary metabolites, putative differences in pigment patterns were analysed by HPTLC and fluorescence photography.

P1.418 - Environmental impacts on secondary metabolite production and differential gene expression in the amphibian gut fungus *Basidiobolus*

K. Syring[1], *G. Rodriguez*[2], *G. Neuhaus*[2], *L. Vargas Gastelum*[1], *K. McPhail*[2,3], *J. Spatafora*[1]
[1]Oregon State University, Department of Botany and Plant Pathology, Corvallis, United States, [2]Oregon State University, College of Pharmacy, Corvallis, United States, [3]University of Utah, Department of Medicinal Chemistry, Salt Lake City, United States

Basidiobolus is a genus of fungi with a complex life cycle of contrasting environmental niches with unique hyphal and spore morphotypes. It spends part of its life cycle as a free-living filamentous saprobe on detritus and in the anaerobic digestive systems of herptile species in a yeast-like palmella stage. Previous studies on *Basidiobolus* genomes found a strong signal for biologically active specialized metabolites (SM) such as cyclic peptides, which are thought to provide an adaptative advantage. To elucidate variation in SM under contrasting environmental variables and gain insight into their ecological function, we compared LC-MS/MS data from different strains of *Basidiobolus* grown on multiple media types under aerobic and anaerobic conditions. PCA analysis revealed variations in SM production across different strains and conditions, with aerobic conditions resulting in greater variation of SM within and between species compared to anaerobic conditions. This suggests anaerobic conditions may result in more conserved metabolism within the gut environment. Of particular interest, an eight-residue cyclic peptide was detected, with one putative biosynthetic gene cluster (BGC) matching within the genomic data. To explore differential expression (DE) related to life stages, we selected an isolate with relevant BGCs for a preliminary RNA-seq DE study. The experiment focused on anaerobic and aerobic conditions, introducing pH as a variable. We will present data and analyses exploring the production of SM and differential expression as a function of environmental variables relevant to the life history of *Basidiobolus*, and the predicted BGCs responsible for their biosynthesis.

P1.419 - Identification of potential regulators of the transcription factor AmyR using the screening method based on growth defects caused by *brlA* overexpression in *Aspergillus nidulans*

K. Gomi[1], *T. Shintani*[1], *D.M. Jeong*[1], *J. Tokashiki*[1]
[1]Tohoku University, Graduate School of Agricultural Science, Sendai, Japan

Amylolytic gene expression is regulated by the transcription factor AmyR in *Aspergillus* species, but the factors involved in the functional regulation of AmyR have not been elucidated. Therefore, to identify unknown potential regulators of AmyR, we constructed an *Aspergillus nidulans* strain that overexpressed *brlA*, which is involved in conidiation, under the control of the α-amylase gene promoter. The resulting strain showed a significantly restricted growth in the presence of isomaltose, an inducer of amylase production. Using this strain as a parent, spontaneous mutant strains that recovered growth were isolated on isomaltose-containing agar medium. Consequently, we successfully identified a novel sugar transporter involved in

isomaltose transport/sensing through whole genome sequencing of the spontaneous mutants. On the other hand, the screening method we developed can be applied to find unknown factors other than the transporter/sensor, such as cofactors and modifying enzymes, involved in the functional regulation of AmyR. For this purpose, using *A. nidulans* ABPU1/ΔligD (*wA3, argB2, biA1, pyroA4, pyrG89, ΔligD::ptrA*) as a host, we constructed a strain in which two copies of *brlA* overexpression cassette were integrated into the *argB* and *biA* loci, and the *amyR* and isomaltose transporter/sensor genes were integrated into the *pyrG* and *pyroA* loci, respectively. As in the previous study, this strain showed a significantly poor growth in the presence of isomaltose. We could isolate several spontaneous mutant strains that restored growth on isomaltose agar medium using this strain as a parent, and attempted to identify mutated genes by whole genome sequencing.

P1.420 - Mutation of nitrogen source assimilation in industrial strains of *Aspergillus oryzae*

S. Miki[1,2], K. Sakai[1], T. Nakagawa[2], T. Tanaka[1], L. Liu[1], H. Yamashita[2], K.-I. Kusumoto[1]
[1]Osaka University, Department of Biotechnology, Graduate School of Engineering, Donated Lab of Breeding Engineering for Koji Mold, Suita, Japan, [2]Higuchi Matsunosuke Shoten Co., Ltd., Osaka, Japan

Aspergillus oryzae mutants defective in nitrogen source assimilation are known to grow poorly on Czapek-Dox (CD) medium. In this study, we found an industrial strain of *A. oryzae* that grew very poorly on a CD medium containing sodium nitrate as a nitrogen source. We used media with various nitrogen components to examine the steps affecting the nitrogen source assimilation pathway of this strain. The strain grew well on the CD medium supplied with nitrite salt or ammonium salt, suggesting that the strain was defective in nitrate assimilation step. To ascertain the gene causing the defect of nitrate assimilation, a gene expression vector harboring either *niaD* or *crnA* of *A. oryzae* RIB40 was introduced into the industrial strain. The industrial strain containing the *crnA* vector recovered its growth. This is the first report that a mutation of *crnA* causes poor growth on CD medium in an industrial strain of *A. oryzae*, and *crnA* can be used as a transformation marker for *crnA* deficient strains.
This work was published in Journal of Bioscience and Bioengineering, 137(4), Pages 231-238 (2024)
[#]The first two authors contributed equally to this work.

P1.421 - Characterization of a new glycerol transporter GlpA from *Aspergillus niger*

L. Nummela[1,2], C. Lyra[1,2], H.N. Maina[2], J.T. Pronk[3], R. Mans[3], M.R. Mäkelä[1,2]
[1]Aalto University, Espoo, Finland, [2]University of Helsinki, Helsinki, Finland, [3]Delft University of Technology, Delft, Netherlands

A recent extensive *in silico* study revealed numerous putative polyol transport protein coding gene candidates in fungi, including glycerol transporters[a]. However, the physiological and

functional roles of glycerol transporters in filamentous fungi, such as *Aspergillus niger*, remain poorly understood. Here, we characterized the first known glycerol transporter, GlpA, from *A. niger*.
In *A. niger*, a Δ*glpA* strain exhibited reduced growth and glycerol consumption on solid and in liquid media, respectively, compared to the reference strain. In addition, the deletion mutant showed slower uptake of other polyols, including xylitol, mannitol and galactitol. This suggests a central role for GlpA in glycerol uptake in *A. niger*.
To also study the function of GlpA with minimal interference of other transport proteins, we heterologously expressed it in a *Saccharomyces cerevisiae* strain lacking all hexose and disaccharide transporters, and disaccharide hydrolases[b]. A *glpA*-GFP fusion was constructed to confirm the correct localization of GlpA within the yeast plasma membrane by fluorescent microscopy. The growth of the recombinant *S. cerevisiae* strain was tested on different hexoses and polyols and showed a slightly increased growth rate on polyols compared to the negative control.
This study highlights the physiological importance of GlpA in both glycerol and polyol uptake in *A. niger* and advances our understanding of polyol transporters in filamentous fungi.
[a] Xu *et al. Biores Technol.* 391:1–7 (2024)
[b] de Valk SC *et al. Biotechnol. Biofuels* 15:47 (2022)

P1.422 - Regulation of itaconic acid accumulation by the extracellular phosphate ion concentration in *Aspergillus terreus*

L. Karaffa[1], *Á.P. Molnár*[1], *I. Bakondi-Kovács*[1], *A. Márton*[1], *V. Bíró*[1], *E. Fekete*[1]
[1]University of Debrecen, Debrecen, Hungary

Itaconic acid is a five-carbon, unsaturated, weak diprotic acid. Its unique chemical properties are roundly exploited by the polymer industry. On industrial scale, itaconic acid is produced from carbohydrates by large-scale submerged fermentation of the filamentous Ascomycete fungus *Aspergillus terreus*. Itaconic acid overflow requires low pH, high dissolved oxygen levels, high initial carbon source concentration, as well as growth-limiting concentrations of nitrogen and manganese(II) ions. The inhibitory effect of manganese(II) ions is particularly critical, as concentrations as low as >5 ppb reduce itaconic acid accumulation by 20%. In this study, fully optimized itaconic acid fermentations performed in 6-L scale bioreactors – where all cultivation conditions including initial phosphate concentration were optimal for maximal volumetric yield – were compared with fermentations where initial phosphate concentrations were set higher or lower than the optimal value. Fermentations were performed on D-xylose or D-glucose as sole carbon sources. We demonstrate that phosphate ion limitation facilitates itaconic acid accumulation on at least three different grounds, i.e., (1) shifting the carbon flux between biomass and product formation in favour of the latter, (2) attenuating the inhibitory effect of manganese(II) ions, and (3) increasing the expression and activity of the cyanide-resistant alternative oxidase.

P1.423 - Dissection of the septal pore apparatus in the agaricomycete *Coprinopsis cinerea*

B. Sokolowska[1], *T. Gassler*[1], *M. Pilhofer*[2], *S. Pöggeler*[3], *M. Künzler*[1]
[1]ETH Zurich, Institute of Microbiology, Zurich, Switzerland, [2]ETH Zurich, Institute of Molecular Biology & Biophysics, Zurich, Switzerland, [3]University of Göttingen, Göttingen, Germany

Vegetative mycelia of agaricomycetes can be exceptionally large and long-lived. The hyphae of these fungi are compartmentalized by cross-walls (septa) containing pores with thickened cell walls (dolipores) and sophisticated cap structures, referred hereafter as septal pore apparatus. This apparatus ensures that nutrients and signals are distributed between the hyphal compartments via cytoplasmic bulk flow. Like in ascomycetes, the apparatus can also block cytoplasmic bulk flow between compartments by septal pore plugging e.g. as a response to hyphal damage and maybe also internal cues. Since the septal pore apparatus ultimately controls the cytoplasmic bulk flow in a fungal mycelium, it is an essential organelle for multicellular fungi. In contrast to multicellular ascomycetes, however, very little is known about the molecular composition of the septal pore apparatus and the mechanism of septal pore plugging in multicellular basidiomycetes. We set out to determine the function, the structure and the protein composition of the septal pore apparatus in the model agaricomycete *Coprinopsis cinerea* using a combination of microscopic, genetic and biochemical approaches. These findings will contribute to our understanding of cytoplasmic bulk flow dynamics within coenocytic systems.

P1.424 - The mechanisms behind *Aspergillus fumigatus* augmented virulence upon pollution exposure

J. Jorge[1], *T. Martins*[1], *P. Gonçalves*[1], *D. Mil-Homens*[2], *C. Silva Pereira*[1]
[1]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal, [2]Institute for Bioengineering and Biosciences (iBB) and Institute for Health and Bioeconomy (i4HB), Instituto Superior Técnico, University of Lisbon, Lisboa, Portugal

Prof. Silva Pereira lab has been studying the impact of acute exposure of a fungal metacommunity to pollutants. Our studies, revealed that exposure to the model pollutant Pentachlorophenol (PCP) led to the production of airborne fungal spores that were virulent, while spores from unpolluted conditions were not virulent. *Aspergillus fumigatus* strains isolated from pre-mortem larvae were moderately to highly virulent when came from polluted conditions or benign when collected from unpolluted conditions. Taxonomic and functional traits of the strains could not separate virulent from not virulent strains. In this work, we aimed to gain a deeper knowledge about the genetic regulatory mechanisms contributing to rise of fungal virulence in a polluted niche. We screened a transcription factor knock-out *A. fumigatus* library containing 397 single deletion mutants and identified the ones that most impact tolerance to PCP. These mutants (twelve) were selected to test *in vivo* infection capacity using *Galleria mellonella*. Health indexes, growth in solid medium and the spore's germination fitness were evaluated, which allowed us to reduce the number of mutants to four. Confirmation of the mutation by PCR and the number of colony forming units inside the larvae were evaluated as well. Two mutants were

chosen to perform RNA-seq experiments in the presence and absence of PCP in order to link a set of responsive genes/pathways to virulence. Results are being analyzed, but seem promising and will be validated by generating several deletion mutants (up to 10 of the most promising genes) and by their phenotypic characterization.

P1.425 - Deciphering the fungal-fungal and fungal-bacterial interactions in wood decay using omics approach

M. Qurashi[1], *M. Erjama*[1], *J. Österman-Udd*[1], *T. Lundell*[1]
[1]University of Helsinki, Department of Microbiology, Helsinki, Finland

Forests store enormous amounts of carbon in the form of deadwood, which is a physically and chemically demanding environment and substrate for microbes. Filamentous fungi, especially of *Basidiomycota* class *Agaricomycetes*, have been studied in respect to degradation of deadwood via white rot, brown rot, intermediate or soft rot type of wood decay. During this process, fungi interact with other fungi and bacteria inhabiting the same environment. Bacteria from *Acidobacteria, Pseudomonadota, Bacillota*, and *Actinobacteria* have been reported to exist in the same environments with wood decay fungi, but these interactions are not known yet. The current study aims to use omics approach, specifically meta-genomics and transcriptomics, together with metabolomics to study metabolic pathways and changes in gene and enzyme expression incurred due to these interactions. Another aim is to explore naturally present microbiomes of fungi and bacteria in forest deadwood samples.
We cultivated five species of wood decay fungi representing different decay types and substrate specificities (*Fomitopsis pinicola*, *Fomitopsis betulina*, *Fomes fomentarius*, *Phlebia radiata* and *Schizophyllum commune*) individually and in combinations to analyze their growth, hyphal interactions and metabolism on different media and wood substrate. Transcriptomal changes were analysed by RNA-Sequencing, and metabolic activities by enzyme assays and chromatography. Selected fungi and well-known bacterial isolates are likewise co-cultivated to monitor their genetic and biochemical responses. These simulations will provide us information on the biological events on-going during wood degradation processes. Microbial co-cultures may also contribute new natural products and bioactive secondary metabolites to be further investigated for potentiality towards applications.

P1.426 - Regulation of the citrate exporter-encoding *cexA* during *Aspergillus niger* citric acid fermentation

L. Karaffa[1], *A. Márton*[1], *V. Bíró*[1], *A. Tsang*[2], *C.P. Kubicek*[3], *E. Fekete*[1]
[1]University of Debrecen, Debrecen, Hungary, [2]Concordia University, Montreal, Canada, [3]TU Wien, Vienna, Austria

Two critical parameters of the *Aspergillus niger* citric acid fermentation are the high (14%) glucose levels and the suboptimal (<5 ppb) concentration of manganese(II) ions in the culture broth. To investigate the requirement for manganese deficiency, we compared the transcriptome of a hyper-producer *A. niger* strain at Mn(II) ion deficient (5 ppb) and Mn(II) ion sufficient (100

ppb) conditions. Mn(II) deficiency triggered a 110-times upregulation of the citrate exporter-encoding gene *cexA*. To test whether *cexA* upregulation is a derepression, we grew *A. niger* at both manganese deficiency and sufficiency, but with only 1% glucose. No citric acid accumulated and no *cexA* transcript was detected independently of the concentration of manganese(II) ions, suggesting that the metabolism under manganese deficiency may create a metabolite which induces *cexA*. We surmised that this could be citrate itself. To test this, we grew *A. niger* on 1% glucose, and pulsed the culture with citric acid, which led to expression of *cexA* independently of manganese(II) ion deficiency or sufficiency. We conclude that manganese(II) ions are not repressors of *cexA* transcription, but its upregulation is triggered by the accumulation of citric acid or a metabolite related to its metabolism.

P1.501 - Homothallic or heterothallic: a genomic investigation into the sexual capabilities of *Clonostachys rosea*

M. Karlsson[1], *A. Wilson*[2], *E. Piombo*[1], *M. Brandström Durling*[1], *S. Chaudhary*[1], *H. De Fine Licht*[2], *M. Dubey*[1]
[1]Swedish University of Agricultural Sciences, Forest Mycology and Plant Pathology, Uppsala, Sweden, [2]University of Copenhagen, Plant and Environmental Sciences, Copenhagen, Denmark

Modes of reproduction and sexual strategies strongly influence the genetic diversity and evolutionary potential of a species. The ascomycete fungus *Clonostachys rosea* is reported to be homothallic (sexually self-fertile), although a rapid decay of genome-wide linkage disequilibrium is also reported, which is not in line with an obligate homothallic mode of reproduction. To investigate this phenomenon, we identified the mating-type (*mat*) locus in 63 genome-sequenced *C. rosea* strains under the hypothesis that each strain contains genes from both *mat* idiomorphs. Eleven strains indeed contained both *mat1-1* and *mat1-2* genes, suggesting homothallism. However, most strains harboured either *mat1-1* or *mat1-2* genes and co-existed in North America, Europe and China, suggesting heterothallism. The *mat* locus of heterothallic strains was highly conserved, and the linkage disequilibrium half decay distance was 625 bp, suggesting sexual outcrossing. The presence of conserved *mat1-1* or *mat1-2* idiomorphs in strains of other *Clonostachys* species shows that heterothallism is the ancestral state. A phylogenetic analysis of 2800 single-copy orthologues revealed that homothallic and heterothallic strains clustered in two separate, well-supported clades, indicating a single lineage of homothallic *C. rosea*, followed by inter-continental dispersal. We discuss the evolutionary, genomic and applied consequences of this unique mixed-mode type of sexual reproduction.

P1.502 - Metabolite-mediated interactions of the mycoparasitic fungus *Trichoderma atroviride* with plant pathogenic and plant benefical microbes

S. Zeilinger[1], *D. Flatschacher*[2], *A. Eschlböck*[2], *A. Oberdanner*[2], *R. Schuhmacher*[3]
[1]University of Innsbruck, Department of Microbiology, Innsbruck, Austria, [2]University of Innsbruck, Innsbruck, Austria, [3]BOKU University, Tulln, Austria

Plant-beneficial microbes are realistic alternatives to synthetic chemicals for the protection of plants against pathogens. Common to all biocontrol products consisting of plant-protecting bacteria and/or fungi is the production of specialized metabolites as important determinants of their activity.
We used the mycoparasitic fungus *Trichoderma atroviride* as a model to study its interaction with fungal plant pathogens as well as with plant beneficial bacteria with a special focus on the role and effect of secreted metabolites. One of the main bioactive metabolites produced by *T. atroviride* is 6-pentyl-alpha-pyrone (6-PP). Deletion of the *pks1* gene responsible for 6-PP biosynthesis resulted in a significantly reduced inhibitory activity of *T. atroviride* against fungal plant pathogens evidencing a role of 6-PP in the mycoparasitic interaction. Accordingly, 6-PP localized ahead of the hyphal growth front in the pre-contact interaction zone of a *T. atroviride-B. cinerea* co-culture.
Co-cultivation with plant beneficial bacteria resulted in growth inhibition of *T. atroviride* in most of the interactions tested. Especially *Bacillus subtilis*, *Streptomyces spp.*, and *Pseudomonas spp.* significantly reduced radial growth of the fungus. Interestingly, the observed inhibitory effect of *P. protegens* was similar between the wild type strain and deletion mutants defective in the biosynthesis of DAPG, pyoluteorin, pyrrolnitrin, and orfamide A, indicating that additional substances contribute to the antifungal activity of this plant-protecting bacterium. On the other hand, metabolites secreted by *T. atroviride* inhibited bacterial growth which was most evident with *B. subtilis* and *P. putida.* Comparative metabolite fingerprinting revealed co-culture specific changes in metabolite production.

P1.503 - Characterization of the antifungal effect of *Bacillus amyloliquefaciens* against *Fusarium oxysporum*

A. Yáñez-Vilches[1], *F.M. Laddaga*[1], *C. Ruiz-Roldán*[1], *A. Di Pietro*[1]
[1]Universidad de Córdoba, Genetics, Córdoba, Spain

The soil-borne ascomycete *Fusarium oxysporum* comprises a large number of clonal isolates that cause devastating vascular wilt disease on more than 150 different crops. A promising alternative to chemical control is the use of biocontrol agents (BCAs) that inhibit the pathogen or improve plant defence. The bacterial BCA strain *Bacillus amyloliquefaciens* BO7 secretes an antifungal lipopeptide (LP) with a strong inhibitory effect against *F. oxysporum* both in vitro and in the rhizosphere of tomato plants (Romano et al. 2011, J Nat Prod). The aim of this study is to understand the inhibitory mechanism of the LP for its use in biocontrol and the development of new control strategies against *Fusarium* wilt. For that purpose, **t**he morphogenetic effect of BO7 LP on *Fusarium* was analyzed by light microscopy and through a variety of phenotypic assays such as drop tests or cellophane penetration assay. We found that the LP triggers a rapid response in *Fusarium* and exhibit a synergistic effect when applied in combination with cell wall destabilizing agents and affects β-tubulin. The morphological and developmental changes elicited by the LP negatively affect the invasive ability of the fungus, causing reduced pathogenicity (Vitullo et al. 2012, Plant Pathol.). In conclusion, *B. amyloliquefaciens* BO7 produces lipopeptides that reduce the pathogenicity of *F. oxysporum* making it a highly promising BCA.

P1.504 - Effect of volatile organic compounds from bacterial biocontrol agents against the tomato vascular wilt pathogen Fusarium oxysporum

C. Ruiz Roldán[1], *C. Navarro Laguna*[2], *M.J. Cardador Dueñas*[3], *L. Arce Jiménez*[3], *A. Di Pietro*[2]
[1]Univeristy of Cordoba, Faculty of Sciences, Genetics, Cordoba, Spain, [2]University of Cordoba, Faculty of Sciences, Genetics, Cordoba, Spain, [3]University of Cordoba, Faculty of Sciences, Analytical Chemistry, Cordoba, Spain

Chemical fungicides play an important role in the prevention of plant diseases caused by fungal pathogens, but their use entails important disadvantages. Currently, biological control with bacterial biocontrol agents (BCAs) is one of the most promising alternatives. Recent research suggests that volatile organic compounds (VOCs) produced by rhizosphere microorganisms play important roles in signaling and biocontrol. In this study, we examined the effect of VOCs from two rhizosphere isolates of the genus *Pseudomonas* (PICF6 and PICF7) on *Fusarium oxysporum* (Fo), a fungal phytopathogen that causes vascular wilt on a wide variety of crops. Using a sandwich plate assay, we detected a significant inhibition of microconidia germination by VOCs emitted by PICF6 and PICF7. Some of the emitted VOCs were identified by gas chromatography coupled to ion mobility spectrometry (GC-IMS) and mass spectrometry (GC-MS). Germination inhibition tests with some of the pure identified compounds confirmed their inhibitory effect on spore germination. Currently, we are testing the effectiveness of single VOCs in preventing vascular wilt disease caused by Fo. Our results suggest the existence of VOC-mediated molecular communication mechanisms between *Pseudomonas* and *Fusarium* that could be of interest for application in biological control.

P1.505 - Mycovirus infections of *Aspergillus fumigatus* are common

B. Auxier[1], *F. Reyes Marquez*[1]
[1]Wageningen University & Research, Laboratory of Genetics, Wageningen, Netherlands

There has been a recent rise in interest regarding mycoviral infections of various fungal species, including the human pathogen *Aspergillus fumigatus*. Such viral infections are thought to be potential modulators of virulence or other host interactions in this species. RNA-sequecing of *A. fumigatus* isolates has shown infection of viruses from the families Chrysoviridae, Polymycoviridae, Partiviridae and Narnaviridae among others. Despite several studies of reference isolates and clinical samples, the occurrence of these viruses in natural populations of *A. fumigatus* was unclear. Here, we sampled >400 isolates of *A. fumigatus* collected during routine air sampling in the Netherlands. To detect infections, we designed degenerate primers targeting conserved regions of the RNA-dependent RNA polymerase for four different viruses, and screened pools of isolates. We found that infection from these viruses was relatively common, for example ~2% infection with viruses related to AfuPMV-1. Sequencing of this conserved polymerase region showed abundant genetic variation, with the expected strong purifying selection on this core enzyme. The common occurrence of these viruses raises questions about transmission biology for both the fungus and the virus, as widespread

heterokaryon incompatibility is thought to limit the spread between individuals and the sexual cycle has been demonstrated to prevent mycoviral transmission in other *Aspergillus* species. Based on these results, the potential use of these viruses to limit fungal virulence should explore non-natural conditions.

P1.506 - Biological control of Colletotrichum acutatum with endophytic bacteria from olive drupes

A.G. Papageorgiou[1], *D.I. Tsitsigiannis*[1]
[1]Agricultural University of Athens, Department of Crop Science, Athens, Greece

Olive anthracnose (OA), caused by fungi of the *Colletotrichum* species, poses a significant threat to olive cultivation worldwide, leading to severe yield losses and degradation of olive oil quality. Among the *Colletotrichum* species, strains belonging to the *C. acutatum* species complex are dominant in Greece. The continuing withdrawal of active substances, combined with the adverse effects of chemical fungicides on the environment, underscores the need to explore more sustainable ways of managing OA. This study aimed to explore potential biological control agents and assess their impact on the severity of OA. As part of this objective, 210 endophytic bacterial isolates were tested for their antagonistic activity against *C. acutatum* through multiple and dual culture assays. Several isolates showed satisfactory inhibition of mycelial growth and were further tested in situ for their effect on disease severity and conidial production on detached olive drupes (*cv.* Kalamon). Statistically significant differences were observed in all bacterial treatments compared to the control, and the isolates identified through 16S rRNA gene sequencing. The four most effective isolates were further evaluated in artificially inoculated olive trees at the fruit ripening stage, under field conditions, for two consecutive years. Notably, isolate Π8 (*Serratia* spp.) reduced disease severity by 89.31%, while the other three isolates showed promising results. Field applications were also conducted during the flowering stage in olive cultivars *cv*. Kalamon and *cv*. Amfissis, where the bacterial isolates significantly reduced anthracnose severity in young fruits, with reductions up to 50% in *cv*. Kalamon and 36.8% in *cv*. Amfissis.

P1.507 - Endophytic bacteria from olive drupes as plant defense inducers against *Colletotrichum acutatum* in olive trees

A.G. Papageorgiou[1], *M. Varveri*[1], *D.I. Tsitsigiannis*[1]
[1]Agricultural University of Athens, Department of Crop Science, Athens, Greece

Olive anthracnose is one of the most important diseases affecting olive drupes worldwide and is caused by fungi belonging to the genus *Colletotrichum*. The predominant strains are associated with *C. acutatum* and *C. gloeosporioides* species complexes. Due to the withdrawal of several fungicides and the risk of pathogen resistance, finding more sustainable control measures of the disease, such as using biological control agents and plant-resistance inducers is considered essential. Some biological agents can act as inducers of plant defense mechanisms, while they can also combine more than one mode of action, which makes them more suitable for agricultural

use. This study aimed to evaluate antagonistic endophytic bacteria from olive drupes against *C. acutatum* for their ability to induce plant defense mechanisms. The experiments were conducted on young olive seedlings pre-treated with beneficial bacteria and/or artificially inoculated with the pathogen. The expression of ten defense genes was evaluated by RT-qPCR. All four tested bacterial strains showed increased expression of plant defense genes associated with Pathogenesis-related proteins (*PR10, Mpol*) compared to the controls, while the application of bacteria K13 (*Bacillus methylotrophicus*), B1 (*B. amyloliquefaciens*) and P8 (*Serratia* sp.) also triggered genes involved in biosynthetic pathways of phenylpropanoids and salicylic acid. Finally, *B. amyloliquefaciens* strain B1 also induced increased expression of the lipoxygenase (*LOX*) gene involved in the jasmonic acid biosynthetic pathway.

P1.508 - Innovative low-cost biopolymers from *Ixora coccinea* extracts: a new approach to antifungal control

P. Solis-Manrique[1], *A. Zapata-Dominguez*[1], *P. Ortiz-Montalvo*[1], *I. Cano-Parra*[1], *J. Gonzalez*[1], *Y. Pech-Canche*[1], *M. Reyes-Rodriguez*[1], *B. Millan-Chiu*[2], *S. Rincon-Arriaga*[1], *D. Magaña-Ortiz*[1]
[1]Tecnológico Nacional de México, Campus Mérida, Chemical, Biochemical and Environmental Engineering Department, Mérida, Mexico, [2]Universidad Nacional Autónoma de México, CONACyT—Centro de Física Aplicada y Tecnología Avanzada, Jurquilla, Mexico

Invasive fungal infections (IFIs) represent a critical challenge in health services, particularly for patients with compromised immune systems, including those receiving chemotherapy, organ transplants, or suffering from chronic diseases. Unfortunately, available therapies for IFIs have limited efficacy and cause severe side effects in mammalian cells.
The main purpose of this research was to evaluate antifungal activity of *Ixora coccinea*, a medicinal plant highly appreciated in Asian culture; however, its efficacy for IFIs has been inconsistently documented. To address this, ethanolic extracts of leaves (EE) was used to establish the minimum inhibitory concentrations (MIC) in macrodilution method for different species of fungi. Furthermore, two distinct biopolymer formulations were developed: one based on carboxymethyl cellulose (CMC) and another on citric pectin, each incorporating extracts of *Ixora coccinea*. These biopolymers were developed to facilitate the release of active compounds, targeting fungal pathogens effectively.
The MIC results exhibited notable antifungal activity, with 8.8 mg/mL against *Aspergillus foetidus*, 8.7 mg/ml against *Trichoderma longibrachiatum*, and in the case of *Candida* species isolated from human tissues the values were in the range of 5 to 10 mg/mL of EE. Also, it was determined that both biopolymer formulations present inhibition at a concentration of 14.8 mg/mL for *Aspergillus foetidus* and 10 mg/mL for *Trichoderma longibrachiatum*.
These results confirmed the antifungal effects of ethanolic extracts of *Ixora coccinea* and the possibility to formulate innovative biopolymers at low cost. Future research may develop better formulations, highly compatible with human tissues and with minor side effects in comparison to current antifungals.

P1.509 - Elucidating the role of oxidative stress-responsive genes in the biocontrol activity of *Papiliotrema terrestris*

G. Barone[1], *R. Castoria*[1], *F. De Curtis*[1], *M. Morel-Rouhier*[2], *G. Ianiri*[1]
[1]University of Molise, Department of Agricultural, Environmental and Food Sciences, Campobasso, Italy, [2]Université de Lorraine, Faculté des Sciences et Technologies, Nancy, France

Understanding the mode of action of biocontrol agents (BCAs) is foundational to promoting their use in sustainable agriculture. Reactive oxygen species (ROS) are involved in a number of biological processes, including microbe-plant interactions and plant immunity. ROS are produced by the plant/fruit tissues following damage and by fungal plant pathogens as a virulence mechanism, while yeast BCAs must be able to resist to ROS to be effective antagonists. Among the yeast BCAs, *Papiliotrema terrestris* gained attention for its control of fungal plant pathogens both in postharvest and in the field. RNAseq analysis during the tritrophic interaction *P. terrestris* – fungal pathogen – host revealed that competition for nutrients and oxidative stress response are the most important mechanisms operated by the BCA to counteract fungal infections. Here we focused on the ROS-responsive genes that are expected to play a role in the antagonistic activity of *P. terrestris*. Our analysis identified a set of genes, including a glutathione transferase, whose function in the antagonistic activity of *P. terrestris* has been evaluated through the generation of specifically targeted mutants that were subjected to extensive *in vitro* and *in vivo* phenotypic characterization. The findings of this study will contribute to potentiate the understanding of biocontrol systems aiming at their practical utilization in organics agriculture.

P1.510 - The bioactive substances as an alternative for synthetic fungicides and their impact on the diversity of soil fungi

P. Tarnawska[1], *A. Kalwasińska*[1], *K. Pałubicka*[2], *K. Dembińska*[1], *M. Swiontek Brzezinska*[1]
[1]Nicolaus Copernicus University, Department of Environmental Microbiology and Biotechnology, Toruń, Poland, [2]Nicolaus Copernicus University, Department of Conservation and Restoration of Paper and Parchment, Toruń, Poland

Diseases brought by parasitic fungi may arise due to intensive agricultural plant cultivation. To limit this effect, this study aimed to assess the new formulation's impact on fungal communities in arable soil and develop a defense against fungal pathogens for plant seedlings using natural ingredients. For testing against critical fungal pathogens — *Fusarium solani*, *F. oxysporum*, *F. culmorum*, *Botrytis cinerea*, and *Alternaria alternata*, three biocidal compounds — rutin, quercetin, and p-coumaric acid (p-CA), found in plants belonging to the *Brassicaceae* family were chosen. The synthetic fungicide Porter 250 EC was used to compare its effectiveness. The most effective antifungal activity was shown by p-CA among natural origin. When p-CA-based preparation was applied to the seeds of common crop plants, most tested pathogens showed a significant reduction in growth. When applied to soil, the p-CA-based formulation and Porter 250 EC significantly changed the fungal community structure at any time studied (T0, T14, and T28). They had an overall similar effect on soil fungal communities. Less than 2% of all ASVs were

affected by changes in the fungal community composition. During the fourth week of treatment, the formulation's effects on the soil microbiota were most noticeable.
In comparison to soil samples treated with Porter 250 EC, two ASVs linked to the plant pathogens *Botrytis* and *Chromelosporium*, as well as an unidentified ASV from *Diversisporales*, which includes the arbuscular mycorrhizal fungi (AMF), were significantly reduced in p-CA-treated soil samples. It was confirmed that p-CA-based preparations can be used instead of commercial fungicides.

P1.511 - Fungicidal properties of phenolic compounds against phytopathogenic fungi

K. Pałubicka[1], *M. Swiontek Brzezinska*[2], *J. Karbowska-Berent*[1]
[1]Nicolaus Copernicus University, Department of Conservation and Restoration of Paper and Leather, Toruń, Poland, [2]Nicolaus Copernicus University, Department of Environmental Microbiology and Biotechnology, Toruń, Poland

Farmers and gardeners are increasingly turning to natural pesticides that are less harmful to the environment and plants themselves. The fungicidal potential of a group of phenolic substances naturally occurring in plants has been identified. In this study, the fungicidal potential of p-coumaric acid, ferulic acid, caffeic acid, gallic acid, and tannic acid was tested against phytopathogens such as *Fusarium solani*, *F. culmorum*, *F. oxysporum*, *Botrytis cinerea*, *Alternaria alternata*, *Phoma lingam*, and *Sclerotinia sclerotiorum*. To identify the most effective compound, the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of these substances were determined. The most potent bioactive substances were selected for further studies. The next step involved creating an emulsion based on the most active phenolic compound, combined with emulsifiers and adjuvants, followed by the determination of MIC and MFC against the same plant pathogens. The final stage of the research evaluated the effects of seed coating on rapeseed, cucumber, and cabbage. The results showed that p-coumaric acid and ferulic acid had the best antifungal effects at the lowest concentrations, while the other three substances demonstrated fungicidal properties at higher concentrations. Emulsions based on p-coumaric acid or ferulic acid with emulsifiers and adjuvants were more effective at lower concentrations than pure bioactive substances. Additionally, coating rapeseed with an emulsion containing p-coumaric acid and the emulsifier ROCAnol DB7W provided the best pathogen inhibition. Coating cucumber seeds with ferulic acid-based emulsions also showed strong effectiveness in inhibiting mold growth."

P1.512 - Phenolic compounds as an alternative to fungicides for ground cucumber (*Cucumis sativus* L.)

K. Dembińska[1], *K. Pałubicka*[2], *M. Michalska-Sionkowska*[1], *M. Wróbel*[1], *A. Richert*[3], *M. Swiontek Brzezinska*[1]
[1]Nicolaus Copernicus University, Department of Environmental Microbiology and Biotechnology, Toruń, Poland, [2]Nicolaus Copernicus University, Department of Conservation and Restoration of Paper and Leather, Toruń, Poland, [3]Nicolaus Copernicus University, Department of Genetics, Toruń, Poland

Fungal pathogens are responsible for 70-80% of crop losses attributed to microorganisms. These pathogens can impair seed germination, negatively influence nutrient availability, and alter seed color. Currently, there is a growing demand for the development of fungal pathogen control methods aligned with sustainable and organic agriculture. One promising group of compounds that could be employed to prevent fungal diseases in plants are phenolic compounds. These substances are derived from plants, where they are synthesized under stress conditions. Phenolic compounds are involved in pigmentation, contribute to astringency, and serve as protective agents against UV radiation, insects, and other parasites.
To evaluate the potential of phenolic compounds in biocontrol, eight plant-derived substances (eugenol, thymol, carvacrol, cinnamaldehyde, geraniol, citral, tannic acid, and caffeic acid) were tested against fungal pathogens isolated from cucumber leaves. The minimum fungicidal concentration (MFC) of each compound was determined for the identified pathogens, which were subjected to molecular identification. The lowest MFC values were obtained for cinnamaldehyde, which was selected for further testing. The antifungal efficacy of an emulsion containing cinnamaldehyde was assessed both directly on cucumber seeds and as a foliar application on leaves.
The results indicate that cinnamaldehyde has potential as a fungicidal agent in cucumber cultivation; however, further analyses, including field trials, are necessary.

P1.513 - Tramesan, an exo-polisaccharide from *Trametes versicolor,* as resistance inducer in *Arabidopsis thaliana*

G. Gramegna[1], *M. Rizzato*[1], *E. Romanelli*[1], *M. Beccaccioli*[1], *V. Cecchetti*[1], *M. Reverberi*[1]
[1]Sapienza Università di Roma, Dipartimento di Biologia Ambientale, Rome, Italy

Fungal pathogens cause huge loss in crop production reducing the yield and quality of agricultural products. The intensive use of agrochemicals to counteract these pathogens leads to the emergence of resistant pathogens strains and poses several risks for the environment and human health.
The European regulation 128/2009 governing the use of pesticides has recently removed several products from the market, promoting the employment of natural compounds in agriculture.
In this context, Tramesan, an exo-polysaccharide derived from the basidiomycete *Trametes versicolor*, is emerging as a natural alternative to protect crops.
In fact, it has been previously demonstrated that Tramesan, in addition to inhibits aflatoxin biosynthesis by the mycotoxigenic fungus *Aspergillus flavus*, enhances the resistance of wheat against Septoria Leaf Blotch Complex by inducing plant defense responses.
To better understand the mechanisms of this elicitation, we explored the mode of action of Tramesan in the model plant *Arabidopsis thaliana*. In particular, we observed that treatment with Tramesan enhances the resistance to *Botrytis cinerea* in Arabidopsis leaves. Therefore, we evaluated both the direct antifungal effect of Tramesan as well as its ability to induce plant immune responses, such as induction of defense genes, H_2O_2 accumulation and hormones production. These analyses indicate that Tramesan has only a slight inhibitory effect on Botrytis growth while it has the ability to induce Arabidopsis defense responses to the fungus.

Moreover, our results suggest that Tramesan could act as a priming agent, by enhancing chitin induced gene expression in Arabidopsis seedlings.

P1.514 - Biocontrol of phytopathogenic fungi using wild cyanobacteria biomass

D. Burokienė[1], *D. Čepukoit*[1], *J. Koreivienė*[2], *I. Sokė*[1], *S. Jaseliūnaitė*[1]
[1]Nature Research Centre, Laboratory of Plant Pathology, Vilnius, Lithuania, [2]Nature Research Centre, Laboratory of Algology and Microbial Ecology, Vilnius, Lithuania

Plant pathogenic microorganisms pose a major threat to agricultural crops and their products. The damage caused by these organisms causes economic losses for the Lithuanian agricultural sector and has a negative impact on global trade. Their activities also contribute to climate change. Farmers are increasingly using synthetic pesticides to protect their crops from pathogens. However, this practice can increase environmental pollution and endanger human and animal health. The aim of this study is to identify sustainable and environmentally friendly methods to control pathogenic microorganisms that can be used in the production of biopesticides as a sustainable biocontrol tool.
To achieve this goal, cyanobacteria from blooming water bodies that have a negative impact on Lithuanian aquatic ecosystems are analyzed. The study evaluates cyanobacteria as a potential source of biopesticides by analyzing the impact and efficacy of their biomass and biological extracts on pathogenic organisms. The antagonistic activity of cyanobacterial biocomponents against 15 different agricultural pathogens, including fungi (with emphasis on the genera *Alternaria, Botrytis, Chalaropsis, Diaporthe, Fusarium, Hymenoscyphus, Mycocentrospora, Rhizoctonia*) and oomycetes (*Phytophthora*), is analyzed. The most effective identified strains of cyanobacteria are proposed for the production of biopesticides.
This research is funded by the Lithuanian Research Council (project No P-MIP-24-699).

P1.515 - Exploring biocontrol potential of non-aflatoxigenic *Aspergillus flavus* isolates in aflatoxins mitigation: Insights into population dynamics and genetic profiles

M. Varveri[1], *V. Mpartzis*[1], *S. Dimitropoulou*[1], *M. Kaminiaris*[1], *D. Tsitsigiannis*[1]
[1]Agricultural University of Athens, Department of Crop Science, Laboratory of Plant Pathology, Athens, Greece

Eco-friendly strategies for effective aflatoxin mitigation seem to be a necessity to ensure food and feed safety worldwide. The most effective approach for preharvest aflatoxin management is the use of non-aflatoxigenic *Aspergillus flavus*strains. Several conducted experiments showcased aflatoxin inhibition in rates above 90%, by selected Greek endemic non-aflatoxigenic *A. flavus* strains based on *in-vitro*, *ex-planta* and *in-field* experiments in maize kernels and pistachios. Additionally, the biocontrol potential of 14 selected most promising non-aflatoxigenic strains was further confirmed, during a three-year field experiment in maize, presenting significant reductions in aflatoxin contamination in rates up to 100%, as compared to the control.

Adaptability to the local environment was subsequently investigated, by subjecting selected isolates in Vegetative Compatibility Analysis (VCA). Results demonstrated that the pistachio Greek non-aflatoxigenic *A. flavus* isolates ranked in 56 distinct VC groups, while the maize isolates in 23 VCGs, proving their widespread distribution in Greek territory. Moreover, a plausible overlapping of maize and pistachio VCGs was examined to identify population patterns between the two plant hosts. A study on Cluster Amplification Patterns among the 14 selected non-aflatoxigenic isolates was carried out, towards the exploration of deleted genes among the aflatoxin, cyclopiazonic acid and sugar clusters. Results revealed a great genetic diversity within non-aflatoxigenic strains, varying from single to multiple gene deletions in the clusters of interest. The findings of this study contribute to the establishment of biological control systems, aiming to the prevention of food crises and the reassurance of safe food trade free of aflatoxins.

P1.516 - Endophytic mycobiome of conifer trees promotes seedling growth and mitigates negative effects of root and stem rot pathogen (*Heterobasidion* spp.)

F. Asiegbu[1], *K. Wang*[2], *Z. Wen*[1]
[1]University of Helsinki, Department of Forest Sciences, Helsinki, Finland, [2]Fujian Agriculture and Forestry University, College of Forestry, Fuzhou, China

Mycobiome of forest trees play diverse functional roles in host fitness including growth promotion and increased defence. However, little is known about the impact on transcriptome and metabolites of the mycobiota during tripartite interaction with host and pathogen. To understand the transcriptional regulation of endophytic mycobiota during co-infection, conifer tree seedlings were infected with either ectomycorrhiza *Suillus luteus*, or *Tricholoma matsutake* or dark septate endpphyte (DSE) *Phialocephala sphaeroides*, or with root pathogen *Heterobasidion spp* or with both. The results showed that all the endophytes promoted the root growth of the seedlings. The DSE *P. sphaeroides* showed low but stable transcripts secretion (a decrease of 40%) during interaction with *P. abies* and conifer pathogen. By contrast, *H. parviporum* transcripts were significantly reduced (92%) in tripartite interaction with the endophyte and host. The *P. sphaeroides* transcripts experienced a shift from cell growth to anti-stress and antagonistic responses, while it repressed the ability of *H. parviporum* to access carbohydrate nutrients by suppressing its lignocellulose degrading enzyme machinery. The pathogen secreted cysteine peptidase to restrict free growth of *P. sphaeroides*. The expression of plant growth promotion genes by *P. sphaeroides* and pathogen *H. parviporum* were equally detected. This was supported by presence of tryptophan-dependent indolic in culture of *P. sphaeroides*. Norway spruce and Arabidopsis seedlings treated with *P. sphaeroides* culture filtrate exhibited auxin-like phenotypes, such as enhanced root hairs, and root elongation. The endophyte repressed the expression of host defense-related genes but increased the transcripts of host genes involved in plant hormone signal transduction.

P1.517 - Transcriptional dynamics of carbohydrate-active enzymes in the mycoparasite *Clonostachys rosea*

L. Atanasova[1]
[1]University of Natural Resources and Life Sciences, Vienna (BOKU), Department of Biotechnology and Food Sciences, Vienna, Austria

Biocontrol strategies utilizing mycoparasitic fungi have gained significant attention in sustainable agriculture. *Clonostachys rosea*, a versatile fungus capable of mycoparasitism, saprotrophy, and biotrophy, shows promise as a biocontrol agent against various plant pathogens. We employed transcriptional profiling to investigate *C. rosea*'s gene expression patterns under distinct conditions: two stages of mycoparasitism (against its natural host *Fusarium culmorum*), saprotrophy (growth on cellulose and wheat straw), and biotrophy (interaction with living plant roots). Our analysis focused on carbohydrate-active enzymes (CAZymes) and revealed distinct transcriptional patterns across the three nutritional modes, with involvement of an array of auxiliary activities enzymes, particularly those with an oxidative function. Our findings provide insights into the adaptive mechanisms of *C. rosea*, highlighting the role of CAZymes in its versatile lifestyle. This research advances our understanding of fungal-fungal and fungal-plant interactions of mycoparasites, and will further focus on the functional characterization of key CAZymes identified, with the aim of understanding *C. rosea*'s life style.

P1.518 - Exploring the evolution of biocontrol yeast *Aureobasidium pullulans* across Europe: a multi-locus analysis

N. Khomutovska[1], *A. Di Francesco*[2], *J.A. Stenberg*[1]
[1]Swedish University of Agricultural Sciences, Department of Plant Protection Biology, Lomma, Sweden, [2]Udine University, Department of Agriculture, Food, Environmental and Animal Sciences, Udine, Sweden

Investigating the functional diversity of beneficial wild microorganisms is crucial for identifying effective biocontrol agents for sustainable management of phytopathogens in agriculture. The black yeast-like fungus *Aureobasidium pullulans* is a potent biocontrol agent, and a model organism for studying the evolution of functional traits involved in biocontrol across diverse European environments.
This study assessed the genetic and biocontrol diversity of *A. pullulans* strains associated with the wild strawberry across Europe. We analyzed over 200 strains using a combined approach, including multi-locus sequencing of the internal transcribed spacer (ITS) and elongase (ELO) genes. We employed phylogenetic methods and comparative analysis with reference sequences from existing databases to explore strain diversity. Biocontrol efficacy against *Botrytis cinerea* was also evaluated, revealing regional strain-specific variation.
Our findings underline the importance of integrating genetic and functional analyses to guide the regional selection of effective biocontrol strains for sustainable agriculture.

P1.601 - The development of a Tet-off system in Mucorales unveils the crucial role of DNA 6mA in pathogenesis

C. Lax[1], *M. Sanchís*[2], *Y. Ahmiane*[2], *E. Navarro*[1], *J. Capilla*[2], *F.E. Nicolás-Molina*[1], *V. Garre*[1]
[1]University of Murcia, Genetics and Microbiology, Murcia, Spain, [2]Universitat Roviria i Virgili, Ciències Mèdiques Bàsiques, Reus, Spain

The scarcity of available molecular tools in early-diverging fungi (EDF) has hindered the study of crucial aspects of their biology compared to higher fungi (or Dikarya). Some species of Mucorales are opportunistic human pathogens causing a lethal fungal disease known as mucormycosis. To unravel the molecular mechanisms underlying this virulence, tools are needed to precisely characterize the involvement of specific genes in the infection process. In this study, we adapted a tetracycline-inducible (Tet-off) expression system to characterize genes in in vivo infection models. The development of this system was made possible by the previous development of genetic modification tools in the fungus *Rhizopus microsporus*. Only the precise adaptation of the components of this system to the particularities of this fungus enabled their correct expression and function. To validate the developed system, we used the *mta1* gene encoding an adenine methyltransferase responsible for 6mA deposition, an essential epigenetic modification in the DNA of this fungus. In vitro assays demonstrated the effect of tetracycline in regulating this gene under the control of this system. In vivo murine infection studies further demonstrated the functionality of the system and the crucial role of adenine methylation in the infection process. The tool developed here can be a key platform for characterizing genes that are determinants in the pathogenesis of Mucorales.

P1.602 - Identification and characterization of compounds that inhibit *Candida albicans* morphogenesis

N. Case[1], *Y. Guo*[1], *M. Yoshimura*[2], *T. Sonoda*[2], *T. Kaizuka*[2], *M. Itou*[2], *H. Hirano*[2], *Y. Yashiroda*[2], *H. Osada*[2], *M. Yoshida*[2], *C. Boone*[1], *L. Whitesell*[1], *N. Robbins*[1], *L. Cowen*[1]
[1]University of Toronto, Toronto, Canada, [2]RIKEN, Wako, Japan

The limited armamentarium of clinical antifungals necessitates the exploration of novel approaches. For the human fungal pathogen *Candida albicans*, the morphogenetic transition between yeast and filamentous states is tightly linked to virulence. Thus, targeting filamentation may serve as a promising anti-virulence strategy. To this end, we developed a high-throughput and quantitative, dual-strain screen strategy to distinguish molecules that inhibit filamentation from those that inhibit growth. Using this approach, we screened ~50,000 compounds and identified 259 putative inhibitors of *C. albicans* filamentation, 16 of which were prioritized for their ability to inhibit filamentation but not growth. We subsequently focused on three structurally diverse compounds (UT1, NPD1, and NPD2) and through epistasis experiments we predict the target(s) of all three compounds are either downstream of a central regulator of filamentation, namely protein kinase A, or act on a parallel pathway. While mode of action studies are ongoing, western blot analysis determined that protein levels of Efg1, a key transcription factor downstream of protein kinase A, are reduced upon UT1 treatment under

filament-inducing conditions. Experiments also suggest NPD2 may act as a cationic amphiphilic drug with different cellular targets important for either filamentation or growth at neutral and alkaline pH, respectively. Lastly, we found both NPD1 and NPD2 inhibit biofilm formation, suggesting potential therapeutic utility. Together, this work identifies novel inhibitors of *C. albicans* filamentation, with implications for the development of anti-virulence therapies.

P1.603 - Comparative genomic and phenotypic analysis of Candida albicans strains isolated from different niches within the human body

P. Rintarhat[1], *T. Kim*[1], *D. Croll*[2], *W. Jung*[1]
[1]Chung-Ang University, Systems Biotechnology, Anseong, Korea, Republic of, [2]University of Neuchâtel, Evolutionary Genetics, Neuchâtel, Switzerland

Candida albicans is a commensal yeast residing in the human gastrointestinal tract but can transition from a harmless organism into an opportunistic pathogen under specific conditions. Previous studies have suggested that genetic and phenotypic variations in *C. albicans* clinical isolates may influence its ability to cause disease. In this study, we hypothesized that *C. albicans* residing in different body niches may adapt distinctly, which could impact their genotypes and phenotypes, including the pathogenic potential of the fungus. *C. albicans* strains were obtained from different body niches: fecal samples and gut mucosa of patients with ulcerative colitis or healthy individuals, as well as blood isolates from patients with candidemia. Phenotypic characteristics such as drug susceptibility to multiple antifungal agents, hyphal formation, biofilm production, and virulence were investigated. Our findings revealed distinct phenotypic traits among the *C. albicans* strains. Strains isolated from the mucosa of healthy individuals exhibited decreased biofilm formation and increased survival rates in the *Galleria* model, while strains from the blood of patients displayed reduced hyphal formation and lower larval survival rates. Genomic analyses, including genome-wide association studies and copy number variation analysis, are currently underway, and the results will be presented. Overall, our data suggests that niche-specific environments may drive variation in *C. albicans* isolates.

P1.604 - Understanding *Rhizopus v*irulence in mucormycosis

A. Guerrero[1], *D. Stark*[2], *D. E. Corzo-León*[3], *J. King*[2], *E. R. Ballou*[3], *J. K. Uehling*[1]
[1]Oregon State University, Botany and Plant Pathology, Corvallis, United States, [2]University of Sheffield, Biosciences, Sheffield, United Kingdom, [3]University of Exeter, Exeter, United Kingdom

Mucormycosis is a dangerous emerging infectious disease for which there are few effective treatments. *Rhizopus microsporus* (Mucorales, Mucoromycota) is among the fungi that cause mucormycosis. This fungus and its pathogenic relatives frequently harbor Burkholderiaceae-related endobacteria (BRE). It has been demonstrated that BRE maintain the genetic and functional capacity to produce secondary metabolites. These products have the potential to manipulate host immune defenses, facilitate fungal invasion and pathogenesis. Some evidence suggests that endosymbionts of clinical *Rhizopus* strains may impart similar benefits by

weakening human immune systems. However, not all clinical *Rhizopus* strains contain endosymbionts. Thus, it is unclear how and to what degree endobacteria contribute to fungal pathogenicity in humans. Our goal is to understand how bacterial – fungal dynamics relate to virulence. We will leverage a library of dozens of clinically and environmentally derived *Rhizopus* strains to meet this goal. The proven ability to mix and match endosymbionts, recent developments in CRISPR genome editing, and the application of robust virulence assays will allow us to test specific fungal genes and bacteria for contributions to pathogenicity. We expect this research to provide insights into the role of endobacterial and fungal virulence factors in the development of mucormycosis, ultimately improving our biological understanding of a deadly and increasingly common disease.

P1.605 - Elucidating the antifungal modes of action of G-quadruplex-stabilising ligands in A. fumigatus

I. Storer[1], *G. Middleton*[1], *E. Barclay*[2], *J. McColl*[3], *B. Lezé*[1], *Z. Waller*[4], *S. Bidula*[1]
[1]University of East Anglia, School of Chemistry, Pharmacy and Pharmacology, Norwich, United Kingdom, [2]John Innes Centre, Bioimaging Facility, Norwich, United Kingdom, [3]University of East Anglia, Henry Wellcome Laboratory for Cell Imaging, Norwich, United Kingdom, [4]University College London, School of Pharmacy, London, United Kingdom

Invasive aspergillosis, caused by fungal pathogens in the genus *Aspergillus*, causes almost 2 million deaths each year. Due to the emergence of antifungal resistance, the drugs we use to treat fungal infections are becoming increasingly ineffective. Therefore, new drugs with novel mechanisms of action are urgently needed. We have shown that ligands that stabilise G-quadruplexes (G4s), four-stranded secondary structures found in DNA and RNA, prevent the growth of *A. fumigatus, Candida* spp., and dermatophytes. Here, we show that the G4-stabilising ligand, PhenDC3, increased the number of stable G4s in A. fumigatus RNA. Notably, spores treated with PhenDC3 became swollen, but did not germinate. This increase in spore size was associated with a significant increase in the thickness of the cell wall. Similarly, another G4-stabiliser, pyridostatin, prevented germination and spore swelling. TEM imaging indicated that PhenDC3 could significantly impact organelle organisation. These impacts were explored further by imaging the nuclei, mitochondria, cell membrane, peroxisomes, and vacuoles. Finally, we investigated transcription using RNAseq and uncovered differential expression of genes associated with primary metabolism upon PhenDC3 treatment. These genes are predicted to contain G4s by prediction software, suggesting G4 sequences in these genes were stabilised by PhenDC3, preventing transcription. This work describes the first steps in identifying the target or targets of G4-stabilising ligands PhenDC3 and pyridostatin to guide the design of fungal-specific DNA/RNA-binding antifungal agents.

P1.606 - Impact of multi-fungicide resistance on the fitness of *Aspergillus fumigatus*

S. Kanellopoulos[1], *T. Bayraktar*[1], *D. Aanen*[1], *E. Snelders*[1], *B. Auxier*[1]
[1]Wageningen Univeristy, Genetics, Wageningen, Netherlands

Aspergillus fumigatus is a ubiquitous fungus that proliferates in plant degrading material. It easily distributes through the wind-borne dispersal of its asexual spores. These spores can infect people, leading to various diseases, which are best treated with clinical antifungal compounds. Increasingly *A. fumigatus* strains are detected that exhibit multi-fungicide cross-resistance to clinical and agricultural antifungals, challenging treatment. Strains of *A. fumigatus* that are resistant to agricultural fungicides, seem to be genetically clustered, separate from susceptible strains. Resistance to a compound is usually associated with a fitness penalty that is often mitigated with compensatory mutations, but little is known about the existence of any compensatory mutations in the wider context of multi-fungicide resistance in *A. fumigatus*. In the current project, we investigate the divergence between resistant and sensitive isolates due to a potential reduced fitness of the sexual offspring. We hypothesize that the independent segregation of compensatory mutations from antifungal resistance genes reduces the fitness of hybrid offspring. In the scope of this research, the mutations that have been correlated are responsible for multi-fungicide resistance are experimentally characterized by introducing them to susceptible *A. fumigatus* with CRISPR/Cas9 knock-ins. Finally, the CRISPR/Cas9 mutants will be tested for their fitness and virulence with a competition model and *Galleria mellonella* as a host. The outcome of this research can provide information on how broad fungicide use with multiple different classes of antifungal compounds can create reproductive barriers, leading to divergence within this species.

P1.607 - Genetic and phenotypic diversification of Aspergillus fumigatus during persistent infection

S. SahoShibata[1], *T. Arai*[1], *L. Alsharqi*[2], *A. Watanabe*[1], *D. Hagiwara*[3], *D. Armstrong-James*[2], *H. Takahashi*[1]
[1]Medical Mycology Research Center, Chiba University, Chiba, Japan, [2]Imperial College London, London, United Kingdom, [3]Tsukuba University, Tsukuba, Japan

Aspergillus fumigatus is a ubiquitous fungus and the primary causal agent of aspergillosis. It infects millions of people annually, particularly those with compromised immune systems. Mortality rates range from 40% to 90% among high risk groups. During infection *A. fumigatus* has been shown to accumulate genomic mutations, leading to phenotypic changes, such as drug resistance, during infection. However, the mechanisms underlying genetic and phenotypic diversification of pathogens during persistent infections remain poorly understood. This lack of understanding complicates the development of effective treatment strategies. This study analyzed 28 strains collected over 8-years from a single patient, using whole-genome sequencing and SNP comparison to characterize genetic and phenotypic diversity. Phenotypic characterization involved examining six parameters to clarify morphological and physiological features. As a

result, four genotypic groups with distinct phenotypes were identified. Strains with different genotypes coexisted within the patient over the course of the infection. This study highlights the importance of accounting the emergence of phenotypically diverse populations when establishing treatment regimens. Further research is needed to understand pathogen diversification within the host body during infection.

P1.608 - Translation regulation in response to host stresses in the pathogenic yeast *Candida glabrata*

A. Rana[1], *A. Thakur*[1]
[1]Regional Centre For Biotechnology, Faridabad, India

Candida glabrata, an opportunistic fungal pathogen of humans, exhibits heightened stress tolerance, drug resistance, and pathogenicity. While significant progress has been made in understanding the mechanisms behind these efficient stress adaptations, the role of translation regulation still needs to be fully elucidated. Our study found that two significant host-induced stresses, oxidative stress and amino acid starvation, reduce global translation in *C. glabrata* by activating the Gcn2 kinase, which subsequently activates Gcn4, a master transcription factor. Our findings demonstrate that Gcn2 and Gcn4 are critical for *C. glabrata's* stress adaptation and survival in the host environment. RNA-sequencing of *gcn4* mutants subjected to stress revealed significant alterations across numerous metabolic pathways, including proline metabolism. Metabolic flexibility is necessary for adaptation to the host's stressful niches, with proline abundantly present in the host systems. Many pathogenic fungi utilise proline as nitrogen and carbon sources, underscoring the importance of proline catabolism. Through this study, we show that proline utilisation depends on the Gcn2 -Gcn4 pathway, with Gcn4 directly regulating the major players of the proline pathway. Disruptions in proline metabolism further compromise *C. glabrata's* virulence and survival in the host. Overall, this research highlights the pivotal role of translational regulation in *C. glabrata's* adaptation to host-induced stresses and its pathogenicity.

P1.609 - Endoplasmic Reticulum function contributes to caspofungin tolerance in *Cryptococcus neoformans*

X. Qu[1], *G. Hu*[1], *J. Kronstad*[1]
[1]Michael Smith Laboratories, University of British Columbia, Microbiology and Immunology, Vancouver, Canada

Echinocandin drugs are antifungal agents that target the cell wall by inhibiting β-1,3-glucan synthesis. Widely used in clinical settings, echinocandins are effective against Aspergillus and Candida species. However, they are not effective against *Cryptococcus neoformans*, the fungus responsible for life-threatening meningoencephalitis in immunocompromised individuals. Caspofungin does inhibit *C. neoformans* β-1,3-glucan synthase at high concentrations, but not achievable in a clinical setting. The mechanisms leading to echinocandin tolerance in *C. neoformans* are emerging and are associated with calcineurin signaling, and cell wall and membrane composition. The synergistic effects of caspofungin have been studied in combination

with various inhibitors in *C. neoformans* including FK506 (calcineurin inhibitor), manumycin (disrupt Ras pathway signaling), clorgyline (monoamine oxidase), 2BP (palmitoyltransferase inhibitor), and SDS. One hypothesis suggests that the polysaccharide capsule or cell wall components may block permeability to caspofungin, preventing it from reaching its target enzyme. To test this hypothesis, we examined the impact of other inhibitors that influence capsule. Previous studies revealed that *C. neoformans* strains exhibit reduced capsule formation when exposed to tunicamycin, a protein glycosylation inhibitor. Our experiments revealed a synergistic effect between caspofungin and tunicamycin, with a significant reduction in both capsule and chitin levels when the drugs were combined. We propose that tunicamycin disrupts capsule formation to enable caspofungin to penetrate the polysaccharide structure, target the cell wall, and inhibit growth. Our analysis offers further evidence of the endoplasmic reticulum's role in capsule formation and provides new insights into the connection between caspofungin treatment and ER function in *C. neoformans*.

P1.610 - G-quadruplexes as novel antifungal targets

G. Middleton[1]
[1]University of East Anglia, Chemistry, Pharmacy and Pharmacology, Norwich, United Kingdom

Fungal infections are responsible for 3.75 million deaths a year.[1] The rapid and global emergence of azole resistance means that new agents with novel mechanisms of action are urgently required. G-quadruplexes (G4s) and intercalated motifs (i-motif) are two examples of four-stranded secondary structures found in DNA and RNA. They play key regulatory roles in the genome and thus have emerged as antimicrobial targets. However, their roles in pathogenic fungi are currently unknown.[2] We found that the G4-stabilising compounds, PhenDC3 and pyridostatin (PDS), inhibit the growth and metabolism of various pathogenic *Aspergillus* and *Candida* species. Surprisingly, these stabilisers had increased potency against *A. fumigatus* isolates that had developed resistance to at least one azole. Crucially, resistance to PhenDC3 did not appear by passage 10, double the number of passages which induced azole resistance, and no cross-resistance to other antifungals emerged following repetitive PhenDC3 exposure. PhenDC3 could also synergise with amphotericin B and was protective in an invertebrate model of fungal infection. Finally, we show that PhenDC3 had low cytotoxicity and genotoxicity towards human A549 lung carcinoma cells and primary vascular smooth muscle cells. These insights suggest that the development of new drug molecules targeting G4s represents a promising future therapeutic option to overcome drug-resistant fungal infections.

P1.611 - Aspergillus fumigatus conidial surface-associated proteome reveals factors for fungal evasion and host immunity modulation

C. Pinzan[1], *C. Valero*[2], *P. de castro*[3], *J. Silva*[1], *K. Earle*[2], *H. Liu*[4], *M. Horta*[1], *O. Kniemeyer*[5], *T. Krüger*[5], *A. Pschibul*[5], *D. Cömert*[5], *T. Heinekamp*[5], *A. Brakhage*[5], *J. Steenwyk*[6], *M. Mead*[7], *N. Hermsdorf*[5], *S. Filler*[4,8], *N. Garzon*[1], *E. Delbaje*[1], *M. Bromley*[2], *H. Cabral*[1], *C. Diehl*[1], *C. Angeli*[9], *G. Palmisano*[9], *A. Ibrahim*[4,8], *D. Rinker*[7], *T. Sauters*[7], *K. Steffen*[7], *A. Gumilang*[7], *A. Rokas*[7], *S. Gago*[2], *T. Reis*[1,10], *G. Goldman*[1,10]

[1]University of Sao Paulo, Ribeirao Preto, Brazil, [2]University of Manchester, Manchester, United Kingdom, [3]University of Sao Paulo, Ribeirão Preto, Brazil, [4]Harbor-UCLA Medical Center, Torrance, United States, [5]Friedrich Schiller University, Jena, Germany, [6]University of California, Berkeley, United States, [7]Vanderbilt University, Nashville, United States, [8]David Geffen School of Medicine at UCLA, Los Angeles, United States, [9]University of Sao Paulo, Sao Paulo, Brazil, [10]National Institute of Science and Technology in Human Pathogenic Fungi, Sao Paulo, Brazil

The section *Fumigati* is composed of *Aspergillus* fungal species presenting variable pathogenicity levels. *Aspergillus fumigatus*, an opportunistic pathogen, belongs to this section and is responsible for approximately 70% of cases of invasive pulmonary aspergillosis (IPA). The establishment of IPA depends on the inhalation of asexual spores (conidia) that trigger host infection. Thus, conidia represent the first point of contact between the fungus and human cells and, therefore, are important for the establishment of IPA. Despite its importance in the initial steps of IPA, there is scarce information about conidial surface proteins of *A. fumigatus* involved in fungal evasion and host immunity modulation. We analysed the conidial surface proteome (surfome) of *A. fumigatus*, two closely related non-pathogenic species, *Aspergillus fischeri* and *Aspergillus oerlinghausenensis*, as well as pathogenic *Aspergillus lentulus*, to identify such proteins. From 62 proteins exclusively detected on the *A. fumigatus* surfome, we constructed null mutants for 42 genes encoding these proteins. Deletion of 33 of these genes altered the fungal susceptibility to macrophage, epithelial cells and cytokine production. The gene encoding a putative glycosylasparaginase was characterized in detail and demonstrated its importance in modulating the levels of host proinflammatory cytokines and contributing to virulence in an immunocompetent murine model of IPA. Other genes are also in the process of being characterized. In summary, our results suggest that the conidial surfome of *A. fumigatus* encompasses proteins that are important for evasion and modulation of the immune response at the onset of fungal infection.
Funding: FAPESP, Brazil and NIH-NIAID, USA

P1.612 - Biological boundary conditions regulate the internalization of Aspergillus fumigatus conidia by alveolar cells

N. Schiefermeier-Mach[1], *J. Polleux*[1], *L. Heinrich*[1], *L. Lechner*[1], *S. Perkhofer*[1]
[1]Health University of Applied Sciences Tyrol/FH Gesundheit Tirol, Research and Innovation Unit, Innsbruck, Austria

Biological boundary conditions, such as cell shape, density, extracellular matrix composition, substrate stiffness and mechanical forces, play a critical role in regulating cellular behaviour, particularly in complex environments like the lung. The unique geometry of the alveoli and the mechanical forces during respiration generate distinctive boundary conditions that influence cellular responses in health and disease. While these factors have been studied in bacterial infections, their role in pulmonary fungal infections remains unexplored.
We hypothesize that boundary conditions influence the host's ability to internalize and process fungal conidia. In this study, we used A549 Lamp1-NeonGreen cells as a model for alveolar epithelial cells. Micropatterned substrates of different shapes and sizes were employed to explore

how boundary conditions impact the uptake and trafficking of *A. fumigatus* conidia. We observed a non-homogeneous distribution of fungal conidia, with notable differences across micropattern shapes, cell densities and substrate coatings. Constraining cells in specific geometries altered mechanical tension and cytoskeletal dynamics, affecting phagolysosomal internalisation efficiency. Additionally, modifying the extracellular matrix composition influenced integrin signalling and conidia trafficking routes. Our results suggest that the mechanical and biochemical environment of alveolar cells is a key determinant of pathogen internalization and trafficking. This study underscores the importance of boundary conditions in shaping lung host-pathogen interactions.

P1.613 - Antifungal mode of action of NFAP2 in Candida albicans based on transcriptomic data

K. Dán[1,2], *N. Zsindely*[3], *K. Laczi*[1], *C. Papp*[1], *A. Farkas*[4], *L. Bodai*[3], *L. Galgóczy*[1]
[1]University of Szeged, Faculty of Science and Informatics, Department of Biotechnology and Microbiology, Szeged, Hungary, [2]University of Szeged, Faculty of Science and Informatics, Doctoral School of Biology, Szeged, Hungary, [3]University of Szeged, Faculty of Science and Informatics, Department of Biochemistry and Molecular Biology, Szeged, Hungary, [4]HUN-REN Biological Research Centre, Institute of Plant Biology, Szeged, Hungary

The prevalence of fungal infections caused by antifungal drug-resistant strains is continuously increasing. Therapeutic application of a small molecular weight, cationic and disulfide bridge-stabilized extracellular antifungal protein, the NFAP2, secreted by *Neosartorya* (*Aspergillus*) *fischeri* NRRL 181 may overcome this problem. NFAP2 is a promising drug candidate due to its ability to inhibit the growth of both planktonic and biofilm-forming cells of various *Candida* species *in vitro* and *in vivo*. However, its antifungal mechanism remains unclear. Our previous observations suggest that NFAP2 disrupts the cell membrane when applied at its minimum inhibitory concentration (MIC). Below the MIC, NFAP2 is taken up by *Candida albicans* cells, localizes intracellularly and exerts long-term antifungal effects by interfering with cell functions. Understanding its mode of action is crucial to facilitate reliable therapeutic application of NFAP2. In this study, we explored the transcriptomic changes in *C. albicans* following treatment with sublethal concentrations of NFAP2 to gain insight into its long-term antifungal effects. The transcriptomic data showed that genes involved in membrane transport and metabolic processes were down-regulated, while genes associated with stress responses related to cell wall integrity, starvation and (negative) regulation of filamentous growth were up-regulated. These findings support our previous observations in *C. albicans*, such as slow growth kinetics in the presence of NFAP2 and inhibition of biofilm formation.
Present work of K.D. was financed by the NKFIH, MEC_R 149040 project. Present work of L.G. was financed by the Hungarian National Research, Development and Innovation Office - NKFIH, FK 134343 and K 146131 projects.

P1.614 - Comparative genomic and phenotypic analysis of *Candida albicans* strains isolated from different niches within the human body

P. Rintarhat[1], *T. Kim*[1], *D. Croll*[2], *W. Jung*[1]
[1]Chung-Ang University, Systems Biotechnology, Anseong, Korea, Republic of, [2]University of Neuchâtel, Evolutionary Genetics, Neuchâtel, Switzerland

Candida albicans is a commensal yeast residing in the human gastrointestinal tract but can transition from a harmless organism into an opportunistic pathogen under specific conditions. Previous studies have suggested that genetic and phenotypic variations in *C. albicans* clinical isolates may influence its ability to cause disease. In this study, we hypothesized that *C. albicans* residing in different body niches may adapt distinctly, which could impact their genotypes and phenotypes, including the pathogenic potential of the fungus. *C. albicans* strains were obtained from different body niches: fecal samples and gut mucosa of patients with ulcerative colitis or healthy individuals, as well as blood isolates from patients with candidemia. Phenotypic characteristics such as drug susceptibility to multiple antifungal agents, hyphal formation, biofilm production, and virulence were investigated. Our findings revealed distinct phenotypic traits among the *C. albicans* strains. Strains isolated from the mucosa of healthy individuals exhibited decreased biofilm formation and increased survival rates in the *Galleria* model, while strains from the blood of patients displayed reduced hyphal formation and lower larval survival rates. Genomic analyses, including genome-wide association studies and copy number variation analysis, are currently underway, and the results will be presented. Overall, our data suggest that niche-specific environments may drive variation in *C. albicans* isolates.

P1.615 - *Aspergillus* species epidemiology is driven by climate, soil and fungicides

T. Easter[1], *M. Bromley*[1], *J. Rhodes*[2], *M. Brockhurst*[3], *N. van Rhijn*[1,3]
[1]University of Manchester, Manchester Fungal Infection Group, Manchester, United Kingdom, [2]Radboud University Medical Centre, Department of Medical Microbiology, Nijmegen, Netherlands, [3]University of Manchester, Microbial Evolution Research Manchester, Manchester, United Kingdom

Aspergillosis is a severe fungal infection caused by members of the *Aspergillus* genus, primarily *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus flavus*. These soil-based saprotrophs are responsible annually for more than 4 million life-threatening infections and an economic burden of over $10 billion. While *A. fumigatus* is the primary cause of aspergillosis in Europe and the Americas, previous data from our lab have shown that *A. flavus* is more common in Asia and Africa. As the climate changes and global temperatures rise, the impact on the epidemiology of *Aspergillus* species is unknown. To further study the effect of changing environments on *Aspergilli*, we aimed to establish an environmentally relevant soil microcosm. Using CRISPR-Cas9 mutagenesis, we generated genetically barcoded pools of *Aspergillus* species to perform Bar-seq. Bar-seq is a next generation sequencing approach to quantify each fungal isolate within a pooled inoculum before and after growth in our soil microcosms. First, we assessed fitness of

n=25 barcoded *A. fumigatus* isolates for their fitness in a compost soil microcosm model. We measured antifungal and fungicide concentrations in these composts and correlated that with fitness and drug resistance. Our results showed that fungicides could be detected in all commercial compost, and a fitness signature of drug resistance could be found. This soil microcosm model will allow us to test more soil-based variables over longer periods of time, and gain a better understanding of how antifungal resistance can develop alongside changing environments.

P1.616 - The role of rhizoferrin in growth and virulence of *Rhizopus microsporus*

U. Binder[1], *J. Scheler*[2], *C. Lax*[3], *B. Sarg*[2], *F. Nicolas*[3], *T. Telser*[2], *B. Zelger*[2], *I. Bauer*[2], *K. Faserl*[2], *C. lass-Flörl*[2], *V. Garre*[3]
[1]Medical University Innsbruck/Institute of Hygiene and Medical Microbiology, Innsbruck, Austria, [2]Medical University Innsbruck, Innsbruck, Austria, [3]Universidad de Murcia, Murcia, Spain

Mucormycosis, an invasive fungal infection caused by Mucorales fungi, presents a significant threat particularly for patients with conditions such as uncontrolled diabetes, hematological malignancies, and COVID-19 co-infections. Iron acquisition is vital for Mucorales pathogenicity, with elevated serum free iron levels intensifying their virulence. Investigating virulence factors to potentially find new drug targets is urgently needed for this group of fungi.
Fungi secrete siderophores to enable chelation and uptake of ferric iron. For clinically relevant mucormycetes it has been shown that a polycarboxylate siderophore, rhizoferrin, is secreted. This study aims to elucidate the role of the rhizoferrin synthetase encoding gene (rfs) and its product rhizoferrin, in the growth and virulence potential of Rhizopus microsporus.
Assessment of rhizoferrin production via chrome azurol S (CAS)-assays and High-performance liquid chromatography (HPLC), growth assays under varying iron or (xeno)siderophore availabilities were conducted for both *R. microsporus* wild type and rfs-deletion mutants. Galleria mellonella larvae were utilized to study virulence potential of wt versus deletion strains with or without addition of (xeno)siderophores or co-infection with Pseudomonas aeruginosa.
Rfs deletion resulted in non-detectable amounts of rhizoferrin and significantly reduced virulence potential in the *Galleria* mellonella infection model. Further, rfs deletion strains were unable to form hyphae within Galleria larvae and growth reduction/unability under low iron conditions was evident. Currently investigations are carried out to determine if virulence and germination can be restored in the presence of iron or (xeno)siderophores, applied directly or vi co-incubation with Pseudomonas.

P1.617 - Deciphering the interactions between *Candida albicans* biofilm, extracellular vesicles, and antifungal treatment

J. Karkowska-Kuleta[1], *K. Kulig*[1], *K. Kowalik*[1,2], *D. Satala*[1], *O. Barczyk-Woznicka*[3], *E. Karnas*[4], *E. Pyza*[3], *E. Zuba-Surma*[4], *M. Rapala-Kozik*[1]
[1]Jagiellonian University, Department of Comparative Biochemistry and Bioanalytics, Faculty of Biochemistry, Biophysics and Biotechnology, Krakow, Poland, [2]Jagiellonian University,

Doctoral School of Exact and Natural Sciences, Faculty of Biochemistry, Biophysics and Biotechnology, Krakow, Poland, [3]Jagiellonian University, Department of Cell Biology and Imaging, Institute of Zoology and Biomedical Research, Krakow, Poland, [4]Jagiellonian University, Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Krakow, Poland

The formation of structured communities by pathogenic fungi is strongly contributing to the increased tolerance to antifungal treatment, which currently is a considerable problem related to the prevalence of infections caused by *Candida* yeasts. Recently, it was revealed that fungal extracellular vesicles (EVs)—nanometer-sized structures with varied cargo surrounded by a lipid bilayer—may play a significant role in the reduced susceptibility to antimycotics. *Candida* EVs are produced both by planktonic cells and biofilms, and are comprehensively involved in fungal biology and the pathogenesis of candidiasis. In our research we demonstrated that *C. albicans* EVs derived from an established mature biofilm can support the formation of a newly initiated biofilm in the presence of the antifungal drug caspofungin. Furthermore, EVs produced by *C. albicans* yeast cells grown at subinhibitory concentrations of three selected antimycotics—fluconazole, amphotericin B, and caspofungin—showed some variations in terms of size and protein content. EVs produced in the presence of the latter antifungal drug also had a noticeable effect on *C. albicans* biofilm formation, increasing biofilm thickness significantly more than EVs produced in the absence of antifungals. Importantly, the presence of caspofungin, fluconazole, and amphotericin B induced a significant increase in EVs production by fungal cells, compared to drug-free conditions. This indicates the role of EVs in the response of pathogen cells to treatment and highlights the importance of understanding EVs functionalities in antifungal resistance.
This work was supported in part by the National Science Centre, Poland (grant number 2021/43/D/NZ6/01464 awarded to JKK).

P2.101 - High-efficiency multiplex gene targeting in *Colletotrichum* fungi using CRISPR-Cas9 co-editing and dual-marker exchange

N. Kumakura[1], *K. Yonehara*[1,2], *K. Shirasu*[1,2]
[1]RIKEN, Center for Sustainable Resource Science, Yokohama, Japan, [2]The University of Tokyo, Bunkyo, Japan

Colletotrichum fungi cause significant crop losses globally, emphasizing the need to elucidate their infection mechanisms for effective control strategies. Although gene targeting via donor DNA and homology directed recombination (HDR) is feasible in these fungi, it remains hindered by low efficiency, and the number of genes that can be disrupted per strain is limited to 2–3, depending on the availability of selectable markers. To address these limitations, we previously developed a CRISPR-Cas9 system to enhance HDR efficiency, coupled with a *URA3*-based marker recycling method for multiplex gene disruption. This approach, however, was labor-intensive, enabling only single-gene disruptions per transformation with a complex selection marker recycling process. In this study, we established an efficient multiplex gene-targeting method in *Colletotrichum orbiculare* and *C. higginsianum* by integrating CRISPR-Cas9-mediated co-editing with a marker exchange technique. With this co-editing strategy, we

achieved the simultaneous disruption of up to four genes in a single transformation. Additionally, our marker exchange approach, which alternates between two selectable markers, eliminates the need for marker removal after each use, enabling sequential transformations in a simplified, streamlined manner. This advanced method significantly accelerates reverse-genetic identification of virulence factors and deepens our understanding of the infection mechanisms in *Colletotrichum* fungi.

P2.102 - Phyllospheric non-phytopathogenic bacteria promotes the virulence of the Brassicaceae anthracnose fungus *Colletotrichum higginsianum*

K. Tanaka[1], *Y. Hayashi*[1], *S. Yamazaki*[1], *C. Tanaka*[1], *F. Ishida*[1], *M. Yamaguchi*[1], *N. Takeshita*[2], *S. Tanaka*[1]
[1]Setsunan University, Faculty of Agriculture, Hirakata, Japan, [2]University of Tsukuba, Tsukuba, Japan

Phyllospheric microbiomes are primarily dominated by non-phytopathogenic bacteria. However, their influence on the pathogenicity of plant-pathogenic fungi remains poorly understood. In this study, we isolated cultivable bacteria from the leaves of *Arabidopsis thaliana* and investigated their effects on the pathogenicity of the Brassicaceae anthracnose pathogen *Colletotrichum higginsianum* (Ch). When the isolated bacteria were co-inoculated with Ch, the bacterium *Chitinophaga* sp. significantly promoted lesion formation by Ch. Although there were no significant differences in plant gene expression changes between inoculation with Ch alone and co-inoculation with *Chitinophaga* sp., we observed that the presence of *Chitinophaga* sp. led to the formation of secondary appressoria capable of penetrating plant cells, thereby increasing the frequency of successful penetration. The formation of secondary appressoria was also induced by *Chitinophaga pinensis* and *Flavobacterium* sp., both members of the Bacteroidetes, but not by *Escherichia coli*, *Brucella* sp., or *Cupriavidus* sp., which are members of the Proteobacteria. Furthermore, secondary appressoria formation was induced by the culture supernatant of *Chitinophaga* sp., with its activity remaining unaffected by heat treatment, suggesting that non-proteinaceous molecules may be responsible for this activity. Additionally, the biomass of *Chitinophaga* sp. increased on lesions caused by Ch, indicating that it utilized the exudates from plant cells killed by Ch infection for its growth. Our findings suggest that phyllospheric non-phytopathogenic bacteria may exploit fungal infections to enhance their own growth.

P2.103 - Host cell death triggered by plant immunity during the necrotrophic phase of hemibiotrophic fungal pathogen infection

K. Yonehara[1,2], *N. Kumakura*[2], *B. Cole*[3], *H. Morinaka*[4], *K. Sugimoto*[4], *G. Coaker*[5], *K. Shirasu*[1,2]
[1]The University of Tokyo, Department of Biological Science, Tokyo, Japan, [2]RIKEN, Plant Immunity Research Group, Center for Sustainable Resource Science, Yokohama, Japan, [3]DOE Joint Genome Institute, Lawrence Berkeley National Lab, Berkeley, United States, [4]RIKEN, Cell Function Research Team, Center for Sustainable Resource Science, Yokohama, Japan, [5]University of California, Davis, Department of Plant Pathology, Davis, United States

Hemibiotrophic fungal pathogens infect living host tissues during the early biotrophic phase and spread within dead host tissues in the later necrotrophic phase. While previous studies suggest that toxic molecules produced by fungi are involved in the host cell death during the necrotrophic phase, it remains unclear whether the host immune system also plays a role in this process. Here, using *Colletotrichum higginsianum*, a hemibiotrophic fungal pathogen infecting *Arabidopsis thaliana*, we demonstrated that plant immune system contributes to the host cell death during the necrotrophic phase. Our cytological analysis revealed that, in wild-type *A. thaliana*, hypersensitive-response-like cell death, marked by trypan blue staining, occurs in mesophyll cells distant from invaded epidermal cells during the necrotrophic phase. However, this trypan-blue stained mesophyll cell death was absent in a mutant plant lacking a key immune component, while allowing a significant increase in fungal growth. These findings suggest that *A. thaliana* recognizes *C. higginsianum* in epidermal cells and activate a signaling pathway that triggers cell death in distant mesophyll cells. To test this hypothesis, we performed single-nucleus RNA-seq on infected leaves from both wild-type and the mutant plants, identifying candidate genes that contribute to mesophyll-specific cell death. Our study provides insights into how the plant immune system mediates host cell death during the necrotrophic phase of hemibiotrophic pathogens and proposes a potential mechanism for this process.

P2.104 - *Zymoseptoria tritici* effectors structurally related to killer proteins KP4 and KP6 are toxic to fungi, and define extended protein structural super families

K. de Guillen[1], A. Padilla[1], J. Gracy[1], L. Mammri[1], P. Barthe[1], F. Hoh[1], M. Lahfa[1], J. Rouffet[2], Y. Petit[2], T. Kroj[3], M.-H. Lebrun[4]
[1]Centre de Biologie Structurale, Université Montpellier, INSERM U1054, CNRS UMR 5048, Montpellier, France, [2]Université Paris-Saclay, INRAE, UR BIOGER, Palaiseau, France, [3]PHIM Plant Health Institute, University of Montpellier, INRAE, CIRAD, Institut Agro, IRD, Montpellier, France, [4]Université Paris-Saclay, CNRS, CEA, UMR I2BC, Genomics, Gif sur Yvette, France

Fungal effectors play crucial roles in plant infection. Despite their low sequence identity, their classification into families was recently improved using structural homology. In this study, we have elucidated the structures of the two effectors Mycgr3-91409 and Zt-NIP1 from the wheat fungal pathogen *Zymoseptoria tritici* using X-ray crystallography and NMR. These analyses revealed that these effectors shared structural homology with, respectively, KP6α and KP4 killer toxins, encoded by UmV dsRNA viruses infecting the corn fungal pathogen *Ustilago maydis*. Orthologs and paralogs of Zt-Mycgr3-91409 and Zt-NIP1, renamed Zt-KP6-1 and Zt-KP4-1, were identified in *Zymoseptoria*, but not in other fungi, with the exception of ECP2 effectors related to Zt-KP4-1. A novel pipeline relying on Foldseek and cysteine-pattern constrained HMM searches generated a comprehensive inventory of KP4 and KP6 proteins in fungi and plants. Their structure-based classification revealed, respectively, four and three super families and 37 and 26 meta-clusters for KP4 and KP6, respectively. This classification creates a unifying framework for the investigation of these large effector families. Since Zt-KP6-1 and Zt-KP4-1 were toxic to fungi, these results highlight the importance of structure determination of effectors for the prediction of their biological function.

P2.105 - Within-field *Zymoseptoria tritici* surveys reveal high variability in effector haplotypes

A. Tobian Herreno[1], *I. Siepe*[2], *P. Huang*[3], *R. Stam*[1]
[1]Christian-Albrechts Universität zu Kiel, Phytopathology, Kiel, Germany, [2]BASF SE, Limburgerhof, Germany, [3]BASF corporation, Durham, United States

Zymoseptoria tritici is a cosmopolitan wheat pathogen and causal agent of for Septoria tritici blotch (STB), with a hemibiotrophic lifestyle. It is characterised by a high mutation rate and mixed reproductive system, which challenges disease management. The high pathogen variability enables it to partially or completely evade multiple resistance genes like Stb6, Stb7 and Stb9 in different varieties across multiple nations implementing resistance-based programs. This study analyses whole-genome sequence data from three datasets to explore field-level genetic diversity: one from a newly sampled UK field population and two publicly available datasets from fields in the US and Switzerland.
Admixture analysis revealed evidence for two genetic populations, separated at the continental level: the US and the UK and Switzerland. The population genetic analyses of the independent field populations, including analyses of minor allele frequency distribution and clonality, revealed no clear within-field structure. Most SNPs occur at low frequencies, yet the European fields showed higher clonality.
A PCA analysis based on the core genome supported metapopulation separation at the regional level. Interestingly, however, within-field effector diversity, crucial to virulence, showed no segregation for effector genes. Moreover, effector haplotypes do not segregate between the fields, and multiple effector haplotypes were detected within fields, including different haplotypes in what we define as clonal isolates. This suggests that within and between-field selection on effectors differs from the general selection pressure on *Z. tritici* isolates.

P2.106 - The host-specificity factor SOVIG9 of *S. reilianum* affects phytoalexin induction in *Sorghum bicolor*

L.D. Dittiger[1], *S. Chaudhary*[1], *J. Schirawski*[1]
[1]Friedrich Schiller University Jena, Department of Genetics, Jena, Germany

Sporisorium reilianum is the causal agent of head smut in sorghum and maize. It exists in two host-specific formae speciales, *Sporisorium reilianum* f. sp. *reilianum* (SRS) and *Sporisorium reilianum* f. sp. *zeae* (SRZ) that are pathogenic on sorghum and maize, respectively. While SRS-infected sorghum shows almost no visible symptoms in early infection stages, sorghum inoculation with SRZ induces heavy defence responses including the generation of reddish-brown phytoalexins (PA). We identified an effector protein of SRS, SOVIG9, that suppresses the PA response of sorghum. Transcriptome comparison of wildtype- and SRSΔSOVIG9 deletion strain-inoculated sorghum leaves showed upregulation of defence-related sorghum genes, including PA biosynthesis and receptor-like kinase genes. Currently, we identify functional domains of the effector protein as well as plant protein interactors to elucidate the molecular function of SRS-SOVIG9 in the suppression of the sorghum PA response.

In SRZ, a second shortened splice-variant of SOVIG9 is expressed. When this short SRZ splice variant is used to complement SRSΔSOVIG9 deletion strains, the fungus induces a strong PA response similar to complementation strains carrying non-functional, C-terminally tagged versions of the SRS effector that are recognized as avirulence proteins in sorghum. Therefore, the PA response-suppressing effector SOVIG9 seems to have been subject to forma specialis-specific alternative splicing, generating an effector version in SRZ that is recognized by the sorghum defense proteins and contributes to the inability of SRZ to cause disease on sorghum.

P2.107 - Targeting pathogen evolution: a forward genetics approach to investigate gain of virulence in *Zymoseptoria tritici* against the resistant wheat cultivar Cougar

L.E Lamberte[1], *N. Kettles*[1], *T. Wood*[2], *K. Kanyuka*[2], *M.C McDonald*[1], *G.J Kettles*[1]
[1]University of Birmingham, School of Biosciences, Birmingham, United Kingdom, [2]Niab, Cambridge, United Kingdom

Wheat is a cornerstone of global food security yet is vulnerable to diseases such as Septoria tritici blotch (STB), caused by the fungal pathogen *Zymoseptoria tritici*. This disease leads to significant yield losses through necrotic leaf lesions that inhibit photosynthesis. Host genetic resistance to *Z. tritici* can be improved through incorporation of major disease resistance genes, including *Stb* genes, into new wheat varieties. However, the rapid evolution of *Z. tritici* often leads to the breakdown of such resistance, as was demonstrated for the UK wheat cultivar Cougar in 2015. One of the objectives of our project, SeptPROTECT, is to identify the *Z. tritici* gene(s) that facilitate increased virulence on the previously resistant Cougar wheat variety.

We employed the “ZymoSoups” forward genetics approach to randomly mutagenise the IPO323 strain avirulent on Cougar and generate gain-of-virulence (GoV) mutants. GoV mutants recovered from two soups were subjected to whole-genome sequencing. Analysis using GATK and SnpEff identified mutations across the genome, including deletions and high-impact SNP variants. Several candidate genes with potential roles in virulence have been identified, based on their predicted functions and genomic locations. Ongoing functional assays aim to determine the contribution of these genes to the pathogen’s ability to overcome resistance mechanisms. In parallel, we also employ a classical genetic approach through quantitative trait locus (QTL) analysis, leveraging both established genotypic data and newly derived populations to map effector gene loci associated with virulence. Future work will focus on gene knockout experiments to elucidate the function of candidate virulence genes.

P2.108 - Genomic features of the two life-cycle forms of Scots Pine Blister Rust fungus, *Cronartium pini*

Å. Olson[1], *P. Gomez-Zapata*[1], *C. Tellgren-Roth*[2], *J. Kaitera*[3], *B. Samils*[1], *J. Stenlid*[1], *M. Seidl*[4], *M. Durling Brandström*[1]
[1]Swedish University of Agricultural Sciences, Forest Mycology and Plant Pathology, Uppsala, Sweden, [2]Uppsala University, Immunology, Genetics and Pathology, Uppsala, Sweden, [3]Natural Resources Institute Finland, Oulu, Finland, [4]Utrecht University, Theoretical Biology and Bioinformatics, Utrecht, Netherlands

Scots Pine Blist Rust is caused by two life-cycle forms of *Cronartium pini*. The microcyclic autoecious form of *C. pini* (syn. *Peridermium pini*), which is spread directly from pine to pine and the macrocyclic heteroecious form of *C. pini* (syn. *Cronartium flaccidum*) which has five spore stages and alternates between two different hosts. Important alternate host species are in the genera *Melampyrum*, *Vincetoxicum* and *Paeonia*. How these two life-cycle forms are related to each other is still an enigma? Our hypothesis is that the heteroecious form (*C. flaccidum*) is the ancestral form and a genetic change has happened that resulted in the lifestyle switch. Using population genomic analyses on a collection of *C. pini* strains from Scandinavia, we examined the differences between the life-cycle forms. We analysed the differences in the distribution, infection biology and genomic features. Genomic regions which differ in diversity and heterozygosity were identified and described. These insights advance our understanding of the biology of rust fungi and evolution of correlated species. Furthermore, the observed differences between the life-cycle forms have broader implications for pathogen evolution and virulence in rust fungi.

P2.109 - Comparative genomic approaches on germ tube length of *Sphaerulina musiva* under Alkene treatment enlightened a cysteine-rich chaperone protein

A. Lassagne[1], *M. Lamascus*[1], *J. Anderson*[1], *J. LeBoldus*[1]
[1]Oregon State University, Botany and Plant Pathology, Corvallis, United States

Sphaerulina musiva (syn = *Septoria musiva*) is the causal agent of Septoria leaf-spot and stem canker disease of *Populus* species and their hybrids. This fungus is endemic to the range of *Populus deltoides* in the eastern USA and has been introduced to the Pacific Northwest where it can infect the native host *Populus trichocarpa*. Molecular interactions between the host and the pathogen remain poorly understood. Greenhouse, field, and lab experiments have revealed a correlation between cuticular wax composition and susceptibility to this disease. *Populus* accessions with a greater abundance of alkenes in their cuticle are more susceptible to leaf spot disease. Alkene treatment of the *S. musiva* grown on Petri-dishes significantly increases the germ tube length relative to untreated accessions. Furthermore, the growth answer of germ tube under the alkene treatment is isolate dependent. Comparative genomics approaches on S*phaerulina musiva* isolates revealed one SNP associated to germ tube length trait on chromosome 11. The closest gene of the association encoded a molecular chaperone DnaJ with a Hsp cysteine-rich

domain. This protein is known to regulate the activity of the Hsp70 chaperone that performs many roles in the cell as coordinating responses to stress and targeting selected proteins for degradation.

P2.110 - Revisiting the evolution and function of NIP2 parologs in the *Rhynchosporium spp.* complex

R. Darma[1], D. Yu[1], M. Outram[1], Y.-C. Sung[1], E. Hill[1], D. Croll[2], S. Williams[1], B. Ovenden[3], A. Milgate[3], P. Solomon[1], M. McDonald[4]
[1]The Australian National University, Canberra, Australia, [2]University of Neuchatel, Neuchatel, Switzerland, [3]NSW Department of Primary Industries, Wagga Wagga, Australia, [4]University of Birmingham, School of Biosciences, Birmingham, United Kingdom

The fungus *Rhynchosporium commune*, the causal agent of barley scald disease, contains a paralogous effector gene family called *Necrosis-Inducing Protein 2* (*NIP2*) and *NIP2-like protein* (*NLP*). However, the function and full genomic context of these paralogs remains uncharacterised. Here we present a highly contiguous long-read assembly of *R. commune* WAI453. Using this assembly, we show that the duplication of the *NIP2* and *NLP* gene families is distributed throughout the genome and pre-dates the speciation of *R. commune* from its sister species. Some *NIP2* paralogs have subsequently been lost or are absent in the sister species. The diversity of these paralogs was examined from *R. commune* global populations and their expression was analysed during *in planta* and *in vitro* growth to analyse the importance of these genes during infection. The majority of *NIP2* and *NLP* paralogs in WAI453 genome were significantly upregulated during plant infection suggesting that the *NIP2* and *NLP* genes harbour virulence roles. An attempt to further characterise function of NIP2.1 by infiltrating purified protein into barley leaves did not induce necrosis questioning its previously reported role as an inducer of host cell death. Together these results suggest that the *NIP2* effector family does play a role during infection of barley, however the exact function of *NIP2*, like many effectors, remains uncharacterised.

P2.111 - Identifying peptides translated from putative short open reading frames with LC-MS and de novo sequencing in wheat leaf rust

C. Rampitsch[1], Z. Yao[1], S. Djuric-Ciganovic[1], M. Lubberts[2]
[1]Agriculture and Agrifood Canada, Molecular Genetics, Morden, Canada, [2]Agriculture and Agrifood Canada, Bioinformatics, Summerland, Canada

Puccinia triticina (Pt) causes leaf rust on wheat. This disease occurs annually and potentially results in large yield losses. The potential roles of peptides (smaller than 10 kDa) has generally not been examined in plant pathogenic fungi, although peptides transcribed from short open reading frames have been reported from model fungi. This research investigates the presence of such peptides in *Pt* and their possible role(s) in the wheat-rust interaction. We have used top-down LC-MS analyses to detect novel peptides, which are sequenced *de novo* in an approach which eliminates the need for a database. This is important because sORF databases do not exist

and would be difficult to create. For LC-MS we evaluated several approaches, including N-terminal proteome enrichment; C4 RP-HPLC; and SEC-HPLC. We now use SEC-HPLC to enrich peptides, digest these with trypsin and then analyzed by LC-MS in a high-resolution Orbitrap mass spectrometer. Automated *de novo* sequencing, is used to obtain candidate peptide sequences and these are queried against wheat and rust genomic sequences to eliminate fragments resulting from protein turnover or breakdown. To find potential short open reading frames, peptides are mapped back on to the genomic sequence of *Pt*, while accounting for both codon redundancy, reading frames and potential *de novo* sequencing errors (e.g. AB to BA reversals). We have found several candidate peptides with no significant homology to the *Pt* nor to the wheat genomes, but which match short open reading frames on the *Pt* genome. The most recent research progress will be presented.

P2.112 - The maize late wilt pathogen Magnaporthiopsis maydis interspecies interactions, and its combined influence with Fusarium verticillioides

O. Degani[1,2], *G. Shofman*[1,2]
[1]MIGAL – Galilee Research Institute, Plant Sciences, Kiryat Shmona, Israel, [2]Tel Hai College, Faculty of Sciences, Tel Hai, Israel

Maize late wilt disease, caused by the fungus *Magnaporthiopsis maydis*, poses a significant threat to susceptible crops. This study reveals that the population of *M. maydis* in Israel displays diverse levels of aggressiveness, which are unrelated to geographic distribution. Additionally, some subspecies groups specialize in growth disruption, while others cause wilting, indicating biotrophic and necrotrophic variation. While weak pathogenic strains affected susceptible cultivars, resistant cultivars were only impacted by highly aggressive isolates, resulting in a 7% reduction in growth and 11% mortality at harvest. Unexpectedly, a mixed inoculum of the two most virulent isolates during sprouting led to reduced disease severity. However, by harvest, this trend reversed, and adding a weakly virulent strain to the mix caused a more severe reduction in growth (23%) and health (71%), with a high level of *M. maydis* infection. Compared to *Fusarium verticillioides*, another post-flowering stalk rot pathogen, *M. maydis* exhibited greater aggressiveness, with 40% plant survival and up to 1,000 times higher DNA levels. Co-inoculation of the two pathogens increased the number of healthy plants from 10% (for *M. maydis* alone) to 30%. Sequential infection with *F. verticillioides* first reduced symptoms and *M. maydis* infection, though plant growth remained poor. This study improves our understanding of the late wilt pathogen's complex inter- and intraspecies interactions and its destructive threat to maize crops.

P2.113 - Compensatory pathogenicity mechanisms in *Parastagonospora nodorum* lacking major necrotrophic effectors

S. Morikawa[1], *C. Verdonk*[1], *H. Phan*[1], *J. Debler*[1], *C. Turo*[1], *K. Clarke*[1], *D. Jones*[1], *B. Henares*[1], *C. Mousley*[2], *K.-C. Tan*[1]
[1]Centre for Crop and Disease Management, School of Molecular and Life Sciences, Perth, Australia, [2]Curtin Health Innovation Research Institute, Curtin Medical School, Perth, Australia

The fungus *Parastagonospora nodorum* causes septoria nodorum blotch (SNB) of wheat by secreting a suite of proteinaceous necrotrophic effectors (NEs) to induce tissue necrosis upon infection. Tox effectors only induce necrosis/chlorosis on wheat cultivars that possess matching dominant susceptibility genes (Snn). It has been demonstrated that multiple NE-Snn interactions dictate the outcome of SNB through additive but also epistatic interactions. In this study, we have generated a *P. nodorum* mutant (*toxa13*) that lacked major NE genes; *SnToxA*, *SnTox1* and *SnTox3*. Surprisingly, the virulence of *P. nodorum toxa13* is comparable to the wildtype on modern bread wheats despite ablating three NE-Snn interactions. This suggests that other functionally redundant pathogenicity mechanisms compensate for the loss of the three major effectors. A comparative RNA-Seq study revealed that the NE gene *SnTox267* and two phytotoxic secondary metabolite (SM) gene clusters were highly up-regulated in *toxa13* in-planta. Furthermore, several candidate NE genes, uncharacterised SM gene clusters and signal transduction genes were up-regulated and may contribute to maintaining the virulence of *toxa13*. Characterisation of these genes for their role in the sustained virulence of *P. nodorum toxa13* will be discussed.

P2.114 - Order from disorder: a structurally disordered domain of PnPf2 is essential for regulation of virulence factors in a phytopathogenic fungus

C. Verdonk[1], *S. Morikawa*[1], *E. John*[2], *C. Mousley*[3], *B. Hernares*[1], *K.-C. Tan*[1]
[1]Curtin University, Centre for Crop and Disease Management, Perth, Australia, [2]Institute of Crop Science and Resource Conservation, University of Bonn, Department of Plant Pathology, Bonn, Germany, [3]Curtin University, Curtin Health Innovation Research Institute & Curtin Medical School, Perth, Australia

Septoria nodorum blotch (SNB) is a devastating disease of wheat and is caused by the fungal pathogen *Parastagonospora nodorum*. The primary cause of SNB is proteinaceous effectors, which interact with wheat host sensitivity genes. These effectors are controlled by DNA-binding regulatory proteins called transcription factors (TF), of which the Zn2Cys6 zinc-finger type is one of the most abundant in fungi. These zinc-finger-containing TFs possess a DNA-binding domain (DBD) and central “middle homology domain” (MHD). In *P. nodorum* the TF PnPf2 is a positive regulator of major necrotrophic effector genes required for host-specific virulence on wheat. PnPf2 is a DBD-MHD protein with a disordered C-terminus tail which has low homology with its orthologs. We demonstrated that both the DBD and MHD are essential for PnPf2 function but are not the main drivers of pathogenicity. Instead, only the disordered C-terminus tail is seemingly required for both full effector activation, as well as disease symptoms on specific wheat cultivars. We also show the PnPf2 MHR interacts with genes related to transcription via a modified two-hybrid methodology. Identification of PnPf2-interacting partners has highlighted a potentially novel mechanism for gene regulation by these ubiquitous TFs. By understanding the structural and biological mechanisms of PnPf2, we can untangle the complexity surrounding effector regulation in necrotrophs and other phytopathogenic fungi.

P2.115 - The gene *CgEP4* encodes an effector that plays a key role in *Colletotrichum graminicola* virulence in maize

P. García-Rodríguez[1], *L. Rodríguez-Mónaco*[1], *F.B. Cuevas-Fernández*[1], *S. Becerra*[1], *S. Gutiérrez-Sánchez*[1], *A. Jerez-Vanegas*[1], *F. Rogério*[1], *J. Ortíz-Álvarez*[2], *R. Baroncelli*[3], *E.A. Espeso*[4], *M.R. Thon*[1], *S.A. Sukno*[1]
[1]Universidad de Salamanca - Instituto de Investigación en Agrobiotecnología (CIALE), Villamayor, Spain, [2]Programa "Investigadoras e Investigadores por México" Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCyT), Ciudad de México, Mexico, [3]University of Bologna, Department of Agricultural and Food Sciences (DISTAL), Bologna, Italy, [4]Centro de Investigaciones Biológicas (CIB) Margarita Salas - Consejo Superior de Investigaciones Científicas (CSIC), Department of Molecular and Cellular Biology, Madrid, Spain

Colletotrichum ranks among the most important fungal pathogens, affecting nearly every major crop worldwide. The diseases it causes significantly impact agriculture and the economy, reducing crop yields and threatening global food security. *Colletotrichum graminicola* is the causal agent of maize anthracnose. Its genome contains multiple genes encoding effector proteins that modify plant defense mechanisms to facilitate host infection. Previously, we identified *CgEP1*, a putative effector-encoding gene, through phylogenetic and transcriptomic analyses. Deletion of this gene resulted in a significant reduction of virulence in maize leaves. *CgEP4* is a paralog of *CgEP1* and encodes a protein composed of two alpha-helix. Evolutionary analyses reveal that *CgEP4* originated from a duplication event before the *Graminicola* species complex diverged. Gene expression analyses showed that *CgEP4* is upregulated during the late biotrophic stage of infection. Knockout mutants and complemented strains of *CgEP4* were created and then tested in pathogenicity assays. The results show that *CgEP4* is key to *C. graminicola* infection in maize, affecting both leaf blight and stalk rot disease forms. In addition, phenotypic characterization of the *CgEP4* null mutant showed reductions in spore formation, *in vitro* germination, and growth under diverse stress conditions. Pathogenicity assays revealed that the *CgEP4* mutant had reduced penetration efficiency, with a stronger callose response in maize leaves compared to the wild type. CgEP4 has a nuclear localization prediction but experiments to determine it are underway. In conclusion, our findings provide important insights into the role of *CgEP4* in *C. graminicola* pathogenicity, suggesting potential targets for improved disease control.

P2.116 - Mycosporines in spore-type specific development and corn anthracnose spreading

D. Rathi[1], *S. Lewandowski*[1], *D. Nordzieke*[1]
[1]Georg-August University Göttingen, Institute of Microbiology and Genetics, Department of Genetics of Eukaryotic Microorganisms, Göttingen, Germany

Colletotrichum graminicola is a hemibiotrophic plant pathogen that induces anthracnose in *Zea mays*. This fungus produces two types of asexual spores: falcate and oval conidia, which exhibit

distinct gene expression patterns, generate distinct metabolites, and undergo unique developmental processes. Interestingly, falcate conidia produces mycosporine molecule, whereas, oval conidia release cell fusion signals.
In cyanobacteria, two parallel biosynthetic pathways were identified for the production of mycosporines. Outgoing from these known genes, we have successfully identified homologs in *C. graminicola*, which are organized in a gene cluster. Interestingly, none of the two pathways from cyanobacteria are fully present in *C. graminicola*, but we have identified genes from both, indicating a merge of both pathways during evolution. Our initial tests involving three putative genes (*Cgddgs*, *CgATP-grasp*, *Cgo-met*) have demonstrated significant impact on germination and germling fusion compared to the wild type (CgM2). Although our initial tests for leaf infection (5dpi) did not reveal any difference between mutants and wild type strains, we identified a regulatory role of mycosporines for the generation of appressoria. Additionally, we are now knocking out the remaining gene of the biosynthesis pathway (*Cgdahp*), also generating double mutant strains, to get an insight into the full biosynthetic pathway in *C. graminicola*. Furthermore, we have initiated a comprehensive biochemical analysis of mycosporine production to gain in-dept understanding of the intermediate molecules formed. This approach will provide us with valuable insights into the pathogenicity and development of various spore types, shedding light on how these molecules influence fungal growth and infection processes.

P2.117 - Small-spored *Alternaria* infecting wild tomatoes show genetic and chemical diversity

T. Schmey[1,2], *A. Adeyemo*[3], *M. Schmidt-Heydt*[3], *R. Stam*[1]
[1]Christian-Albrechts-Universität zu Kiel, Phytopathology, Kiel, Germany, [2]Technische Universität München, Phytopathology, Freising-Weihenstephan, Germany, [3]Max-Rubner-Institut, Department of Safety and Quality of Fruit and Vegetables, Karlsruhe, Germany

Wild tomato species in South America offer an exciting wild plant pathosystem. These crop wild relatives occur in habitats as different as the Andes and the Atacama desert. Filamentous fungi of the genus *Alternaria* cause serious foliar and post-harvest diseases on cultivated tomatoes worldwide.
We found that several sections of *Alternaria*, but predominantly the small-spored *Alternaria* section *Alternaria*, infect the leaves of eight wild tomato species in Chile and Peru. After a barcoding study showed the broad distribution of small-spored *Alternaria*, we chose a subset of these fungi for whole genome sequencing. The full genomes of the pathogens revealed high genomic diversity, not only for isolates sampled from different hosts and habitats over great distances, but also on a smaller scale. Some of the wild isolates produce high levels of well-described mycotoxins, whereas others show reduced levels or complete absence. With comparative genomic approaches, we aim to connect this observed variation in mycotoxin production to the adaptation of the pathogen to host and habitat.
Since small-spored *Alternaria* like *A. alternata* are an increasing problem for tomato growers, the adaptive potential of this pathogen is of interest to crop protection and plant breeding, as well as researchers investigating fungal genome evolution.

P2.118 - Genomic characterization of pathogenicity genes in Colletotrichum species affecting apple orchards in Northern Italy

S. Iacono[1], *M. Calì*[1], *C. Fiorenzani*[1], *A. Menicucci*[1], *E. Cappelletti*[1], *A. Prodi*[1], *C. Ratti*[1], *R. Baroncelli*[1]
[1]University of Bologna, Department of Agricultural and Food Sciences, Bologna, Italy

Apple cultivation is a cornerstone of northern Italy's agricultural economy, but fungal diseases pose a significant threat to orchard productivity, affecting both fruit quality and yield. Among the most detrimental fungal pathogens are species of *Colletotrichum*, responsible for Apple Bitter Rot (ABR) and Glomerella Leaf Spot (GLS). Previous studies from our group have identified pathogenic *Colletotrichum* species in apple orchards, belonging to three distinct species complexes and exhibiting substantial intraspecific diversity. Some of these species can cause both ABR and GLS, while others are opportunistic pathogens. This diversity presents an ideal platform for investigating the genetic elements associated with pathogenicity. This study aims to identify and characterize pathogenicity genes in *Colletotrichum* species associated with apple diseases, to inform the development of targeted disease management strategies. Seven isolates, representing the pathogen's genetic diversity in northern Italy and capable of causing both ABR and GLS, were selected for genomic analysis. DNA was extracted using a modified CTAB protocol, and genomes were sequenced using Illumina technology. Genome assembly was conducted with SPAdes, followed by gene prediction and annotation. These newly sequenced genomes, along with publicly available data, were used in a comparative genomics framework to identify and analyze putative pathogenicity genes. Comparative genomic analyses are currently ongoing, and results will be presented.

P2.119 - Tomato rot by *Rhizopus microsporus* alters native fungal community composition and secondary metabolite production

M. Napo[1], *A. Kock*[1], *K. Alayande*[1], *J. Uehling*[2], *T. Pawlowska*[3], *R. Adeleke*[1]
[1]North-West University, Microbiology, Potchefstroom, South Africa, [2]Oregon State University, Botany and Plant Pathology, Oregon, United States, [3]Cornell University, School of integrative Plant Science, Plant pathology, and Plant-Microbe Biology, Ithaca, New York, United States

Rhizopus microsporus (Mucoromycota) is a prevalent causative agent of spoilage in horticulture commodities. This thermophilic filamentous fungus harbours a bacterial endosymbiont, *Mycetohabitans*, that causes rice seedling blight and interferes with the growth of other fungi via the production of secondary metabolites that inhibit essential cellular processes. However, the factors contributing to tomato rot remain unclear. In the present study, two *R. microsporus* cultures were isolated from rhizosphere soil of *Fouquieria splendens* (Fouquieriaceae) in the Nama Karoo ecoregion of South Africa. We screened the fungal isolates for putative endobacteria using the polymerase chain reaction for 16S rRNA gene in fungal DNA extracts. *Mycetohabitans* were identified using phylogenetic analysis. *R. microsporus* isolates were cured of *Mycetohabitans* to generate cured lines. Both wild-type and cured strains were used for pathogenicity tests in tomato fruits in accordance with Koch's postulate. We conducted ITS

rRNA gene metabarcoding on tomato DNA extracts to examine the native endophytic fungal community shift caused by these inoculations, followed by multi-analyte LC-MS/MS to analyse the secondary metabolites produced by these communities. The pathogenicity test showed that the endobacterium-containing *R. microsporus* W2-50 was able to cause tomato spoilage. This was accompanied by decreased relative abundance of *Alternaria* spp. and an increase in the relative abundance of *Penicillium* spp. that may have facilitated the observed spoilage. No spoilage nor *Penicillium* spp. were observed in the tomatoes treated with the cured line, suggesting that the wild-type W2-50 may facilitate fruit spoilage, possibly through successful colonisation or toxin production by its endosymbiont.

P2.120 - Zymoseptoria tritici KP6 effectors interact with PR-14 (lipid transfer proteins)

E. Thynne[1], *M. Abukhalaf*[2], *K. Seong*[3], *K. Krasileva*[3], *A. Tholey*[2], *E. Stukenbrock*[1]
[1]Christian-Albrechts University, Botanical Institute, Kiel, Germany, [2]Christian-Albrechts University, Institute for Experimental Medicine, Kiel, Germany, [3]University of California, Berkeley, Department of Plant and Molecular Biology, Berkeley, United States

Zymoseptoria tritici is a significant fungal pathogen of wheat, responsible for the leaf disease, septoria tritici blotch (STB). Despite the importance of *Z. tritici* as a pathogen, relatively little is known about its molecular interactions with wheat during infection. Before inducing necrotic STB symptoms, *Z. tritici* grows asymptomatically through the leaf tissue. During this phase, infected wheat expresses a number of defence-related proteins, including pathogenesis-related (PR), which can enhance signalling responses or directly inhibit pathogen growth. However, despite the presence of these proteins, *Z. tritici* can develop and reproduce. We have identified a number of *Z. tritici* effectors that directly interact with PR proteins. Among these are *Z. tritici* effectors that belong to the killer-like protein 6 (KP6) structural family of effectors described to have antimicrobial activity. We found that *Z. tritici* KP6 effectors can interact with PR-14 proteins (lipid transfer proteins; LTPs). Currently, we are exploring the role that KP6 effectors have in inhibiting PR-14 antimicrobial activity, and a putative interaction between these proteins that leads to a non-host immune response when heterologously expressed in the model plant species, *Nicotiana benthamiana.*

P2.121 - Genomics approach in analysis of *Fusarium* apple fruit rot

M. Petres[1], *D.H. Ayhan*[2], *M. Grahovac*[1], *D. Budakov*[1], *M. Loc*[1], *T. Dudas*[1], *L.-J. Ma*[2]
[1]University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia, [2]University of Massachusetts Amherst, Department of Biochemistry and Molecular Biology, Amherst, MA, United States

A well-known phytopathogen of cereals and maize *Fusarium graminearum* has recently been detected, along with *F. avenaceum*, as a causal agent of stored apple fruit rot. The significance of these species is evident not only in the yield loss of stored apples, but also in the increased risk of mycotoxin contamination in infected fruits.
In our previous research, we assembled *F. graminearum* genome (strain TaB10 from Serbia), and

compared it with the reference genome of the strain PH-1, and the global genome comparison showed high percentage of synteny. Despite the high similarity of two genomes a total of 67 unique genes of the TaB10 genome were identified by combining different approaches. Further analyses revealed enrichment of uniquely found genes in effector proteins, particularly in apoplastic effectors, which may be significant for pathogenicity in apples.
These results emphasize the crucial role of genomic research in revealing the pathogenic processes of *F. graminearum*. Future research will focus on identification of genes underpinning pathogenic interaction between *Fusarium* and apple fruit utilizing RNA sequencing and gene knockout using CRISPR/Cas9, evaluating eco-friendly control methods including bioagents and physical measures, and exploring advanced molecular strategies like RNAi for effective pathogen management.
This work was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Project No. 451-03-66/2024-03/200117) and by the United States Fulbright Scholar Program.

P2.122 - Disruption of the infection process of *Botrytis cinerea* and *Ciborinia camelliae* using common plant secondary metabolites

G. Ricardo[1], *N. Kondratev*[1], *S. Sofkova-Bobcheva*[1], *P. Dijkwel*[1]
[1]Massey University, School of Agriculture and Environment, Palmerston North, New Zealand

As a consequence of current agricultural processes, the emergence of new fungal-related diseases and resistant strains is inevitable. This causes not only a burden to public health but also food security. The Sclerotineaceae fungal family contains various plant pathogens such as the generalist *Botrytis cinerea*, the causative agent of grey mould which is responsible for the loss of millions of dollars in crop yield. In addition, it contains specialists like *Ciborinia camelliae*, which causes Camellia Petal Blight uniquely on Camellia blooms. We have previously shown that *Camellia lutchuensis* has an inherent resistance to *C. camelliae* as a result of an early activation of the phenylpropanoid pathway upon infection. We tested if the external application of these secondary metabolites could inhibit the infection of susceptible *Camellia* hybrid 'Nicky Crisp' with *C. camelliae*, and also of *Arabidopsis thaliana* with *B. cinerea*. Nine phenylpropanoids were applied by means of droplet incubation, whole petal/leaf sprays, and by feeding through the vascular system. The alterations in the infection process were visualized using non-destructive and destructive methods and ultimately five compounds effectively inhibited mycelial growth and lesion progression. Spores of both fungi were unable to germinate, mycelium development was impaired, and the capability to penetrate the upper epidermis decreased significantly. The effects that these compounds have at the level of gene expression are currently being evaluated via transcriptomics. From a literature analysis coupled with a membrane attachment assay, we hypothesise that several phenylpropanoids compromise spore cell wall integrity.

P2.123 - Impact of *Fusarium* species composition and incidence on onion basal rot in Northeastern Israel

O. Degani[1,2], *E. Dimant*[1], *E. Margalit*[3]
[1]MIGAL—Galilee Research Institute, Plant Sciences, Kiryat Shmona, Israel, [2]Tel-Hai College, Faculty of Sciences, Tel-Hai, Israel, [3]Israel Ministry of Agriculture and Rural Development, Extension Service, Bayit Dagan, Israel

Fusarium basal rot (FBR) places a significant limitation on onion (*Allium cepa*) production worldwide. The disease can be observed throughout the entire crop cycle. This research explored the composition and incidence of *Fusarium* species involved in FBR outbursts in two onion fields in northeast Israel, one in Galilee (Hula Valley) and the second in the Golan Heights, where the disease incidences reached 8%. Using colony morphology, microscopic taxonomic keys, and molecular methods, a new, unreported *Neocosmospora* (previously *Fusarium solani*) species complex (SC, mostly *N. falciformis*) was discovered as a wildly spread member of the *Fusarium* pathobiome community. This SC was also less virulent in seed germination (42–52% higher sprout biomass, $p < 0.05$) and bulb pathogenicity tests (41–45% less necrotic) than *Fusarium acutatum*. Whereas the Galilee yellow Orlando (Riverside) onion cultivar bulbs sampled were colonized by *Neocosmospora* SC (70%) and two other, less abundant species, *F. oxysporum* f. sp. *cepae* and *F. acutatum* (15% each), the Golan Heights field's *Fusarium* community showed host specificity. In the Golan Heights field, *F. oxysporum* f. sp. *cepae* inhabited the red Ha2 onion cultivar bulbs, whereas *F. acutatum* colonized the yellow Ha1 cultivar (40% and 50% prevalence along with *Neocosmospora* SC). A better understanding of this disease complexity, affected by different *Fusarium* species and with a divergence in host susceptibility and virulence, is critical for developing disease management strategies. Since each *Fusarium* species reacts differently to pest control treatments, changes in the species composition may require specifically adapted management solutions.

P2.124 - Aetiology, epidemiology and environmental influences on almond anthracnose in Portuguese orchards

M. Ramos[1], *P. Arsénio*[1], *R. Baroncelli*[2], *P. Talhinhas*[1]
[1]Instituto Superior de Agronomia, LEAF- Linking Landscape, Environment, Agriculture and Food Research Centre and Associate Laboratory TERRA, Lisboa, Portugal, [2]Università di Bologna, Dipartimento di Scienze e Tecnologie Agro-Alimentari, Bologna, Italy

Almond anthracnose, caused by *Colletotrichum* spp., is a disease that affects the fruit, resulting in sunken, circular, orange-coloured lesions, producing white mycelium and spore masses, ultimately leading to kernel mummification. This disease is becoming increasingly significant in Alentejo, Portugal, due to the recent intensification and large-scale establishment of almond plantations. To assess the impact of the disease, including severity, incidence, and species identification of *Colletotrichum* in almond orchards, in relation to the surrounding landscape and production systems, several almond orchards in Alentejo were sampled between 2022 and 2024. Plant material was collected for the epidemiological, etiological, and genetic characterisation of

the disease. The results showed that anthracnose prevalence and incidence were higher in hedged almond orchards compared to those under the intensive system. The cultivars with the highest incidence were 'Soleta,' 'Guara' and 'Belona' although the severity of fruit infections did not significantly differ between cultivars. It was found that almond anthracnose in Alentejo is mostly caused by *C. godetiae* and to a lower extent by C. *acutatum* and by a second lineage of *C. godetiae* (members of the acutatum species complex). These species are not host-specific, indicating potential pathogen movement between wild species and agricultural crops, such as apple, strawberry, blueberry, peach, loquat, and olive. Further research on almond anthracnose will enhance understanding of how inoculum spread, and disease incidence are influenced by environmental, meteorological, ecological, and agronomic factors. This knowledge will contribute to the development of more informed protection strategies, supporting the sustainable intensification of almond cultivation.

P2.125 - New experimental tools for investigating the pathogenicity and endophytic behavior of Diplodia sapinea

A.G. Oostlander[1], *L. Brodde*[2], *M. von Bargen*[1], *B. Slippers*[3], *R. Enderle*[4], *Y. Becker*[4], *U. Brandt*[5], *C. Grobler*[3], *L. Well*[1], *J. Stenlid*[2], *J. Oliva*[2], *M. Elfstrand*[2], *A. Fleißner*[5]
[1]Technische Universität Braunschweig, Braunschweig, Institute of Genetics, Braunschweig, Germany, [2]Swedish University of Agricultural Sciences, Department of Forest Mycology and Plant Pathology, Uppsala, Sweden, [3]University of Pretoria, Forestry and Agricultural Biotechnology Institute (FABI), Pretoria, South Africa, [4]Julius Kühn Institute (JKI)—Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany, [5]Technische Universität Braunschweig, Institut of Genetics, Braunschweig, Germany

Diplodia sapinea is a globally widespread fungal pathogen and endophyte of conifers, causing significant forest damage, especially under climate change conditions. Common symptoms of infection include shoot dieback, cankers, root disease, and tree death. Despite extensive and successful research on this pathogen, key aspects of its interactions with plants remain unresolved. To better understand the biology and infection mechanisms of *D. sapinea*, we developed laboratory-based methods for its cultivation and genetic manipulation. We first established an efficient protocol for producing and storing highly viable pycnidiospores, which were then used to infect *Pinus sylvestris* seedlings. Inoculation on wounded plants led to the most pronounced disease symptoms, while non-wounded plants exhibited high rates of asymptomatic infection, indicating an endophytic lifestyle. This experimental setup could be valuable for future studies on the switch between *D. sapinea*'s endophytic and pathogenic states. Additionally, we designed a more time- and space-efficient infection assay using two-week-old pine seedlings, which does not require greenhouse facilities.
Furthermore, we developed a protocol for *Agrobacterium*-mediated transformation of *D. sapinea*, enabling molecular genetic studies for the first time. This method allows for gene knockout experiments and the expression of fluorescently labeled proteins, facilitating live-cell imaging. In a proof-of-concept study, we successfully visualized the growth of *D. sapinea* in planta using a fluorescently tagged strain. Together, these tools provide a powerful platform for studying the

transition from endophytic to pathogenic behavior in *D. sapinea*, which will aid in developing better strategies to control this pathogen and mitigate its impact on forest health.

P2.126 - Functional adaptation of a transcriptional activator effector family in smut fungi

W. Zuo[1], *G. Doehlemann*[1]
[1]University of Cologne, CEPLAS & Institute for Plant Sciences, Cologne, Germany

Ustilago maydis causes common smut disease on maize. In contrast to other smut fungi that infect the host systemically and only cause symptoms on inflorescence, it induces localized host cell division to produce tumors on the seedling leaves. The role of effector adaptation in this change of pathogenesis remains unclear.
In our previous study, we identified the effector UmSts2 (small tumor on seedlings2) as a novel transcription activator from *U. maydis* that activates the expression of maize leaf developmental regulators, thereby causing the host cell division and tumor formation. Interestingly, Sts2 has undergone neo-functionalization between *U. maydis* and its closest pathogenic relative *Sporisorium reilianum*.
Here, we investigated the transcriptional activation activity and virulence function of all Sts2 orthologs from smut fungi. Our findings indicated that Sts2 orthologs represent a transactivator effector family in smut fungi, and that neo-functionalization has occurred in *U. maydis* enabling UmSts2 for leaf tumor formation. Further analysis of SrSts2 from *S. reilianum* through RNA-seq suggested its role in regulating host carbohydrate metabolism and kinase activity, which may imply the ancestral function of Sts2. Additionally, by using AlphaFold structure prediction, we identified and experimentally validated the functional determinants between SrSts2 and UmSts2, which could be the potential targets for adaptation. Our findings elucidate the evolutionary trajectory of an effector family and its role in shaping pathogenesis during speciation.

P2.128 - Loop of unknown origin – Discovery of a novel 24 kb circular element harbouring viral-like genes in *Ascochyta* pathogens

J. Debler[1], *R. Lee*[1], *B. Henares*[1], *L. Kamphuis*[1], *K. Singh*[1]
[1]Curtin University, Centre for Crop and Disease Management, Bentley, Australia

Ascochyta blight (AB), caused by the necrotrophic and hemibiotrophic fungal pathogens *Ascochyta rabiei and Ascochyta lentis*, is the most damaging fungal disease of chickpea and lentil, respectively.
By sequencing isolates of both pathogens from around the world we discovered a circular contig encoding virus-like genes in some isolates from geographically distant regions and separated by over 30 years. Interestingly some versions of the element are closer related between isolates of the two pathogens than within the same species hinting at a potential horizontal transfer. Which is curious since the pathogens are very specific to their respective host plant.

P2.129 - BLASTOR: Investigating TOR-related signalling nodes to combat the rice blast fungus

N. Yakobov[1], *L. Bonadei*[1], *A. Gillet*[2,1], *R. Loewith*[1]
[1]Université de Genève, Department of Cellular and Molecular Biology, Genève, Switzerland,
[2]Centre Médical Universitaire de Genève, Genève, Switzerland

Fungal blast diseases including rice blast, wheat stem rust, corn smut, soybean rust and potato late blight present major global food security concerns. It is projected that mitigation of these fungal plant pathogens would provide enough food to feed more than 600 million people. *Magnaporthe oryzae* is the causative agent of the most widespread and serious disease of cultivated crops, severely impacting cultivars in the Poaceae family. To gain entry to plant hosts, airborne conidia attach to the hydrophobic and nutrient-free leaf surface where they develop a specialized infection structure, the appressorium. During differentiation the 3-celled conidium undergoes autophagy mediated cell death while the remaining cell becomes an appressorium – a highly pressurized structure designed to provide the mechanical force needed to breach the leaf cuticle. Despite growing efforts over the past three decades, the molecular mechanisms underlying appressorial formation are far from being fully understood. Better understanding of these molecular mechanisms could identify vulnerable signalling nodes and thus generate novel strategies to tackle blast disease. In this vein, we propose to investigate the role that Target Of Rapamycin (TOR) signalling plays in appressorium formation in pathogenic fungi. Appealingly, critical components of the otherwise well conserved TOR signalling network that are present in fungi, but absent in plants, and could thus represent suitable targets for effective control of blast disease.

P2.130 - The Vap1-Vip1-Vip2 complex of V. dahliae controls resting structure formation, secondary metabolism and virulence

R. Harting[1], *A. Nagel*[1], *E. Bastakis*[1], *N. Wiegräbe*[1], *M. Bromm*[1], *O. Valerius*[1], *K. Schmitt*[1], *Y.-Y. Chen*[1], *A.P. Chavez Rodriguez*[1], *S.A. Braus-Stromeyer*[1], *G.H. Braus*[1]
[1]Georg-August-Universität Göttingen, Göttingen, Germany

Verticillium dahliae together with its host plant *Solanum lycopersicum* (tomato) is a well-established pathosystem. The fungus causes Verticillium wilt, which is difficult to control by fungicides. We investigate on control mechanisms that regulate fungal growth, development, secondary metabolism and virulence. The velvet domain family proteins (Vel1-3 and Vos1) comprise an important group of transcriptional regulators, which are involved in these processes. We identified direct targets of Vel1 and Vel2 using a combination of chromatin immunoprecipitation DNA-Sequencing (ChIP Seq) and RNA Sequencing (RNA Seq) and found a transcriptional feedback loop between the velvet proteins and the membrane-associated protein Vap1. Using GFP pulldown experiments, the two interacting methyltransferases Vip1 and Vip2 were identified, suggesting that a similar complex as known from *Aspergillus spp*. is formed. Further experiments with deletion strains showed that this complex is involved in the regulation

of resting structure formation, secondary metabolism and virulence, underlining its important function of the regularly network controlling fungal development and pathogenicity.

P2.131 - Biotechnological production of antifungal proteins (AFPs) in plants for crop and postharvest protection

A. Bugeda[1], X. Shi[1], L. Castillo[1], J.F Marcos[2], J.J. López-Moya[1], P. Manzanares[2], M. Coca[1]
[1]Centre for Research in Agricultural Genomics (CRAG, CSIC-IRTA-UAB-UB), Barcelona, Spain, [2]Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Food Biotechnology, Paterna, Spain

Fungi are the major plant pathogens and pose a serious threat to food security and safety. Antifungal proteins (AFPs), which are small, cationic, cysteine rich proteins produced by filamentous fungi, show great potential against economically relevant phytopathogenic fungi. However, their application in agriculture requires efficient, economic, and safe production systems. This study focuses on the *Penicillium expansum* PeAfpA and *Penicillium digitatum* PdAfpB proteins, particularly outstanding for their potent and specific activity against important phytopathogens. Both AFPs were produced through a tobacco mosaic virus-based expression vector in *Nicotiana benthamiana* plants, combining signalling sequences for apoplastic and vacuolar compartmentalization. Introduction of a vacuolar signalling peptide at the C-termini of the AFPs in combination with an apoplastic N terminal signalling significantly enhanced 9-fold and 3-fold PeAfpA and PdAfpB yields, respectively, compared to constructs with only the apoplastic N-terminal signalling. Transmission electron microscopy and immunogold labelling confirmed the localization of AFPs in both the apoplast and the vacuole, highlighting its compatibility with vacuolar environments. We demonstrate that PeAfpA- and PdAfpB-enriched plant extracts are equally active to their counterpart fungal proteins, without requiring further purification steps and reducing downstream processing, and consequently production costs. We also show that these plant extracts are effective in controlling *Botrytis cinerea* gray mold in tomato plants and fruits, *Magnaporthe oryzae* blast disease in rice leaves, and *Fusarium proliferatum* infection in rice seeds. Our findings represent a significant advance in the use of PeAfpA and PdAfpB as environmentally friendly and effective “green fungicides” for crop and postharvest protection.

P2.132 - The evolution of pathogenicity in toxin-dependent necrotrophic plant pathogens

M. Kodama[1], T. Arie[2], H. Kaminaka[3], Y. Takano[4], T. Tsuge[5]
[1]Tottori University, Faculty of Agriculture, Tottori, Japan, [2]Tokyo University of Agriculture and Technology, Tokyo, Japan, [3]Tottori University, Tottori, Japan, [4]Kyoto University, Kyoto, Japan, [5]Chubu University, Kasugai, Japan

The tomato pathotype of *Alternaria alternata* (*A. arborescens*) produces host-specific AAL-toxin and induces apoptotic cell death on tomato cells. The AAL-toxin biosynthetic (*ALT*) gene cluster homologues are found in *Fusarium* spp., *Aspergillus niger* and *Cochliobolus heterostrophus*. The

ALT cluster in the tomato pathotype might be acquired by horizontal transfer of the entire cluster genes from those pathogenic fungi. The *ALT* cluster locates only on a conditionally dispensable chromosome (CDC) found in the tomato pathotype. The CDC in the tomato pathotype strains from different geographical origins was identical although the genetic backgrounds of the strains differed. The results imply that the CDC has a different evolutionary history from the essential or core chromosomes in the same genome. We propose a hypothesis whereby the ability to produce a toxin and to infect a plant is distributed among *A. alternata* strains by horizontal transfer of an entire pathogenicity chromosome (CDC). This could provide a possible mechanism by which new toxin-producers (plant pathogens) arise in nature. The tomato pathotype could infect *lag1* homologue (*loh2*) mutants of *Arabidopsis*, which have a defect in AAL-toxin resistant gene (*Asc1*). The nonhost resistance to *A. alternata* consists of multilayered defense systems that include pre-invasion resistance including *PEN2* and *PEN3* and an postinvasion resistance. In addition, the tomato pathotype can completely overcome the nonhost resistance if the plant is sensitive to the AAL-toxin. These results suggest that HST-producing ability may have evolved in the necrotrophic *Alternaria* pathogens as a pathogenic strategy to disrupt the nonhost resistance response in plants.

P2.133 - A novel species-specific chloroplastic effector of *Colletotrichum graminicola* suppresses the early immune response in maize

F.B. Cuevas-Fernández[1], R. Baroncelli[2], S. Becerra[1], P. García-Rodríguez[1], J.M. Sanz-Martín[1], J. Ortíz-Álvarez[3], S. Gutiérrez-Sánchez[1], L. Rodríguez-Mónaco[1], T.R. Boufleur[4], E.P. Benito[1], R. O'Connell[5], E.A. Espeso[6], M.R. Thon[1], S.A. Sukno[1]
[1]Universidad de Salamanca - Instituto de Investigación en Agrobiotecnología (CIALE), Villamayor, Spain, [2]University of Bologna, Department of Agricultural and Food Sciences (DISTAL), Bologna, Italy, [3]Programa "Investigadoras e Investigadores por México" Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCyT), Ciudad de México, Mexico, [4]University of Sao Paulo - Luiz de Queiroz College of Agriculture, Department of Plant Pathology and Nematology, Piracicaba, Brazil, [5]Université Paris-Saclay, INRAE, UR BIOGER, Palaiseau, France, [6]Centro de Investigaciones Biológicas (CIB) Margarita Salas - Consejo Superior de Investigaciones Científicas (CSIC), Department of Molecular and Cellular Biology, Madrid, Spain

Maize anthracnose disease threatens crop yields and food security, making its study vital for agricultural sustainability. Effectors play a crucial role in the interaction between plants and pathogens. Understanding their function can lead to the development of disease-resistant crop varieties. The effector protein 3 (CgEP3) of *C. graminicola* is a novel species-specific effector that enhances virulence in the early stages of infection and localizes to the chloroplasts of maize cells *in vivo*. Phylogenetic analysis indicates that *CgEP3* likely originated from the duplication of an ancestral gene that initially functioned as a nucleoside-phosphorylase, which later evolved to encode an effector protein. Synteny analysis suggests that rapid genomic evolution, possibly driven by ectopic recombination, contributed to the formation of the new gene, *CgEP3*. Transcriptional profiling and live cell imaging revealed that *CgEP3* is specifically expressed in conidia and appressoria, indicating its functional importance at the early stages of infection.

Mutants lacking *CgEP3* showed significantly reduced lesion size, impaired appressorial penetration, and decreased biomass accumulation during infection, although internal appressorial pressure and development remained normal. Complementation of *CgEP3* null mutants restored the wild-type phenotype. An RNA-seq analysis was performed to understand plant responses to the effector during maize leaf colonization by the fungus. CgEP3 repressed several plant defense genes, particularly those associated to chloroplast pathways, leading us to propose a model of action. Therefore, *CgEP3* counteracts maize's pre-invasion defense mechanisms, enabling faster fungal colonization. To our knowledge, this is the first known species-specific effector characterized in *C. graminicola*.

P2.134 - Unraveling the regulation of *Parastagonospora nodorum* effectors important in the infection of wheat

G.K. Kariyawasam[1,2], N.A. Wyatt[3], Z. Liu[2], J.D. Faris[4,5], T.L. Friesen[4,2]
[1]University of Tennessee, Entomology and Plant Pathology, Knoxville, United States, [2]North Dakota State University, Plant Pathology, Fargo, United States, [3]Edward T. Schafer Agricultural Research Center, USDA-ARS, Sugarbeet Unit, Fargo, United States, [4]Edward T. Schafer Agricultural Research Center, USDA-ARS, Cereal Crop Unit, Fargo, United States, [5]North Dakota State University, Plant Science, Fargo, United States

Effectors are small, secreted proteins that are beneficial to the pathogen during plant infection. *Parastagonospora nodorum*, which causes septoria nodorum blotch of wheat, produces multiple necrotrophic effectors that induce programmed cell death (PCD) in wheat genotypes that carrying the corresponding susceptibility genes. Five necrotrophic effectors including *SnToxA*, *SnTox1*,*SnTox267*, *SnTox3* and *SnTox5* have been cloned and functionally characterized. However, little is known about the role of non-PCD inducing effectors during infection. Therefore, in this study the proteome of *P. nodorum* strain Sn2000 was screened using signalP v5.0 and effectorP v3.0. RNA-Seq data were generated for samples collected at 0, 4, 12, 24, 48, 72, and 96 hours post inoculation of the SnTox5-producing isolate Sn2000 on the wheat differential line LP29, which carries the host susceptibility gene *Snn5*. *In silico* analysis predicted a total of 563 effectors whereas RNA-Seq analysis supported *in planta* expression of 435 of those predicted effectors. Out of 435, 250 effectors yielded at least 100 reads across RNA-Seq libraries and were therefore used in downstream analysis. InterProScan screening of these effectors revealed that these predicted effectors function as cell wall degrading enzymes, chitin binding proteins, proteases, necrotrophic effectors, enzymes involved in nutrient-breakdown, proteins that provide protection from reactive oxygen species and other host defense mechanisms. These results, together with temporal gene expression analysis, suggest that these non-PCD inducing effectors not only play a critical role in adhesion, penetration, and colonization, but also provide protection to the fungus in adverse conditions created by the host during the defense response.

P2.135 - Identification of accessory chromosome regions involved in the pathogenicity in the banana wilt pathogen, *Fusarium oxysporum* f. sp. *cubense* race 1

H. Ashikawa[1], A. Sagimori[2], M. Yachinaka[1], M. Matsui[1], K. Tobata[3], M. Yamazaki[3], L.M. Aragon Caballero[4], M. Kodama[5], T. Kashiwa[6], T. Arazoe[7], S. Asai[8], K. Komatsu[1], T. Arie[1]
[1]Tokyo University of Agriculture and Technology (TUAT), Graduate School of Agriculture, Fuchu, Japan, [2]Tokyo University of Agriculture and Technology (TUAT), Faculty of Agriculture, Fuchu, Japan, [3]Tokyo University of Agriculture and Technology (TUAT), United Graduate School of Agricultural Science, Fuchu, Japan, [4]Universidad National Agraria La Molina (UNALM), Plant Pathology, Lima, Peru, [5]Tottori University, Faculty of Agriculture, Tottori, Japan, [6]Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan, [7]Tokyo University of Science, Department of Applied Biological Science, Faculty of Science and Technology, Noda, Japan, [8]RIKEN CSRS, Tsurumi, Yokohama, Japan

Fusarium oxysporum f. sp. *cubense* (*Foc*) is a soilborne fungal pathogen causing the banana wilt (or, Panama disease) on banana (*Musa* spp.). The whole genome information of *Foc* race 1 160527 (*Foc*1) isolated in Okinawa, Japan (Asai 2019) and its comparison with other *F. oxysporum* isolates suggested that the contig 2 and contig 12 in *Foc*1 are accessory chromosome regions. We generated accessory region-loss mutants in *Foc*1 by treatment with 15 mg/ml benomyl, a cell-division inhibitor. Both of Dctg2#3 and Dctg2#2 mutants losing the entire contig 2 and the 2.5 Mb region of contig 2, respectively, lost their pathogenicity on banana cv. Shima-banana, whereas the mutant Dctg12#1 losing a part of the contig 12 showed no difference in pathogenicity compared to *Foc*1 wild type. The growth of Dctg2#2 and Dctg2#3 on PDA medium was not significantly different with those of *Foc*1 wild type. These suggested that the 2.5 Mb-region of the contig 2 of *Foc*1 is involved in pathogenicity on banana. The 2.5 Mb-region of the contig 2 contains the putative effector genes such as *SIX1*s, *SIX6*s, *SIX9* and *SIX13*s.

P2.136 - Fusarium verticillioides G protein-coupled receptors required for pathogenicity and fumonisin production

D. Brown[1], R. Proctor[1], H.-S. Kim[1], M. Busman[1]
[1]USDA-ARS, Mycotoxin Prevention and Applied Microbiology Research Unit, Peoria, United States

G protein-coupled receptors (GPCRs) are integral membrane proteins in eukaryotes that transduce chemical signals from the environment into the cell. In fungi, GPCRs influence diverse processes, including growth, development and pathogenesis. Contamination of maize with the mycotoxin fumonisin is a problem worldwide and is caused primarily by *Fusarium verticillioides*, which can also cause seedling, stalk and ear rots of maize. How *F. verticillioides* evades plant defenses to grow in planta, why it causes disease under some conditions but not others, and why it produces fumonisins are poorly understood. To address these questions, we examined the relationships of oxylipin-responsive GPCRs to pathogenesis and fumonisin production in *F. verticillioides*. Exposure of *F. verticillioides* to the oxylipins 9-HODE and 13-

HODE led to the upregulation of hundreds of genes, including multiple GPCR genes. Deletion analysis of three oxylipin-responsive GPCR genes revealed that all three were required for wild-type levels of seedling disease on maize and fumonisin production in culture. The three GPCR genes were also found to be required for induction of fumonisin gene expression by the plant defense oxylipin jasmonic acid. These findings revealed important links between GPCR-mediated detection of oxylipins and pathogenicity and fumonisin production in *F. verticillioides*.

P2.137 - Interactions of the fungal pathogen *Ramularia collo-cygni*

M. Burt[1], *I. Durand*[2], *S. Karki*[2], *R. Alexander*[1], *A. Feechan*[1]
[1]Heriot-Watt University, Institute of Life and Earth Sciences, Edinburgh, United Kingdom,
[2]University College Dublin, School of Agriculture and Food Science, Dublin, Ireland

Ramularia collo-cygni (*Rcc*) is the causal agent of Ramularia leaf spot (RLS), an economically important disease in barley. The resulting total yield losses can be as high as 20% with the average in Scotland being 0.4 tonnes/ha which equates to the loss of £10 million a year.
In pathogenic fungi, damage to the plant structure or fungal molecules in the apoplastic space can trigger pathogen-triggered immunity (PTI). Fungi have developed a mechanism to overcome and interfere with PTI by secreting a range of fungal effectors. In return, host plants have evolved to directly or indirectly perceive pathogen effectors via resistance proteins, triggering effector triggered immunity (ETI).
In European countries, chemical control of RLS has declined in efficiency. Resistance to *Rcc* is a key factor in barley cultivation and breeding. Studies looking for host resistance to *Rcc* in barley cultivars found no varieties with substantial varietal resistance available in the commercial market. *Rcc* has also been found to infect some wheat cultivars although this is usually asymptomatic. .
Currently we are investigating and comparing the interactions of *Rcc* in three cereal crops;, barley, wheat and tritordeum (a hybrid of wheat and barley). We will use previously generated transcriptomic data to identify genes involved in plant immunity that are differentially expressed in both wheat and barley in response to Rcc. These genes will be investigated as part of any conserved immune response to *Rcc* in barley, wheat and tritordeum. This work may have future applications to understand resistance or susceptibility to *Rcc*.

P2.138 - From the identification of new sources of resistance to late effectors to their introgression into modern oilseed rape varieties

A. Jiquel[1], *C. Rabeau*[1], *J. Gervais*[1], *A. Geistodt-Kiener*[1], *R. Delourme*[2], *J. Mas*[3], *P. George*[3], *A. Wagner*[3], *A. Fior*[3], *S. Faure*[3], *M.-H. Balesdent*[1], *T. Rouxel*[1]
[1]INRAE Bioger, Palaiseau, France, [2]INRAE IGEPP, Le Rheu, France, [3]INNOLEA, Mondonville, France

Leptosphaeria maculans is a phytopathogenic fungus being responsible for stem canker on *Brassica napus*. This disease is mainly controlled by plant genetic resistance: specific or quantitative resistance. During its long infectious cycle, alternating biotrophic, necrotrophic, and

endophytic stages, *L. maculans* colonizes asymptomatically the stems of oilseed rape, producing specific late effectors. We hypothesized that quantitative resistance partly relies on gene-for-gene interactions, with fungal effectors produced during stem infection being recognized by resistance proteins. We exploited the repertoire of 'late' effectors to identify corresponding genes in *Brassica* that could contribute to quantitative disease resistance. Using an innovative strategy of early expression of late effector genes, we validated that the interaction between the late effector LmSTEE98 and the resistance RlmSTEE98 obeys a typical gene-for-gene interaction, occurring during the stem colonization by *L. maculans*, that contributes partly to quantitative resistance, in controlled conditions. We then used the same strategy to search for new sources of resistance after selecting ten of the most relevant late effectors. Our screening approach of 130 diversified genotypes of *B. napus*, allowed us to identify new sources of resistance, displaying diversified interaction phenotypes. Our results demonstrate the existence of unsuspected sources of resistance that are potentially more durable than the classic major genes expressed earlier in the fungal cycle. Now, the next steps of this project are further validation of their efficacy in the field by introgression into modern genotypes and validation of the quantitative resistance markers.

P2.139 - Conserved function of a *Fusarium* homolog of the *Verticillium* effector *TRADE* for induction of host cell identity switches

G. Gupta[1], *J.-N. Lübbers*[1], *K. Subieta*[1], *C. Warning*[1], *T. Teichmann*[1,2], *V. Lipka*[1,2]
[1]Georg August University Göttingen, Germany, Department of Plant Cell Biology, Goettingen, Germany, [2]Georg August University Göttingen, Germany, Central Microscopy Facility of the Faculty of Biology & Psychology, Goettingen, Germany

Xylem-colonizing pathogens such as *Verticillium* and *Fusarium* infect a variety of economically important crops, causing tremendous annual yield loss. Contrary to the majority of *Verticillium* isolates that cause wilt on their host plants, chlorosis-inducing isolates do not negatively affect the water status of their host plants. We recently showed that in contrast to *Verticillium* wilt strains, the genomes of chlorosis class strains harbor genes encoding a secreted TRANSDIFFERENTIATION EFFECTOR (**TRADE**) protein. TRADE is capable to enter plant host cells and to trigger cellular reprogramming and host identity switches of bundle sheath and xylem parenchyma cells into novel xylem elements in Arabidopsis.
Interestingly, we found a homolog of *TRADE* in *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (*Forc*), $TRADE_{Fo}$, whose gene product shares 90.72% identity with the TRADE protein from *Verticillium dahliae*, suggesting acquisition via horizontal gene transfer. Infection experiments with *Forc* on its host plant cucumber showed macroscopic vein-clearing symptoms, which turned out to reflect transdifferentiation of bundle sheath cells into novel xylem elements at the microscopic level. This suggests a conserved function of the TRADE protein in *Verticillium* and *Fusarium*. We generated stable transgenic Arabidopsis lines producing fluorescent protein-labelled $TRADE_{Fo}$ in order to functionally characterize the effector protein in more detail. Transgenic lines exhibited macroscopic vein-clearing and microscopic cell identity switches into novel xylem elements. These findings were consistent with results obtained with heterologously expressed and purified $TRADE_{Fo}$ protein in Arabidopsis well plate growth assays, corroborating the conserved function of the $TRADE_{Fo}$ protein as an inducer of host cell identity switches.

P2.140 - Regulation of the development of two types of plant surface penetration structures in *Botrytis cinerea*

F. Pieterse[1,2], *L. Fokkens*[1], *R. Smith*[2], *E. Saccenti*[2], *J. van Kan*[1]
[1]Wageningen University, Laboratory of Phytopathology, Wageningen, Netherlands,
[2]Wageningen University, Laboratory of Systems and Synthetic Biology, Wageningen, Netherlands

The plant pathogenic fungus *Botrytis cinerea* has two distinct structures for penetrating the host surface. The unicellular appressorium facilitates the penetration of a single host cell, while a multi-cellular branched infection cushion penetrates at multiple sites and has a great capacity for the enzymatic destruction of the host surface. Which of the two strategies *B. cinerea* uses can be critical for the outcome of an infection. It is unclear however, which chemical signals and molecular mechanisms determine whether *B. cinerea* forms one structure or the other. We show how the presence of cutin wax monomers and spermine-like poly-amine groups presented on a surface promote early appressorium development, which reduces the amount of infection cushions formed at a later stage. Based on these experiments, we plan to define two experimental conditions where either appressoria or infection cushions are predominantly developed, preferably with synchronous dynamics. By sampling the transcriptome and phospho-proteome over time in these conditions, we plan to untangle the signaling events and gene regulation events downstream of the chemical signals sensed by the fungus. Using a network inference approach, this could give insight into the regulatory landscape of appressorium and infection cushion development. This network analysis will provide targets for further reverse genetic analyses. By comparing the regulatory network in *B. cinerea* to the regulatory network present in *Magnaporthe oryza*, we plan to study how conserved the regulation of appressorium development is between different plant pathogenic ascomycetes.

P2.141 - Investigating host targets of Zinc Finger Fold (ZiF) effectors in *Magnaporthe oryzae* for rice blast disease

L. Huang[1], *X. Yan*[1], *J.C. de la Concepcion*[2], *M.J. Bautista*[1], *I. Saado*[3], *J. Bennett*[3], *M. Banfield*[3], *N.J. Talbot*[1]
[1]The Sainsbury Laboratory, Norwich, United Kingdom, [2]Gregor Mendel Institute, Vienna, Austria, [3]John Innes Centre, Norwich, United Kingdom

Rice and wheat blast diseases, caused by *Magnaporthe oryzae*, result in significant crop yield losses. In rice, the *Pii* resistance gene, in conjunction with OsExo70F2/F3, recognize the pathogen's effector protein AVR-Pii. Interestingly, most *M. oryzae* lineages lack the *Avr-Pii* gene, potentially as an evolutionary strategy to evade recognition by *Pii*. Recent structural analysis of the AVR-Pii-OsExo70F2 complex revealed a zinc-finger fold (ZiF) domain within AVR-Pii. This discovery initiated a genomic survey of various *M. oryzae* strains, identifying 10 distinct tribes of ZiF-containing effector proteins. Preliminary experiments showed that many common ZiF effector tribes do not bind OsExo70F3, suggesting they may target other host proteins. Our research aims to identify host targets of ZiF effectors in rice-infecting *M. oryzae*

strains. We have selected six Zif effectors for detailed functional characterisation based on their presence across diverse *M. oryzae* strains and their potential involvement in host-pathogen interactions. To investigate their roles in pathogenicity, we will generate knockout mutants of these ZiF effectors using targeted CRISPR-Cas9 technology. In parallel, we will generate transgenic rice lines expressing ZiF effectors tagged with GFP or TurboID. These tagged effectors will facilitate identification of their host interacting partners through immunoprecipitation and subsequent mass spectrometry analysis. By isolating protein complexes associated with these effectors, we aim to uncover their targets in rice, elucidating the molecular mechanisms that underlie *M. oryzae* infection. This comprehensive characterisation of ZiF effector function will provide new insight into how *M. oryzae* manipulates host cellular processes to evade plant defence.

P2.142 - Effector-mediated suppression of host immunity by *Zymoseptoria tritici*: insights into early infection strategies

H. Ali[1], *E. Thynne*[2], *G. Kettles*[1]
[1]University of Birmingham, Biosciences, Birmingham, United Kingdom, [2]Christian-Albrechts University, Botanical Institute, Kiel, Germany

Zymoseptoria tritici is a major fungal pathogen and a significant threat to wheat production. Despite its economic importance, the molecular interactions between *Z. tritici* and wheat remains poorly characterised. This is particularly true for the early phase of colonisation, when the fungus needs to gain entry to the leaf mesophyll through stomata without stimulating host immunity. In this study, we utilised a library of previously cloned *Z. tritici* candidate effectors[1] to identify proteins that could (i) suppress pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI), or (ii) interfere with stomatal dynamics. Candidate effectors were transiently expressed in *Nicotiana benthamiana* and their ability to inhibit reactive oxygen species (ROS) production was assessed following treatment with bacterial and fungal PAMPs. Interference with stomatal dynamics was assessed by leaf thermal imaging and direct observation. Our screening identified 17 effectors with strong PTI-suppression phenotypes, indicating that suppression of ROS production is important for fungal colonisation. Furthermore, we identified 11 effectors that altered stomatal aperture, several of which also suppressed ROS production. One of these effectors (*Zt-LRR),* is predicted to have structural similarity to several plant LRR-RLKs previously shown to be involved in host defence, suggesting a potential mode of action. These results provide insights into the mechanisms of immune suppression employed by *Z. tritici* and highlight the reliance of this pathogen on a diverse array of effectors to overcome host defences during the early infection stages.

References: 1. https://doi.org/10.1111/nph.14215

P2.143 - Determining the Sln1-mediated-turgor sensing complex and phosphohistidine landscape in the blast fungus *Magnaporthe oryzae*

F. Dos Santos Barbosa[1], *L. Ryder*[1], *N. Cruz-Mireles*[1], *R. Kalagiri*[2], *T. Hunter*[2], *F. Menke*[1], *N. Talbot*[1]
[1]The Sainsbury Laboratory, University of East Anglia, Norwich, United Kingdom, [2]Salk Institute, Tony Hunter Lab, San Diego, United States

Host entry by the rice blast fungus *Magnaporthe oryzae* requires formation of a specialised infection structure called an appressorium. Appressoria generate enormous turgor pressure of up to 8 MPa, to breach the host leaf cuticle. This invasive force is directed by septin-mediated cytoskeletal reorganisation and actin-dependent protrusion of a rigid penetration hypha. Turgor generation in the appressorium is sensed by the Sln1 turgor sensor kinase, a histidine-aspartate kinase, which localises to the appressorium pore in a pressure-sensitive manner. Mutants lacking *SLN1* produce hypermelanised appressoria and excess turgor, leading to aberrant septin ring organisation and impaired appressorium repolarisation and plant infection. In yeast, Sln1 was previously characterised as an osmosensory histidine-aspartate kinase that initiates a phosphorelay system which regulates the high osmolarity glycerol (HOG1) MAPK pathway in response to hyperosmotic stress. In *M. oryzae,* Sln1 has been recruited to coordinate several pathways involved in turgor generation including melanin biosynthesis, the protein kinase C cell-integrity pathway, and the cAMP-dependent protein kinase A signalling pathway. Direct tracking of histidine phosphorylation in Sln1 targets has been historically challenging due to the acidic and heat labile nature of phosphohistidine, and lack of suitable detection tools. However, the groundbreaking recent development of 1-pHis and 3-pHis antibodies has provided a means to study histidine phosphorylation. Our research aims to identify and characterise downstream phosphorylation targets of Sln1 *M. oryzae.* aiming to elucidate how histidine phosphorylation regulates the transition from turgor generation to host penetration during appressorium-mediated plant infection.

P2.144 - Rice blast diversity in the Yuanyang Terraces

P.-M. Marty[1], *H. Adreit*[1], *S. Ravel*[1], *S. De Mita*[1], *E. Fournier*[1]
[1]PHIM Plant Health Institute, Univ Montpellier, INRAE, CIRAD, Institut Agro, IRD, Montpellier, France

Pyricularia oryzae is a phytopathogenic fungus responsible for the severe rice blast disease. Traditional rice culture of the Yuanyang terraces (YYT) of the Yunnan province of China maintained high diversity with more than 180 landraces and extensive repertoires of immune receptors. Blast incidence is low in YYT and we previously shown that generalist lifestyle with intermediate performance was selected for in *P. oryzae* populations from YYT. We aim to better understand how *P. oryzae* populations evolve when facing such a diverse host environment. To achieve this goal, the first step is to characterize the genetic diversity of *P. oryzae* in the YYT and to understand the demographic history of this pathogen.
Four *P. oryzae* lineages infecting rice were previously described worldwide and South-East Asia

appeared as a hot-spot of diversity. We used 94 Illumina genomes of *P. oryzae* from the YYT. An additional set of 150 *P. oryzae* genomes representative of the global diversity of *P. oryzae* were included, resulting in a dataset containing ca. 250 isolates and 35 biallelic kSNPs without missing data. Genetic network and principal component analyses were performed to delineate genetic lineages. We showed that three out of the four previously described worldwide lineages coexisted with four YYT endemic lineages. The latter were highly differentiated from worldwide lineages and showed low nucleotide diversity. LD decay analyses and *in vitro* cross experiments suggest that *P. oryzae* reproduces asexually in the YYT. Altogether, these results suggest a complex demographic history still to be disentangled.

P2.145 - Unravelling transcriptomic dynamics of fungal-host interaction in the *Botrytis cinerea* - strawberry pathosystem

N. Mariz Ponte[1], *T.R. Fernandes*[1], *S.M. Carvalho*[1]
[1]GreenUPorto—Sustainable Agrifood Production Research Centre/Inov4Agro, DGAOT, Faculty of Sciences of the University of Porto, Vairão, Portugal

Botrytis cinerea is a devastating fungal pathogen causing grey mould (GM) disease in numerous crops of agronomic importance, including strawberry. GM leads to severe fruit losses, both during pre- and post-harvest, also threatening fruit quality. Although disease symptoms mostly appear after harvest, *B. cinerea* usually infects strawberry plants at flowering or unripe fruit stages, remaining in a symptomless quiescent phase for long periods before causing rapid decay of tissues. Most studies have focused on the mechanisms controlling the physiological switch from quiescent to necrotrophic behavior. However, what drives and keeps *B. cinerea* into quiescence until fruit ripening remains largely uncharacterized. This study aims to explore fungal-host crosstalk during both quiescent and necrotrophic infection stages through a global expression profiling in unripe and ripe infected strawberry fruits. Our preliminary results on gene ontology (GO) analysis indicated that photosynthesis-associated genes are downregulated in ripe infected fruits compared to unripe, 72 hours post-infection (hpi). Furthermore, KEEG analysis revealed that *B. cinerea* infection leads to an upregulation of the monoterpenoid biosynthesis and glutathione pathways, and triggers suppression of pyruvate biosynthesis in ripe infected fruits. Additionally, the ongoing analysis of *B. cinerea* transcripts will uncover the uncharacterized mechanisms inhibiting pathogenicity in unripe fruits, where the fungus is in a quiescent phase. These results could strongly contribute for developing new sustainable strategies to mitigate GM disease.

P2.146 - Building a genetic map of aflatoxin biosynthesis to accelerate novel strategies for transgenic and biological Control

B. Bluhm[1], *T. Marshall*[1], *K. Swift*[1]
[1]University of Arkansas Division of Agriculture, Department of Entomology and Plant Pathology, Fayetteville, United States

Conventional breeding has revolutionized modern corn production but has not yet provided reliable resistance to aflatoxin. Transgenic approaches can accelerate the deployment of aflatoxin resistance, but a key bottleneck is an incomplete understanding about which fungal genes should be targeted. Relatedly, one of the most effective tools currently available to combat aflatoxin is biological control via the application of atoxigenic strains of *Aspergillus flavus*. Molecular dissection of chemical communication among strains could enable development of novel inhibitors of aflatoxin biosynthesis; however, fungal genes underlying biological control have not been identified. The overarching goal of this project is to globally map the genetic regulation of aflatoxin biosynthesis and atoxigenic suppression in *A. flavus*. To this end, we are taking a two-pronged approach. First, we are harnessing naturally occurring genetic and phenotypic diversity to dissect the regulation and suppression of aflatoxin biosynthesis via population genetics. In a complementary approach, we are utilizing a blend of forward and reverse genetics to identify genes involved in the regulation of aflatoxin biosynthesis. Results from these parallel activities will be integrated to provide a comprehensive genetic map of aflatoxin biosynthesis in *A. flavus*.

P2.147 - Optimization of the heterologous production of the antifungal protein PeAfpA from *Penicillium expansum* in a stirred-tank bioreactor

S. de Ovalle[1], *S. Garrigues*[1], *M. Gomez-Romero*[1], *A. Abad-Fuentes*[2], *J.V. Mercader*[2], *J.F. Marcos*[1], *P. Manzanares*[1]
[1]Institute of Agrochemistry and Food Technology (IATA-CSIC), Food Biotechnology, Paterna (Valencia), Spain, [2]Institute of Agrochemistry and Food Technology (IATA-CSIC), Food preservation and safety, Paterna (Valencia), Spain

The use of chemical fungicides is the primary method employed to protect crops from fungal infections. Antifungal proteins (AFPs), which are small, cationic, cysteine-rich proteins secreted by filamentous fungi, are emerging as promising alternatives for crop and postharvest protection. The PeAfpA protein produced by the phytopathogen *Penicillium expansum* has demonstrated *in vivo* inhibitory activity in various plant pathosystems. Despite its potential, large-scale application is limited due to the mycotoxigenic nature of the producing strain.
This study presents the optimization of PeAfpA production by *Penicillium chrysogenum* PCMG0052 strain at a semi-pilot scale in 0.5 L bioreactors. Firstly, we developed a competitive enzyme-linked immunosorbent assay (ELISA) for PeAfpA quantification in culture supernatants avoiding the protein purification steps. Optimized ELISA conditions resulted in an IC_{50} value of 8.8 ng/mL and a calculated limit of detection (LOD) value of 0.4 ng/mL. Optimal conditions for PeAfpA production in PcMM medium at 25°C were pH 8, 300 rpm, 20% dissolved oxygen, and a 10% v/v inoculum of a 48h pre-grown culture. Under these conditions, PeAfpA yield increased from the initially reported 5 mg/L in Erlenmeyer flasks to 60 mg/L after 7 days of growth. Moreover, PeAfpA-enriched culture supernatants showed antifungal effect against the relevant citrus phytopathogenic fungus *Penicillium digitatum in vitro* (MIC: 3 µg/mL), suggesting the applicability of enriched supernatants for fungal control without requiring further purification steps, reducing downstream processing and production costs. These results represent a significant advance in the efficient production of PeAfpA, enhancing its potential for industrial application as novel biofungicide.

P2.148 - Heterologous expression and characterization of defensins from *Solanum lycopersicum* L

R. Papp[1], *G. Váradi*[2], *Z. Kele*[2], *A. Borics*[3], *P. Poór*[4], *G.K. Tóth*[2,5], *L. Galgóczy*[1], *L. Tóth*[1]
[1]University of Szeged, Faculty of Science and Informatics, Department of Biotechnology and Microbiology, Szeged, Hungary, [2]University of Szeged, Albert Szent-Györgyi Medical School, Department of Medical Chemistry, Szeged, Hungary, [3]HUN-REN Biological Research Centre, Institute of Biophysics, Szeged, Hungary, [4]University of Szeged, Faculty of Science and Informatics, Department of Plant Biology, Szeged, Hungary, [5]University of Szeged, MTA-SZTE Biomimetic Systems Research Group, Szeged, Hungary

The prevention of crop losses caused by fungicide-resistant plant pathogenic fungi is a huge challenge for agriculture every year. This problem is further complicated by the European Union (EU) regulations, that several chemical-based fungicides have been withdrawn from the market. In accordance to the new EU directives, safely applicable bio-based fungicides must be introduced to the pesticide market. Natural antifungal defensins produced by plants are considered as potential plant and crop protective biocompounds. However, plants synthesize them in small amount, therefore their agricultural application requires industrial bulk production by a heterologous expression system. In the present work, we established *Pichia pastoris*-based heterologous expression systems for bulk production of two *Solanum lycopersicum* L. defensins (K4CBP6 and B1N680), which allowed the large-scale production of them. Mass spectrometry, reversed-phase high performance liquid chromatography, and circular dichroism spectroscopy indicated that both recombinant defensins are properly matured, have an ordered, disulphide-bridge stabilized tertiary structure with secondary structural elements that are characteristics of plant defensins. The antifungal effects of the recombinant K4CBP6 and B1N680 were investigated. Both defensins effectively inhibited the growth of different *Fusarium*, *Botrytis cinerea* and *Cladosporium herbarum* isolates. These suggest that *P. pastoris* can be applicable for the heterologous production of tomato plant defensins, which can be suitable biofungicides for agriculture.
The presentation of research work of L.T. at this conference was supported by the NKFIH, MEC-R 149039 project. Present work of L.G. was financed by the Hungarian National Research, Development and Innovation Office - NKFIH, FK 134343 and K 146131 projects.

P2.149 - Population genomics of the laminated root rot fungus *Coniferiporia*

L. Vargas Gastelum[1], *S. McMurtrey*[2], *J. LeBoldus*[1]
[1]Oregon State University, Botany and Plant Pathology, Corvallis, United States, [2]USDA-Agricultural Research Service, Hood River, United States

The wood-inhabiting fungi *Coniferiporia sulphurascens* and *Coniferiporia weirii* are classified as aggressive pathogenic basidiomycetes, causing laminated root rot (LRR) disease in economically important conifer species worldwide. While it has been suggested that these two pathogens were introduced to North America from Eurasia 7.5 million years ago, they are considered endemic to

western North America's mixed conifer forests. *C. sulphurascens* and *C. weirii* differ in their host specificity, with the former being predominantly associated with *Pseudotsuga menziesii* and *Abies grandis*, and the latter being primarily associated with *Thuja plicata* and *Callitropsis nootkatensis*. Although much is known about the biology and spatial distribution of *Coniferiporia*, little is known about the processes that affect the population dynamics of the pathogen. The successful management of LRR requires a more comprehensive understanding of disease transmission dynamics and the formation of new infection centers. In order to gain a deeper insight into the demographic and population structure of *C. sulphurascens* and *C. weirii*, 85 whole genome sequences of *C. sulphurascens* and 5 whole genome sequences of *C. weirii* were produced using Illumina HiSeq 3000 technologies. These sequences were derived from 12 populations across Canada, Japan, Russia, and the United States. A population genomics approach was undertaken utilizing genome-wide single nucleotide polymorphism (SNP) data with the objective of estimating population structure, characterizing gene flow among populations, and to compare the evolutionary history of the two species.

P2.150 - Assessing new SSR markers for population structure and genetic diversity analysis of *Plenodomus tracheiphilus*, causing "mal secco" disease of citrus in Italy

M.T. Valente[1], *L. Orzali*[1], *M. Aragona*[1], *A. Grottoli*[1], *A. De Iudicibus*[1], *S. Bechini*[1], *A. Infantino*[1]
[1]Council for Agricultural Research and Economics (CREA), Rome, Italy

Plenodomus tracheiphilus is a vascular pathogen of citrus, particularly lemons, causing the "mal secco" disease. The aim of the present study was to validate new simple-sequence repeats (SSR or microsatellites) markers to assess genetic variability among the isolates collected in different Italian lemon-producing areas. SSRs have been proven to be the markers of choice in species with reduced genetic diversity, just as *P. tracheiphilus* appears to be from previous studies. Sampling was carried out starting from spring 2024 from symptomatic twigs of trees. Isolations were performed on the basis of one isolate per tree and identification was done by morphological and molecular (qPCR) analysis. A total of 158 monohyphal isolates were obtained from 11 populations in 5 Italian regions. The *P. tracheiphilus* genome, available at the NCBI Genome Database, was used to find SSR motifs by the software KRAIT. A total of 2454 SSR loci were detected (0.16% of total sequence length). Tri-nucleotide repeats constituted the maximum percentage of SSRs counts and lengths followed by di-nucleotide repeats. In the preliminary screening for polymorphic SSRs these two motifs were the most used, as it has been established that longer microsatellites are more likely to be polymorphic than shorter ones. Twenty primer pairs for di-, tri-, tetra-, penta-, and hexanucleotide loci were designed and preliminarily tested on 8 isolates from different sampling areas. Moreover, this project will involve long-read sequencing of the *P. tracheiphilus* genome to generate complete chromosomal assemblies onto which selected microsatellites can be mapped.

P2.151 - Weed seeds as hidden reservoirs: genomic insights into *Fusarium* species and their role in TR4 persistence and evolution in Philippine banana plantations

L.L. Kottenhagen[1], *D.G. Catambacan*[2], *G. Nakasato-Tagami*[1], *M. Cavite*[2], *R.J. Gallardo*[2], *C.M.A. Tupino*[2], *B. Corcolon*[2], *D.W. Etalo*[1], *G.H.J. Kema*[1]
[1]Wageningen University and Research, Laboratory of Phytopathology, Wageningen, Netherlands, [2]Tagum Agricultural Development Company Inc, Research Information and Compliance Division, Panabo City, Philippines

The Philippines ranks as the fourth-largest banana-producing country in Asia and the second-largest global exporter of Cavendish bananas, a crop severely threatened by *Fusarium odoratissimum* Tropical Race 4 (TR4), the causal agent of Fusarium Wilt of Banana. This soil-borne fungus is notorious for its persistence, remaining viable in soil for decades. It is further exacerbated by alternative hosts, such as weeds in banana plantations, enhancing its survival and spread. A preliminary greenhouse study showed that TR4 could reside in alternative hosts and may even transmit to their seeds, underscoring their potential role in pathogen dispersal. In light of these findings, we conducted a field survey in Mindanao, Philippines, to investigate the role of weed seeds as potential reservoirs for *Fusarium* species. A total of 125 weed seed samples were collected from different locations across plantations managed by TADECO, DOLE, and from areas abandoned due to TR4 infestation. Our screening revealed multiple *Fusarium* species, including three isolates of *Fusarium oxysporum*. Given that *Fusarium oxysproum* race 1 and TR4 are genetically distinct, we hypothesized that these weed isolates may represent ancestral forms of the pathogen, providing critical insights into their evolutionary relationships. Whole-genome sequencing of these isolates was conducted using Oxford Nanopore technology to better understand the genetic relatedness of these isolates to pathogenic forms, specifically race 1 and TR4. Further analysis of the genome and accessory regions will help clarify the role of weed seeds in *Fusarium* ecology and evolution and their potential impact on managing Fusarium Wilt in banana plantations.

P2.152 - Pleosporaceae: the evolution of plant pathogens

S. Dool[1,2], *S. Puechmaille3*[3,4]
[1]CIRAD, Plant Health Institute Montpellier (PHIM), Montpellier, France, [2]PHIM, Univ. Montpellier, CIRAD, INRAE, Institut Agro, IRD, Montpellier, France, [3]University of Montpellier, Institut des Sciences de l'Évolution Montpellier (ISEM), Montpellier, France, [4]Institut Universitaire de France, Paris, France

The family Pleosporaceae includes numerous genera containing fungal species with diverse lifestyle strategies, including many significant plant pathogens (e.g. *Bipolaris sp.*). The lack of informative morphological characters has long hampered taxonomic decisions and phylogenetic studies in this group. The advent of molecular data has helped to clarify these aspects, but inter-generic relationships within the group are not fully resolved and have thus far been inferred based on a few genetic markers. Phylogenetic uncertainty prevents studies on the evolution of traits

facilitating pathogenicity. In this study, phylogenetic relationships were studied using whole genome data (n=66) to resolve inter-generic relationships and to shed light on the evolutionary origin of plant pathogens in this family. The evolution of lifestyle, host specialisation and geographic origin were estimated using ancestral state reconstruction. Phylogenetic signal in genomic traits implicated in pathogenicity such as genome size, repetitive and transposable element content were assessed and interspecific syntenic blocks were mapped. These results contribute to our understanding of the evolution of plant pathogens using comparative genomics based on well-resolved phylogenies.

P2.153 - Understanding the virulence of the soilborne hybrid Verticillium longisporum plant pathogen through genomic and evolutionary analysis

M. Orsucci[1], *A. Samaras*[2], *J. Svedberg*[1], *M. Karlsson*[2], *G. Tzelepis*[2], *H. Johannesson*[1]
[1]Stockholm University, Department of Ecology, Environment and Plant Sciences, Stockholm, Sweden, [2]Swedish University of Agricultural Sciences, Forest Mycology and Plant Pathology, Uppsala, Sweden

Verticillium longisporum is a soil-borne fungal specie that causes Verticillium wilt disease in members of the Brassicaceae family. This species has a wide distribution and is known to have a severe negative impact on rapeseed yield worldwide. *Verticillium longisporum* is an allodiploid species that has arisen from three events of hybridization, resulting in three distinct lineages. A1/D1, A1/D2, and A1/D3. The species A1, an unknown ancestor, is common to all three lineages of *V. longisporum*. This pathogen has been comparatively understudied in comparison to the parental species, *V. dahliae*. This is partly due to the complexity of its genome (diploid, multiple origins, etc.) and the lack of effective control methods. Therefore, a more profound comprehension of its evolutionary history and genomic constitution may prove instrumental in the formulation of novel management strategies. In this study, we employ a range of analytical techniques to examine the distinctions between the *V. longisporum* lineages and to ascertain any correlations between virulence and genotype. A virulence assay was conducted on 100 strains isolated from regions where Brassicaceae plants are cultivated. Significant differences in virulence were observed between strains, depending on their geographical origin. Genome sequences were generated from a number of strains using long-read sequencing methods, which revealed a mosaic structure and breakdown of collinearity, even within the same lineage. This raises an open question concerning the evolution of asexual species and the understanding of the dynamic genome evolution through rearrangements.

P2.154 - Identifying pathogenicity determinants of *Plenodomus tracheiphilus* via comparative *in vitro* and *in vivo* RNASeq analysis

A. Grottoli[1], *M. Aragona*[1], *L. Pirone*[1], *A. Haegi*[1]
[1]Council for Agricultural Research and Economics (CREA), Rome, Italy

'Mal secco' disease, caused by the fungus *Plenodomus tracheiphilus*, poses a significant threat to lemon cultivation in Italy and the broader Mediterranean region. The pathogen infects the vascular system of lemon trees, leading to wilting, dieback and eventual death, resulting in substantial economic losses. Understanding the molecular mechanisms underlying the pathogenicity of *P. tracheiphilus* is crucial for developing effective and sustainable control strategies. This study aims to evaluate the gene expression profile of *P. tracheiphilus* using RNA sequencing (RNASeq) of an *in vitro* assay. The fungus was cultured in liquid medium supplemented with lyophilized twigs and leaves of the susceptible lemon cultivar 'Femminiello' to mimic host-pathogen interactions and induce the expression of pathogenicity-related genes. Total RNA was extracted from these cultures, and high-throughput RNASeq was performed to identify differentially expressed genes in response to the presence of lemon tissue. Transcriptomics data were used to annotate the reference genome of *P. tracheiphilus* RNASeq data obtained will be compared to RNASeq datasets present in the literature and obtained from *in planta* interaction between the pathogen and the host, to identify common and unique genes associated with pathogenicity. By identifying key determinants of pathogenicity, such as effector proteins and other virulence factors, this research enhances our understanding of the molecular interactions between *P. tracheiphilus* and its host. The findings may lead to the development of targeted interventions, support breeding programs for disease-resistant lemon varieties, and contribute to more effective management strategies for mal secco disease.

P2.155 - Identification and characterization of core *Cercospora beticola* biotrophic effectors through pan-genomic analysis

N. Wyatt[1], *R. Jaswal*[2], *L. Barnabas Ebinezer*[2], *G. Secor*[2], *M. Bolton*[1]
[1]USDA-ARS, Sugarbeet Research Unit, Fargo, United States, [2]North Dakota State University, Plant Pathology, Fargo, United States

Cercospora beticola, causal agent of Cercospora leaf spot (CLS), is the most prominent foliar disease of sugar beet. The infection caused by *C. beticola* can significantly deteriorate sugar beet foliar tissue leading to severe yield losses. Despite being a pathogen of high economic importance, very limited information is available regarding the molecular mechanisms deployed by C. *beticola* to manipulate the sugar beet host. Recently, we sequenced a large globally diverse population of *C. beticola* to identify effector genes that are conserved among the species and leveraged RNAseq data to select potential effectors that were transcriptionally active during the biotrophic phase of *C. beticola* infection, resulting in 28 candidates. Effectors candidates were subjected to structural prediction via Alphafold2 and structural homologs were identified using a combination of Foldseek and Dali revealing homology to diverse enzymes such as hydrolases, metalloproteases, superoxide dismutase, cellulases, ribotoxins, as well as uncharacterized functions. Candidate effectors are currently being characterized via targeted gene knockouts and transient expression in in *Nicotiana benthamiana* using to screen for cell death suppression. Further, i*n planta* localization studies of fluorescently tagged candidates is underway to identify the cellular location of these effector genes.

P2.156 - Sources of genomic diversity in *Sclerotinia sclerotiorum* using a population from western Canada

B. Lone[1], *H Garg*[1], *K.D. Puri*[2], *J. Durkin*[1], *J. Adam*[1], *M. Harrington*[1], *D. Liabeuf*[3], *A. Davies*[1], *D.D. Hegedus*[1], *A.G. Sharpe*[4], *K. Gali*[5]
[1]Agriculture and Agri-Food, Saskatoon Research and Development Centre, Saskatoon, Canada, [2]University of Saskatchewan, Crop Development Centre, Department of Plant Sciences, Saskatoon, Canada, [3]Driscoll's, Watsonville, California, United States, [4]University of Saskatchewan, Global Institute for Food Security, Saskatoon, Canada, [5]University of Saskatchewan, Crop Development Centre, Department of Plant Sciences, Saskatoon, Canada

Sclerotinia sclerotiorum was characterized for genomic and pathogenic diversity using a collection of isolates from three Provinces in western Canada. New simple sequence repeat (SSR) markers representing all 16 fungal chromosomes were used to genotype 127 isolates, and showed each was an unique haplotype. Analysis of linkage disequilibrium rejected random genomic recombination consistent with a self-fertile life style. Mycelium compatibility tests among 133 isolates revealed a higher frequency of compatible isolates in a province with more susceptible crops species in rotation and wetter growing season. Furthermore, this population was dominated by clones of mutually compatible isolates as well as long stings of pairwise compatible isolates in contrast to the other provinces, likely because of frequent mycelium interaction on plants combined with more life cycles over time. Interestingly, clones of mutually compatible isolates had 86-95% similar SSR haplotype, while incompatible isolates were dissimilar and had numerous private SSR alleles. The dataset seems to have captured isolates at various stages of divergence, beginning with clonal isolates having similar SSR haplotypes, followed by stepwise divergence into compatible isolates forming strings, pairs of compatible isolates, and ending with incompatible isolates with unique SSR haplotypes. The most likely sources of genomic diversity in *S. sclerotiorum* in this study likely comprise slippage during DNA replication and point mutation affecting individual nucleotides, which are particularly frequent in SSRs and accumulate with each cell division. It is conceivable that genetic information passes from one mycelium compatible isolate to another by hyphal anastomosis, but over time, certain genetic factors prevent further compatibility, after which isolates become distinct haplotypes where polymorphisms continue to accumulate. In addition, it was clear that physical separation contributed to divergence, seen as low (11%) mycelium compatibility between isolates from different Provinces compared to higher (35-61%) compatibility between isolates within each Province. A phylogenetic tree based on SSR haplotype grouped isolates into 17 sub-populations. Aggressiveness was tested by inoculating one isolate from each sub-population onto six *Brassica napus* lines with quantitative resistance. Subsequent analysis of variance was significant for isolate, line and isolate by line interaction. These 17 isolates represent the genomic and pathogenic diversity in western Canada and are suitable for resistance screening in plant breeding programs. Reference: Buchwaldt et al. 2022. Sources of genomic diversity in the self-fertile plant pathogen, *Sclerotinia sclerotiorum*, and consequences for resistance breeding PLOSONE. https://doi.org/10.1371/journal.pone.0262891

P2.157 - A study of population genetics in fungi of the genus *Diaporthe*

D. Čepukoit[1], *I. Sokė*[1], *S. Jaseliūnaitė*[1], *D. Burokienė*[1]
[1]Nature Research Centre, Laboratory of Plant Pathology, Vilnius, Lithuania

The fungus genus *Diaporthe* Nitschke is one of the most widespread and damaging fungi, affecting a large number of host plants worldwide. It can cause significant damage through canker, leaf spot, rot and even plant death, resulting in both economic and ecological losses. However, there are no comprehensive studies on *Diaporthe* in Lithuania yet.
The aim of this study was to assess the diversity and population genetics of *Diaporthe* species associated with Fabaceae plants. Between 2017 and 2022, 149 woody plants were collected, with *Diaporthe* identified as the most abundant fungus. 339 fungal isolates were obtained from 89 plants. The genetic diversity of the populations and mating types of isolates of the genus *Diaporthe* was analysed. The mating type was determined by PCR reactions using the degenerate primers specific for the MAT1-1-1 and MAT1-2-1 genes. To perform RAPD analysis, PCR was performed with an M13 primer. For the ISSR analysis, a PCR with five primers was performed. The most informative primer, UBC-880, was selected for further analysis.
RAPD analysis of *Cytisus scoparius* populations revealed a high degree of genotypic diversity, suggesting asexual reproduction. In contrast, no significant population structure was detected in *Caragana arborescens* and *Caragana frutex* by RAPD analysis, although ISSR analysis showed different results. Ongoing research is focusing on the genetic and vegetative compatibility of *Diaporthe* species occurring in *Robinia pseudoacacia* and *Robinia ambigua* to further explore the genetic structure of these fungal populations.

P2.158 - Pilidium lythri: from tropical origins to temperate invasion - genomic sequencing and de novo assembly

D. Siegieda[1], *J. Panek*[1], *E. Hannula*[2], *M. Frąc*[1]
[1]Institute of Agrophysics, Polish Academy of Sciences, Lublin, Poland, [2]University of Leiden, Leiden, Netherlands

Pilidium lythri belongs to the phylum Ascomycota, class Leotiomycetes, order Chaetomellales, and family Chaetomellaceae. This pathogen is known to attack both wounded and intact fruits, with a particular affinity for strawberry plants, which are its primary host. While *P. lythri* has predominantly been identified as a phytopathogen in tropical and Mediterranean/subtropical climate zones, our team recently molecularly identified it on symptomatic strawberry plants in the temperate climate of Poland.
The ongoing dissemination of this phytopathogen into new areas poses a significant threat to global agricultural production (due to reduced yields) and consumer safety (due to the potential production of allergens and/or toxins). Despite this, only a few scientific reports have been published on the pathogen, primarily focusing on obtaining its genetic fingerprint and characterizing its phylogenetic relationships within the genus using the LSU region. However, a complete genome sequence of this fungus is not yet available in bioinformatics databases.

Our research focused on hybrid sequencing and *de* novo assembly of the pathogen's genome using short-read Illumina MiSeq and long-read ONT MinION technologies. The characterization of *P. lythri* remains incomplete, and a comprehensive investigation of its traits using advanced bioinformatics tools is crucial to safeguarding global agricultural production and consumer safety.

This research was funded by National Science Centre, Poland, PRELUDIUM21, contract number: 2022/45/N/NZ9/02089
The scholarship at the University of Leiden was financed by the Polish National Agency for Academic Exchange, contract number: BPN/BEK/2023/1/00125/U/00001

P2.159 - Identification of inoculum reservoirs of the causal agent of almond anthracnose (*Colletotrichum godetiae*)

M. Ramos[1], *R. Maurício*[2], *P. Talhinhas*[1]
[1]Instituto Superior de Agronomia, LEAF- Linking Landscape, Environment, Agriculture and Food Research Centre and Associate Laboratory TERRA, Lisboa, Portugal, [2]Instituto Superior de Agronomia, Lisboa, Portugal

The productivity of almonds in Portugal has significantly increased due to its cultivation under intensive irrigation systems in the Alentejo region. However, this system also increased the incidence of almond anthracnose, which is primarily caused by *Colletotrichum godetiae* but can also be caused by *C. acutatum*, *C. fioriniae*, *C. nymphaeae*, and *C. simmondsii*. These pathogens are polyphagous and primarily dispersed by rain splashes, allowing them to move between plants. Consequently, weeds in almond orchards may contribute to the survival and dispersal of the inoculum during the almond tree's dormant period (Autumn-Winter). This study investigated how *C. godetiae* interacts with various species of Alentejo spontaneous flora and how these species may influence the maintenance and spread of inoculum and the disease. After inoculating a collection of plants with a suspension of *C. godetiae* conidia, it was observed that the fungus can cause symptoms and signs on *Lathyrus tingitanus* and on *Trifolium pratense* and act as an epiphyte with the ability to sustain and multiply conidia in species such as *Cichorium intybus*, *Conyza* sp., *Medicago* spp., *Picris echioides*, *Polygonum aviculare*, *Scorpiurus sulcatus*, *Taraxacum* sp., and *Trifolium vesiculosum*, thus contributing to inoculum survival and multiplication. Conidia germinated and produced appressoria on *Andryala integrifolia*, *Torilis arvensis*, and *Rumex pulcher* but no further development was detected, suggesting that these plants may limit the pathogen's spread. A deeper understanding of the susceptibility of characteristic flora in almond orchards will help optimize vegetation management between and around the rows, aiming to reduce potential inoculum reservoirs.

P2.160 - Recent advances in dissecting the molecular basis of the *Zymoseptoria tritici* - wheat interaction

E. Hill[1], *N. Tasneem*[1], *E. Cross*[1], *D. Bhatta*[1], *B. Ovenden*[2], *N. Yang*[2], *S. Williams*[1], *A. Milgate*[2], *P. Solomon*[1]
[1]The Australian National University, Research School of Biology, Canberra, Australia, [2]New South Wales Department of Primary Industries and Rural Development, Wagga Wagga, Australia

The Network for Extracellular Plant Immunity (NEPI) is a consortium of researchers in Australia focussed on understanding how plants perceive and respond to pathogens in the extracellular space (apoplast). A key program within NEPI focusses on understanding the interaction of *Zymoseptoria tritici* with its wheat host. *Zymoseptoria tritici* undergoes an extended latent phase during its interaction with wheat where it grows for up to 14 days within the apoplast asymptomatically. During the latent phase, the pathogen and the host undergo a complex interaction whereby the pathogen seeks to evade the myriad of host defence responses. A key mechanism employed by wheat to resist the infection by *Z. tritici* is to perceive specific pathogen proteins (avirulence proteins) by receptors (resistance proteins) and initiate a defence response. To date, all identified resistance proteins harbour an extracellular domain strongly suggesting that resistance to *Z. tritici* is mediated by perception in the apoplast. I will present recent data from NEPI that has made progress towards understanding the molecular basis of this interaction, particularly at the protein level.

P2.161 - Improvement of olive anthracnose management in prefecture of Chania, Crete

A. Papageorgiou[1], A. Karagianni[2], C. Steppas[3], M.M. Mathioudakis[2], D. Tsitsigiannis[1], A.L. Lagopodi[3]
[1]Agricultural University of Athens, Faculty of Plant Sciences, Plant Pathology Laboratory, Athens, Greece, [2]Institute of Olive Tree, Subtropical Crops & Viticulture (ELGO-DIMITRA), Plant Pathology Laboratory, Chania, Greece, [3]Aristotle University of Thessaloniki, School of Agriculture, Plant Pathology Laboratory, Thessaloniki, Greece

Temperatures' rise combined with high relative humidity, led to serious outbreaks of the olive anthracnose disease in Greece, in previous years. In the frame of the GleoliveTreat project, an integrated olive anthracnose management is currently implemented in Chania prefecture of Crete. It includes an integrated management model adapted to the local conditions indicated by meteorological data, and the pathogen's population profile in the area of action, specific phenological stages of olive, in pilot groves (conventional, organic). In particular, sprays are applied from the beginning of blooming and during autumn, using suggested commercial formulations. Additionally, combined cultural and hygiene measures are suggested. The effectiveness of the treatments is evaluated annually. According to the results so far, for the period from spring 2023 to winter 2024, it can be concluded that: 1. The weather conditions did not favor the appearance of olive anthracnose, but in combination with entomological infestations the development of rots from other fungi was not prevented with most predominant species in genera *Fusarium* and *Alternaria*. 2. The causative species of anthracnose, endemic to the region is *Colletotrichum acutatum*. However, this pathogen participated in the complex of olive fruit rots causes by only 3% of the total isolates performed. 3. The sprays applied, did not significantly reduce the rates of rotting, which is possibly due to the use of formulations that targeted *Colletotrichum* species, while other pathogens prevailed during the application period. 4. The implementation of an integrated treatment system and the parallel treatment of entomological infestations is extremely difficult due to the natural topography of the area, where the access to mechanical equipment is impossible, making it imperative to investigate additional means of treatment. Precision agriculture approaches seem to be the most appropriate tools to manage

anthracnose and other olive fruit rots in mountainous areas' olive groves.
Acknowledgments: This research has been co-financed by the European Agricultural Fund for Rural Development of the European Union and Greek national funds through the Action "Rural Development Program for Greece 2014-2020", under the call (project code: M16ΣΥΝ2-00188)

P2.162 - Transcriptomic impact of positive-sense RNA mycoviruses on Botrytis cinerea life cycle and pathogenicity in grapevine

J. Pardo-Medina[1], *J. Méndez-García*[1], *V. López-Alejandre*[1], *J. Rodriguez-Romero*[1,2], *M.A. Ayllon*[1,2]
[1]Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid (UPM)-Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA/CSIC), Madrid, Spain, [2]Universidad Politécnica de Madrid, Biotechnology - Plant Pathology, Madrid, Spain

The phytopathogenic fungus Botrytis cinerea is the causative agent of gray mold disease, which affects many economically important crops, including grapevine. Managing this disease is challenging due to the rapid development of resistance to antifungal treatments, making alternative control strategies essential. Mycoviruses—viruses that infect fungi—have shown potential to alter fungal physiology and pathogenicity, sometimes inducing hypovirulence or an endophytic lifestyle by modulating fungal fitness or interactions with host plants.
Understanding the molecular mechanisms by which mycoviruses reduce host virulence could aid in the development of novel disease control approaches.
In this study, we compare a virus-free wild-type strain of B. cinerea with two strains infected by distinct positive-sense single-stranded RNA
mycoviruses: a virus that does not produce observable changes in fungal phenotype, and another virus that induces hypovirulence. Using dual transcriptomic analysis, we examined gene expression profiles of each fungal strain at several developmental stages in vitro (conidia, germlings, vegetative growth, and asexual development) and at different time points during whole-plant grapevine infection. We also analyzed grapevine transcriptomes to assess the plant's response to each fungal strain.
Our goal is to identify differentially expressed genes involved in mycoviral response in B. cinerea, specifically those associated with hypovirulence, as well as genes in grapevine that may indicate altered pathogenicity and immune responses linked to the hypovirulent mycovirus.

P2.163 - Pyricularia oryzae shows high metabolic dynamics during plant infection

J. Li[1], *N.J Talbot*[2], *M.-H. Lebrun*[3]
[1]Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands, [2]Norwich Research Park, Norwich, United Kingdom, [3]Universite Paris-Saclay, Palaiseau, France

Pyricularia oryzae, the rice blast fungus, causes a destructive plant disease that poses a serious threat to global rice (*Oryza sativa*) production. Many studies addressed the effectors involved in pathogenesis, but the metabolic changes of *P. oryzae* during plant infection remain poorly

understood. Here we used a set of temporal RNA-seq data, including eight different time points that are key stages for disease development, to identify the dynamics of primary metabolism of *P. oryzae*.
We identified 131 sugar metabolic genes and revealed major temporal changes of these genes during rice infection. In addition, not only sugar metabolic genes, but CAZy genes involved in plant biomass conversion and sugar transporters exhibited similar temporal expression patterns. This revealed the substrates used by the fungus at different stages of infection as well as the degradation pattern of the polysaccharides present in rice. Interestingly, key enzymes of the secondary metabolism showed a similar expression profile. The data was compared to plant infection transcriptome datasets of *Zymoseptoria tritici*, *Colletotrichum higginsianum* and *Colletotrichum graminicola* to compare their metabolic transcriptome patterns.
This is the first comprehensive study of primary metabolism, sugar transport and CAZy genes in plant pathogenic fungi during plant infection and has revealed a new level of understanding into their physiology.

P2.164 - The contribution of Killer Protein-like 4 in *Fusarium graminearum* inter-specific competitive dynamics

A. Petrucci[1], *I. Vicente*[2], *R. Baroncelli*[3], *A. Susca*[4], *G. Vannacci*[1], *S. Sarrocco*[1]
[1]University of Pisa, Department of Agriculture, Food and Environment (DAFE), Pisa, Italy, [2]University of Salamanca, Department of Microbiology and Genetics, Institute for Agribiotechnology Research (CIALE), Salamanca, Spain, [3]University of Bologna, Department of Agricultural and Food Sciences (DISTAL), Bologna, Italy, [4]National Research Council, Institute of Sciences of Food Production, Bari, Italy

Fungi play critical roles in ecosystems, continuously interacting with other organisms. These interactions often lead to interspecific competition for specific niches, facilitated by the production of specialized metabolites, including toxic compounds.
One example is killer proteins (KPs), a family of secreted toxic compounds that inhibit cell growth in target organisms. Within the *Fusarium* genus, homologues of KP4-encoding genes are present in 12 species (KP4-like), with 4 to 6 genes per genome. *Fusarium* is a major group of plant pathogenic fungi responsible for several diseases, including Fusarium Head Blight (FHB) of wheat. FHB is caused by nearly 20 different species, with *F. graminearum* being the most aggressive. As a result, competition among *Fusarium* species frequently occurs to establish the predominant causal agent.
The *F. graminearum* genome contains four homologous genes encoding KP4-like domains (*FgKP4L-1*, *FgKP4L-2*, *FgKP4L-3*, and the heterodimeric *FgKP4L-4*), all located on chromosome 1. Transcriptomic analysis of interactions with the biocontrol agent *Trichoderma gamsii* revealed upregulation of all four genes during the initial sensing phase of the non-self interaction on PDA plates, compared to self-interaction. Similarly, these genes were upregulated during interactions on wheat spikes. *FgKP4L*-encoding genes were also involved during interactions with other plant pathogenic *Fusarium* species on both PDA and wheat spikes.
Our results suggest a possible role for these genes in mediating interspecific interactions between *F. graminearum* and other species. The modulation of FgKP4L-encoding genes appears to be finely tuned according to the competing organism.

P2.165 - Host specificity of *Sporisorium reilianum* is linked to clustered effector genes

S. Chaudhary[1], *L.D. Dittiger*[1], *B. Bhawna*[1], *J.A.R. Hernandez*[1], *M.A.A.N. Khan*[1], *J. Schirawski*[1]
[1]Friedrich Schiller University, Department of Genetics, Jena, Germany

Sporisorium reilianum is a biotrophic, phytopathogenic basidiomycetous fungus and causes head smut disease in maize and sorghum. This soil-borne pathogen exists in two *formae speciales, S. reilianum* f. sp. *reilianum (SRS) and S. reilianum* f.sp. *zeae (SRZ)* that can cause disease on sorghum or maize, respectively. To identify the factors determining host-specific plant infection, we used a classical genetics approach of hybridization combined with next-generation sequencing and GWAS analysis. This resulted in the discovery of a cluster encoding nine effectors (SOVIG1-SOVIG9). Deletion of all nine genes from SRS led to a significant reduction in virulence on sorghum, while SRZ cluster deletion strains were fully virulent on maize. Of nine individual SRS gene deletion strains, those lacking SOVIG3, SOVIG4, and SOVIG7 were most severely affected in virulence on sorghum. All three effector proteins were shown to contain a functional secretion signal peptide, were the highly expressed cluster-encoded proteins, and localized to the cytoplasm and nucleus of the plant cell when transiently expressed in *Nicotiana benthamiana*. By yeast two-hybrid screening, we identified SAUR36 (Small Auxin Up RNA 36), the Ubiquitin ligase ATL39 (Arabidopsis Toxicos en Levadura 39) and the cell-death affecting protein LOL3 (LSD-One-Like 3) as interaction partners of SOVIG3, SOVIG4, and SOVIG7, respectively. Transcriptome analysis supported the suggestion that the three unrelated effector proteins have different functions in host specific virulence. Clustering of effector genes may support infection-specific upregulation.

P2.166 - Increased copper-resistance in the *Fusarium oxysporum* species complex

R. Palos-Fernández[1], *E. Jiménez-Jiménez*[1], *J. Dijkstra*[2], *A. van Westerhoven*[3,2], *M.F Seidl*[3], *G.HJ Kema*[2], *A. Di Pietro*[1], *M. Sánchez López-Berges*[1]
[1]Universidad de Córdoba, Genética, Córdoba, Spain, [2]Wageningen University & Research, Laboratory of Phytopathology, Wageningen, Netherlands, [3]Utrecht University, Theoretical Biology and Bioinformatics, Department of Biology, Utrecht, Netherlands

Fungi colonize different niches, from soil and plants to humans, obtaining nutrients from diverse sources while implementing regulatory mechanisms to rapidly respond to limiting or excessive conditions. Copper is an essential micronutrient but toxic at high concentrations. It is used as an agricultural fungicide and deployed by macrophages during anti-fungal immune bombardment. Thus, fungi face selective pressure for increased copper-tolerance both in agricultural and clinical settings.
Copper-excess is sensed by the conserved transcription factor Ace1, which transcriptionally activates the copper-exporting ATPase Crp1. Loss of Ace1 or Crp1 leads to severe copper-sensitivity and attenuation of virulence in human pathogens. Here we surveyed copper-tolerance in different isolates of *Fusarium oxysporum* (*Fo*), a fungal pathogen that causes vascular wilt

disease in many important crops and opportunistic infections in humans. Most of the plant and clinical *Fo* isolates were highly copper-tolerant compared to other plant or human pathogens such as *F. graminearum*, *F. verticillioides* or *Aspergillus fumigatus*. Interestingly, increased copper-tolerance in *Fo* appears to correlate with the presence of a cluster of two copper-related genes located on accessory regions, one of them encoding a transcription factor with homology to Ace1. The role of this cluster is supported by the finding that, unlike in other fungi, inactivation of Ace1 in *Fo* f. sp *lycopersici 4287* does not affect copper-tolerance and *crp1* expression. Furthermore, *Fo* f. sp. *cubense* mutants that have lost an accessory chromosome harboring the cluster become highly copper-sensitive. Our results suggest that accessory genes mediate copper-tolerance in *Fo* and may impact fungal pathogenesis.

P2.167 - A smut hybrid provides insights into effector gene regulation and tumor formation of *Ustilago maydis*

J. Werner[1], *W. Zuo*[1], *K. Stein*[1], *G. Saridis*[1], *G. Doehlemann*[1]
[1]University of Cologne, Biology, Cologne, Germany

Most smut fungi cause systemic infections, leading to disease symptoms primarily in the inflorescences. One notable example is *Sporisorium reilianum*, which infects both maize and sorghum. In contrast, the model organism among the smuts *Ustilago maydis* produces localized tumors on all areal parts of maize. The genomes of *S. reilianum* and *U. maydis* are of similar size and exhibit conserved synteny and mating systems. Infection of the same host renders them suitable candidates for interspecific hybridization.
To investigate how these species, with their different effector gene expressions, contribute to virulence, we exchanged the mating genes of *U. maydis* with those of *S. reilianum*, resulting in a recombinant *U. maydis* X *S. reilianum* hybrid (rUSH). rUSH infection on maize exhibited no tumor formation and an *S. reilianum*-like phenotype in the leaf and ear, without the formation of teliospores. An RNA-seq analysis revealed that the one-to-one effector orthologs were differentially expressed with distinct expression patterns: *cis*, *trans*, and hybrid-specific. Within the hybrid-specific expression patterns, we discovered two novel *U. maydis* virulence factors. Importantly, we identified a conserved transcription factor that enables rUSH to induce tumor formation on maize. Subsequent RNA-seq analysis helped to identify *U. maydis* effector genes putatively involved in tumor formation, which could not be discovered in previous studies. Our results represent a significant advance in understanding of *U. maydis*-induced tumor formation and will enhance our knowledge of the molecular mechanisms underlying the differences in disease development observed in *U. maydis* and *S. reilianum*.

P2.168 - Exploring Zymoseptoria tritici AvrStb6 antimicrobial activity using computational and biochemical approaches

L. Florez[1,2], *E. Stuckenbrock*[1,2]
[1]Max Planck Institute for Evolutionary Biology, Environmental Genomics, Plön, Germany,
[2]University of Kiel, Botanical Institute, Kiel, Germany

The molecular interactions between plants and pathogens are crucial for advancing agricultural science and addressing global food security. *Zymoseptoria tritici*, the causative agent of septoria tritici blotch (STB), is one of the most destructive fungal pathogens of wheat in Europe. Despite its significance, the molecular mechanisms underlying its interactions with wheat remain poorly understood. The wheat resistance gene Stb6, which encodes a wall-associated kinase (WAK), mediates resistance against *Z. tritici* isolates expressing the effector AvrStb6. The direct interaction between Stb6 and AvrStb6 has yet to be demonstrated, suggesting the involvement of an additional factor in Stb6-mediated recognition. Unlike Stb6, AvrStb6 function remains largely uncharacterized. AlphaFold3 structural predictions suggest that AvrStb6 (pLDDT 79.84) shares high structural similarities with cysteine-rich proteins from predatory bacteria, and AMAPEC –an antimicrobial effector prediction software– assigns a high probability (0.9904) for AvrStb6's antimicrobial activity. *In vitro* growth inhibition assays demonstrate selective antimicrobial activity of AvrStb6 against certain Gram-negative bacteria. Further structural analysis revealed two positive-charged poles in AvrStb6 protein structure, common features of pore-forming antimicrobial peptides. Liposome-based assays demonstrated AvrStb6's capacity to form pores in cell membranes, providing insights into its potential mechanism of action as an antimicrobial effector within the wheat apoplast microbiome. This study presents the first evidence of *Z. tritici* AvrStb6's antimicrobial activity. Whether this antimicrobial function is linked to its recognition by Stb6 and the plant immune response remains an open question.

P2.169 - Investigating the role of fungal NLR genes in fungal-bacterial interactions

J.A Rodgers[1], *L.E Lamberte*[1], *G.J Kettles*[1]
[1]University of Birmingham, Birmingham, United Kingdom

Biocontrol of Fungal Infections, Plant Pathogenic Fungi NOD-like receptor or nucleotide-binding and leucine-rich repeat domain (NLR) genes are found in large families in plants and animals. They are activated by pathogen-associated molecules such as virulence factors (effectors) and stimulate immune responses involving host cell death. Whilst their role in plant and animal immunity is well known, how they contribute towards a fungal immune system is unclear. *Zymoseptoria tritici* causes septoria tritici blotch disease of wheat and is one of the most devastating wheat-infecting pathogens, responsible for losses of 5-10% in Europe annually. *Z. tritici* is an appealing system to study fungal NLRs as it is genetically tractable and has a relatively small NLR complement compared to other ascomycetes. Our goal is to investigate how NLRs contribute towards interactions between *Z. tritici* and other microorganisms in the environment.
The *Z. tritici* IPO323 genome encodes 20 NLRs based on presence of NB-ARC or NACHT domains, with the majority (17/20) containing NACHT domains. In common with other fungi, some *Z. tritici* NLRs contain C-terminal ankyrin repeat or WD40 domains. However, the majority of N- and C-terminal domains have no obvious function. Using published RNAseq data, we selected the four most highly-expressed NLRs for gene knockout and functional characterisation. To this end, we are developing *in planta* and *in vitro* co-culturing assays with anti-fungal bacteria such as *Bacillus velezensis*. These assays will be used to assess resistance to

bacterial attack, and ability to colonise plants of wild-type and NLR knockout mutants. Our most recent data will be presented.

P2.170 - Lem2-mediated tethering of telomeres to the nuclear periphery is essential for *Ustilago maydis* pathogenesis

E. Sanz Marti[1], *J.I. Ibeas Corcelles*[1], *R. Ramos Barrales*[1]
[1]Universidad Pablo de Olavide/ Centro Andaluz de Biología del Desarrollo, Departamento de Biología Molecular e Ingeniería Bioquímica, Sevilla, Spain

The transcriptionally silent heterochromatin is predominantly located at the nuclear periphery, anchored mainly by lamina-associated proteins (LAPs). This peripheral localization of heterochromatin is essential for its complete silencing and has important implications in cell fate and development. However, its impact on fungal pathogenesis remains unexplored. In this study, we investigate the role of the LAP homolog Lem2, involved in the perinuclear tethering of centromeres and telomeres and their silencing in yeast, in the pathogenesis of *Ustilago maydis*. Our results demonstrate that Lem2 is essential for virulence, specifically for plant penetration, as most of the Δ*lem2* mutant filaments become arrested at the appressorium stage, a specialized structure involved in penetration. We found that the Δ*lem2* mutant exhibited impaired nuclear migration and telomere detachment from the nuclear periphery. Interestingly, nuclear migration issues are partially restored when we artificially tether telomeres to the nuclear periphery in a Δ*lem2* mutant, suggesting that telomere attachment to the nuclear envelope is essential for nuclear migration from the appressorium to the penetrating filament. To investigate why telomere detachment prevents nuclear migration, we performed a transcriptomic analysis during filamentation and found that the Δ*lem2* mutant shows upregulation of G1-S specific genes and several kinases involved in cell cycle checkpoints that could explain the nuclear migration block. Overall, our data not only enhances our understanding of fungal virulence but also opens new avenues for exploring how chromatin location can shape pathogen behavior.

P2.201 - Modification of *Fomes fomentarius* cell wall properties for the removal of beta lactam antibiotics from wastewater streams

S. Starke[1]
[1]TU Berlin, Applied and Molecular Microbiology, Berlin, Germany

Filamentous fungi, particularly the basidiomycete species like *Fomes fomentarius*, can efficiently convert lignocellulosic biomass into valuable materials. These fungi grow rapidly, can be cultivated on a large scale and produce a large volumetric output of material, making them ideal for creating scalable, low-cost, biodegradable composites (Pohl et al., 2022). These materials are promising for construction, as well as thermal and acoustic insulation, while offering a sustainable alternative to conventional oil based products and concrete (Stelzer et al., 2021). However, there has been limited research on engineering these living materials using standardized biotechnological methods that are commonly applied to ascomycete fungi.
This study lays the foundation for using *Fomes fomentarius* in engineered living materials

(ELMs) by modifying its cell wall to remove beta-lactam antibiotics from wastewater, addressing the rising concern over antibiotic resistance (Wang et al., 2020). Fusion proteins, composed of a beta-lactamase and a glucan-binding protein, expressed and purified in *E. coli*, are used to coat the cell wall of *F. fomentarius*. Specific and stable binding of the fusion proteins to the *F. fomentarius* cell wall is confirmed through confocal fluorescence microscopy and SDS-PAGE. Furthermore, the capability to inactivate ampicillin is measured by HPLC. This study proves how the surface properties of fungal-based biomaterials can be modified with selected characteristics to widen its applicability as a filter material for wastewater treatment.

P2.202 - Activity-based profiling of *β*-mannanases in *Aspergillus niger*

M. Tedeschi[1,2], *V.A.J. Lit*[2], *P. Kooloth Valappil*[1], *M. Arentshorst*[1], *J. C. R. Brzoskowski*[2], *T. Gote*[2], *B.I. Florea*[2], *Z. Armstrong*[2], *H.S. Overkleeft*[2], *A.F.J. Ram*[1]
[1]Leiden University, Institute of Biology Leiden, Microbial Sciences, Fungal Genetics and Biotechnology, Leiden, Netherlands, [2]Leiden University, Leiden Institute of Chemistry, Bio-organic Synthesis, Leiden, Netherlands

β-Mannanases are endo-acting glycosyl-hydrolases (GHs) capable of hydrolyzing *β*-1,4 linkages of mannan-containing polysaccharides. *Aspergillus* species are amongst the main producers of these enzymes, which have applications in many industrial sectors. A specific *β*-mannanase of *Aspergillus niger* (*An*ManA) was successfully identified and characterized by activity-based protein profiling (ABPP). ABPP is an effective tool to study GHs in complex mixtures. ABPP relies on a simple yet powerful concept in which activity-based probes (ABPs) irreversibly inhibit an enzyme by covalently binding to its active site. ABPs are endowed with a reporter entity (either a fluorophore like Cy5 or a capture agent like biotin) which allows detection and isolation of the enzyme bound to the ABP. In this study ABPs for *β*-mannanases were developed. Mannobiose and mannotriose were chosen as recognition elements, and an epoxide was employed as electrophilic warhead. The synthesized ABPs were evaluated on *A. niger* secretomes obtained from cultivations on mannan-containing substrates. *An*ManA was pulled-down from the secretome with a biotinylated ABP and identified by LC-MS. The ABPs were also employed to test the temperature and pH stability of the labelled enzymes directly in the secretome. Furthermore, *An*ManA was overexpressed in a genetically modified strain of *A. niger*. The recombinant mannanase was purified from the secretome of the overexpressing strain. Finally, the active site nucleophile of ManA was experimentally identified by ABPP using LC-MS/MS. This research highlights the utility of ABPP in the identification and characterization of fungal mannan-degrading GHs and its applicability in the field of industrial biotechnology.

P2.203 - Mutations in the AmyR transcription factor leading to constitutive expression of starch degrading enzymes in *Aspergillus niger*

J.L. van Dam[1], *P.K. Valappil*[1], *M. Arentshorst*[1], *R. Rijnders*[1], *L. Kruithof*[1], *A. Faber*[1], *R.J.M. Lubbers*[1], *J. Visser*[1], *P.J. Punt*[1,2], *A.F.J. Ram*[1]
[1]Leiden University, Microbial Sciences, Leiden, Netherlands, [2]Ginkgo Bioworks NL, Zeist, Netherlands

The expression of starch degrading enzymes (SDEs) by filamentous fungi is tightly controlled. In *Aspergillus niger* the expression of SDEs is induced by maltose and glucose and dependent on the AmyR transcription factor. Detailed knowledge on the mechanism(s) that keep AmyR inactive during non-inducing conditions and result in its activation in the presence of inducer, are lacking.
To fill this gap, we have developed dual and single reporter strains to study the regulation of SDEs. Promoter sequences of the glucoamylase (*glaA*), acid amylase (*aamA*) and alpha-glucosidase (*agdA*) genes were used to make reporter strains using both the acetamidase gene (*amdS*) and the luciferase gene (lux_{613}). The dual reporter strains containing the *PaamA-amdS* and *PaamA-lux*) reporters were selected to isolate constitutive mutants by screening for mutants that grow well on acrylamide plates under non-inducing conditions. Trans-acting mutants were identified using the luciferase reporter and/or by performing AZCL-amylase plate assays.
In total six mutants were identified that had point mutations in the *amyR* gene resulting in specific amino acid changes. The mutations and resulting amino acid changes were not confined to a specific region of the AmyR protein but scattered over the AmyR protein sequence. Reintroduction of the strongest AmyR mutation in a strain expressing multiple copies of the *glaA* gene confirmed that this mutation in AmyR leads to a constitutively active AmyR transcription factor. This finding is currently exploited to increase production of other homologous and heterologous proteins in *A. niger*.

P2.204 - Precision fermentation for reimagined cheese

S. Obermaier[1], *B. Bernardi*[1], *L. Matyika*[1], *B. Jöhnk*[1]
[1]Formo Bio GmbH, Frankfurt am Main, Germany

The environmental impact of the current food system is a growing concern. Livestock farming uses over 80% of global farmland, with the dairy industry alone responsible for 4% of global greenhouse gas emissions. To address this, the food industry must expand the animal-free dairy market. While plant-based alternatives are more sustainable, replicating the taste and texture of traditional cheese remains a challenge. Precision fermentation, a refined brewing process, uses microorganisms to produce specific molecules. This technology can be applied to produce virtually any product of choice at industrial scale, including proteins for animal-free foods.
At Formo, we are reimagining traditional cheese products using precision-fermented protein ingredients. In particular, we are producing recombinant milk proteins in a variety of expression hosts. Filamentous fungi are particularly promising due to their ability to secrete recombinant proteins in high yields. However, they also present challenges such as limited genetic accessibility, high proteolytic activity, and secretion-inhibiting pellet morphology. To overcome these hurdles, we implemented a series of genetic modifications that resulted in a strain with enhanced genetic accessibility, reduced proteolytic activity, and a dispersed growth phenotype. With this modified base strain we screened multiple expression constructs in microtiter plates and identified modifications which substantially improved protein expression.
Harnessing fungi for food ingredient production through precision fermentation is a game-

changing approach to creating sustainable, fair, and delicious products for the future of our food system.

P2.205 - Screening edible fungal strains for antioxidant ergothioneine production in brewery side-streams

E. Jamil[1,2], *T.-T. Bui*[1], *S. Shabeer*[1], *S. Doyle*[1], *O. Bayram*[1]
[1]Maynooth University, Biology, Maynooth, Ireland, [2]University of Pavia, Molecular Medicine, Pavia, Italy

Ergothioneine (EGT; 2-mercaptohistidine trimethylbetaine) is a naturally occurring antioxidant and anti-inflammatory amino acid found in various foods, particularly mushrooms. It accumulates in key tissues such as the brain, liver, and red blood cells, offering protection against oxidative stress and reducing the risk of chronic diseases, including cardiovascular conditions, neurodegenerative disorders, and age-related ailments. Despite its important role in biological systems, ergothioneine synthesis is exclusive to bacteria and fungi, while other organisms acquire it from the soil or by consuming fungi. This study aimed to identify edible fungal strains with high EGT synthesis potential and explore their application in producing valuable secondary products from whisky distillery waste. Here, we screened edible fungal strains for high ergothioneine synthesis using Thin Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC) for quantification. Several strains demonstrating high ergothioneine production were subsequently adapted to a brewery waste environment for production of functional foods and to treat the brewery side-streams to reduce their environmental impact. This approach aims to achieve the dual benefit of waste treatment and ergothioneine production, providing a sustainable biotechnological solution. The current state of the project will be presented.

P2.206 - Advancing CRISPR-Cas9 tools to mitigate the impact of a non-native fungal pathogen on managed ecosystems

J. Sparks[1], *T. Rush*[1], *C. Sawyer*[1], *M. Drott*[2], *K. Sondreli*[3], *W. Alexander*[1], *D. Jacobson*[1], *C. Eckert*[1], *P. Abraham*[1], *J. LeBoldus*[3], *J. Tannous*[1]
[1]Oak Ridge National Laboratory, Oak Ridge, United States, [2]USDA, St. Paul, United States, [3]Oregon State University, Corvallis, United States

The invasion of non-native fungal pathogens can severely disrupt ecosystem structure, biodiversity, and function. To preserve biodiversity and ecosystem health, it is crucial to understand how these pathogens establish and spread, as well as to develop effective strategies for mitigating their impacts. *Sphaerulina musiva*, a fungal pathogen originally native to Eastern North America, exemplifies an invasive species spread by human activities. Recently introduced to the Pacific Northwest, *S. musiva* has caused significant damage to susceptible *Populus* species, which serve as both a key bioenergy crop and a keystone species in forest ecosystems. Within the Secure Ecosystem Engineering and Design (SEED) Science Focus Area at ORNL, our goal is to uncover the genetic factors driving the establishment, spread, and impact of *S. musiva* in *Populus*

ecosystems managed by the Department of Energy. To achieve this, we are advancing CRISPR genetic tools for *S. musiva*, aiming to enhance basic research by elucidating gene functions and exploring molecular mechanisms of pathogenicity, while also driving applied research efforts to develop CRISPR-enabled technologies for disease control and sustainable population management. This research supports innovative genetic approaches to protect bioenergy crops and preserve ecosystem integrity, ensuring the long-term sustainability of *Populus* populations in both natural and managed environments.

P2.207 - Development of Serine Recombinase-Assisted Genome Engineering (SAGE) in Fungi

K. Davis[1], *C. Eckert*[1], *A. Guss*[1], *J. Tannous*[1]
[1]Oak Ridge National Laboratory, Oak Ridge, United States

Filamentous fungi and yeasts play vital roles both ecologically and industrially. Metabolic engineering of these organisms to improve these roles is limited by several factors including the scarcity of tools available, especially for non-model filamentous species. Other confounding factors include low homologous recombination (HR) rates compared to non-homologous end joining (NHEJ) as well as the lack of naturally occurring, autonomously replicating plasmids. The implementation of the Serine Recombinase-Assisted Genome Engineering (SAGE) system offers a solution to most of these challenges, as it enables the integration of up to 12 DNA constructs in a site-specific and iterative manner. Furthermore, this tool obviates the need for replicating plasmids and intricate, long donor DNA sequences that precisely correspond to the break site, a process that can be costly and time-consuming. Additionally, the SAGE system allows the reuse of selectable markers by employing a secondary serine-integrase. This tool is versatile and can be utilized for different applications including promoter library construction, gene function analysis, and metabolic pathway engineering. In this study, we demonstrate the ability to implement this technology in the industrial workhorse *Asperiglus niger*, hereby establishing its proof of principle for applicability in fungi. We are currently applying SAGE to engineer industrial and environmental fungi across various species within the fungal kingdom. We anticipate that this system will expand the capability to engineer filamentous fungi and yeasts to improve or introduce traits for ecological and industrial applications.

P2.208 - Fungal Cutinase revisited: Expression and characterization of novel *Fusarium* Cutinases in *Aspergillus niger* and application in bioremediation

M. Arentshorst[1], *J.-A. Verschoor*[1], *S. Seekles*[1], *T. Regensburg*[1], *K. van den Hondel*[1], *A. Ram*[1], *H. de Winde*[1]
[1]Leiden University, Institute Biology Leiden, Fungal Genetics and Biotechnology, Leiden, Netherlands

Background and aims
Cutinases (EC 3.1.1.74) are widely distributed in fungi, bacteria and plants and act on the natural

substrate cutin, a waxy aliphatic polyester acting as protection barrier of plants. In addition, cutinases have been reported to hydrolyze artificial polyesters and toxic xenobiotics such as polyethylene terephthalate (PET), polycaprolactone (PCL), polylactic acid (PLA), and polyhydroxybutyl succinate (PBS). Moreover, cutinases can act as promising stereoselective catalysts in (trans)esterification reactions with high selectivity. Hence, cutinases are powerful tools in synthetic biology and bioremediation and deserve further investigation towards a broader range of ester-containing substrates.

Methods and Results
62 putative endophytic fungal strains were isolated from plants. ITS-sequencing clearly placed the majority of these strains in the genus *Fusarium*. Full genome analysis of the most closely related strains revealed the presence of 3 putative cutinase genes. Cutinase activity was confirmed via para-nitrophenyl butyrate (pNPB) assay. Two strains with considerable cutinase activity were chosen for further analysis. Those strains each contained two different cutinases, with one of each being highly identical. The 3 resulting cutinases were expressed in *Aspergillus niger* using an in-house developed CRISPR/Cas9-based multicopy integration system. Enzyme purification was followed by extensive biochemical analysis of the purified cutinases on various natural and non-natural substrates.

Conclusions
We have identified and characterized 3 novel cutinase enzymes from *Fusarium* spp. The enzymes show different activities on different natural and non-natural substrates, thereby strengthening the plea for an important role of cutinases in synthetic biology and bioremediation.

P2.209 - Reducing proteolytic activity of the filamentous fungus *Thermothelomyces heterothallica* C1

M. Vitikainen[1], *M. Wiebe*[1], *R. Tchelet*[2], *N. Valbuena Crespo*[2], *M. Emalfarb*[2], *M. Saloheimo*[1]
[1]VTT Technical Research Centre of Finland Ltd, Espoo, Finland, [2]Dyadic International, Inc., Jupiter, United States

Thermothelomyces heterothallica C1 is a well-known industrial enzyme production host able to reach enzyme titers over 120 g/l in a 6-7-day process. We have further developed the C1 Technology and are exploiting the excellent protein production efficiency of this system for low-cost manufacturing of different types of therapeutic and vaccine proteins.
One of the major bottle necks in recombinant protein production is the proteolytic activity by host cell proteases. Therefore, one of our goals in C1 strain development is reducing proteolytic activity to achieve higher production levels and better quality of the recombinant proteins. The work was initiated by characterization of C1 extracellular proteases using several techniques. Based on the characterization results, a set of proteases was selected, sequentially deleted and the effect of deletions was tested on several target proteins resulting in multiple protease deletion strains, depleted up to fifteen proteases.
As a result, we observed step-wise improved production and increased stability of several target proteins. We also identified two proteases responsible for degradation of monoclonal antibodies in C1 and by deleting those proteases were able to produce stable mAbs over 20g/l. Recently, we

have identified a protease responsible for clipping the C-terminal His-tag from a target protein produced in C1. Another new approach is expressing an intracellular protease in a secreted form which enables testing degradation of target proteins prior constructing C1 production strains. To summarize, our work in reducing proteolytic activity has successfully generated a set of C1 production strains for efficient production of therapeutic proteins.

P2.210 - Improvement of monoclonal antibody production in *Thermothelomyces heterothallica* C1 by over-expression of folding and secretion related genes

M. Mäkinen[1], *A. Aalto*[1], *T. Pakula*[1], *M. Wiebe*[1], *A. Huuskonen*[1], *M. Vitikainen*[1], *S. Havukainen*[1], *R. Tchelet*[2], *N. Valbuena Crespo*[2], *M. Emalfarb*[2], *M. Saloheimo*[1]
[1]VTT Technical Research Centre of Finland Ltd, Espoo, Finland, [2]Dyadic International Inc., Jupiter, United States

The thermotolerant filamentous fungus *Thermothelomyces heterothallica* C1 has been developed into a highly productive expression system capable of secreting over 120 g/l of its native enzymes, and for efficient production of heterologous proteins like antibodies and vaccine candidates. Over 20 g/l titers of monoclonal antibodies (mAbs) have been achieved but some challenges remain including high variation in productivity between mAbs and compromised production levels in glycoengineered strains producing humanized N-glycans.
To address these challenges, the bottlenecks of antibody production were investigated with transcriptome analysis in strains producing five mAbs with different secretion efficiencies and in native glycan producing and glycoengineered strains. The mAb heavy and light chain transcripts were among the most abundant ones in the transcriptome, suggesting that the bottleneck of production is downstream from transcription. Genes involved in folding, glycosylation and transport of proteins in the secretory pathway were induced in the mAb-producing strains, with higher induction in the strains expressing the more challenging mAbs. Transcriptome data was utilized in selecting genes for over-expression in a glycoengineered antibody producing strain to enhance mAb production. Over-expression of 12 genes with different functions in the secretory pathway was found to improve mAb production. Among these genes are secretion related genes *erv29, erv46* and *pmr1* and glycosylation related UDP galactose transporter gene. By transforming a mAb-producing strain with the 12 genes as a pool we have generated C1 strains able to produce mAbs with humanized glycans at up to 9 g/l levels in a 7-day bioprocess.

P2.211 - Identification and characterization of enzymes involved in the tannic acid and gallic acid metabolism of *Aspergillus niger*

R. Lubbers[1], *S. Prins*[1], *N. Dimitrov*[1], *J. van Dam*[1], *F. Calcan*[1], *M. Arentshorst*[1], *A. Tsang*[2], *A. Ram*[1]
[1]Leiden University, Institute Biology Leiden, Microbial Sciences, Fungal Genetics and Biotechnology, Leiden, Netherlands, [2]Concordia University, Department of Biology, Montreal, Canada

Tannic acid is a plant polymeric aromatic compound consisting of multiple gallic acid (3,4,5-trihydroxybenzoate) molecules bound to glucose. Certain fungi, such as *Aspergillus niger*, are able to degrade plant biomass by secreting enzymes. For tannic acid, specific tannases are released by *A. niger* that can hydrolyze gallotannins resulting in the release of gallic acid and glucose. Gallic acid is a valuable aromatic compound with many pharmacological and industrial applications but is also a source of pollution. Therefore, understanding the gallic acid metabolic pathway can be of great interest to utilize tannic acid to create gallic acid or to bioremediate gallic acid pollutants. In *A. niger*, gallic acid is further metabolized intracellular as carbon source. Recently, we identified a repressor/regulator (TanX/TanR) complex involved in the regulation of tannases and the gallic acid metabolism.
In this presented work, we used whole genome transcriptomics on the *tanX* deletion strain, in combination with RNA extracted from a tannic acid grown *A. niger* culture to identify genes involved in the metabolism of gallic acid. Four highly induced candidate genes were selected, and deletion strains were made using CRISPR/CAS9. Phenotypic analysis of the strains on multiple aromatic compounds showed growth defects on tannic acid and gallic acid, indicating that these genes encode enzymes required for gallic acid utilization. To biochemically characterize these four enzymes, the genes encoding the enzymes were overexpressed as His-tagged-proteins and complementation studies confirmed that all enzymes were functional. The enzymes were successfully purified and currently analyzed for their activity on gallic acid.

P2.212 - Development of a synthetic biology toolkit for heterologous gene expression in the oyster mushroom, *Pleurotus ostreatus*

P. Prasongpholchai[1], *T. Fu*[1], *D. Alkhder*[1,2], *D. Taylor*[1], *F. Alberti*[1]
[1]University of Warwick, School of Life Sciences, Coventry, United Kingdom, [2]University of Leicester, Leicester Medical School, Leicester, United Kingdom

Basidiomycota fungi are prolific producers of high-value metabolites including terpenoids, alkaloids and sugar derivatives. Although many fungal-derived natural products are known today, the production of these compounds in the native hosts is limited primarily due to the low/cryptic expression level of biosynthetic genes under the laboratory conditions. In recent years, new genetic and cultivation-based strategies for engineering of Basidiomycota fungi have become available, yet these are often species-specific, and genetic engineering techniques require a sophisticated knowledge of the biology of the producing organisms. Heterologous expression of Basidiomycota biosynthetic pathways in Ascomycota fungi such as *Aspergillus* spp. is feasible, however, more often they are unable to process numerous introns found within Basidiomycota genes.
As such, we aim to develop a robust synthetic biology toolkit for the heterologous expression of Basidiomycota genes in a tractable basidiomycete, *Pleurotus ostreatus* (oyster mushroom), for which several protocols for introducing exogenous DNA as well as genetic manipulation have been established. To date, we have tested *P. ostreatus* strains N001 (dikaryon), PC9 and PC15 (monokaryons) for growth on selected culturing media and performed RNA-seq analyses to identify genes which are highly and constitutively expressed in selected growth conditions. We are currently testing their respective promoters and terminators to express reporter genes in *P. ostreatus*.

We hope that the toolkit would benefit both the synthetic biology community as well as fungal natural products researchers in order to exploit Basidiomycota genomes as the source for the production of high-value compounds and further.

P2.213 - Exploring the structural, biochemical and functional diversity of glycoside hydrolases family 12 from *Penicillium subrubescens*

P. Sun[1,2], *M. Peng*[2], *M.A Kabel*[1], *R.P. de Vries*[2]
[1]Wageningen University & Research, Laboratoray of Food Chemistry, Wageningen, Netherlands, [2]Westerdijk Fungal Biodiversity Institute, Fungal Physiology, Utrecht, Netherlands

Glycoside hydrolases (GHs) play an essential role in plant biomass degradation and modification for sustainable production of biochemicals. The filamentous Ascomycete fungus *Penicillium subrubescens* contains a higher number of GH12 candidates compared to related species. Therefore, we aimed to compare *P. subrubescens* GH12s for their ability and substrate specificity for plant cell wall polysaccharide degradation and species' potential as a source of novel enzymes for plant biomass valorization. Our re-evaluated phylogenetic analysis of fungal GH12 members showed that the *P. subrubescens* GH12s were located in different (new) clades. Biochemical characterization marked *Ps*EglA as an endoglucanase and four other *P. subrubescens* GH12s (i.e., *Ps*XegA–D) as xyloglucanases. Interestingly, structural features of *Ps*XegD and *Ps*XegE were more comparable to Basidiomycete GH12 xyloglucanases with a unique open substrate-binding cleft. *Ps*UegA displayed dual xyloglucanase and endoglucanase activity, and also showed distinct structural features. Comparative transcriptome analysis supported the functional diversity of *P. subrubescens* GH12s in plant biomass degradation. The gene encoding *Ps*UegA was expressed under diverse conditions suggesting a scouting role for this enzyme.

P2.214 - The multipurpose cell factory *Aspergillus niger* can be engineered to produce hydroxylated collagen

T. Morris[1], *T.C. Cairns*[1], *V. Meyer*[1]
[1]Technische Universität Berlin, Berlin, Germany

Advances in tissue printing and wound healing necessitate a continuous global supply of collagen. Microbial systems are highly desirable to meet these demands as recombinant collagenous proteins can be guaranteed as free from animal viruses. The filamentous cell factory *Aspergillus niger* has been instrumental for decades in the production of organic acids, enzymes and proteins, yet this fungus has not been explored for recombinant collagen production. In this study, we conducted extensive genetic engineering and fermentation optimization to provide proof of principle that *A. niger* can produce hydroxylated collagen.
We used a modular cloning system to generate a suite of cassettes encoding numerous n-terminal secretion signals, biodesigned/native collagen genes and, additionally, various prolyl-4-hydroxylases (P4H) for protein hydroxylation. These were expressed in a previously constructed *A. niger* isolate which is capable of producing the crucial P4H cofactor ascorbic acid. We conducted a wide range of media optimization studies to increase collagen production and

hydroxylation levels. Additionally, we deleted an endopeptidase encoding gene, which was likely responsible for degrading secreted collagen. These studies generated an isolate capable of secreting partially hydroxylated collagen to titres of approximately 5mgL^{-1}. Comparative transcriptomic analyses are currently ongoing to identify further candidate genes for genetic and metabolic engineering approaches.

P2.215 - Characterization of GH67 and GH115 α-1,2-glucuronidases (AGUs) for improvement of xylooligosaccharide production

L. Li[1], *P. Sun*[2], *M.A. Kabel*[2], *R.P. Vries*[1]
[1]Utrecht University & Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands,
[2]Wageningen University & Research, Laboratory of Food Chemistry, Wageningen, Netherlands

Xylan, one of the most abundant components of renewable biomass, is a complex heterogeneous polysaccharide composed mainly of a xylosyl backbone with substitutions. Complete saccharification of xylan requires the coordinated action of several hemicellulases. However, our knowledge of these enzyme systems is incomplete and many putative enzymes identified in fungal genomes have not been characterized. In this study, we characterized a set of α-1,2-glucuronidases (AGUs) that remove (4-O-methyl)-glucuronic acid from xylosyl units. AGUs are so far classified in Glycoside Hydrolase (GH) family 67 and 115. We identified putative AGU-encoding genes in several fungal species, such as *Aspergillus oryzae* and *Penicillium subrubescens* and expressed these in *Pichia pastoris*. The produced enzymes were purified and characterized for their biochemical properties, substrate specificities, product profiles and synergy with xylanases. This project deepens our understanding of the diversity of AGUs and explores their potential to improve xylooligosaccharide production.

P2.216 - Identification and functional analysis of the novel isomaltose sensor/transporter involved in the activation of the transcription factor AmyR in *Aspergillus nidulans*

D.M. Jeong[1], *S. Fujita*[1], *J. Tokashiki*[1], *T. Shintani*[1], *K. Gomi*[1]
[1]Tohoku university, Sendai, Japan

Aspergillus oryzae, or koji mold, is known for its ability to produce high levels of hydrolytic enzymes including amylolytic and proteolytic enzymes, which are used in various industrial applications such as Japanese fermented foods and beverages. In *Aspergillus*, the regulation of amylolytic enzyme gene expression is controlled by the fungal-specific transcription factor AmyR. The induction of AmyR activity is triggered by isomaltose most effectively compared to glucose or maltose. However, the mechanism of AmyR induction in the presence of isomaltose is scarcely studied. Previously, we identified the most putative isomaltose sensor/transporter, AN5050 in *Aspergillus nidulans* through SNP analysis, and showed that deletion of *AN5050* resulted in a significant reduction of amylolytic gene expression in the presence of isomaltose[1)]. In this study, AmyR and AN5050 were tagged with fluorescent proteins to explore their subcellular localization. The AN5050-complemented strain with mCherry showed growth

recovery on starch media. Fluorescence analysis revealed that AmyR tagged with eGFP exhibited a significantly delayed nuclear localization in the absence of AN5050. Furthermore, this nuclear localization was observed only when isomaltose was used as an inducer of AmyR, rather than glucose, demonstrating that AN5050 functions as an isomaltose sensor/transporter, although the presence of another minor isomaltose sensor/transporter. Additionally, AN5050 was found to be localized in the transmembrane region. Interestingly, the plasma membrane localization of AN5050 was not affected by the presence of glucose, suggesting that AN5050 does not undergo endocytic degradation.
1) Jeong et al., The 32nd Fungal Genetics Conference, 293A (2024).

P2.217 - Uncover the potential of secreted Luciferases expressed in Aspergillus niger: fusion-proteins and high-throughput screening

K.J. Ost[1], *M. Arentshorst*[2], *A.F.J. Ram*[2], *M.E. Dirks-Hofmeister*[1]
[1]Osnabrück University of Applied Sciences, Food Biotechnology, Osnabrück, Germany, [2]Leiden University, Molecular Microbiology and Biotechnology, Leiden, Netherlands

Aspergillus niger is a filamentous fungus extensively utilized in industrial biotechnology for the production of enzymes, organic acids, and other metabolites. The increasing demand for enhanced protein production and efficient secretion pathways necessitates the development of novel screening methods. Although luciferase technology has been employed in mammalian cells and bacteria for an extended period, only recent advancements have expanded its applicability in fungal biology to investigate gene expression, signal transduction, and metabolic processes. The research landscape is shaped not only by optimization efforts and persistent challenges, but high-throughput methods are currently a primary focus, particularly for the screening of traits pertinent to industrial enzyme production.
In this study, we report successful heterologous expression and secretion of extracellular luciferases in *A. niger*. Using a luciferase-based high-throughput screening assay in 96-well plates, a sensitive method for evaluating the differences in secretion or production efficiency was introduced. This will be highly valuable for screening genetic modifications, for example, across mutant libraries of secretion signals, in future applications. Moreover, fusion of luciferases with homologous or heterologous proteins offers a straightforward approach for determining the secretion and production efficiencies of proteins, both without any enzymatic activity and with activity that is challenging to measure.
The findings of this study indicate that this novel assay addresses the limitations of conventional screening methodologies and may significantly enhance the application of luciferase technology in filamentous fungi. Moreover, the results demonstrate the potential for subsequent research to expand this approach, facilitating improved production systems for *A. niger*.

P2.218 - Exploring biological constraints in the production of disordered material proteins in the filamentous fungus *Trichoderma reesei*

E. Sveholm[1], *H. Mattila*[1], *A. Westerholm-Parvinen*[1], *T. Paasela*[1]
[1]VTT Technical Research Centre of Finland Ltd, Espoo, Finland

Certain natural proteins have superior properties in strength, toughness, and elasticity making them ideal for creating sustainable, biocompatible, and biodegradable materials from renewable sources. Novel designer proteins with improved mechanical properties have been developed but less attention has been given to the major bottleneck for the utilization of these proteins in applications: achieving cost-effective production in heterologous hosts. Disordered material proteins, in particular, are difficult to produce across various hosts, yet the reasons for this remain unclear. To shed light on the major problems and uncover the biological limitations hindering the high-level protein production of disordered proteins we are investigating how the filamentous fungus *Trichoderma reesei* responds to producing material proteins spider silk ADF3 and resilin. *T. reesei* is important industrially used production host known for its superior protein secretion capabilities. Here, we present initial experiments for cultivating material protein-producing strains in Ambr® multi-parallel bioreactors and analysis of transcriptional changes at different stages of the cultivation. We aim to identify single genes or entire pathways that are misregulated during material protein production, providing new targets for strain engineering.

P2.219 - Improved hyphal dispersion strain of *Aspergillus oryzae* with decreased wall-growth in the liquid fermentation

S. Susukida[1], *K. Muto*[1], *K. Miyazawa*[1], *A. Yoshimi*[2,3], *F. Hasegawa*[4], *K. Abe*[1]
[1]Tohoku University, Graduate School of Agricultural Science, Sendai, Japan, [2]Kyoto University, Graduate School of Agriculture, Kyoto, Japan, [3]Kyoto University, Graduate School of Global Environmental Studies, Kyoto, Japan, [4]National Institute of Technology and Evaluation, Tokyo, Japan

Aspergillus oryzae has been widely used in the industrial production of enzymes. In liquid fermentation, the mycelial morphology increases the viscosity of culture liquid, causing poor mixing. In addition, mycelial growth on the bioreactor wall (wall-growth) leads to unstable operation and reduces liquid volume, which can limit yields. Previously, we have shown that the hyphal dispersed mutant of *A. oryzae* lacking both α-1,3-glucan and galactosaminogalactan (AGΔ-GAGΔ) exhibited reduced culture viscosity and increased recombinant enzyme production. However, wall-growth remained a challenge. Hydrophobin RolA, an amphipathic protein on the cell surface of *A. oryzae*, makes surfaces of hyphae and conidia hydrophobic and contributes to their attachment on solid surfaces. In this study, we generated a strain deficient in the hydrophobin RolA (AGΔ-GAGΔ-RolAΔ) using the AGΔ-GAGΔ strain as the parental strain, aiming to reduce wall-growth and improve productivity.
When cultured in Sakaguchi flasks with 100 mL minimal medium, the AGΔ-GAGΔ-RolAΔ strain reduced wall-growth by 32% and increased recombinant enzyme activity by 13% compared to the AGΔ-GAGΔ strain after 72 hours. The liquid volume decreased less in the AGΔ-GAGΔ-RolAΔ strain, with the final volume being 6% greater, leading to a 16% increase in total enzyme activity. In a 5 L bioreactor, wall-growth was reduced by 20% in the AGΔ-GAGΔ-RolAΔ strain compared to the AGΔ-GAGΔ strain at 72 hours of fermentation. These findings suggest that the loss of RolA reduced wall-growth and increased culture volume at the end of the fermentation, resulting in the enhancement of total enzyme production.

P2.220 - Engineering of the sugar transport system of Saccharomyces cerevisiae to increase fermentative efficiency of glucose and xylose

I.C. Taveira[1], *R.N. Silva*[1]
[1]University of São Paulo (USP), Biochemistry and Immunology, Ribeirão Preto, Brazil

The heterologous expression of xylose transporters, along with the xylose metabolic pathway in *Saccharomyces cerevisiae*, has been explored to enhance the fermentative performance in second-generation (2G) ethanol production. However, due to the intrinsic nonspecificity of sugar transporters, competition between sugars (e.g., xylose and glucose) occurs during transport, leading to reduced pentose transport efficiency and lower ethanol productivity. This study aimed to optimize xylose consumption by genetically enhancing *S. cerevisiae* and constructing modified transporters with improved xylose transport capacity. *S. cerevisiae* (PE-2 ΔURAΔTRIP) was transformed with heterologous sugar transporters, and sugar consumption was analyzed. Mutant transporters were designed using the Robetta online server to improve xylose selectivity. Molecular docking analysis was performed with two different software programs to identify mutations that could enhance xylose selectivity and to compare the efficiency of both programs as screening tools. Expression cassettes were built to heterologous expression in *S. cerevisiae* hxt null. The expression of MFS1_WT led to improved xylose transport compared to yeast strains without heterologous transporters. *In silico* analysis shows that three mutations in MFS1 exhibited a promising phenotype, demonstrating a higher affinity for xylose and reducing glucose inhibition. These mutations were selected for *in vivo* expression in *S. cerevisiae* and were successfully constructed and sequenced. This study shows that MFS1 expression increases the bioconversion of xylose to ethanol in *S. cerevisiae*. This is the first to use docking analysis to screen for modified high-affinity sugar transporters, identifying transporters with enhanced affinity for xylose.

P2.221 - Harnessing *Pichia pastoris* for the production of hepatitis B virus D1 sub-genotype antigens

B. Ozcan[1], *B. Gundogdu*[1], *G. Bayram Akcapinar*[1]
[1]Acibadem Mehmet Ali Aydinlar University, Institute of Health Sciences, Department of Medical Biotechnology, Istanbul, Turkey

Hepatitis is a form of liver damage commonly seen in the World as well as Turkey that can be caused by viral and non-viral origin. The main cause of the disease is a DNA virus belonging to the double-stranded *Hepadnoviridae* family known as Hepatitis B Virus (HBV). Although today, safe hepatitis B vaccines are available for people of all age groups, cases where the vaccine is not 100% effective and cases with immunity escape variants can still be observed. The methylotrophic yeast *Pichia pastoris* uses methanol as a carbon source and an inducer via the AOX promoter. *Pichia pastoris* as a microbial cell factory, has an efficient expression system with its high capability to secrete recombinant proteins.
Within the scope of our study, DNA regions of D1 sub-genotype encoding the Major S protein, Medium S protein (M protein), immune escape variant of S protein, immune escape variant of M

protein belonging to the Hepatitis B virus were expressed recombinantly in *Pichia pastoris*. Vaccine candidates were expressed in shake flask as an extracellularly and then, expressed in a bioreactor more than 1 mg/ml yield. Characterized with BCA assays, Tris-Glycine SDS-PAGE and Western Blotting. At the same time, samples were concentrated as a 5X via SartoFlow TFF 10 kDa cut-off filtering system. Additionally, the pellets of variants with problems in extracellular production were exploded and analyzed whether there was intracellular production. Then, after purification and characterization studies, preliminary toxicology profiles of recombinant proteins were determined through cytotoxicity tests on Hep-G2 cells.

P2.222 - Effects of the polar growth-related genes on the growth and the regulation of cellulase expression in Neurospora crassa

L. Qin[1], *Y. Chen*[1], *X. Jiang*[1]
[1]Fujian Normal University, Fuzhou, China

Saprophytic filamentous fungi are the primary decomposers of lignocellulose in nature, possessing strong protein secretion capabilities that are often utilized for the production of enzymes and recombinant proteins. The protein secretion in filamentous fungi occurs mainly through the hyphal tips. Therefore, it is generally agreed that restricting the polar growth and increasing branching of hyphae is an effective method to enhance protein secretion. Our laboratory screened a large number of mutants related to polar growth and discovered that the deletion strains of *pla-7*, *kin-1* and *ede-1* encoding phospholipase D, kinesin, and endocytic protein in *Neurospora crassa*, respectively, exhibited significantly increase in hyphal branching and biomass accumulation under sucrose carbon sources. Although deletion of these genes hindered cellulase induction on cellulose in shake flasks, mis-expressing *clr-2* restored cellulase production in Δ*pla-7*, Δ*kin-1* and Δ*ede-1* strains, and we surprisedly found that these strains constitutively expressing *clr-2* demonstrated higher protein secretion levels under both sucrose and cellulose carbon sources. We further utilized global transcriptional profiling combined with genetic and physiological analyses to investigate how these genes affected the induction and secretion of cellulases. *pla-7*, *kin-1*, and *ede-1* are localized to the plasma membrane, and these genes not only affected the transcription of *clr-2* at the transcriptional level, but also participated in vesicle formation and trafficking as well as vesicle fusion to the plasma membrane. Our data provide the possibility to develop the model organism *N. crassa* suitable for bioreactor fermentation to produce secreted proteins.

P2.223 - High-throughput screening of filamentous fungi using solid substrates in droplets

C. Leal Alves[1,2], *M. Valkonen*[3], *A. Dilokpimol*[3], *Z. Deng*[1,2], *S. Shih*[1,4]
[1]Concordia University, Department of Electrical and Computer Engineering, Montréal, Canada, [2]Concordia University, Department of Biology, Montréal, Canada, [3]VTT Technical Research Centre of Finland Ltd, Espoo, Finland, [4]Concordia University, Centre for Applied Synthetic Biology, Montréal, Canada

Filamentous fungi like *Aspergillus oryzae* and *Trichoderma reesei* are crucial in bioprocessing due to their high protein secretion. However, improving enzyme production remains challenging. Traditional methods for screening mutant libraries are slow, labor-intensive, and costly. Droplet microfluidics offers a solution, enabling high-throughput screening of up to 500,000 conditions per day using minimal volumes (µL) of expensive enzyme substrates. One key challenge in applying droplet microfluidics to filamentous fungi is their complex morphology. Fungal hyphae are typically confined in nanoliter (~20nL) droplets for only 36 hours, which is insufficient for proper spore germination and enzyme production, which usually takes about 72 hours. To address this, we introduced solid supports within the droplets – colloidal chitin and cellulose, mimicking solid-state fermentation. This enabled us to culture *A. oryzae* and *T. reesei* in 2 nL droplets for up to 120 hours, stabilizing growth, reducing droplet instability, and ensuring reliable enzyme secretion. We further developed an automated microfluidic platform equipped with fluorescence optical fibers and a low-voltage (~105V RMS) sorter to minimize droplet breakup. Using this system, we successfully screened *A. oryzae* mutants on colloidal chitin, identifying strains with up to a fivefold increase in α-amylase production when cultured in bench bioreactors. On-going work is focusing on screening *T. reesei* for enhanced cellulase production using colloidal cellulose, highlighting the potential of different solid substrates in microdroplet technology for high-throughput fungal screening.

P2.224 - A CRISPR/Cas9-based multicopy integration system for increased glucoamylase production in *Aspergillus niger*

J.L. van Dam[1], *S. Forrer*[1], *M. Arentshorst*[1], *P.K. Valappil*[1], *J. Visser*[1], *R.J.M. Lubbers*[1], *A.F.J. Ram*[1]
[1]Leiden University, Microbial Sciences, Leiden, Netherlands

The filamentous fungus *Aspergillus niger* is well known for its high protein secretion capacity and is therefore a preferred host for protein production. Glucoamylase is one of the highest expressed genes in *A. niger* and the promoter and terminator regions of glucoamylase are often used to drive the expression of (heterologous) genes of interest. Moreover, the introduction of multiple copies of such gene constructs is known to further boost product yields.
To increase glucoamylase production in *A. niger*, we have designed a CRISPR/Cas9-based gene targeting method to integrate up to six copies of the *glaA* gene to predetermined sites in the genome. Genes encoding extracellular enzymes such as alpha-amylase and alpha-glucosidases or proteases (PepA and PepB), were deleted and replaced by a Glucoamylase Landing Site (Gla_LS). Each Gla_LS consists of the *glaA* promoter and the *glaA* terminator region. In between the *glaA* promoter and *glaA* terminator regions a unique DNA sequence was introduced for which a unique Cas9 compatible guide RNA was designed. A strain lineage in a non-homologous end joining mutant background was made in which up to six Gla_LS were constructed. As a proof of principle, an *A. niger* strain in which six copies of the glucoamylase gene were introduced was subsequently analyzed for glucoamylase production.
We successfully used the expression platform to generate glucoamylase hyperproducing strains of *A. niger*. The expression platform is currently exploited for the expression of heterologous proteins.

P2.301 - Effects of *hap2* deletion on *mnp/vp* transcription in *Pleurotus ostreatus* grown on lignocellulosic substrates

K. Kayama[1,2], *T. Nakazawa*[2], *I. Yamaguchi*[2], *M. Kawauchi*[2], *M. Sakamoto*[2], *Y. Honda*[2]
[1]Institut Pasteur, Department of Mycology, Paris, France, [2]Kyoto University, Graduate School of Agriculture, Kyoto, Japan

The regulatory mechanisms governing the expression of lignin-modifying enzyme (LME) genes in white-rot fungi remain largely unexplored. Although molecular cloning studies have identified CCAAT boxes frequently located in the 5´-upstream regions of these genes, their role in transcriptional regulation is not well understood. To investigate this, gene deletants of *hap2*, which encodes a protein homologous to a component of the CCAAT-binding Hap complex, were characterized in *Pleurotus ostreatus*. Significantly reduced Mn^{2+}-dependent peroxidase activity and lignin-degrading capacity were observed in *hap2* deletants compared to the parental strain 20b when grown on ethanol/toluene-extracted and non-extracted beech wood sawdust (BWS) medium supplemented with 1.3% (w/w) wheat bran, respectively. Real-time PCR analysis revealed that *vp2* transcript levels were significantly lower in *hap2* deletants compared to strain 20b when cultured on three lignocellulose-based media [extracted BWS, holocellulose, or Avicel supplemented with extracted wheat bran], but not on YMG agar plates [0.4% (w/v) yeast extract, 1% (w/v) malt extract, 0.4% (w/v) glucose, and 2% (w/v) agar]. Furthermore, glutathione *S*-transferase pull-down and electrophoretic mobility shift assays demonstrated that recombinant *P. ostreatus* Hap2, Hap3, and Hap5, expressed in *Escherichia coli*, form a complex that binds to the CCAAT sequence in the 5´-upstream region of *vp2 in vitro*. Additionally, a significant decrease in *vp2* transcript level was also observed in *hap3* or *hap5* deletants grown on extracted BWS. These findings suggest that Hap2, as part of the CCAAT-binding complex, plays a crucial role in the transcriptional upregulation of *vp2* in *P. ostreatus* when grown on lignocellulosic substrates.

P2.302 - Amplicon sequencing, isolation, and fermentative evaluation of yeast collected from diverse glacial microhabitats of the Svalbard archipelago

A. Triska[1]
[1]Clemson University, Biological Sciences, Clemson, United States

Arctic glaciers are home to a great number of psychrophilic and psychrotolerant fungi, including yeasts capable of ethanol fermentation. Such yeasts may be valuable in wine production as co-fermenting partners to *Saccharomyces cerevisiae* or as solo fermenters, as they may be capable of tolerating different conditions than *S. cerevisiae*, imparting aromas and structural components, or reducing the need for sulfur and other additives for a more sustainable winemaking process. While non-*Saccharomyces* yeast now see wide commercial use in wineries, Arctic yeasts largely remain unexplored. This study sought to explore the diversity and fermentative capabilities of glacial yeasts from the Svalbard archipelago to determine their potential applications in commercial winemaking, and more broadly, to develop an efficient wine-focused bioprospecting pipeline involving amplicon sequencing, culturing, and ethanol production trials simulating real-

life winemaking. Environmental samples were collected from glacial surface snow, cryoconite holes, and meltwater in the Sarkofagen mountain area and analyzed using ITS1/2 amplicon sequencing. Samples were found to contain a diverse range of ascomycetous and basidiomycetous yeast, including one, *Cladophialophora humicola*, never before documented in Svalbard. Culturable isolates identified as potentially capable of ethanol production were used in a winemaking trial with Sangiovese grape must, with one *Cryolevonia* isolate producing a wine of 1.6% abv under low-temperature conditions. This study highlights the potential for Arctic yeasts to be used in wine production, the simplicity and low cost with which their capabilities might be tested in a scaled-down winemaking environment, and the complementarity of amplicon sequencing and culturing in elucidating fungal biodiversity.

P2.303 - Entomopathogenic activity of *Trichoderma erinaceum* against *Tetranychus urticae*: growth stimulation in *Capsicum chinense* (Habanero pepper) to reduce the impact of synthetic insecticides

D. Magaña-Ortiz[1], *S. Tum-Rodríguez*[1], *J. Martinez-Contreras*[1], *C. Cervantes-Basto*[1], *S. Solis-Pereira*[1], *G. Rivera-Muñoz*[1], *J. Ferraez Balam*[1], *G. Lizama-Uc*[1], *E. Ruiz-Sánchez*[2]
[1]Tecnologico Nacional de Mexico/Campus Merida, Chemistry, Biochemistry and Environmental Engineering Department, Merida, Mexico, [2]Tecnológico Nacional de México, Instituto Tecnológico de Conkal, División de Estudios de Posgrado e Investigación, Conkal, Mexico

In Latin America, most of agriculture activities are associated with the use of synthetic insecticides (SI), especially those that high impact in soil, water and human tissues. Biological control based on microorganisms is an alternative to reduce the contamination caused by SI application in tropical crops.
Habanero pepper (*Capsicum chinense*) is a vegetable with high demand in food and chemistry industries, however, this species is affected by plagues such as two-spotted spider mite, *Tetranychus urticae*. Therefore, the main purpose of this work was to determine the effect of the application of spores of *Trichoderma erinaceum* against pest *Tetranychus urticae* and the interaction of this fungus with *Capsicum chinense.*
The application of *Trichoderma erinaceum* showed effective results against the pest *Tetranychus urticae* using a spore concentration of $1x10^5$ on leaves, giving a percentage of mite mortality of 56% after 3 days of exposure compared to the control (saline solution); while, in the greenhouse assays, spraying spores on mature plants, a mortality of 66% of adult mites was determined after 9 days of exposure in comparison with controls. Thus, entomopathogenic activity was detected in this strain.
Moreover, when roots of *Capsicum chinense* were inoculated with spores of *Trichoderma erinaceum* an increment in biomass of up to 13.38% was observed in comparison to the control plants without any treatment. The results indicated that this novel strain of *Trichoderma* may be employed to effective biocontrol of *T. urticae* reducing the use of SI that impact ecosystems and consequently, human health.

P2.304 - On the Trail of Sustainable Wood Recycling: Insights from *Trametes sanguinea*-Mediated Wood Degradation

A. Dulaj[1]
[1]Institute for Biotechnology and Drug Research (IBWF), Mainz, Germany

Lignin and hemicellulose, key components of plant cell walls, are crucial for structural integrity but are difficult to be microbially degraded. Lignin, a complex and rigid polymer, strengthens the cell wall and resists microbial breakdown. Hemicellulose, a more flexible heteropolymer, links lignin and cellulose and is easier to hydrolyze. This study explores an eco-friendly method for degrading wood waste through fungi to enable its reuse in various applications.
White-rot fungi, particularly *Trametes sanguinea*, are known for their degradation potential due to their many enzymatic systems. Genome sequencing of *T. sanguinea* revealed that there are seven distinct laccase enzymes in *T. sanguinea* that are likely involved in wood degradation. The degradation efficacy of *T. sanguinea* was examined when supplemented with various mediators, including 1-hydroxybenzotriazole (HBT), gallic acid (GA), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), bipyridine (bpy), vanillic acid, and acetosyringone. A comprehensive analytical approach was employed, utilizing scanning electron microscopy (SEM), Fourier transform infrared spectroscopy, and ultraviolet spectroscopy to assess structural and chemical modifications in supplemented wood powder samples. SEM analysis revealed significant fragmentation and increased porosity in samples supplemented with HBT and GA. FTIR indicated the disruption of lignin and hemicellulose linkages, evidenced by changes in the C=O and C-O stretching regions. UV spectroscopy confirmed the formation of new functional groups, particularly with HBT and GA. Among the mediators tested, usage of HBT and GA lead to the highest enzymatic activity and degradation effect on the treated wood powder highlighting their potential to enhance fungal-mediated wood degradation for sustainable recycling processes.

P2.305 - Evaluation of the white rot fungus *Phlebia radiata* for the application in the re-valorization of softwood Kraftlignin

L. Schön[1]
[1]Institute for Biotechnology and Drug Research (IBWF), Mainz, Germany

Fungi, particularly wood-degrading species, play a crucial role in ecosystems by breaking down complex organic materials such as lignin. White rot fungi, like *Phlebia radiata*, are of particular interest due to their lignin-modifying enzymes (LMEs), including laccases, manganese peroxidases, and lignin peroxidases. These enzymes are highly efficient in degrading lignin, making them promising candidates for biotechnological applications such as managing industrial wastes like Kraftlignin (KL).
KL, a byproduct of the pulping process, is produced in large quantities and often remains underutilized, i.e.: being burned for energy rather than valorized into high-value products. In this study, we aimed to address this issue by screening nearly 100 fungal species for their ability to produce LMEs, using ABTS, Poly-R, and Azure B as substrates. Among the top performers was *P. radiata*, a white rot fungus with a strong potential for lignin degradation. In a liquid fermentation, supplemented with KL, the degradation capabilities of *P. radiata* were further investigated. Culture filtrate extracts were analyzed using high-performance liquid

chromatography (HPLC) to assess the extent of KL degradation. After a two-week fermentation period a decrease in signals linked to KL could be seen in the chromatograms. Additionally, novel signals indicated the formation of degradation products such as vanillic acid.
P. radiata showed significant potential in the re-valorization of KL, offering a sustainable approach to converting industrial waste into valuable chemical products. This study highlights the importance of fungal biotechnology in developing innovative solutions for waste management in the paper industry.

P2.306 - Degradation of the hazardous chemical 2-mercaptobenzothiazole by filamentous fungi

K. Heeb[1]
[1]Institute for Biotechnology and Drug Research (IBWF), Mainz, Germany

2-Mercaptobenzothiazole (2-MBT) is used as a vulcanisation accelerator in the production of rubber products. It is environmentally hazardous and potentially carcinogenic. We found that the filamentous fungi *Agrocybe pediades* and *Alternaria alstroemeriae* are capable of degrading 2-MBT in liquid culture. Therefore, *A. pediades* and *A. alstroemeriae* are of significant interest for bioremediation of soil and industrial wastewater.
A. pediades and *A. alstroemeriae* were cultivated in various complete media with 30 mg/L 2-MBT. After a maximum of seven days, 2-MBT was completely degraded. Through protein purification using hydrophobic interaction chromatography, we isolated a protein fraction that achieved 50 % degradation of 2-MBT after 16 hours. Further analysis of this fraction offers potential for identifying proteins responsible for 2-MBT degradation.
We are further interested in elucidating the exact degradation pathway of 2-MBT, as little is known about this process in fungi. A potential metabolite with a mass of 151 Da was identified by HPLC mass spectrometry after cultivation of *A. pediades* in medium supplemented with 2-MBT. This mass might correspond to 2-hydroxybenzothiazole, marking the first description of this metabolite in the fungal 2-MBT degradation pathway.
In future, RNA sequencing will be conducted of fungi incubated with 2-MBT, compared to those incubated without 2-MBT. This approach aims to provide deeper insights into the gene regulatory processes essential for 2-MBT degradation. This may facilitate upscaling of the degradation process.

P2.307 - Streptomyces small laccase expressed in Aspergillus niger as a new addition for the lignocellulose bioconversion toolbox

P. Punt[1], *A. Sidar*[1], *G. Voshol*[2], *E. Vijgenboom*[1], *A. El-Masoudi*[1]
[1]Leiden University, Microbial Biotechnology, Leiden, Netherlands, [2]GenomeScan, Leiden, Netherlands

Laccases are multi-copper oxidases that are usually composed of three Cu-oxidase domains. Domain one and three house the copper binding sites, and the second domain is involved in forming a substrate-binding cleft. However, Streptomyces species are found to have small

laccases (SLAC) that lack one of the three Cu-oxidase domains. This type of SLAC with interesting bioconversion activities have not been reported in Aspergillus niger. In our research, we explored the expression and engineering of the SLAC from Streptomyces leeuwenhoekii C34 in A. niger. Genes encoding two versions of the SLAC were expressed. One encoding the SLAC in its native form and a second encoding the SLAC fused to two N-terminal CBM1 domains. The latter is a configuration also known for specific yeast laccases. Both SLAC variants were functionally expressed in A. niger as shown by in vitro activity assays and proteome analysis. Laccase activity was also analyzed toward bioconversion of lignocellulosic rice straw. From this analysis it was clear that the SLAC activity improved the efficiency of saccharification of lignocellulosic biomass by cellulase enzyme cocktails.

P2.308 - Co-application of spent mushroom compost and arbuscular mycorrhizal fungi on field wheat enhances yield, but reduces mineral nutrient concentration of grain

J. Carrara[1], *A. Smith*[2], *W. Heller*[1]
[1]United States Department of Agriculture, Agricultural Research Service, Wyndmoor, United States, [2]Rodale Institute, Kutztown, United States

The development of farming management practices that enhance crop yield while maintaining soil health is the foremost objective of the regenerative agriculture movement. One avenue to achieve this goal is using biofertilizers and alternative soil amendments to supplement or replace agrichemicals. We inoculated wheat (*Triticum aestivum*) with a commercial arbuscular mycorrhizal fungal (AMF) inoculant containing the species *Rhizophagus irregularis* and/or with a spent mushroom compost amendment and compared these treatments to untreated plants in a field trial. AMF benefit the yield and nutritional quality of many crops by enhancing access to mineral nutrients and water. Mushroom compost is a biproduct of the edible mushroom industry comprised of a variety of organic materials. We hypothesized that mushroom compost amendment would have synergistic effects with AMF on wheat production and nutrient uptake. We found that mushroom compost addition, regardless of AMF inoculation, enhanced grain yield by ~40%, but reduced AMF root colonization level by ~25-40%. Additionally, despite yield increases, mushroom compost addition reduced grain phosphorus (P), potassium (K), and magnesium (Mg) concentrations by ~10% and boron concentration by ~45%. In fact, grain P, K, and Mg concentrations were all correlated with mycorrhizal colonization level. Collectively, these results suggest that while mushroom compost additions enhanced grain yield, this may have led to a mineral nutrient ‘dilution effect’ exacerbated by negative impacts on mycorrhizal abundance and community composition. This work highlights the importance of examining nutritional quality in addition to yield when testing the efficacy of soil amendment strategies.

P2.309 - Harnessing filamentous fungi for enzyme cocktail production through rice bran bioprocessing

A.M. Yélamos[1], *J.F. Marcos*[1], *P. Manzanares*[1], *S. Garrigues*[1]
[1]Institute of Agrochemistry and Food Technology, Food Biotechnology, Paterna (Valencia), Spain

Valorization of agri-food residues has garnered significant interest for obtaining value-added compounds. Rice milling by-products, such as rice bran, have limited commercial value and may pose environmental challenges. Filamentous fungi are recognized for their ability to grow on agri-food residues and for their capacity to produce large amounts of metabolites and enzymes of industrial interest. Here, we used filamentous fungi to produce enzyme cocktails from rice bran, which, due to its polysaccharide composition —primarily cellulose and xylan— serves as an ideal substrate for the production of cellulases and xylanases. To this end, sixteen fungal strains isolated from rice bran were identified, which belonged to the genera *Aspergillus*, *Penicillium*, and *Mucor*. The *Aspergillus* species were the most efficient cellulase and xylanase producers, especially *A. niger* var. *phoenicis* and *A. amstelodami*. Additionally, *A. terreus*, *A. tritici* and *A. montevidensis* stood out as xylanase producers, while *P. parvofructum* was a good cellulase producing strain. Finally, *A. niger* var. *phoenicis* followed by *A. terreus* showed the highest specific enzymatic activities α- and β-D-galactosidase, α-L-arabinofuranosidase, α- and β-D-glucosidase, and β-D-xylosidase. Proteomic analysis of *A. terreus*, *A. niger* var. *phoenicis*, and *P. parvofructum* exoproteomes revealed differences in enzyme production for rice bran degradation. *A. niger* had the highest levels of xylanases and cellulases, while *P. parvofructum* excelled in proteases, starch-degrading enzymes and antifungal proteins. Our results demonstrate that fungi can effectively valorize rice bran by producing enzyme cocktails of industrial interest, as well as bioactive peptides, in a cost-efficient manner, aligning with the circular bioeconomy framework.

P2.310 - AMF inoculation enhances nutrient uptake and growth of industrial fiber hemp

W. Heller[1], *J. Carrara*[1], *D. Panday*[2], *A. Ghalehgolabbehbahani*[2], *D. Meyers*[3], *Z. Stansell*[4], *T. Gordon*[4]
[1]USDA-ARS, Eastern Regional Research Center, Sustainable Biofuels and Co-Products Research Unit, Wyndmoor, United States, [2]Rodale Institute, Kutztown, United States, [3]University of Massachusetts Amherst, Department of Plant Biology, Amherst, United States, [4]USDA-ARS, Plant Genetic Resources Unit, Geneva, United States

Recent deregulation of the cultivation of industrial hemp (*Cannabis sativa*) for fiber, seed, and medical uses has sparked interest in the development of plant germplasm and agricultural practices for this versatile crop. Although mycorrhizal symbioses are known to be important soil health factor affecting the agricultural productivity of diverse crops, the fungal communities associated with hemp specifically have not been well characterized. In order to identify these fungal associations, over 250 root samples from 2023 and 2024 were screened for mycorrhizal colonization by root staining and microscopy and separately analyzed using monospecific qPCR for relative quantitation of DNA from 11 different species of arbuscular mycorrhizal fungi (AMF). Plant samples were provided from trials being conducted by the USDA-ARS hemp germplasm repository, and from a commercial hemp variety trial being conducted by the Rodale Institute. From this field data, three AMF species, *Rhizophagus irregularis*, *Rhizophagus intraradices*, and *Funnelimormis mosseae*, were identified as species which commonly associate with hemp plant roots, and a replicated controlled greenhouse inoculation trial was conducted in

summer 2024 to identify the potential benefits of AMF application on plant nutrient uptake and growth. Under nutrient limiting conditions, AMF inoculation enhanced the uptake of nitrogen, phosphorus, and several plant micronutrients, and above ground biomass was increased by up to 300%. These results provide promise for the future development of AMF biofertilizers for the industrial hemp industry.

P2.311 - The enzymatic repertoire of the white-rot fungus *Dichomitus squalens* for the degradation of lignin-carbohydrate complexes

A. Zhang[1], *K. Hildén*[1], *M. Lahtinen*[2], *P. Kilpeläinen*[3]
[1]University of Helsinki, Department of Microbiology, Helsinki, Finland, [2]University of Helsinki, Department of Food and Nutrition, Helsinki, Finland, [3]Natural Resources Institute Finland (Luke), Helsinki, Finland

The efficient use of plant biomass side streams is the key towards the development of efficient and environmentally friendly processes for sustainable technologies and materials. Second generation biofuel plants use lignocellulosic waste streams as substrates, but after saccharification of the fermentable carbohydrates a new pool of lignin rich material is generated in these biorefineries. Lignin-carbohydrate complexes (LCCs) are carbohydrate moieties covalently bound to lignin and they have proven to be a real bottleneck in the total biorefinery concept to fully utilize biomass, affecting the selection of extraction and purification processes for both lignin and polysaccharide fractions.
Basidiomycetous white rot fungi are the most efficient degraders of lignocellulose with a unique ability to mineralize the recalcitrant lignin polymer. As a typical white-rot fungi, Dichomitus squalens is able to degrade effectively all the wood polymers, i.e. cellulose, hemicelluloses and lignin, due to its wide enzyme repertoire. To clarify the role of individual enzymes and their combinations in degradation of LCCs, we have analysed D. squalens secretomes in submerged cultivations with lignin rich xylan and galactoglucomannan as carbon sources. These substrates were obtained from aspen and spruce wood biomass by extraction with pressurized hot water. Secretome analyses showed the expression of several CAZymes, both putative and annotated. Our results lead to the identification of candidate proteins involved in degradation of LCCs, which can separate polysaccharide and lignin moieties for further processing. Functional analyses of selected enzymes will also be presented.

P2.312 - Establishing *Aspergillus niger* as a production system for azaphilone colourants from fungi

F. Hoberg[1], *L. Lietz*[1], *C. Freidank-Pohl*[1], *V. Meyer*[1]
[1]Technische Universität Berlin, Applied and Molecular Microbiology, Berlin, Germany

Colour is an immanent attribute of almost all items of our daily live. However, most colourants used are of synthetic origin and based on non-renewable petroleum products. Many of these possess irritant, toxic or carcinogenic properties. Thus, the production, dyeing process and use pose health risks to humans when exposed in large quantities. In addition, nature and existing

ecosystems can be massively damaged. Due to political bans and social rethinking, demand for natural alternatives has been growing.
Fungi produce a wide range of secondary metabolites (SMs), potentially serving as natural colourants in various industries. In contrast, parallel production of mycotoxin, a relatively slow growth rate or SM production just under specific, potentially unfavourable, conditions, make them irrelevant for an industrial production. A solution is the shift of production into an industrially established heterologous host, i.e. *Aspergillus niger*. *A. niger* has proven to be an efficient heterologous producer for non-ribosomal peptide based SMs with high titers. Further, *A. niger* is capable to provide high quantities of polyketide precursors, rendering it an optimal choice for heterologous gene cluster integration.
Given the capacity of *A. niger* to produce polyketide pigments in high quantities, we aim to establish *A. niger* as a heterologous producer of azaphilones, a promising class of natural colourants within the yellow to red colour spectrum, from the genus *Monascus*. Different strategies of gene expression are investigated and benchmarked against optimal cultivation conditions for *Monascus* species. Results will be presented accordingly.

P2.313 - Development of a PEG-based transformation protocol for CRISPR-mediated genome editing in *Fomes fomentarius*

C. Regner[1], *S. Elsner*[1], *R. Mangaldzhieva*[1], *C. Freidank-Pohl*[1], *T.C. Cairns*[1], *V. Meyer*[1]
[1]Technische Universität Berlin, Applied and Molecular Microbiology, Berlin, Germany

To establish the basidiomycete *Fomes fomentarius* as a model organism in biomaterial research, we developed a reliable and replicable protocol for genetic modifications. Cultivation and PEG-mediated transformation protocols published for other Basidiomycetes were optimized for the heterokaryotic strain PaPF11, which is the protagonist in many publications on fungal composite materials. These improvements resulted in multiple successful transformations with ectopic integrations of the deletion cassette for *ku70* and further premilinary transformants using a deletion cassette for *ura3*. We achieved a 84-fold increase in protoplast generation efficiency and a remarkable reduction in mycelial debris, allowing us to use 6 x 10^7 protoplasts per transformation sample. The regeneration frequency of 0.15% highlights the need for a tremendous number of protoplasts. A total of 53 putative transformants were subcultured over 2 months and the presence of the deletion cassette was confirmed in 49 of these. The *ku70* locus was confirmed to be unedited for all these transformants. Surprisingly, we observed an increased transformation efficiency for DNA templates with shorter homologous flanks (HFs). While 250bp HFs yielded in 36 colonies, 500bp and 1000bp HFs yielded in 15 and two colonies, respectively. The robustness and replicability of the established protocol allows us to subsequently focus on designing studies to achieve target specificity, perform promoter studies and continue with non-targeted transformations. Having identified the crucial point for optimization will also accelerate the implementation of this protocol for the monokaryotic *F. fomentarius* strain Pdm1.

P2.401 - The impact of 3D genome conformation on chromatin interactions and gene regulation in a major wheat pathogen

I. Glavincheska[1], *C. Lorrain*[1]
[1]ETH Zurich, Department of Environmental Systems Science, Zurich, Switzerland

The folding and dynamics of three-dimensional (3D) genome organization play a crucial role in genome functions in eukaryotes, yet remain largely unexplored in non-model fungi. Hierarchical genome organisation includes the formation of distinct chromatin structures separating active and inactive chromatin, which are regionally subdivided in self-interacting topologically associated domains (TADs). Here, we investigate the genome topology of the fungal wheat pathogen *Zymoseptoria tritici* using Hi-C sequencing integrated with epigenomics and transcriptomics. *Z. tritici* genome exhibits a Rabl-like configuration, with frequent centromere interactions. Interestingly, the accessory chromosomes occupy a distinct nuclear territory and interact solely with the short arm of core chromosomes. This association may contribute to the loss and maintenance of accessory chromosomes, as our data show that the most frequently lost accessory chromosome of *Z. tritici* IPO323, exhibits the weakest inter-chromosomal interactions. We observed strong intra-chromosomal contacts between isochores of constitutive heterochromatin marked by H3K9me3, transposable elements and cytosine DNA methylation. These constitutive heterochromatin clusters could be crucial in restricting heterochromatin spread and silencing transposable elements. We identified approximately 500 TAD-like structures, with distinct epigenetic signatures at the boundary regions. 10% of TADs showed significant gene co-regulation during wheat infection compared to in vitro growth. Additionally, distinct TADs are activated between the biotrophic and necrotrophic infection stage, suggesting a link between 3D genome structure and specific gene regulation in *Z. tritici*. These findings provide new perspectives in the spatial organization of fungal genomes and a foundation for further exploration of the impact of chromatin dynamics in non-model fungi.

P2.402 - Genome-wide exploration of the transcriptional regulatory landscape in the early-diverging fungus *R. microsporus*

C. Lax[1], *L.A. Baumgart*[2], *G. Tahiri*[1], *N. Nicolás*[1], *Y. Zhang*[2], *R.C. O'Malley*[3], *I. Grigoriev*[2], *E. Navarro*[1], *F.E. Nicolás-Molina*[1], *V. Garre*[1]
[1]University of Murcia, Genetics and Microbiology, Murcia, Spain, [2]U.S Department of Energy Joint Genome Institute - Lawrence Berkeley National Laboratory, Berkeley, United States, [3]University of Chicago, Human Genetics, Chicago, United States

Genetic regulation mechanisms are based on complex transcriptional networks that are often difficult to unravel. The study of transcription factor binding sites and their targets has presented challenges in scalability for comprehensive cistrome analysis. The development of the DAP-seq technique has addressed many of these issues, allowing large-scale and genome-wide study of transcription factor binding with high reproducibility. We have applied this technique to the human opportunistic pathogen *R. microsporus*, a mucoralean fungus that belongs to the understudied group of early-diverging fungi. The characterization of genome-wide binding sites

of 58 transcription factors from major families has revealed their binding profiles and recognized sequences, expanding and diversifying the catalog of available fungal motifs. The combination of this information with DNA 6-methyl adenine profiling has allowed us to understand the direct and indirect impact of this epigenetic modification on the regulation of gene expression. The generated data has enabled the identification of transcription factors involved in biologically relevant processes such as zinc metabolism and light response. These results will not only be useful for studying processes in this fungus but may also provide insights into the regulation of other fungi and the conservation of promoter structure across the fungal kingdom.

P2.403 - Unraveling the chromosomal landmarks of the filamentous fungus *Trichoderma reesei*

K. Métivier[1], *H. Mathis*[1], *F. Bidard*[1], *E. Jourdier*[1]
[1]IFP Energies Nouvelles, Rueil-Malmaison, France

Filamentous fungi represent a primary source of enzymes and metabolites employed in a range of industrial sectors. Of these, *Trichoderma reesei* is commonly used to produce second-generation ethanol, where it is employed to generate the cellulase enzymes that are essential for the hydrolysis stage. To enhance the long-term capabilities of fungi, the utilization of genetic tools could prove beneficial. Among them, the creation of an artificial chromosome would facilitate the simultaneous testing of multiple genes, reduce the use of selection markers, and enhance the regulation of gene expression and gene landscape control. However, there is currently no available artificial chromosome for *T. reesei*. To facilitate the construction of an artificial chromosome, it is essential to characterize the components that contribute to chromosome stability, in particular centromeres and origins of replication (ORI).
The aim of this study is to ascertain the precise localization and size of the centromeres and to identify any potential ORI in *T. reesei*. Chromatin immunoprecipitation of eGFP-tagged CenH3, the centromere-specific histone, has enabled the precise description of the centromeres inside the AT- and repeat-rich regions previously known. To identify the ORI, several techniques previously used in other species have been adapted and implemented on *T. reesei*. Loci with the potential to act as ORI have been identified and will require further investigation. The identification of the centromere and the ORI in *T. reesei* will facilitate the construction of an artificial chromosome for this species.

P2.404 - Elucidating the role of ADP-ribosylation signalling in the DNA damage response of *Aspergillus fumigatus*

C.J. Parkin[1], *A. Bainbridge*[1], *J. Rack*[1]
[1]University of Exeter, MRC Centre for Medical Mycology, Exeter, United Kingdom

Aspergillus fumigatus is a saprophytic environmental fungus that, in humans, can cause a range of pathologies due to the inhalation of airborne spores. In immunocompetent individuals, innate immune cells effectively clear fungal conidia from the lungs. However, the accumulation of genetic mutations can enhance fungal survival within the host by promoting resistance,

exaggerating the problem of already limited treatment options.
To prevent excessive DNA damage that could be lethal to the fungus, DNA damage response (DDR) mechanisms are employed to maintain genomic integrity. A key regulator of the DDR in eukaryotic cells, including *A. fumigatus*, is ADP-ribosylation (ADPr), a reversible post-translational modification that plays a crucial role in recruiting DNA repair factors to sites of DNA damage and halting cell cycle progression. We have identified homologs of ADPr 'writer' (*Af*-PARP) and 'eraser' enzymes in *A. fumigatus*. Here we present first results showing the impact of ADP-ribosylation of fungal growth and DNA repair dynamics. To investigate the role of ADPr in *A. fumigatus*, we developed a toolkit to evaluate how the fungus coordinates ADPr-associated DDR. This includes quantifying genomic DNA damage and repair using alkaline gel electrophoresis and optimising fluorescent reporters to dynamically monitor ADPr in live cells. This work will lay the foundation for understanding how DDR is orchestrated in pathogenic fungi, potentially providing new insights into antifungal treatment strategies and mechanisms of drug resistance.

P2.405 - Conserved DNA methylation within Neurospora

J. Svedberg[1], *J. Reimegård*[2], *H. Johannesson*[1]
[1]Stockholm University, Department of Ecology, Environment and Plant Sciences, Stockholm, Sweden, [2]Science for Life Laboratory, ScilifeLab, Department of Cell and Molecular Biology, National Bioinformatics Infrastructure Sweden, Uppsala, Sweden

5-methylcytosine DNA methylation is mostly found in repetitive regions in *Neurospora*, where repeat induced point-mutations lead to the formation of H3K9me3 mediated heterochromatin which in turn induces DNA methylation. There has been little evidence of methylation of genic sequences and of control of gene expression through dynamic methylation throughout the life cycle. Most previous studies on methylation in *Neurospora* have focused on *N. crassa*, and we have gained significant knowledge of the molecular underpinnings of DNA methylation based on work done in this species, but little has been known of DNA methylation in the rest of the genus. In a previous study, we investigated DNA methylation patterns by performing bisulfite sequencing of 10 strains from 5 different *Neurospora* species. Here we use this dataset to investigate patterns of conservation of methylation between these species, and identify an excess of sites where methylation is highly conserved in all species. Unlike most methylated sites, which are found in repeats, these are instead mostly located within genes and they are also associated with a specific G-rich sequence motif. Using transcriptomic data we investigate the association between methylation and gene expression in several different tissues and under several different growth conditions and we also investigate the causes of methylation at conserved sites and to what extent it differs from methylation caused by the canonical pathway described in *N. crassa* through the use of deletion mutants.

P2.406 - Towards understanding mechanisms of de novo epigenetic silencing

F. Carlier[1]
[1]Institut Pasteur, Department of Mycology, Paris, France

In eukaryotes, repetitive DNA can become silenced de novo, transcriptionally or post-transcriptionally, by processes that appear independent of strong sequence-specific cues. The mechanistic nature of such processes remains poorly understood. We found that in the fungus *Neurospora crassa*, de novo initiation of both transcriptional and post-transcriptional silencing was linked to perturbed chromatin induced experimentally at the tetO array. In this system, transcriptional silencing was mediated by classical constitutive heterochromatin. On the other hand, post-transcriptional silencing resembled repeat-induced quelling, but occurred normally when homologous recombination was inactivated. Therefore, this process was named 'recombination-independent quelling' (RIQ). We also found that all silencing of the perturbed tetO array required SAD-6, a fungal ortholog of the conserved SWI/SNF chromatin remodeler ATRX, which was further required to maintain nucleosome occupancy in the face of perturbation.These and other results suggested a model in which the de novo initiation of transcriptional and post-transcriptional silencing is coupled to the remodeling of perturbed chromatin [1]. To better understand the mechanism of this newly described phenomenon, we conducted a forward-genetics screen to identify additional required factors. Among several conserved candidates, our effort pinpointed a variant of histone H4 (hH4v) that was absolutely essential for the initiation of both heterochromatin and RIQ by the remodeling-dependent pathway. Our preliminary analysis indicated that hH4v plays additional roles in regulating the state of chromatin in response to stress throughout the genome.
[1] Carlier et al. (2024). Remodeling of perturbed chromatin can initiate de novo transcriptional and post-transcriptional silencing. PNAS 121, e2402944121.

P2.407 - Analysis of potential DNA 6mA readers in early-diverging fungi

P. Carrillo-Marín[1], *C. Lax*[1], *E. Navarro*[1], *F.E. Nicolás*[1], *V. Garre*[1]
[1]Universidad de Murcia, Departamento de Genética y Microbiología, Facultad de Biología, Murcia, Spain

DNA methylation is a key epigenetic modification, with 5-methylcytosine (5mC) and N6-methyladenine (6mA) being the most notable. Recent studies have revealed the importance of 6mA in certain eukaryotes, such as green algae, ciliates, and Early-diverging fungi. In particular, in the basal fungus *Rhizopus microsporus*, approximately 1.5% of adenines have been observed to be methylated. The identification of 6mA in promoters of transcriptionally active genes has raised interest in its potential function as a regulator of gene expression.
While readers of 6mA in RNA (m6A) have been characterized, with the YTH domain being the most common, only two readers of 6mA in DNA have been characterized: Jumu in *Drosophila melanogaste*r and SSBP1 in humans. In this study, a unique YTH and SSBP1 ortholog, YTHDC1 and SSBP1 respectively, have been identified in R. microsporus. YTHDC1 is particularly relevant as it is the only member of the YTH family localized in the nucleus, and the inability to detect m6A in Mucorales RNA suggests that it may have a distinct functional role in this organism. Additionally, orthologs of YTHDC1 and SSBP1 have been found in other Mucorales, indicating a possible evolutionary conservation of their function.
The generation of *ythdc1* and *ssbp1* mutants could be key to describing how this epigenetic modification regulates gene expression. Furthermore, EMSA assays will demonstrate whether

YTHDC1 and SSBP1 specifically bind to DNA probes containing 6mA, providing evidence for the 6mA-based regulatory mechanisms of gene expression.

P2.408 - Precision regulation of Candida albicans pathogenesis through fine-tuning histone acetylation by Rpd31 and Rpd32

J. Kim[1], *S. Park*[1], *J.-S. Lee*[1]
[1]Kangwon National University, Department of Molecular Bioscience, Chuncheon, Korea, Republic of

Rpd3, a well-characterized Class I histone deacetylase in yeast, has two distinct orthologs in *Candida albicans*, Rpd31 and Rpd32. Despite previous studies on their physiological roles, the reason for the coexistence of these two proteins remains unclear. Furthermore, the transcriptional regulatory mechanisms governed by the catalytic activities of Rpd31 and Rpd32 are yet to be fully understood. In this study, we discovered a complementary interaction between Rpd31 and Rpd32 in controlling gene expression in *C. albicans*. Interestingly, their deletion did not result in a global increase in histone acetylation. Instead, we observed a redistribution of H3 acetylation from promoter-TSS regions into gene bodies. Genes with decreased expression in the absence of Rpd31/32 typically had extended upstream intergenic regions (IGRs) that were highly acetylated, a feature lost when Rpd31/32 were absent. Remarkably, many transcription factors important for morphogenesis in *C. albicans* possess long IGRs, and their transcription was regulated by Rpd31/32. As a result, Rpd31/32 deficiency led to defective hyphal formation and complete loss of pathogenicity in mice. These findings underscore the critical role of Rpd31 and Rpd32 in maintaining precise H3 acetylation patterns on regulatory genes necessary for morphogenesis and virulence within host cells.

P2.409 - Regulation of effector gene expression as concerted waves in *Leptosphaeria maculans*: a two-player game involving a chromatin remodeler and a specific transcription factor

C. Clairet[1,2], *E.J. Gay*[1,2], *A. Porquier*[2], *F. Blaise*[2], *C.-L. Marais*[2], *T. Rouxel*[2], *M.-H. Balesdent*[2], *J.L. Soyer*[2], *I. Fudal*[2]
[1]Paris-Saclay University, Palaiseau, France, [2]INRAE, BIOGER, Palaiseau, France

Leptosphaeria maculans is a phytopathogenic fungus responsible for oilseed rape stem canker that displays a particularly complex lifecycle. Different sets of effector-genes are expressed at each stage of oilseed rape infection. Repeat-rich regions of *L. maculans* genome are enriched in effector-genes specifically expressed during biotrophic stages of infection. These regions show a repressed chromatin state during mycelial growth *in vitro*. We showed the importance of chromatin remodeling in the control of effector-genes expression. As such, the repressive histone modification H3K9me3 deposited by the methyltransferase KMT1, is involved in the regulation of these genes not expressed *in vitro* but highly expressed during infection. However, inactivation of *KMT1* did not de-repress the expression of effector-genes *in vitro* at the same level as observed during infection, suggesting additional actors involved, such as transcription factor(s) (TF). We

investigated the involvement of Pf2, a fungal specific Zn2Cys6 TF, in the control of effector-gene expression. Deletion of LmPf2 lead to a non-pathogenic mutant. Its over-expression was not sufficient to express effector-genes *in vitro*. In contrast, its over-expression in a Kmt1 mutant background induced the expression of effector-genes *in vitro* to the same level as during plant infection. These results demonstrated for the first time a dual control of effector-gene expression involving a chromatin remodeler and an infection specific TF.

P2.410 - Set1-dependent H3K4 modifications regulate inducible gene expression in Candida albicans

J.-S. Lee[1], *J. Kim*[1], *Y.-J. Cho*[1]
[1]Kangwon National University, Department of Molecular Bioscience, Chuncheon, Korea, Republic of

Set1-mediated methylation of histone H3 at lysine 4 (H3K4) is a well-established marker of active transcription in eukaryotes. Interestingly, however, in the absence of Set1, global gene expression remains largely unchanged, with certain genes even exhibiting enhanced expression. Remarkably, these genes are normally repressed and only activated in response to specific external stimuli. Among the genes upregulated in the Δset1 mutants are hypha-specific genes of *Candida albicans*, which become expressed in the absence of Set1, even without external signals. These inducible genes display atypical H3K4 methylation patterns. Genes poised for activation but remaining transcriptionally inactive (i.e., in an 'off' state) possess only H3K4me1, lacking both H3K4me2 and H3K4me3. During the early phase of induction, H3K4 methylation does not contribute to the rapid activation of these genes. Instead, H3K4 acetylation occupies these regions, facilitating a swift transcriptional response. Notably, in the absence of H3K4 methylation, the levels of H3K4 acetylation are significantly elevated, leading to aberrant mRNA expression and subsequent morphological changes, even in the absence of external signals. Upon prolonged exposure to inducing signals, the regulatory landscape shifts—H3K4 acetylation decreases as H3K4me3 levels increase, establishing a positive feedback loop for stable and sustained gene expression. These findings position Set1 as a crucial regulator of transcriptional responses to environmental changes.

P2.411 - Investigating the role of histone post-translational modifications in yeast stationary phase

Z. Bazeem[1]
[1]Trinity College, University of Dublin, Moyne Institute of Preventive Medicine, Department of Microbiology, Dublin, Ireland

The study investigated the role of H3K36me3 and H2Bub during the yeast stationary phase. The *htb-K123R* and *hht-K36A* mutants showed increased formation of (Q) cells compared to the wt. In contrast, the mutant cells had higher viability in the (NQ) state, with the double mutant exhibiting the highest (NQ) cell viability. These results suggest that histone modifications may influence apoptosis/necrosis, leading to more (Q) cells and reduced death in the (NQ) population.

Percoll-gradient centrifugation was utilised to separate (Q) cells at different time points in both wt and mutant strains to analyse gene transcription regulation and q(Q) maintenance further. Furthermore, these histone modifications also appear to be involved in the maintenance of quiescent cells and exiting cells from quiescence. Our findings provide new insights into the functions of these histone PTMs in non-growing cells, expanding our understanding of chromatin regulation and cellular processes during the stationary phase in yeast. By investigating and reporting on the mechanisms of histone modifications, our research provides valuable insights into the maintenance and survival of quiescent cells. These cells have implications for research on aging, cancer, and stem cell biology. Future directions for this research could involve exploring the molecular mechanisms that link H2Bub and H3K36me3 to cell cycle regulation, identifying other histone modifications, and the role of H3K36me retention and H2Bub loss, while relatively unknown, may play a role in cellular quiescence.

P2.412 - A selfish Spore Killer element contributes to mitotic stability of a dispensable fungal chromosome

G. Puebla Planas[1], *D. Hazal Ayhan*[2], *P. Gutierrez Escrivano*[1], *L. Gomez Gil*[1], *C. Lopez Díaz*[1], *S. Janevska*[3], *M. Sanchez Lopez Berges*[1], *L.J. Ma*[2], *A. Di Pietro*[1]
[1]University of Cordoba, Cordoba, Spain, [2]University of Massachusetts Amherst, Massachusetts, United States, [3]Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Jena, Germany

Fungi carry dispensable genomic regions, including entire chromosomes, which are not essential for survival and can be lost at high frequency under certain environmental conditions. One unanswered question is how such dispensable regions persist in the population despite their mitotic instability. Here we studied the mechanisms promoting stability of a dispensable chromosome in the fungal pathogen *Fusarium oxysporum* (*Fo*), which causes vascular wilt disease in more than a hundred different crop species and opportunistic infections in humans. We found that the conserved core chromosome 12 (chr12) is frequently lost during serial passaging on plates in the clinical keratitis *Fo* isolate MRL8996, but not in the tomato pathogenic isolate Fol4287. Sequence analysis revealed that chr12 of MRL8996 lacks a 90 kb region present in Fol4287, which harbors a single copy of Spore Killer (Spok). Spoks are a class of genetic elements that act as meiotic drivers by killing neighboring cells lacking the element. Strikingly, transfer of the single Spok element from chr12 of Fol4287 to fluorescently labelled chr12 of MRL8996 led to a significant reduction in spontaneous chromosome loss events, as determined by flow cytometry. Importantly, no stabilizing effect was observed upon transfer of a Spok allele carrying a point mutation previously shown to abolish the killer activity of Spok *in Podospora anserina*. Our results suggest that selfish meiotic drivers such as Spoks contribute to mitotic stability of dispensable chromosomes in fungi.

P2.501 - Phenotypic and omics analysis of *Marquandomyces marquandii* and *Albophoma yamanashiensis* isolated from sediment samples of Basque estuaries

Z. Agirrezabala[1], *A. Otamendi*[1], *C. Perez-Cruz*[2], *R. Liébana*[2], *L. Alonso-Sáez*[2], *M. Dueñas*[1], *M. Ostra*[3], *A. Lanzén*[4,5], *R. López*[6], *S. Haridas*[7], *O. Etxebeste*[1]
[1]University of the Basque Country, Applied Chemistry, Laboratory of Microbiology, San Sebastian, Spain, [2]AZTI, Marine Research, Sukarrieta, Spain, [3]University of the Basque Country, Applied Chemistry, Laboratory of Analytical Chemistry, San Sebastian, Spain, [4]AZTI, Marine Research, Pasaia, Spain, [5]IKERBASQUE, Bilbao, Spain, [6]University of the Basque Country, Organic Chemistry, San Sebastian, Spain, [7]Energy Joint Genome Institute, Walnut Creek, United States

Marine microorganisms play a crucial role in ecological balance, biogeochemical cycles and food webs. These microorganisms have developed diverse strategies to adapt to challenging conditions, making them a valuable source for new biotechnological products such as pigments, enzymes and bioactive compounds. Despite the ubiquitous presence of fungi in marine environments, research has mainly focused on bacteria. Thus, the study of marine fungi is still a poorly explored area with great potential for discovering innovative biotechnological tools. The present work focused on the isolation and characterization of filamentous fungi from sediment samples collected in Basque estuaries. Their phenotypic characterization led to the identification of strains potentially able to grow on minimal culture medium supplemented with recalcitrant algal polysaccharides or to produce secondary metabolites. Two isolates were selected for genome sequencing and analysis: 1) *Albophoma yamanashiensis* for its apparent ability to grow in minimal culture medium supplemented with commercial fucoidan and 2) *Marquandomyces marquandii* due to its ability to stain the culture medium in yellow, indicative of secretion of pigments and secondary metabolites. Co-culture experiments suggested an inhibitory effect on fungal growth for this secreted fraction, while RNA-seq experiments informed of the set of secondary metabolite gene clusters upregulated under culture conditions inducing pigment secretion. Furthermore, transcriptomic and proteomic experiments on *A. yamanashiensis* samples suggested that, compared to bacteria, fungi use different enzymatic mechanisms to respond to the presence of fucoidan. Overall, results suggest that the isolates of our library could serve as a source of new enzymatic activities and secondary metabolites.

P2.502 - Analysis of the diversity in the *Fusarium oxysporum* and *Fusarium solani* species complexes in indoor environments and clinical isolates

E. Garbe[1], *U. Farooq*[1], *A. Ullah*[1], *S. Janevska*[1], *G. Walther*[2]
[1]Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, (Epi-)Genetic Regulation of Fungal Virulence, Jena, Germany, [2]Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, National Reference Center for Invasive Fungal Infections, Jena, Germany

Fusarium is a highly diverse and ubiquitously distributed fungal genus. In humans it can cause devastating systemic infections in severely sick patients but also superficial infections, such as of the cornea, in otherwise healthy individuals. In Germany, the species causing these infections mainly belong to the *F. solani* (FSSC) and *F. oxysporum* (FOSC) species complexes. Yet, only little is known about the specific ecology and infection mechanisms of these opportunistic species. Interestingly, they are frequently encountered in indoor habitats like bathrooms. Furthermore, previous work found a dominance of specific sequence types (STs) in indoor *Fusarium* isolates, and the presence of potentially virulence-associated accessory chromosomes (ACs) in two genome-sequenced clinical FOSC isolates. Given these aspects, we now investigated: (I) Does FOSC and FSSC species diversity differ in clinical and environmental samples? (II) Do specific STs dominate in those groups? (III) How diverse are ACs in FOSC isolates and are they linked to specific environments? Therefore, we collected clinical and indoor *Fusarium* isolates, performed molecular species identification and sequence typing, screened for known AC sequences in FOSC isolates and used whole-genome sequencing for identification of suspected, hitherto unknown ACs. Overall, we found with few exceptions the same species in both, clinical and indoor samples and dominating STs for *F. veterinarium* (FOSC) and *F. petroliphilum* (FSSC), while other species were more diverse. Also, whole-genome sequencing revealed great variability of AC sequences in FOSC isolates, thereby creating starting points for subsequent investigation of their putative role in virulence and adaptation to specific environments.

P2.503 - A survey conducted to the Madeira Island (Portugal) reveals the presence of 21 species of *Colletotrichum* occurring on nature and on agricultural and ornamental plants

P. Talhinhas[1], *A. Cabral*[1], *T. Nascimento*[1], *A.P. Ramos*[1], *I. Martino*[2], *A. Prodi*[3], *D. Spadaro*[2], *R. Baroncelli*[3], *V. Guarnaccia*[2]
[1]Instituto Superior de Agronomia, Universidade de Lisboa, Lisbon, Portugal, [2]University of Torino, Grugliasco, Italy, [3]University of Bologna, Department of Agri-Food Science and Technology, Bologna, Italy

There are ca. 400 species of *Colletotrichum*, most of which known from few locations and hosts. Some, however, are ubiquitous and polyphagous. The knowledge of the diversity of modern-term *Colletotrichum* species in Europe is lagging behind that from other continents. This study reports on a survey on *Colletotrichum* species occurring on nature and on agricultural and ornamental plants in Madeira island (Portugal). Madeira has a mild climate though conditioned by altitude and topography and its flora presents many endemisms. The climatic conditions of Madeira have long encouraged the cultivation of exotic sub-tropical agricultural crops and ornamental plants, a situation further incited by tourism and by the links to the Madeiran diaspora in different parts of Africa and America. In this study 21 Colletotrichum species were identified, seven in the gloeosporioides complex, six in the acutatum complex, four in the boninense complex, three in the spaethianum complex and one in the trichellum complex. *Colletotrichum* was detected in 45 host species, with fungi from the gloeosporioides complex mostly occurring on exotic agricultural plants (e.g., mango, banana or heliconia), whereas fungi from the acutatum complex were more common on plants from nature and with *C. fioriniae* appearing typically associated to

endemic plants. However, the most common fungus was *C. karsti* (boninense complex), occurring both in nature and on cultivated plants. Most of the fungus-host combinations reported here are new records. This study thus represents an advancement on the understanding of the geographical distribution and host range of *Colletotrichum* in the world.

P2.504 - A nation-wide inventory for Dutch soil fungal diversity

M. Sandoval-Denis[1], H. Kandemir[2,1], M. Hernández-Restrepo[3], D. Vu[3], M. Vermaas[4], J.Z. Groenewald[1], M. Groenewald[3], G.J. Verkley[3], P.W. Crous[1]
[1]Westerdijk Fungal Biodiversity Institute, Evolutionary Phytopathology, Utrecht, Netherlands, [2]Leiden University, Institute of Environmental Sciences (CML), Leiden, Netherlands, [3]Westerdijk Fungal Biodiversity Institute, CBS and NCCB Microbial Genetic Resources, Utrecht, Netherlands, [4]Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands

The ARISE (Authorative and Rapid Identification System for Essential Biodiversity Information, www.arise-biodiversity.nl) consortium aims to catalogue all multicellular life in the Netherlands (including fungi), developing tools for quick identification of these species. This will be accomplished via a digital infrastructure accessible to Dutch researchers, industry, infrastructure developers, nature conservation organizations, policy makers, regional water authorities, and many more. The project was made possible by a subsidy from the Dutch Research Council (NWO), as part of its National Roadmap for Large-Scale Scientific Infrastructure program. DNA barcodes for soil fungi are being generated as one of the components of this project. Launched in 2021, circa 300 soil samples covering 11 of the 12 Dutch provinces have been studied to date for culturable filamentous fungal species. The latter, coupled with additional Dutch soil fungal cultures retrieved from the Westerdijk Fungal Biodiversity Institute biobank (CBS), have resulted in over 5,600 fungal cultures, of which 84 % (representing 485 species from 181 genera) have been confidently identified by means of molecular data and phylogenetic analyses. Moreover, this study revealed that Dutch soils are extremely rich in undescribed fungal species, with 791 cultures (14 % of current total isolations) found to represent novel taxa. The relative abundance and composition of fungal genera differed per province. Preliminary data on filamentous fungal species from Dutch soil are currently represented by over 16,000 DNA barcodes, including nrDNA and protein-coding sequences, which will be released through the ARISE infrastructure and public nucleotide sequence databases.

P2.505 - Can we determine the conservation status of microfungi when increasingly species can only be distinguished based on molecular data? The case of *Colletotrichum*

P. Talhinhas[1]
[1]Instituto Superior de Agronomia, Universidade de Lisboa, Lisbon, Portugal

As biological entities, fungi are part of the planet's biodiversity and deserve to be conserved. In fact, the extinction of fungal species would alter population levels of other organisms. However, fungal conservation is incipient as compared to that of plants and animals, in part because fungi

are often seen as pathogens and therefore undesired, and because they are considered redundant, meaning that multiple species have similar roles in nature. Moreover, fungal conservation faces structural problems, as fungal populations, particularly of microfungi, are difficult to count, as there are no individuals, making standard conservation criteria of little use for these organisms. This problem is aggravated by the fact that fungal species are increasingly being delimited based on molecular, often requiring multiloci analyses, which render metagenomics approaches unfeasible. In the genus *Colletotrichum* currently there are nearly 400 species recognized, half of which have been reported only once and may face conservation problems. Many of these species occur on hosts where other species of *Colletotrichum* also occur, whereas others occur on vaguely defined hosts (genus level or less), making the assessment of their conservation statuses challenging. These examples from the genus *Colletotrichum* can be translated to several other genera. How can taxonomy and conservation mycology evolve in order to solve this paradox?

P2.506 - Mycoflora of date palm Phoenix dactylifera from Native and Naturalized Ranges

M. Alomran[1]
[1]Princess Nourah bint Abdulrahman University,College of Science, Department of Biology, Riyadh, Saudi Arabia

Seeds are an important component of national and international trade, and are directly consumed as food or forage, or used for growing crops. Approximately 90% of all food crops originate from seeds. Dates are important economically, socially and environmentally. The date palm (*P.dactylifera L.*) is native to the arid Arabian Peninsula, North Africa, and the Middle East. During the past three centuries, the date palm has been introduced to many countries where it is not native: Australia, India, Pakistan, Mexico, South Africa, and the United States. Previous studies of seed-borne fungi in date palm were done in Saudi Arabia, using a number of cultivars. The results were thirteen genera of fungi have been isolated from seeds: *Alternaria, Aspergillus, Bipolaris, Chaetomium, Curvularia, Fusarium, Penicillium, Phialophora, Rhizopus, Scytalidium, Thielavia, Trichoderma,* and *Ulocladium*. In this study seeds of three date palm cultivars, Thoory, Halawi and Barhi, were purchased from local market from native range (Saudi Arabia) and naturalized range (United States). Seed borne mycoflora of *P. dactylifera* was tested by using filter paper and fungi transferred to potato dextrose agar for identification at genera level. Fungal isolates were recovered from native range seeds were belonging to three genera: *Chaetomium, Penicillium, and Aspergillus,* and naturalized range seeds were belonging to five genera: *Chaetomium, Penicillium, and Aspergillus,Ulocladium, Alternaria,* and *Chaetomium.* As the results, all genera from native and naturalized ranges were reported in previous studies. Comparison the two ranges, naturalized range had more diversity then the native range.

P2.507 - Population genomic analyses reveal geographic structure in *Fusarium verticillioides*

S. Harding[1], *C. Andorf*[2], *R. Proctor*[1], *H. Kim*[1]
[1]U.S. Department of Agriculture, Peoria, United States, [2]U.S. Department of Agriculture, Ames, United States

Fusarium is among the fungi with the greatest negative impact on agriculture because many species cause severe diseases on diverse crops and/or contaminate crops with mycotoxins. *F. verticillioides* causes maize ear rot and is the primary cause of fumonisin contamination in maize, but isolates can differ markedly in levels of fumonisins they produce. Studies of collections of isolates from individual countries, regions and/or hosts have provided insight into genetic bases for these differences. However, it is not known whether there is substantial variation within *F. verticillioides* related to geographic, climactic and/or host origin. Such information can aid development of broadly effective strategies to reduce *F. verticillioides*-incited fumonisin contamination and ear rot in maize. To address this knowledge gap, we generated genome-wide single nucleotide polymorphisms (SNPs) from 113 *F. verticillioides* isolates collected from 12 countries and representing five continents. We then used the SNP data to estimate intraspecific genetic variation and population structure. We found evidence that the isolates constitute four distinct populations, that admixture of populations has occurred, and that population structure is geographically partitioned. These data provide critical insight into how genetic variation is distributed and shared across continental boundaries, which can potentially inform region-specific control strategies and thus, broadly increase the effectiveness of efforts to control mycotoxin contamination in maize.

P2.508 - Exploring the Potential of Irish Endolichenic Fungi for Sustainable Industrial Enzymes

S. Agrawal[1], *P. Scanlan*[1]
[1]University College Cork, APC Microbiome Ireland and School of Microbiology, Cork, Iceland

Lichens are symbiotic mutualistic associations between a mycobiont and a photobiont, and often host a diversity of microorganisms, including endolichenic fungi (ELF). Despite their ecological significance, and potential for industrial and clinical application, no studies of the diversity and phenotypic properties of ELF communities has been conducted in Ireland.
In this study, we employed pure culture isolation and phenotypic analysis to examine the ELF communities of four lichens—*Parmotrema reticulatum*, *Parmotrema* sp., *Ramalina* sp., and *Phyllopsora* sp.—collected from the Cork region of Ireland. Preliminary analysis revealed that the fungi isolated from our lichen collection belonged to various genera. *Parmotrema reticulatum* and *Parmotrema* sp. were associated with genera *Chaetomium***,** *Rosellinia, Xylaria, Biscogniauxia*,and Hypoxylon whereas *Ramalina* sp. and *Phyllopsora* sp. were primarily associated with *Diplodia*, *Xylaria*, Diaporthe and *Mucor*. Initial phenotypic assays to assess for the production of extracellular amylases, cellulases, and proteases in eleven ELF isolates revealed that six isolates exhibited protease activity, with only two isolates displaying amylase and cellulase activity. Of note, *Hypoxylon rubiginosum* showed the highest activity and produced all three enzymes. Collectively, these data highlight the varying substrate specificities of ELF, reflecting their diverse capacities for carbon assimilation, which may contribute to the ability of lichens to grow in specific environments. Moreover, these preliminary findings underscore the need for further research into the diversity, ecological roles, and potential biotechnological applications of ELF.

P2.509 - Shedding light on the vegetative life stage of the fairy ring fungus *Marasmius oreades*

B. Olsson[1], *M. Hiltunen Thorén*[2], *H. Johannesson*[2], *O. Eriksson*[1]
[1]Stockholm University, Department of Ecology, Environment and Plant Sciences, Stockholm, Sweden, [2]The Royal Swedish Academy of Sciences, Stockholm, Sweden

What it means to be a fungus varies within the lifetime of the individual and over generations. As the life cycle proceeds, ploidy as well as morphology changes, which alter the evolutionary context and ecological functions of the fungus. Historically, fungi have primarily been studied in life cycle stages that can be cultured in the lab, while unculturable and otherwise cryptic life cycle stages have remained unexplored. Consequently, how fungi interact with the surrounding environment during these hidden life cycle stages is not fully understood. The development of metagenomic methods, however, have made the cryptic parts of the life cycle accessible for analysis. The study presented here is an effort to uncover the ecological implications of the vegetative stage of the fairy ring-forming basidiomycete *Marasmius oreades* by utilizing metagenomic tools. Specifically, we want to understand how the growth progression of the fairy ring front is associated with the microbial diversity. To do this, a total of 44 soil samples were collected along transects drawn across two individual *M. oreades* fairy rings and sequenced with metagenomic shotgun sequencing. The idea is to first assess where along the transect the ring-forming fungus is present by searching for *M. oreades* reads. Second, the aim is to describe and compare the microbial composition found outside, at the mycelial growth front and inside of the fairy ring. Ultimately, the goal is to use this comprehensive description of the fairy ring-associated microbial community to decipher the ecological context to which *M. oreades* has adapted.

P2.701 - Iterative CRISPR/Cas9 genome editing to reduce extracellular protease activity for heterologous protein production in *Aspergillus niger*

M. Arentshorst[1], *J. van Dam*[1], *M. Raats*[2], *T. Verschuren*[2], *M. Zhou*[2], *J. Visser*[1], *R. Lubbers*[1], *A. Ram*[1]
[1]Leiden University, Institute Biology Leiden, Fungal Genetics and Biotechnology, Leiden, Netherlands, [2]Avans University of Applied Sciences Breda, Center of Expertise Materials and Energy Transition, Breda, Netherlands

The high protein secretion capacity of *Aspergillus niger* has been well recognized and exploited for the production of homologous and heterologous enzymes and other proteins. Genome mining revealed many extracellular or secretion pathway related (Golgi/vacuolar localized) proteases that are potentially harmful for heterologous protein yields. Inferred from experimental data and computational predictions, 60 possible secretion related proteases were identified.
To build a protein expression platform in *A. niger* and to identify proteases harmful for the production of heterologous proteins, a strain lineage of *A. niger* was developed to effectively express the gene of interest (GOI) combined with reduced protease activity. We used iterative

CRISPR/Cas9-based genome editing to first delete genes encoding the most abundant secreted proteins (glucoamylase, acid amylase and alpha-glucosidase A), and genes involved in acidification (glucose oxidase and oxaloacetate hydrolase). In this non-acidifying background predicted secretion related proteases were deleted. Here we report a strain lineage consisting of 34 strains in which a total of 60 protease encoding genes were deleted. Gene deletions we verified by diagnostic PCR and representative strains from the lineage were genome sequenced to verify the deletions and to asses chromosome stability. Initial gene deletions were made by replacing the gene with a glucoamylase landing site, allowing targeted integration of the GOI. Using this integration system, up to 10 copies of the GOI can be integrated effectively. The strain lineage is a powerful tool to identify secretion related proteases that are harmful for the production of heterologous proteins prone to proteolytic degradation.

P2.702 - Whole genome sequencing of *Coccidioides posadasii* clinical isolates reveals associations with geography and clonal clusters from different patients

K. Jackson[1], *B. Barker*[1,1]
[1]Northern Arizona University, Flagstaff, United States

Coccidioides posadasii is a human fungal pathogen in the order Onygenales. *C. posadasii* is a thermally dimorphic fungi that grows as a mycelia in the environment and as a parasitic endospore filled spherule under infection conditions. *C. posadasii* is a primary pathogen that is geographically restricted to arid and semi-arid regions of the Americas, and is especially prevalent in the state of Arizona. Due to a lack of genetic tools and biosafety concerns, *Coccidioides* is not well understood and is understudied. We sequenced clinical isolates from 85 clinical isolates from North and South America. Strains generally grouped phylogenetically by geography. We next examined the distribution of the 1-1 and 1-2 mating types in each population. Some locations, such as Venezuela, had only one mating type and were clonal while others, including the population from Arizona, had an equal mix of mating types. To more closely examine the Arizona population, we sequenced 300 clinical isolates collected from patients in Maricopa and Pima counties. Isolates showed high admixture and continued to have an equal distribution of mating types. They generally clustered geographically by county, but, intriguingly, there were 10 clonal clusters that contained isolates that were nearly identical. In two clusters, the isolates were collected from patients who reside in different counties; in another cluster, the patients lived 20 miles apart and isolates were collected two months apart. Overall, these data suggest *Coccidioides* recombines in populations with both mating types but can form clonal clusters that may have clinical implications.

P3.101 - The sequestration and spatiotemporal distribution of the germline in a mushroom-forming fungus

P.J. Vonk[1], *M. Hiltunen Thorén*[1,2], *B. Olsson*[1], *J. Reimegård*[3], *M. Ryberg*[4], *H. Johannesson*[1,2]
[1]Stockholm University, Department of Ecology, Environment and Plant Sciences, Stockholm, Sweden, [2]The Royal Swedish Academy of Sciences, Stockholm, Sweden, [3]Uppsala University,

Science for Life Laboratory, Uppsala, Sweden, [4]Uppsala University, Evolutionary Biology Centre, Uppsala, Sweden

Filamentous fungi are modular organisms that can reproduce from different parts of their mycelium. Because this requires the presence of totipotent stem cells in many locations, it is generally assumed that the germline is established in the adult stage in fungi. Contrary to this, we find that in the fairy ring mushroom *Marasmius oreades*, somatic mutations are not transmitted to offspring. Instead, we find that sterile tissues (stipe, cap) from multiple mushrooms share an identical set of mutations, while fertile tissues (lamellae, spores) within the same mushrooms exhibit distinct, shared mutations, creating a clear genetic divide between sterile and fertile tissues. This indicates that in *Marasmius oreades* sequesters its germline prior to mushroom development. These findings challenge our understanding of reproduction and evolution in filamentous fungi and raises many questions on the mechanism and impact of early germline sequestration in a modular organism. We are using a combination of digital PCR and novel fluorescent *in situ* hybridization techniques with single nucleotide sensitivity to determine the spatiotemporal distribution of the germline in the soil and fruiting bodies. This work aims to uncover the mechanisms and evolutionary implications of early germline sequestration in a modular organism.

P3.102 - Identification of two new genes controlling dimorphism in Mucorales

G. Navarro Del Saz[1], *G. Tahiri*[1], *E. Navarro*[1], *V. Garre*[1], *F.E. Nicolas*[1]
[1]Universidad de Murcia, Facultad de Biología, Genética y Microbiología, Murcia, Spain

Mucormycosis is a fungal infection caused by species of the Mucorales order, primarily affecting immunocompromised individuals and showing high mortality rates. Some species, like *Mucor lusitanicus*, exhibit dimorphic development, alternating between yeast and mycelial forms, with the latter being virulent. Genomic studies have identified gene families that are differentially regulated in each form. Notably, two gene families involved in iron uptake were found: ferroxidases (*fet3a*, *fet3b*, *fet3c*) and permeases (*ftr1m* and *ftr1y*), with differential expression depending on the dimorphic form. It was observed that *fet3a* and *ftr1y*, expressed in yeast, share a bidirectional promoter, while *fet3b* and *ftr1m*, expressed in mycelium, are similarly clustered. To investigate gene regulation, a "DNA Pull-Down" assay was performed using protein extracts from both vegetative forms. Candidate proteins were identified, and mutants for two gene coding for an *F-box* and a *kinase protein* (*Mucci31471074* and *Mucci31468915*, respectively) were generated, showing altered yeast development. Phenotypic assays revealed no differences in media with varying iron availability. However, RT-qPCR gene expression analysis of *fet3a*, *fet3b*, *ftr1m*, and *ftr1y* was performed, alongside transcriptomic analyses of both mutants. To confirm the relationship between the *f-box* and *kinase* genes and the observed phenotypes, and to study the subcellular localization of the F-box and kinase proteins, complementation studies with recombinant wild-type genes fused to Cherry were conducted. These findings could be key to developing new treatments for mucormycosis.

P3.103 - Discovery and characterization of the first fungal granulin in *Aspergillus fumigatus*

U. Perez-Cuesta[1], X. Guruceaga[2], S. Cendon-Sanchez[3], E. Pelegri-Martinez[3], A. Martin-Vicente[1], H. Thorn[1,4], J. Xie[1,4], A. Nandi[1], D. Bale[1,4], A. Ramirez-Garcia[3], J. Fortwendel[1,5], A. Abad-Diaz-de-Cerio[3], A. Rementeria[3]
[1]University of Tennessee Health Science Center, Clinical Pharmacy and Translational Science, Memphis, United States, [2]Institute for Multidisciplinary Research in Applied Biology, Public University of Navarre, Pamplona, Spain, [3]University of the Basque Country, Department of Immunology, Microbiology and Parasitology, Leioa, Spain, [4]University of Tennessee Health Science Center, PhD Program in Pharmaceutical Sciences, Memphis, United States, [5]University of Tennessee Health Science Center, Deparment of Microbiology, Immunology and Biochemistry, Memphis, United States

Granulin is a secreted growth factor conserved among eukaryotic organisms. In humans, it is related to neuronal, autoimmune, and cancer diseases because it has a role in survival, growth modulation, migration, inflammation, and wound repair. It was thought that fungi had lost this type of domain since there is no sequence homolog, but in this work, we described a conserved protein in filamentous fungi with the same 3D structure. Different approaches using *Aspergillus fumigatus* mutant strains were employed to demonstrate that human and fungal proteins have similar functions and localization. Phenotypic characterization of the deletion strain revealed that the fungal protein is implicated in cell proliferation, polarization, conidiation, morphology, septation, stress resistance, and cell wall integrity. The absence of the gene produced a significantly lower expression of the cell wall integrity pathway and microtubule and cell end markers-related genes. The protein was found in the secretome being one of the first described extracellular polarization determinants of *A. fumigatus*. Therefore, the protein was localized in the cell membrane during germination and in the external hyphae of solid colonies. Finally, genetic replacement of the fungal protein with human Granulin A confirmed the homology between both proteins since this mutant strain almost phenocopied wild-type strain rescuing the defects observed in the deletion strain.

P3.104 - Early hyphal differentiation in the fruiting pathway of the model basidiomycete *Coprinopsis cinerea*

B. Haase[1], H. Kapli[1], B. Hegedüs[2], L. Nagy[2], U. Kües[1], S. Subba[1]
[1]Georg-August University of Göttingen, Molecular Wood Biotechnology and Technical Mycology, Göttingen, Germany, [2]Biological Research Center, Hungarian Acedamy of Science, Synthetic and Systems Biology Unit, Institute of Biochemistry, Szeged, Hungary

Fruiting body development (FBD) in *Coprinopsis cinerea* is a complex coordinated process regulated by environmental cues like temperature, illumination, darkness, and aeration. The *scl*-gene is required for both the formation of sclerotia from primary hyphal knots (Pk) in darkness and the *cfs*-gene-dependent emergence of light-induced secondary hyphal knots (Sk) committed to FBD. This suggests that the two pathways share Pks as a common origin. Nevertheless,

conclusive cytological evidence remains lacking. Timelapse under the microscope shows the development of Pks and from there to sclerotia or the development of an Sk from a more wide-spread hyphal aggregate that appears to differ from a simple Pk. About 50 mutants from different complementation groups were generated from the self-compatible homokaryon AmutBmut, either defective in the formation of Pk, sclerotia and/or Sk. The recessive mutant Proto159 e.g. is blocked in the formation of localized intense hyphal branching resulting in Pks, thereby supressing sclerotia as well as Sk formation and thus fruiting. Colony growth is somewhat reduced, while clamp cell production and light-induced asexual sporulation are still active as in AmutBmut. The vegetative mycelium has an unusually high laccase activity and forms dark-brown pigments staining hyphae and growth medium. The pleiotropic phenotypes suggest Proto159 to carry a defect in a regulatory gene of expression of morphological and enzymatic genes. A family of *NWD2* genes for NTPases with a NACHT-domain is shown to act as suppressors of the defects. The actual defect responsible for the pleiotropic mutant phenotype will be deduced from comparative genome analyses.

P3.105 - Developmental gene expression and genome evolution in the shift from asexual propagation to sexual resistance in *Neurospora crassa*

J.R. Wang[1], *Y.-W. Wang*[1], *V. Halder*[1], *Z. Wang*[1], *J.P. Townsend*[1]
[1]Yale School of Public Health, Biostatistics, New Haven, United States

The switch from asexual to sexual reproduction is critical for the success of filamentous fungi in responding to diverse environmental challenges. However, standard culturie protocols for the model filamentous fungus *Neurospora crassa* use synthetic agars promoting either the asexual or sexual cycle—leaving the natural switch understudied. To characterize this key developmental process and provide insights into how genomic evolution has shaped it, we sampled RNA from cultures of *N. crassa* (*mat-A*, FGSC2489) on carrot agar, which supports development from the appearance of aerial hyphae through growth of hyphal knots, asexual conidiophores, and formation to maturation of unfertilized sexual protoperithecia. Genes with known functions in asexual and sexual reproduction, such as *ccg-4* and *matA-2* were expressed as expected, upregulated during asexual conidiation and growth of young sexual protoperithecia. Genes involved in DNA methylation and chromatin remodeling showed minimal change across development. Astoundingly, of 312 previously identified transcription factors, expression increased in 92% of genes during asexual maturation and 84% of genes during sexual maturation. In contrast, expression decreased for 44% of genes during early sexual development. This intervening period of downregulation suggests cellular deprogramming during the organism's transition to sexual development. Additionally, compared to genes with phylogenetically older homologs, genes with phylogenetically younger homologs (present only in species diverging more recently from *N. crassa*) showed larger changes in expression across development, especially in late sexual maturation. Our findings provide both confirmatory results and novel explanations of developmental gene regulation and evolution during the asexual-sexual switch in *N. crassa*.

P3.106 - Control of conidiation in the genus *Aspergillus*: on the centrality and specificity of the master regulator BrlA

F. Wang[1], A. Bereziartu[2], P. Wu[1], R. Chen[1], K. Tan[1], E.A. Espeso[3], D. Cánovas[4], C.K.H. Wong[1], O. Etxebeste[2]
[1]Faculty of Health Sciences, University of Macau, Institute of Translational Medicine, Macao, Macao, SAR of China, [2]Laboratory of Microbiology, Faculty of Chemistry, University of the Basque Country, Applied Chemistry, San Sebastian, Spain, [3]Centro de Investigaciones Biológicas Margarita Salas (CSIC), Dept. of Cellular and Molecular Biology, Madrid, Spain, [4]Faculty of Biology, University of Seville, Department of Genetics, Seville, Spain

Asexual spores are the main vehicle for dissemination of fungi. In *aspergilli*, the transcription factor BrlA plays a central role in conidiophore development and conidia production. Several transcriptional activators bind target sequences in the promoter of *brlA* (*brlA^{p}*) and determine its expression patterns before/during development. The 5'-UTR region of *brlA* is also bound by repressors that inhibit its expression at late stages of conidiophore development and activate sexual development. Here, a mutagenesis procedure was followed to generate: 1) *brlA::HA$_{3x}$* strains that included serial deletions of *brlA^{p}* (spanning ~3 Kb upstream of the *brlAβ* start codon); 2) strains in which the hypothetical binding sites of transcriptional regulators binding *brlA^{p}* were deleted; and 3) a strain bearing a mutation corresponding to a Met1Ile substitution in BrlAβ (BrlAβ and BrlAα differ only in the first 23 amino acids). None of these strains showed the *fluffy* aconidial phenotype characteristic of the Δ*brlA* mutant. Only deletion of the *brlA^{p}* region that includes a uORF caused an inhibition of conidiation, although conidiophores with an aberrant morphology of vesicles and metulae were developed. Sexual development was induced prematurely in this strain, suggesting that BrlA activity goes beyond specifically controlling conidiation. ChIP-Seq results of *BrlA::HA$_{3x}$* and *AbaA::HA$_{3x}$* (after 24h of conidial development) support this hypothesis, showing that BrlA/AbaA bind to promoters of genes encoding activities required for polar-growth, development, cell-wall organization and signaling. We also analyzed what could be the cause of the phenotypic difference between Δ*brlA* and *brlA^{p}*-ΔuORF mutants, and identified additional genes necessary for conidiation.

P3.107 - Deciphering the biological role of oxidative chitin-active CAZymes in the life cycle of the plant pathogen *Ustilago maydis*

R.A. Yao[1], S. Vujakovic[2], D. Chaduli[1,3], D. Damoo[2], S. Grisel[1,4], M. Haon[1,4], J. Nilsson[1], D. Collinet[5], E. Danchin[5], M. Kretschmer[2], J.-G. Berrin[1], J. Kronstad[2], B. Bissaro[1]
[1]INRAE, Aix Marseille Univ, BBF, Biodiversité et Biotechnologie Fongiques, Marseille, France, [2]Michael Smith Laboratories and Department of Microbiology and Immunology, University of British Columbia, Vancouver, Canada, [3]INRAE, Aix Marseille Univ, CIRM-CF, Centre International de Ressources Microbiennes-Champignons Filamenteux, Marseille, France, [4]INRAE, Aix Marseille Univ, 3PE, Marseille, France, [5]Institut Sophia Agrobiotech, INRAE, Université Côte d'Azur, CNRS, Sophia Antipolis, France

The fungal cell wall (FCW) plays a crucial role in survival and adaptation of fungi to various environmental conditions (*1*). It is mainly composed of structural polymers such as glucans and chitin, which can be enzymatically modified by carbohydrate-active enzymes (CAZymes; (*2*)) during the fungal life cycle. While the functions of some CAZymes involved in the synthesis or hydrolysis of FCW polysaccharides have been investigated (*3*, *4*), the functions of oxidative CAZymes remains largely unknown. Here, using the plant pathogenic fungus *Ustilago maydis* as a model, we studied the biological role of some of its oxidative enzymes active on chitin: its unique lytic polysaccharide monooxygenase (LPMO; (*5*)) and its two chitooligosaccharide oxidases (*Um*AA7A and *Um*AA7B). To this end, we used (i) bioinformatics to detect horizontal gene transfer (HGT) events, (ii) wet enzymology to probe the enzymes substrate specificity (*6*) and (iii) reverse genetics (using CRISPR-Cas9) to investigate their involvement in fungal growth and plant infection.

References:

1. N. A. R. Gow, M. D. Lenardon, *Nat Rev Microbiol*. **21**, 248–259 (2023).
2. E. Drula *et al.*, *Nucleic Acids Research*. **50**, D571–D577 (2022).
3. T. Langner *et al.*, *Eukaryot Cell*. **14**, 846–857 (2015).
4. Y. S. Rizzi *et al.*, *mBio*. **12**, e03419-20 (2021).
5. A. Munzone, V. G. H. Eijsink, J.-G. Berrin, B. Bissaro, *Nat Rev Chem*. **8**, 106–119 (2024).
6. R. A. Yao *et al.*, *Applied and Environmental Microbiology*. **0**, e00573-23 (2023).

P3.108 - Substrate-driven plasticity in ectomycorrhizal fungi cell wall architecture: new insights into host-fungal interactions

T. Rush[1], *J. Tannous*[1], *I. Black*[2], *B. Davison*[1], *G. Tuskan*[1], *A. Biswal*[2]
[1]Oak Ridge National Laboratory, Bioscience Division, Oak Ridge, United States, [2]University of Georgia, Complex Carbohydrate Research Center, Athens, United States

The fungal cell wall is essential for morphology, rigidity, and adaptation to environmental and host interactions. While its structure is well-characterized in human and plant pathogens, its composition in mutualistic and saprotrophic fungi remains largely unexplored. A previous phylogenetic analysis of 491 fungal genomes revealed divergent enzymes involved in sugar metabolism, suggesting a potential impact on cell wall formation in these fungi. Agaricomycotina, which houses most ectomycorrhizal fungi, are predicted to have cell walls composed primarily of N-acetylglucosamine (GlcNAc), galactose, glucuronic acid, glucose, fucose, mannose, and xylose. To experimentally validate these predictions, we studied three ectomycorrhizal fungi that form nutrient-exchange structures with *Populus*, a bioenergy crop. Our research focused on assessing cell wall composition across different substrates, tracking temporal changes, and evaluating the potential to manipulate cell wall architecture. Using GC-MS and NMR analysis, we confirmed the presence of GlcNAc and previously predicted sugars, and uniquely identified arabinose and rhamnose, marking the first report of these sugars in Agaricomycotina fungi. Moreover, cell wall composition and GlcNAc linkage data varied between species, and with substrate availability, potentially influencing fungal morphology, growth rate, and hyphal development. These findings shed new light on the plasticity of ectomycorrhizal fungal cell walls and their role in host interactions. Future research will explore on how substrate availability affects fungal colonization of *Populus* through alterations in cell

wall architecture. This work could lead to new strategies for manipulating fungal cell walls to enhance biotechnological applications and strengthen plant-fungal symbioses.

P3.109 - The role of Knh1 in conidiogenesis in *Bipolaris maydis*

K. Tsuji[1], *Y. Yamada*[1], *D. Cecilia Ruiz-Nava*[2], *A. Yoshimi*[1,2], *M. Kawauchi*[1], *Y. Honda*[1], *C. Tanaka*[1,2]
[1]Kyoto University, Graduate School of Agricluture, Kyoto, Japan, [2]Kyoto University, Graduate School of Global Environmental Studies, Kyoto, Japan

The polysaccharide β-1,6-glucan is a component of the cell wall of fungi, and its deficiency in budding yeast is lethal. Knh1, a protein with a signal peptide, is known to be involved in its synthesis in budding yeast, but Knh1 homologs have not been studied extensively in filamentous ascomycete fungi. To investigate the functions of Knh1 in the phytopathogenic fungus *Bipolaris maydis*, the causal agent of southern corn leaf blight, we generated a *KNH1* gene-disrupted (Δ*knh1*) strain and performed functional analyses. The Δ*knh1* strain exhibited mycelial growth and pathogenicity similar to the wild-type strain. Interestingly, the conidia of the Δ*knh1* strain were smaller and the number of septa within the conidia was also reduced. Additionally, these smaller conidia showed delayed germination. After being crossed with the wild-type strain, normal pseudothecia and ascospores were produced. For further investigation, we performed a localization analysis of Knh1 using the NeonGreen fusion protein. Our results indicated that the Knh1-NeonGreen signal was localized and evenly distributed in the conidiophores and immature conidia, while concentrated at the conidial septa and their bases in the case of premature and mature conidia. These findings suggest that Knh1 in *B. maydis* is involved in conidiogenesis and septation. Moreover, Kre6, a glycoside hydrolase family 16 (GH16) protein, and its homologs have been shown to participate in β-1,6-glucan synthesis in budding yeast and the plant pathogen *Colletotrichum graminicola*. We are currently generating deletion mutants of Kre6 homolog genes in *B. maydis* and conducting morphological observations of conidia.

P3.110 - Unraveling the role of the transcription factor Con7 in the morphogenesis of Coprinopsis cinerea

H. Wu[1], *M. Virágh*[1], *X. Liu*[1], *Z. Merényi*[1], *B. Hegedüs*[1], *L. Nagy*[1]
[1]HUN-REN Biological Research Centre, Szeged, Hungary

Fungal morphogenesis is a complex process depending on both the genetic constitution and environmental conditions. Mushroom-forming fungi (Agaricomycetes) represents the most morphologically complex multicellular fungi, but knowledge on their morphogenesis remains limited. In this study, we identified a transcription factor Con7, which belongs to the C2H2 family and is a conserved subfamily in both Basidiomycota and Ascomycota. Con7 has only been shown to play a key role in fungal morphogenesis including conidiation and appressorium formation in plant pathogenic fungi in Ascomycota, however its role in Basidiomycota is unexplored. Knocking out con7 using CRISPR/Cas9 in the Basidiomycota model organism Coprinopsis cinerea resulted in a failure of fruiting body differentiation. Histological sections of

the primordium from both wild-type and Δcon7 mutant revealed a deficiency in tissues developing well-defined structural patterns, characterized by a lack of cell expansion in the mutant strain. To identify the genes affected by con7 deletion, we sampled the hyphal knot ring along with mycelium from wild-type and Δcon7 strains after 2 hours of light induction followed by 24 hours in darkness. Transcriptomic analysis of the above samples identified 585 downregulated genes and 380 upregulated genes (fold change > 1, BH adjusted $p < 0.01$), respectively. Downregulated genes were primarily associated with cell wall biosynthesis and modification, signal transduction, communication, and transcriptional regulation. Conversely, upregulated genes were mostly related to metabolism, cell division, proliferation, growth, and lipid metabolism. This study sheds light on the role of con7 in fungal morphogenesis, addressing the gap in our understanding of these processes.

P3.111 - Engineered lifestyle switch in *Botrytis cinerea*

D. Zheng[1], *H. Beenen*[1], *J. van Kan*[1]
[1]Wageningen University and Research, Experimental Plant Science- Phytopathology, Wageningen, Netherlands

Botrytis cinerea, a plant pathogenic fungus that causes severe loss in crop production, performs sexual reproduction. This fungus displays a heterothallic (self-incompatible) lifestyle, which means it requires a compatible partner with opposite mating type for sexual reproduction to occur. We transferred the *B. cinerea* MAT1-2 locus (carrying two genes) to the sequenced reference strain B05.10 (containing the MAT1-1 locus), aiming to generate a homothallic, self-compatible strain. Following self-fertilization of the transformants, abnormal fruiting body morphology and absence of ascospores in the ascus were observed. We examined the genome of the recipient B05.10 strain and noticed that it harbors a SNP in the BcGpr2 gene, resulting in a premature stop codon. BcGpr2 encodes a G-protein coupled receptor that is highly expressed during meiosis and resembles a cAMP receptor in the slime mold *Dictyostelium discoidium*. Its orthologs in *Neurospora crassa* and *Fusarium graminearum* display similar expression patterns as in *B. cinerea*, specifically around the onset of karyogamy and meiosis. Now I am proceeding to repair the SNP in BcGpr2 in the recipient, to examine whether this will restore the production of fruiting bodies and ascospores in the strain that carries all four MAT genes.

P3.112 - Engineering meiotic drive in *Botrytis cinerea*

D. Zheng[1], *J. Dijkstra*[1], *J. van Kan*[1]
[1]Wageningen University and Research, Experimental Plant Science- Phytopathology, Wageningen, Netherlands

Meiotic drive refers to the preferential transmission of a specific allele during sexual reproduction, often observed as spore killing in various fungi. Spore killer genes are widespread and conserved across the fungal kingdom, but it is not present in *Botrytis cinerea*. To date, meiotic drive has not been documented in the sexual reproduction of *B. cinerea*. In this study, we introduced the *Fusarium oxysporum* spore killer gene (FoSPOK4), known to function as a

meiotic drive element, into the *B. cinerea* strain B05.10 (MAT1-1). During sexual reproduction between the transformant and a wild-type MAT1-2 *B. cinerea* strain, we observed strongly reduced and aberrant fruiting body development. The few fruiting bodies that developed contained only low numbers of asci and ascospores with normal morphology. Single ascospores that germinated were propagated to obtain a collection of offspring individuals. About 50% of the offspring inherited the SPOK4 gene from the transformed parent. Also ~50% of the offspring exhibited a loss of sporulation ability, which was inheritable but not linked to the FoSPOK4 gene. We are currently sequencing the genomes of these single ascospore offspring and conducting vegetative compatibility tests to further investigate the genetic factors contributing to the loss of sporulation ability.

P3.113 - TU_MyCo-Vision: a deep-learning cell detection tool to navigate the landscape of morphological diversity in pleomorphic fungi

K. Deopujari[1], *M. Schmal*[1], *R. Mach*[1], *A. Mach-Aigner*[1], *C. Zimmermann*[1]
[1]TU Wien, Institute of Chemical, Environmental and Bioscience Engineering, Vienna, Austria

Phenotypic plasticity in pleomorphic fungi has always intrigued mycologists, it's not only important in understanding basic biology but also has significant implications for clinical mycology and industrial biotechnology. While advances in microscopic imaging aid these studies, operator bias and handling large quantities of data is a challenge.
To address this, we developed a deep-learning object detector: TU_MyCo-Vision, based on Ultralytics YOLOv11 deep-learning object detection model. TU_MyCo-Vision detects 13 distinct cell morphologies in light microscopic images ranging from single-celled to filamentous morphologies. TU_MyCo-Vision is trained using transfer learning, predominantly on *A. pullulans*, as this fungus exhibits a very broad range of cell types and morphologies. Additional data from *A. melanogenum* and *Trichoderma ressei* was included to improve generalisation.
As TU_MyCo-Vision is trained to detect the presence of various cell morphologies, it offers the capability of identifying the physical state of the culture (more filamentous or single-celled), monitoring events in cultures like clumping or budding, monitoring the phenotype switching, etc. It has displayed promising performance when tested for detecting cell-type switching in *A.pullulans* and proved its generalising capabilities when tested on *Komagataella pastoris* and *Candida albicans* images. Importantly, the open cell-class definitions enable TU_MyCo-Vision to be used for various research objectives.
TU_MyCo-Vision, performed well with 72.3% precision, 67.2% recall, mAP50 of 0.72, and mAP50-95 of 0.511. Ultralytics framework along with our data-analysis pipeline makes TU_MyCo-Vision user-friendly. TU_MyCo-Vision only requires a microscope, an imaging device, and a computer, which makes it a versatile tool for studying the morphological changes in pleomorphic fungi.

P3.114 - Disruption of protein kinase A catalytic subunit gene *pkac2* enhances mycelial dispersion and wood degrading enzyme production in *Pleurotus ostreatus*

Y. Otsuka[1], *M. Kawauchi*[1], *S. Endo*[1], *K. Schiphof*[1], *K. Tsuji*[1], *A. Yoshimi*[2,1], *C. Tanaka*[2,1], *S. Yano*[3], *V. Elisashvili*[4], *T. Nakazawa*[1], *T. Irie*[5], *Y. Honda*[1]
[1]Kyoto University, Graduate School of Agriculture, Kyoto, Japan, [2]Kyoto University, Graduate School of Global Environmental Studies, Kyoto, Japan, [3]Yamagata University, Graduate School of Sciences and Engineering, Yonezawa, Japan, [4]Agricultural University of Georgia, Institute of Microbial Biotechnology, Tbilisi, Georgia, [5]School of Environmental Science, University of Shiga Prefecture, Hikone, Japan

Wood degrading enzymes secreted from white-rot fungi have significant potential for enhancing the efficient utilization of lignocellulosic resources. Despite efforts to achieve heterologous expression of these enzymes in various host systems, production of enzymes with high level activity remains unattained. Therefore, there is a need to develop technologies on an industrial scale to produce useful enzymes using white-rot fungi. Recently, mycelial dispersion for high-density cultures has been achieved in ascomycetes by through cell wall modification. In this study, we aim to develop a mycelial-dispersing strain of the white-rot fungus *Pleurotus ostreatus* by disrupting the *pkac2* gene, which encodes the catalytic subunit of protein kinase A, a cell wall synthesis regulator. Liquid cultures of the Δ*pkac2* strains showed very high mycelial dispersibility and were visibly different from the wild-type (WT). Δ*pkac2* strains grew faster in liquid culture and at 5 d mycelial dry weight was approximately twice that of the WT. Microscopic observations highlighted that Δ*pkac2* cell walls were thinner compared to the WT. Furthermore relative amounts of β-glucan, cell surface hydrophobicity, and cell wall stress tolerance were also decreased in Δ*pkac2* strains. Enzyme activity analysis of Δ*pkac2* strains in liquid culture revealed increased activities of cellulase and xylanase, by 10- and 50-fold, respectively when compared to the WT. Transcriptome analysis by RNA-Seq subsequently confirmed elevated expression of the encoding genes. These results suggest that disruption of *pkac2* alters cellulase and xylanase expression and cell wall surface layer structures, resulting in mycelial dispersion.

P3.115 - Duplication of a septin might have contributed to early expansion of fruiting body hyphae in Agaricales

M. Virágh[1], *J.Z. Kristóffy*[1], *D.A. Szafián*[1], *L.G. Nagy*[1]
[1]HUN-REN Biological Research Centre, Szeged, Institute of Biochemistry, Szeged, Hungary

Fungal fruiting bodies (FBs) can expand dramatically via cell expansion. The timing of cell expansion relative to cell proliferation and early tissue differentiation during FB formation varies widely between and within groups. The FBs of the order Agaricales are characterized by an early, moderate and a late, rapid and more extensive cell expansion during development.
Septins were reported to play a role in FB morphogenesis and cell expansion in the model agaric *Coprinopsis cinerea* where Cdc11, the terminal component of the septin heteropolymer has two

copies and only Cdc11b was reported to be differentially regulated during FB formation along with the other core septins.
We showed that *cdc11* duplication occured early during the evolution of Agaricales after the split of Pleurotaceae. Cdc11a resembles the ancestral Cdc11, while Cdc11b accumulated conserved substitutions.
To better understand the function of septins during FB formation we deleted the *cdc11* paralogs and another core septin *cdc10* using CRISPR/Cas9 in *C. cinerea*. We found that the deletion of *cdc11b*, both *cdc11* paralogs and *cdc10* resulted in an aborted FB development. Images of longitudinal median sections of P1 primordia of the deletion strains suggest that septin heteropolymers are needed for the initiation of cap, gill and stipe tissue differentiation and Cdc11a and Cdc11b can complement each other during these processes. On the other hand, Cdc11b-capped heteropolymers are essential for the proper, early anisotropic expansion of FB hyphae.
Our data suggests that *cdc11* duplication in Agaricales might have contributed to an early expansion of FB hyphae during development.

P3.116 - Genetic differentiation in the MAT-proximal region is not sufficient for suppressing recombination in *Podospora anserina*

P. Grognet[1], *R. Debuchy*[2], *T. Giraud*[3]
[1]Université Paris-Saclay, I2BC-CNRS, Gif-sur-Yvette, France, [2]I2BC-CNRS, Gif-sur-Yvette, France, [3]CNRS - Université Paris Saclay, IDEEV - ESE, Gif-sur-Yvette, France

Recombination is advantageous over the long-term, as it allows more efficient selection and purging deleterious mutations. Nevertheless, recombination suppression has repeatedly evolved in sex chromosomes and mating-type chromosomes. Both the evolutionary causes for recombination suppression and the proximal mechanisms preventing crossing overs are, however, still poorly understood. The ascomycete fungus *Podospora anserina* is an excellent model for investigating these questions. A 0.8 Mb region around the mating-type locus is non-recombining, despite being collinear between the two mating types. This fungus is mostly selfing, so that strains are typically highly homozygous, except in the non-recombining region around the mating-type locus, which displays differentiation between mating types. Here, we generated a mutant to test the hypothesis that sequence divergence alone is responsible for recombination cessation. We replaced the MAT-proximal region in the “minus” mating type by the sequence present in the MAT-proximal region in the “plus” mating type in the reference strain, to obtain a strain homozygous in the MAT-proximal region. Crosses showed that recombination was still suppressed in the MAT-proximal region in the mutant strains, indicating that other proximal mechanisms than inversions or mere sequence divergence are responsible for recombination suppression in this fungus. This finding suggests that selective mechanisms likely acted for suppressing recombination, as the neutral model does not seem to hold, at least in this fungus.

P3.117 - Asexual development of *A. nidulans* required the shuttle of the VeA velvet domain regulator from cytoplasm to nucleus and back

A. Strohdiek[1], *A.M. Köhler*[1], *R. Harting*[1], *H. Stupperich*[1], *J. Gerke*[1], *P. Neumann*[1], *Y.L. Ahmed*[1], *R. Ficner*[1], *G.H. Braus*[1]
[1]Georg-August-University Göttingen, Göttingen, Germany

Survival of multicellular organisms requires the functional interplay between complex regulatory networks including gene expression, the ubiquitin proteasome protein degradation system, and intracellular transport control. Velvet domain proteins as VeA, VelB or VosA play key roles in fungal differentiation during early development. Heteromeric VelB-VeA is shuttled in darkness to the nucleus by the importin KapA to induce gene expression for sexual development. Nuclear localizations of VeA or VelB are reduced by light when asexual spore formation is promoted. The trimeric velvet complex VelB-VeA-LaeA further links transcriptional to epigenetic control for the coordination of fungal developmental programs to specific secondary metabolite production. VeA carries three nuclear localization signals NLS1, NLS2 and NLS3, which were analyzed, and all contribute to nuclear import. VeA is nuclear during vegetative growth and has to be exported from the nucleus to promote asexual development. This allows VeA movement between periphery and matrix of nuclei as prerequisite for coordinated development and secondary metabolism. The obtained results illustrate various mutual dependencies of Velvet proteins, which ensure accurate nuclear import, export, and specific protein stability control mechanisms as prerequisites for fungal development and secondary metabolism.

P3.118 - Kinesin-3 motor KIN3 plays a crucial role in sexual development of *Podospora anserina*

F. Hernández-Sánchez[1], *S.K. Schroder*[1], *L. Peraza-Reyes*[1]
[1]Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Dept. Bioquímica y Biología Estructural, CDMX, Mexico

In mycelial Ascomycetes there are two class 3 kinesins, the highly processive motor containing PH and FHA domains, and the non-processive short motor that lack cargo binding domains. The role of these proteins in organelle motility during somatic growth has been extensively studied, but their role during sexual development remains unexplored. *Podospora anserina* has been an excellent model for organelle dynamics during meiotic development, revealing a dynamic distribution of nuclei, peroxisomes and the endoplasmic reticulum (ER), which implies the activity of motor proteins. Here we analyzed the role of *P. anserina* long and short class-3 kinesins KIN2 and KIN3. We found that, while *KIN2* deletion did not significantly alter sexual development, the lack of KIN3 causes sterility. This phenotype was associated with altered meiotic nuclei positioning, misplacement of meiotic spindles, alteration of chromosome sorting and loss of spore individualization. Analysis of KIN3 cell localization showed that this protein localizes to septa in dikaryotic cells prior karyogamy, and to ring-like structures nearby chromosomes at early meiotic development and during ascospore individualization, suggesting a

role in nuclear dynamics. Our results disclose that KIN3 plays a crucial role in sexual development.

P3.119 - Functional characterization of the Target of Rapamycin signalling pathway in the rice blast fungus *Magnaporthe oryzae*

M. Wengler[1], *I. Eisermann*[1], *N. Cruz-Mireles*[1], *F. Menke*[1], *N.J. Talbot*[1]
[1]The Sainsbury Laboratory, Norwich, United Kingdom

The Target of Rapamycin (TOR) signalling pathway plays a critical role in regulating growth and development across eukaryotes, operating through two functionally distinct multiprotein complexes: TORC1 and TORC2. These complexes integrate nutrient signals with fundamental anabolic and catabolic processes. While TOR signalling has been extensively studied in yeast and mammals, its role in filamentous fungal pathogens, such as the rice blast fungus *Magnaporthe oryzae*, remains less well understood. Recent findings show that treatment of *M. oryzae* spores with TOR activators impairs appressorium initiation, while TOR inhibitors induce constitutive autophagy and produce aberrant infection structures. To further explore this pathway, we immunoprecipitated Kog1 and Avo3 orthologs, identifying components of TORC1 and TORC2, providing biochemical evidence for the presence of two TOR complexes in *M. oryzae*. We then performed a comprehensive study of the TOR-dependent phosphoproteome in mycelium under conditions of TOR inhibition, including exposure to rapamycin and Torin1 as well as carbon and nitrogen starvation. The assay's validity was evaluated by monitoring the phosphorylation of ribosomal protein Rps6, a marker of TORC1 activity. Phosphopeptides were enriched on TiO_2 and Fe-NTA columns and analysed through discovery proteomics based on data-dependent acquisition, revealing 9,192 phosphosites across 1,446 proteins. Gene ontology term enrichment analysis highlighted biological processes related to regulation of catalytic activity, regulation of DNA-templated transcription, positive regulation of developmental processes, and protein phosphorylation. This study provides a foundational dataset on TOR-dependent phosphorylation in *M. oryzae*, which we are using to characterize the role of TOR signalling during appressorium development.

P3.120 - Homeodomain transcription factor is essential for toxocyst formation in the oyster mushroom *Pleurotus ostreatus*

S.-C. Juan[1], *Y.-Y. Lee*[2], *Y.-P. Hsueh*[1,2]
[1]Max Planck Institute for Biology, Department of Complex Biological Interactions, Tübingen, Germany, [2]Academia Sinica, Institute of Molecular Biology, Taipei, Taiwan, China

Predation is a common ecological phenomenon across various life forms, with fungi exhibiting diverse strategies to prey on nematodes. The basidiomycete *Pleurotus ostreatus* (oyster mushroom) has evolved toxin-containing structures called toxocysts to immobilize and consume nematodes as a nutrient source, particularly under nutrient-limited conditions. However, the molecular mechanisms governing toxocyst formation remain largely unclear. In this study, we conducted a forward genetic screen to identify mutants deficient in nematode paralysis, referred

to as *lot* mutants (Lost Of Toxicity), and identified a homeodomain transcription factor *HTD1* (Homeodomain transcription factor for Toxocyst Development) required for toxocyst formation. Deletion of the *htd1* gene mirrored the *lot* mutant phenotype, with normal growth but a defect in toxocyst development. Comparative RNA-seq analysis between the *htd1* mutant and the wild-type strain under nutrient-limited conditions revealed significant differential expression, with approximately 700 down-regulated and 280 up-regulated genes. Gene ontology enrichment analysis indicated that most of the down-regulated genes are associated with heme binding, ion binding, and oxidoreductase activity. Notably, three small secreted peptides (SSPs) with intrinsic disordered regions were significantly down-regulated in the *htd1* mutant. GFP-labeled SSPs showed movement toward and accumulation in toxocysts. However, deletion of these three SSPs only partially impeded toxocyst formation, suggesting the possibility of functional redundancy of the SSPs.

P3.121 - Innovative tools for recombinant protein production by Myceliophthora thermophila

L. Wasserer[1], *S. Scheipel*[1], *K. Ebner*[1], *A. Glieder*[1]
[1]Bisy GmbH, Hofstaetten an der Raab, Austria

The filamentous fungus *Myceliophthora thermophila* ATCC42464 (DSM 1799), also known as *Thermothelomyces thermophilus,* is a promising fungal production host for recombinant proteins. The special properties of this fungus, like protein secretion to very high titres, as well as, its capability to produce biologically active complex proteins of eukaryotic origin, makes it an interesting microbial host for biotechnological applications.
For the development and assessment of new expression tools and strains, it is essential to have reliable and easily detectable reporter proteins. In this context, we demonstrated the application of an unspecific peroxygenase (UPO, EC1.11.2.1) for evaluating various constitutive promoters derived from the *M. thermophila* genome. The fungal origin makes UPOs a promising reporter for this host. In addition, this is the first report about producing an enzyme of this class recombinantly by *M. thermophila* and, employing this reporter protein, the toolbox of regulatory elements for this fungal host was expanded by a new promoter engineering approach. After transformation of *M. thermophila* with expression cassettes with the *Hsp*UPO gene of *Hypoxylon* sp. EC38, linked to engineered promoter variants, expression analyses were performed by small scale cultivations and determination of the activity of secreted enzyme using a colorimetric high-throughput assay with ABTS (2,2-azina-bis-(3-ethylbenzothiozoline-6-sulfonic acid)) as a substrate. The screening identified inventive promoter variants that resulted in increased peroxidase activity.

P3.122 - Identification of the causative gene for abnormal appressorium formation accompanied by hyphal elongation in *Bipolaris maydis*

S. Yasumoto[1], *K. Tsuji*[1], *M. Saba*[1], *A. Yoshimi*[1,2], *D. Ruiz-Nava*[2], *M. Kawauchi*[1], *Y. Honda*[1], *C. Tanaka*[1,2]

[1]Kyoto University, Graduate School of Agriculture, Kyoto, Japan, [2]Kyoto University, Graduate School of Global Environmental Studies, Kyoto, Japan

Many plant pathogens, including *Bipolaris maydis*, form infection structures called appressoria during their infection stage to penetrate the host. Appressorium formation is believed to be induced by the recognition of surface hydrophobicity and host-derived substances. The Δ*opy2* strain of *B. maydis* lacks the ability to recognize hydrophobic surfaces and does not form appressoria efficiently on such surfaces; however, appressorium formation is induced when host-derived substances, such as pectin, are added. The mechanism by which pectin recognition induces appressorium formation remains unknown. To help elucidate this mechanism, we obtained a mutant strain, DO2N20-296 (D296), in which appressorium formation is not induced by pectin. Besides this, the D296 strain exhibited straight hyphae with few branches immediately after spore germination when compared to the parent Δ*opy2* strain. Crosses between the D296 strain and the wild-type strain suggested that these traits are controlled by a single gene. Whole-genome comparison between wild-type progeny and mutant progeny revealed that an SNP in a gene encoding an αβ-hydrolase was linked to the observed traits. We named this gene *LAG1*. Introduction of wild-type *LAG1* into the D296 strain confirmed *LAG1* to be linked to the two aforementioned traits. Furthermore, *lag1*-disrupted strains were obtained, in which elongated hyphae similar to those in the D296 strain were observed, appressorium formation on hydrophobic surfaces was significantly reduced, and appressorium formation was not induced by pectin. These results show that *LAG1* is involved in appressorium formation in this fungus.

P3.123 - A HOG/CWIS pathways transcription factor Skn7 regulates cell wall formation through hydrophobin Hydph16 in *Pleurotus ostreatus*

T. Izumi[1], *M. kawauchi*[1], *H. Kojima*[1], *K. Tsuji*[1], *A. Yoshimi*[2,1], *C. Tanaka*[2,1], *T. Nakazawa*[1], *Y. Honda*[1]
[1]Kyoto University, Graduate School of Agriculture, Kyoto, Japan, [2]Kyoto University, Graduate School of Global Environmental Studies, Kyoto, Japan

Recently, we have suggested that the basidiomycete *P. ostreatus* has distinct cell wall structures and regulatory pathways especially for downstream transcription factors when compared with those of ascomycetous model fungi. In this study, we investigated the function of an ortholog gene for Skn7, a transcription factor known to regulate cell wall synthesis under the high-osmolarity glycerol (HOG) and cell wall integrity signaling (CWIS) pathways, in *P. ostreatus*. In liquid medium, isolated Δ*skn7* strains had reduced biomass and shorter aerial hyphae compared to the wild type (WT), whereas almost no growth defects were observed on agar medium. Similar to *skn7* homologues in ascomycetes, Δ*skn7* strains showed increased sensitivity to KCl or sucrose, indicating importance in adaptation to the osmotic stress environment. Transmission electron microscopy observations further revealed that Δ*skn7* cell walls were 37% thinner than those of the WT. Notably, the relative percentages of cell wall polysaccharides showed slight increases in chitin and no changes in glucans, despite qRT-PCR indicating that several glucan and chitin synthesis genes were downregulated. Additionally, expression levels of

the hydrophobin-encoding gene *hydph16* were 66% lower than in the WT. In previous studies, disruption of *hydph16* causes thinner cell walls, suggesting that reduced expression of *hydph16* is at least partially responsible for the thinner cell walls in Δ*skn7*. To our knowledge, this study is the first to suggest that Skn7 is involved in proper cell wall formation through hydrophobin regulation.

P3.124 - Dark stipe mutants in fruiting body development of *Coprinopsis cinerea*

S. Subba[1], *V.S.V. Pulusu*[1], *B. Hegedüs*[2], *L. Chen*[1], *S. Smith*[1], *L. Nagy*[2], *U. Kües*[1]
[1]Georg-August University of Göttingen, Molecular Wood Biotechnology and Technical Mycology, Göttingen, Germany, [2]Biological Research Center, Hungarian Acedamy of Science, Synthetic and Systems Biology Unit, Institute of Biochemistry, Szeged, Hungary

Fruiting body development in the basidiomycete *Coprinopsis cinerea* is strictly regulated by light, temperature, and aeration. The differentiation process begins with the formation of primary hyphal knots (Pks) in the dark, which, when exposed to light, transform into compact secondary hyphal knots (Sks). Further light and dark signals control primordium development (P1 to P5) which takes five days to culminate on day 6 of the process in light-induced karyogamy within the basidia followed by meiosis and basidiospore production. Failure in light signaling or in aeration leads to formation of so-called ‘dark stipes’, under unusual proliferation of stipe tissues and a block in further cap development. Defects in formerly described genes *dst1* (*wc1*) and *dst2* caused P1-induced ´dark-stipe´ phenotypes by defects in blue-light receptors and a FAD/FMN-binding GlcD-type dehydrogenase. A dark-stipe phenotype is also observed in the wildtype under block of aeration. Scavenger experiments of CO_2 with KOH recovered the normal phenotypes in fruiting body development. Two mutants (*dst3*, *dst4*) form P3- and P4-induced ‘dark stipe’ phenotypes under standard fruiting conditions with light and aeration. Genome sequencing revealed missense and early stop codon mutations in enzymes associated with the citrate cycle and of branched amino acid production, as well as in the Zn-binding site of the Csn5 subunit of the Cop9 signalosome, and loss of the start codon in an Arf1-like GTPase, suggesting links to the CO_2 metabolism and also light signaling and regulation. Transformation of the mutants with different wildtype genes (*cs, csn5, pda1, ilv2 and arf1*) are ongoing.

P3.125 - Generation of filamentous *Candida albicans* and *Candida auris* cells in liquid, shaken culture to study the mechanism of action of antifungal proteins

R. Merber[1,2], *E. Kazinczi*[1], *G. Bende*[1], *L. Galgóczy*[1]
[1]University of Szeged, Department of Biotechnology and Microbiology, Szeged, Hungary, [2]University of Szeged, Doctoral School of Biology, Szeged, Hungary

Candida species are the most common human pathogenic fungi causing wide range of infections, from cutaneous to invasive candidiasis. Most of the clinically relevant *Candida* species are able to switch morphology between unicellular yeast and multicellular filamentous types, which

allows forming biofilm in the host and the surface of medical instruments. Biofilm forming filamentous *Candida* cells exhibit reduced susceptibility against antifungal drugs compared to planktonic cells. This observation and the emerging number of (multi)drug-resistant *Candida* isolates generate an urgent need to develop new antifungal compounds with high biofilm inhibition/eradication activity, and different mode of action than that of the conventional ones. Antifungal proteins (AFPs) of filamentous Ascomycetes are promising candidates as they show high anti-*Candida* activity *in vitro* and *in vivo*, and they can inhibit biofilm formation of *Candida* isolates and eradicate developed biofilms. For the therapeutic application of AFPs as anti-*Candida*-biofilm compounds, it is essential to understand their growth inhibitory mode of action on filamentous cells. This goal requires isolation of individual early-stage, easy-to-handle filamentous *Candida* cells, what are not provided by traditional *Candida* biofilm generation techniques. Here, we present protocols for generating individual, early-stage filamentous cells of *Candida albicans* CBS 5982 and *Candida auris* NCPF 8971 isolates in liquid, shaken culture; and for determining the minimum inhibitory concentrations of an anti-biofilm AFP (*Neosartorya fischeri* antifungal protein 2) against these cell types of *Candida*.
Present work of L.G. was financed by the Hungarian National Research, Development and Innovation Office - NKFIH, K 146131 project.

P3.126 - Methods for mating and fruiting body formation of the basidiomycete *Fomes fomentarius* under laboratory conditions

C. Freidank-Pohl[1], *A.J. Gennet*[1], *A. Parakenings*[2], *S. Jung*[1], *V. Meyer*[1]
[1]Technische Universität Berlin, Applied and Molecular Microbiology, Berlin, Germany,
[2]Brandenburgische Technische Universität Cottbus-Senftenberg, Cottbus, Germany

The basidiomycete *Fomes fomentarius* is a promising candidate organism to produce materials from fungal biomass and agricultural side streams. Since this species originates from the subkingdom Dikarya, the natural life cycle is dominated by the dikaryotic state, indicated by the presence of clamp connections between hyphae to maintain the two nuclei in each cell. Although the dikaryotic mycelium displays more rapid growth, the presence of two different versions of the genome makes it more challenging to manipulate the genome of *Fomes fomentarius*. An alternative to dikaryotic state is the isolation of spores from fruiting bodies and the cultivation of the monokaryotic state. However, methods for mating monokaryotic strains of *Fomes fomentarius* have not been reported yet.
We developed a simple assay to perform mating of *Fomes fomentarius* on solid media and compared the growth speed of mono- and dikaryotic strains obtained from these experiments. We can discern the presence of both genome copies in the mated strains by a set of SNPs present in both parental strains. We further investigated the formation of fruiting bodies of *Fomes fomentarius* under laboratory conditions and observed fruiting body formation under cold stress for both birch logs inoculated with *Fomes fomentarius*, as well as bag cultures for biomaterial production established in our lab.
These methods are a prerequisite for the establishment of *Fomes fomentarius* as a polyporales model species and a valuable milestone that enable studies on plant-fungal interactions of genetically altered basidiomycete strains in a laboratory in the future.

P3.127 - Efficient protoplast preparation method of *Aspergillus* section *Nigri* using α-1,3-glucanase and α-1,3-glucan synthesis gene disruption strain

J. Tokashiki[1], *O. Mizutani*[2], *K. Gomi*[1]
[1]Tohoku, Agricultural, Sendai, Japan, [2]University of the Ryukyus, Okinawa, Japan

Transformation of Aspergillus filamentous fungi generally require the preparation of protoplasts. For most filamentous fungal strains including Aspergillus oryzae, Aspergillus nidulans and so on, protoplasts can be easily prepared using a mixture of enzymes such as cellulase, β-glucanase, and chitinase, as expected from the polysaccharides that constitute of the cell walls. However, Aspergillus section Nigri is considered to be difficult to readily form protoplasts compared to A. oryzae and A. nidulans. Here, we compare the protoplast generation methods of several strains from section Nigri between a conventional method and one with α-1,3-glucanase preparation. Consequently, the method using α-1,3-glucanase was applied to prepare protoplasts from the Aspergillus luchuensis RIB2604 strain, which is particularly difficult to form protoplasts. Based on the result, the α-1,3-glucan synthesis gene disruption was suggested to be effective in efficient protoplast preparation. Therefore, α-1,3-glucan synthesis gene was disrupted in A. luchuensis, resulting in easier and efficient protoplast preparation even in the absence of α-1,3-glucanase.

P3.128 - Proteomic analyses of liquid droplets excreted by mushroom forming fungi

S. Subba[1], *M. Unger*[1], *U. Kües*[1]
[1]Georg-August University of Göttingen, Molecular Wood Biotechnology and Technical Mycology, Göttingen, Germany

Exudation in fungi is a common phenomenon. Fungal exudates can contain a variety of compounds, including proteins. We observed in the dung fungus *Coprinopsis cinerea* that growing structures during the complete developmental fruiting pathway will secrete from the pileipellis at the apex of the caps translucent liquid droplets which slowly rolls off the veil cells and collect at stem bases that finally during further development colorize yellowish with fruiting body maturation and eventually roll onto the vegetative mycelium. We believe that these droplets have a kind of lotus effect, cleansing the surface of the structures. Proteomic analysis of these liquid droplets revealed them to contain a water-surface-active protein to positively enhance the Lotus effect. Many more characteristic proteins are specifically secreted into the liquid droplets, many of which have defense functions against various biotic threats, in particular for attack of bacteria, other fungi, viruses, and small animals such as nematodes, mites and insects. Known defensive proteins are for example galectins, members of the ricin family, and the serine protease inhibitor copsin. Other proteins in the droplets also present members of larger protein families and are also candidates for defense with potential bacterial, fungicidal, insecticidal or nematicidal functions. In total, up to 318 proteins in total and 142 different proteins with N-terminal secretion signals were detected in droplets from different primordial stages (P1 to P4). 144 proteins were shared between droplets from all developmental stages.

P3.129 - Phenotypic and genetic variation of *Coprinellus disseminatus*

K. Lakkireddy[1], *W. Khonsuntia*[1], *S. Subba*[1], *U. Kües*[1]
[1]Georg-August University of Göttingen, Molecular Wood Biotechnology and Technical Mycology, Göttingen, Germany

Two *Coprinellus disseminatus* colonies were observed underneath two neighboring trees being root-stressed by continuous building works. A larger colony produced mushrooms in soil, on roots and at the stem base of a *Prunus sargentii* tree. The smaller colony grow in the grass underneath and at the stem base of a *Prunus incisa* tree infected also by other white rots. Fruiting occurred over most months of the year, as long as there was sufficient rainfall to moisture soil and increase air humidity. Precipitation caused drop in temperature by several degrees. It resulted in fruiting taking place at temperatures of 4-5°C and up to >20°C. The length of fruiting body development was influenced by the outer temperature. It took 7-12 days at lower temperature, while the process speeded up to 4-5 days at higher degrees. Depending on outer temperatures, the cap of the larger colony was grey to fully black by producing masses of black basidiospores. In contrast, the caps of the smaller colony were white to slightly grey by lack of spores. The white colony appeared to have a defect during the karyogamy stage, as suggested by cytological analysis of basidia. ITS sequencing revealed an origin of the white-colony from East-Asia and an European origin of the black-caped colony with. The mycelia of the two genetic variants grew best at 25°C and could not tolerate longer incubation at 37°C. The dikaryotic mycelia did not produce any asexual spores on the vegetative mycelia, but dry oidia appeared on mycelia of monokaryons.

P3.130 - The role of RNA editing in ascospore formation and physiology in *Sordaria macrospora*

S. Herzog[1], *M. Kutter*[1], *A. Somarajan*[1], *U. Lipka*[1], *M. Skendrou*[2], *J. Grygosch*[2], *L. Bleeken*[1], *F.K. Krekel*[1], *I. Teichert*[1]
[1]University of Goettingen, Forest Botany and Tree Physiology, Goettingen, Germany, [2]Ruhr-University Bochum, Bochum, Germany

RNA editing, the post-transcriptional selective insertion, substitution or deletion of nucleotides plays a crucial role in functional diversity of gene expression within all domains of life. In filamentous ascomycetes, RNA editing primarily involving adenosine-to-inosine (A-to-I) conversions occur within coding regions of transcripts involved in sexual reproduction. Interestingly, not only amino acid codons but also stop codons tend to be affected by editing leading to a change of TAG or TGA codons to TGG tryptophan codons. The precise mechanisms regulating the process of this form of RNA editing still need further investigation. However, it is suggested to play a crucial role in the formation of ascospores and sexual structures, and providing proteome diversity that benefits the progeny.
To unravel the biological role of A-to-I editing during sexual development in filamentous fungi we analyzed so called *efd* genes with edited transcripts in the ascomycete *Sordaria macrospora*. Indeed, several deletion mutants of *efd* genes like *efd4* and *efd7* showed severe defects in their

ascospore formation and/or discharge. Interestingly, complementation studies with constructs expressing either the edited version (TGG) or non-edited version (TAA) revealed putative functions for the editing sites during reproduction. Very recent results suggest a role for *efd16* in cellular structure and physical properties of sexual reproduction structures. To investigate this further we will apply a novel method measuring structural integrity of ascospores. These data will provide better insight of the impact of A-to-I editing during developmental processes and its role for the adaptive capabilities of fungi.

P3.131 - Evolutionary transcriptomics to understand conidial development and germination of pathogenic and non-pathogenic Aspergillus species

P. Wu[1], *K.H. Wong*[1]
[1]University of Macau, Macao, China

Fungal conidia are the main infectious propagules of many human fungal pathogens. Inhalation of conidia by the host is a major entry route for pathogenic fungi, such as *Aspergillus fumigatus*, that cause lung and systemic infections. In order to cause lung infection, the inhaled conidia have to germinate and grow within the lung of a host. Conidial size and germination are believed to be important attributes of pathogenic species. Therefore, understanding conidia development and germination has important medical implications. In this study, we performed transcription profiling to study conidial development and germination in the human pathogen *Aspergillus fumigatus* and the non-pathogenic research model *Aspergillus nidulans*. Comparative and evolutionary transcriptomic analysis revealed that the transcriptomes of the two species during conidial germination are highly conserved, including numerous ancient genes with a pattern reminiscent of the “Hourglass” evolution model. In contrast, the conidiation process involved many modern genes, conforming to a “Reverse Funnel-like Model” of evolution. These findings suggest that the pathogenic features of *A. fumigatus* may have evolved, at least in part, from conidiation rather than germination. Evolutionary transcriptomic analysis of additional non-pathogenic and pathogenic species will provide further insights into the evolution of fungal pathogenicity. These findings have important implications for combating fungal infections and developing novel antifungal strategies.

P3.132 - Unusual segregation patterns and potential DNA repair mechanism in mRNA-destabilizing mutants of *Neurospora crassa*

H. Haj Hammadeh[1], *U. Brandt*[2], *A. Fleißner*[2], *D. Stainier*[1]
[1]Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany, [2]TU Braunschweig, Braunschweig, Germany

While working on mRNA-destabilizing mutations in the *camK-1* gene in *Neurospora crassa*, we observed an unusual segregation pattern in the progeny that did not conform to Mendel's second law. Specifically, a point mutation was introduced in the *camK-1* gene by substituting AAG with TAG to create a premature termination codon (PTC) mutant. This point mutation was linked to a

selection marker.
When the *camK-1* PTC allele was crossed with a wild-type (WT) allele, the progeny (ascospores) were grown on selection media to isolate those carrying the selection marker linked to the mutation. Two distinct phenotypes were observed: a WT-like phenotype and a "sick" phenotype. Sequencing revealed that the sick strains carried the mutant allele, while the healthy strains, though grown on selection media, exhibited the WT sequence. The ratio of mutant (ATG) to WT (AAG) was 50:50. A plausible explanation for this observation is that the mutant allele was repaired to WT during meiosis. This phenomenon was observed across three generations.
Interestingly, when the *camK-1* PTC allele was crossed with a strain harboring a deletion in *upf-3*, a gene involved in nonsense-mediated mRNA decay, the unusual segregation pattern did not occur, and normal Mendelian segregation was observed. This suggests that RNA degradation may play a role in triggering this type of genetic repair.
The same effect has been shown for in *uth-1*.
Given that *camK-1* and *uth-1* are required for meiosis, we hypothesize that a novel DNA repair mechanism may be acting on point mutations in genes expressed during meiosis.

P3.133 - Hyphal cell fate control by the Ndr kinase Cbk1 in C. albicans

M. Marín-Humanez[1], E. Dueñas-Santero[2], S. Píriz-Antúnez[1], A. Esperilla-Muñoz[1], P. Rojo-Dominguez[1], A. Mangas-Losada[2], C.R Vázquez de Aldana[2], J. Correa-Bordes[1]
[1]Universidad de Extremadura, Ciencias Biolgógicas, Badajoz, Spain, [2]Instituto de Biología Funcional y Genómica, Salamanca, Spain

Candida albicans colonizes the gastrointestinal tract in both yeast and hyphal forms. The hypha is a multicellular structure in which only the apical cell grows and divides, while the subapical cell remains arrested in the G0 phase. This suggests that mitosis in the apical cell is asymmetric, producing two nuclei with distinct fates. Cell fate can be determined by asymmetric segregation of gene expression regulators during mitosis. In *S. cerevisiae*, the Ndr kinase Cbk1 controls cell fate by asymmetrically segregating Ace2. Using GFP-tagged proteins, we found Cbk1 at the tip cell cortex of the hypha and observed an asymmetric distribution of transcriptional regulators along the hypha. The transcriptional activator Ace2 was found to localize to the apical nucleus, while Nrg1, which represses the expression of hypha-specific genes (HSG) in yeast cells, was confined to subapical nuclei. This suggests that HSG expression is limited to the apical cell. Results from FRAP experiments with a pECE1-NeonGreen reporter strain further supported this hypothesis. Interestingly, in *Cbk1-as* hyphae treated with the ATP analogue 1NM-PP1, Ace2 and Nrg1 were distributed isotropically across apical and subapical nuclei. Additionally, the subapical cell was no longer arrested in G0 and exhibited lateral budding. These findings demonstrate that Cbk1 is essential for establishing the spatial asymmetry of transcriptional regulators along the hypha. To further explore its role in determining the fate of apical and subapical cells, RNA-seq data from Degron variants of Cbk1, Nrg1, and Ace2 hyphae, with and without auxin, will be presented.

P3.134 - Antagonistic Pathways of Lipid Transfer Proteins (LTPs) for *de novo* Membrane Assembly in Fission Yeast

M. Basante-Bedoya[1], *S.G. Martin*[1]
[1]University of Geneva, Department of Molecular and Cellular Biology (MOCEL), Geneva, Switzerland

Fungal sporulation involves *de novo* synthesis of the spore plasma membrane, named the forespore membrane (FSM), which surrounds each meiotic nucleus. Biomembrane lipid composition is regulated by synthesis, degradation, and lipid transport. Lipid transfer proteins (LTPs) facilitate non-vesicular lipid transport. In *Schizosaccharomyces pombe*, Ltc1, from the LTP anchored at membrane contact sites (LAM) family, transports ergosterol from the plasma membrane (PM) to the endosomes, and Osh41, from the oxysterol-binding homology (Osh) protein family, conversely promotes PM ergosterol levels [1]. However, neither LTP is essential, suggesting redundancy with other transport mechanisms, and their role in sporulation remains unknown.
We have undertaken a systematic exploration of the function of LTPs in fission yeast. Our findings on Osh-family LTPs show that: (i) Concurrent Ltc1/Osh3 or Osh41/Osh2 deletions are synthetically lethal, indicating redundancy; (ii) Deletion of neither *osh41* nor *osh42* causes major effect on sporulation, yet *osh41*Δ*osh42*Δ double mutant exhibits complete failure of spore formation; (iii) *osh3* deletion partially restores *osh41Δ* mutant PM-ergosterol levels and circumvents the sporulation failure of *osh41*Δ*osh42*Δ cells, suggesting antagonistic functions between Osh41/Osh42 and Osh3. Our results highlight both functional redundancy and antagonistic functions among lipid transfer protein families.
[1] Marek, M., Vincenzetti, V. & Martin, S. G., J. Cell Biol. 219, e202001147 (2020).

P3.201 - Role of checkpoint kinases in regulating *Neurospora* circadian clock under DNA damage stress

Y. Yang[1], *Z. Duan*[1], *X. Liu*[1]
[1]Institute of Microbiology, Chinese Academy of Sciences, Beijing, China

Circadian rhythms allow organisms to adjust to daily environmental fluctuations. The interplay between the circadian clock, the cell cycle, and DNA repair has been extensively documented, yet the epigenetic control of the circadian clock by the DNA damage response remains relatively unexplored. Here, we showed that checkpoint kinases CHK1 and CHK2 regulate chromatin structure under DNA damage stress in *Neurospora crassa* to maintain robust circadian rhythms. Under DNA damage stress, deletion of *chk1* and *chk2* disrupted the rhythmic transcription of the clock gene *frq* by suppressing the rhythmic binding of the transcription activator WCC at the *frq* promoter, as the chromatin structure remained condensed. Mechanistically, CHK1 and CHK2 interacted and bound at the *frq* promoter to phosphorylate H3T11, promoting H3 acetylation, especially H3K56 acetylation, to counteract the histone variant H2A.Z deposition, establishing a suitable chromatin state to maintain the robust circadian rhythm despite DNA damage. Additionally, a genome-wide correlation was discovered between H3T11 phosphorylation and

H3K56 acetylation, showing a specific function at the *frq* promoter that is dependent on CHK1 and CHK2. Furthermore, transcriptome analysis revealed that CHK1 and CHK2 are responsible for robust rhythmic transcription of metabolic and DNA repair genes under DNA damage stress. These findings highlight the essential role of checkpoint kinases in maintaining robust circadian rhythms under DNA damage stress.

P3.202 - Genomic variations causing mating preferences of wild fission yeast strains

S.J. Seekles[1], *S.G. Martin*[1]
[1]University of Geneva, Department of Molecular and Cellular Biology, Geneva, Switzerland

How do yeast cells select a partner? The choice of a mating partner is a critical life event, determining the course of evolution as well as genetic isolation and eventually speciation. To probe the basis of mate preference in fission yeast, the in- and out-breeding ratios between twenty-four non-clonal wild *Schizosaccharomyces pombe* isolates was determined. We compared the mating behavior of homothallic strains (capable of switching mating-type) with the behavior of mating-type locked heterothallic strains and determined the isolates with altered mate choice preferences. Through the available genome sequences, we determined if components of the pheromone signaling pathway (e.g. Mam2, Map3, Sxa2) were potentially causing the altered mating behavior. From a subset of isolates with altered mating preferences the genomic variants were pinpointed by bulk segregant analysis, QTL mapping and/or genome wide association studies (GWAS).
One isolate, *S. pombe variant kambucha*, showed higher sensitivity to pheromone and the capability to mate potential partners from larger distances. *S. pombe variant kambucha* was the preferred mate in all mating competition assays. Moreover, lab strain 972 preferred mating cells of *S. pombe variant kambucha* over compatible cells of itself. Candidate genomic variants for the mating behavior of *S. pombe variant kambucha* included point mutations in *rgs1* and *ste6*. Furthermore, we are testing causality of these genomic variations and hope to describe which molecular systems influence mate choice preferences in fission yeast.

P3.203 - Using the CRISPR/Cas9 system for GFP-tagging of endogenous genes in *Neurospora crassa*

S. Grüttner[1]
[1]CAU Kiel/Botanical Institute, Kiel, Germany

Neurospora crassa is a widely used eukaryotic model organism due to its diverse biology and rapid growth. Traditional genetic manipulation methods, such as homologous recombination (HR), are time-consuming and labor-intensive[1]. Conversely, the RNA-guided CRISPR/Cas9 system provides high efficiency, is easy to handle, and allows precisely targeted mutagenesis. This system has already been successfully applied in some filamentous fungi[2]. We have developed a user-friendly CRIPSR/Cas9 system for *N. crassa* by integrating the *cas9* sequence into the fungal genome and introducing guide RNA (gRNA) as naked RNA via electroporation

into the cell[3]. This method was utilized to generate loss-of-function mutants of *N. crassa* and to simultaneously edit two genes. Here we show, that our system is also suitable for GFP-tagging of endogenous genes, and it effectively increases the efficiency of HR compared to the HR efficiency without the Cas9-induced dsDNA break. Compared to traditional methods, the user-friendly and effective system provides a rapid and efficient method for generating loss-of-function and knock-in mutants in *N. crassa*.

References:

1. Wang, S. *et al.* Molecular tools for gene manipulation in filamentous fungi. *Appl Microbiol Biotechnol* **101**, 8063–8075 (2017).
2. Schuster, M. & Kahmann, R. CRISPR-Cas9 genome editing approaches in filamentous fungi and oomycetes. *Fungal Genetics and Biology* vol. 130 43–53 Preprint at https://doi.org/10.1016/j.fgb.2019.04.016 (2019).
3. Grüttner, S. & Kempken, F. A user-friendly CRISPR/Cas9 system for mutagenesis of *Neurospora crassa*. *Sci Rep* **14**, (2024).

P3.204 - Targeted screening of the budding yeast genome reveals *PCP1*, a mitochondrial serine protease on chromosome VII, is highly susceptible to position effects

J. Arnone[1]
[1]Le Moyne College, Department of Biological and Environmental Sciences, Syracuse, United States

Genomic organization results in position effects influencing gene expression throughout a genomic locus. Early characterization of this phenomenon came from the discovery that reporter genes integrated adjacent to heterochromatic regions are rapidly silenced by the ‘Telomere Proximal Effect’ which is widely conserved in eukaryotes. Genome wide screening of position effects on gene expression has revealed significant variance in the levels of expression of identical reporter constructs, based on their integration site, that occurs on a locus-by-locus basis. Our present focus is to screen the genome for this phenomenon, focusing on regions of interest for biotechnological applications – specifically metabolite synthesis, fermentation, and alternate carbon sources for energy. We report the results of a targeted screen: our focus was on the gene set that is linked to increased survival to temperature extremes and those necessary for aerobic respiration and the utilization of ethanol for energy. Our screen revealed that there are multiple loci that are susceptible to position effects only revealed under stress conditions. The *PCP1* locus exhibited the most severe phenotype and was extensively dissected. We find this locus is susceptible to position effects by the deletion of either of the flanking genes, *MDR1* and *GTF1*. In fact, *MDR1* and *GTF1* null mutants completely phenocopy *PCP1* deletion, specifically changes in metabolism and stress tolerance. Our current aim includes characterizing additional phenotypes – including accelerated aging, analysis of conservation, and identification of the mechanism underlying this relationship at this locus.

P3.205 - Distinct trafficking routes of polarized and non-polarized membrane cargoes in *Aspergillus nidulans*

G.M. Sagia[1], X. Georgiou[1], G. Chamilos[2,3], S. Dimou[1], G. Diallinas[1,3]
[1]University of Athens, Department of Biology, Athens, Greece, [2]University of Crete, School of Medicine, Heraklion, Greece, [3]Foundation for Research and Technology, Institute of Molecular Biology and Biotechnology, Heraklion, Greece

Membrane proteins are sorted to the plasma membrane (PM) via Golgi-dependent trafficking. However, our recent studies challenged the essentiality of Golgi in the biogenesis of specific transporters. Here, we investigate the trafficking mechanisms of membrane proteins by following the localization of the polarized R-SNARE SynA versus the non-polarized transporter UapA, synchronously co-expressed in wild-type or isogenic genetic backgrounds repressible for conventional cargo secretion. In wild-type, the two cargoes dynamically label distinct secretory compartments, highlighted by the finding that, unlike SynA, UapA does not colocalize with the late-Golgi. In line with early partitioning into distinct secretory carriers, the two cargoes collapse in distinct ERES in a sec31ts background. Trafficking via distinct cargo-specific carriers is further supported by showing that repression of proteins essential for conventional cargo secretion does not affect UapA trafficking, while blocking SynA secretion. Overall, this work establishes the existence of distinct, cargo-dependent, trafficking mechanisms, initiating at ERES and being differently dependent on Golgi and SNARE interactions.

P3.206 - Genome wide exploring the autophagy-related genes in filamentous fungi and the functional characterization of *atg1* and *atg5* in *Neurospora crassa*

M. Yuan[1], Q. Liu[1], J. Li[1], C. Tian[1]
[1]Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, China

Autophagy is a membrane-dependent degradation process that is well understood in yeast and animal cells, but the molecular basis of autophagy in filamentous fungi including the genes network and detail functions are still poorly understood. We will try understanding the autophagy pathway from genome wide exploring the genes those are involved in filamentous fungi using comparative genomics and functional genomics. The transcriptome data show that the genes for ribosome biogenesis, amino acid synthesis and DNA replication is significantly suppressed and that the expression of genes related to DNA repair is significantly induced in macroautophagy-related genes *atg1* and *atg5* deficient *Neurospora crassa*. Macroautophagy deficient cells display accumulation of stacked ring-like membrane structures, impaired mycelium viability and sporulation ability, as well as significantly changed hyphae morphology. These results indicate that autophagy genes are deeply involved in cell development and hypha growth in filamentous fungi, and could be the good targets to engineer for industrial filamentous fungi in future.

P3.207 - Use of the GCaMP reporter to investigate calcium dynamics in *Candida albicans*

C. Parkin[1], E. Agnew[1], C. Giuraniuc[2], S. Nye[3], M. Cruz Almeida[4], C. Bedford[1], D. Richards[3], A. Brand[1]
[1]University of Exeter, MRC Centre for Medical Mycology, Exeter, United Kingdom, [2]University of Aberdeen, School of Natural and Computing Sciences, Aberdeen, United Kingdom, [3]University of Exeter, Department of Physics and Astronomy, Exeter, United Kingdom, [4]University of Aberdeen, Institute of Medicine, Medical Sciences & Nutrition, Aberdeen, United Kingdom

Calcium signalling is essential for diverse stress responses in the fungal pathogen, *Candida albicans*, yet our understanding of calcium dynamics in real time and in single cells has been limited due to the lack of reporter constructs in this and other fungi. We have developed the GCaMP6 calcium reporter for use in *C. albicans*, and used it to reveal not only differential cytoplasmic calcium flux responses to diverse stressors but also to characterise the 'adaptation factor' of signalling mutants to determine their relevance in stress response pathways. Image analysis tools revealed considerable population heterogeneity and allowed us to define 5 fluorescent output types as reporters of differential stress response mechanisms. Recent work has identified altered Ca^{2+}-GCaMP spike shape in Ca^{2+}-pump and channel mutants, suggesting perturbation of cytoplasmic [Ca^{2+}]. GCaMP has also allowed us to characterise the Ca^{2+} response to deletion of the plasma-membrane flippase, Neo1, and to investigate the Ca^{2+}-related function of a previously-undescribed TRP channel in *C. albicans*. Application of the GCaMP reporter has proved it to be a highly informative molecular tool and has revealed the complexity of Ca^{2+} homeostasis and signalling dynamics in this fungal pathogen.

P3.208 - Molecular interactions and functional role of the essential protein TEA-5 in polarized growth of *Neurospora crassa*

R.R. Mouriño-Pérez[1], O.A. Callejas-Negrete[1], A.N. Espino-Vazquez[2], P.A. Lopez-García[1]
[1]Centro de Investigación Científica y de Educación Superior de Ensenada, Department of Micorbiology, Ensenada, Mexico, [2]Universidad Autónoma de Baja California, Instituto de Investigaciones Oceanológicas, Ensenada, Mexico

This study examines the roles and molecular interactions of three proteins in the TEA complex of *Neurospora crassa*: TEA-1, TEA-4, and TEA-5, homologous to Tea1/TeaA, Tea4/TeaC, and Mod5/TeaR in *S. pombe*/*A. nidulans*., with emphasis in TEA-5. This complex is crucial for interactions between microtubules and the actin cytoskeleton at growth sites. While TEA-1 and TEA-4 mutants showed no impact on fungal growth or morphogenesis, the deletion of *tea-5* proved lethal at the ascospore stage, calling for the study of a heterokaryotic Δ*tea-5*^{Het} mutant. This strain displayed significant reductions in elongation rate (43%), biomass (57%), and conidia production (68%), alongside a two-fold increase in branching rate compared to the wild type. The Δ*tea-5*^{Het} mutant also had a disorganized cytoskeleton and a small, unstable Spitzenkörper. To further investigate TEA protein interactions, Co-Immunoprecipitation (Co-IP) experiments were

performed with GFP-Trap and analyzed through LC-MS/MS. Results confirmed physical interactions among TEA-1, TEA-4, and PP1 (homologous to Dis2 in *S. pombe*) and an association between TEA-1 and microtubules. Additional novel interactions of TEA-4 and TEA-5 with proteins linked to cell wall biogenesis and vesicle trafficking were also identified. These findings underscore TEA-5 essential role in *N. crassa*'s polarized growth, with interactions among TEA proteins and regulatory molecules like PP1 being vital for microtubule-actin dynamics.

4o

P3.209 - A comparative evaluation of CRISPR-Cas9 allele editing systems in *Candida auris*: challenging research in a challenging bug

D. Sofras[1], *H. Carolus*[1], *A. Subotic*[1], *C. Lobo Romero*[1], *C. Ennis*[2], *C.J. Nobile*[2], *J.M. Rybak*[3], *P. Van Dijck*[1]
[1]KU Leuven, Department of Biology, Leuven, Belgium, [2]University of California Merced, Department of Molecular and Cell Biology, Merced, United States, [3]St. Jude Children's Research Hospital, Memphis Tennessee, United States

Candida auris is an emergent fungal pathogen of significant interest for molecular research because of its unique nosocomial persistence, high stress tolerance and common multidrug resistance. In this work, we compare four systems designed to introduce the genetic elements necessary for the production of Cas9 and the guide RNA molecule into the genome of *C. auris*, replacing the *ENO1*, *LEU2* and *HIS1* loci respectively, while the fourth system makes use of an episomal plasmid named *EPIC*. We observed that the editing efficiency of all four systems was system and strain background-dependent. Alarmingly, we did not detect correct integration of linear CRISPR cassette constructs in integration-based systems, in over 4,900 screened transformants. Still, all transformants, whether correctly edited or not, grew on selective nourseothricin media, suggesting common random ectopic integration of the CRISPR cassette. Although the plasmid-based *EPIC* system showed a low transformation yield compared to the other systems, it has the highest editing efficiency with an average of 57.1% correct transformants. To improve editing efficiencies of integration-based systems, we deleted *KU70* and *LIG4* to improve homology-directed repair. However, no improved editing or targeting efficiencies were detected in *ku70*Δ*, lig4*Δ, or *ku70*Δ*/lig4*Δ backgrounds. Our research highlights important challenges in precise genome editing of *C. auris* and sheds light on the advantages and limitations of several methods with the aim to guide scientists in selecting the most appropriate tool for molecular work in this enigmatic fungal pathogen.

P3.210 - Rust fungi (Pucciniales) exhibit diploid nuclei throughout their life cycles

R. Carvalho[1], *L. Morais-Cecílio*[1], *S. Tavares*[1], *T. Ribeiro*[1], *H. Azinheira*[1], *A.P. Ramos*[1], *M.d.C. Silva*[1], *M. Monteiro*[2], *J. Loureiro*[3], *P. Talhinhas*[1]
[1]Instituto Superior de Agronomia, Universidade de Lisboa, Lisbon, Portugal, [2]Instituto Gulbenkian de Ciência, Oeiras, Portugal, [3]Universidade de Coimbra, Centre for Functional Ecology, Coimbra, Portugal

Fungal life cycles are characterised by predominant haploid stages, while in most cases diploid nuclei occur only in a single cell, following karyogamy between haploid nuclei and immediately followed by meiosis. Rust fungi are also reported as obeying to this general rule, with the basidium as the single diploid cell. However, using flow cytometry, the presence of 1C, 2C and a low proportion of 4C nuclei was detected in different stages of the urediniosporic cycle of several rust fungi, but also on the pycnial and aecial stages. The employment of FISH using an rDNA 45S probe on sorted nuclei for each of the populations confirmed that the 1C population corresponds to haploid G1 nuclei, the 2C population comprises a mixture of haploid G2 and diploid G1 nuclei and the 4C population contains diploid G2 nuclei. These results suggest the presence of replicating diploid nuclei in all stages of rust life cycle and not just on teliospores. This unexpected phenomenon seems to be transversal to the Pucciniales, as it is described from 34 Pucciniales species, but not from neighbouring taxa. This could represent an undescribed nuclear life cycle common to an entire order of fungi.

P3.211 - The BEM46 protein at the crossroads of polarity maintenance, auxin biosynthesis and endophytic growth of *Neurospora crassa*: a working model

K. Kollath-Leiss[1], *H. Winter*[1], *F. Kempken*[1]
[1]CAU zu Kiel, Botanical Genetics and Molecularbiology, Kiel, Germany

The BEM46 protein is conserved across eukaryotes and shares homology with human ABHD12, a lipase linked to neurological disorders [1–3]. Investigations in *Drosophila melanogaster* [4], *Arabidopsis thaliana* [5], and our research in *N. crassa* suggest an elusive function of the protein in polarity maintenance. In *N. crassa*, BEM46 affects ascospore and conidiospore germination and is localized to the endoplasmic reticulum (ER) and eisosomes [6]. The protein interacts with the tryptophan synthase and influences the fungus' complex auxin biosynthesis [7]. Whole transcriptome and *in silico* analyses suggest BEM46's potential lipase activity, which is being investigated *in vitro*. Additionally, eisosome formation and the endophytic behavior of *N. crassa* in different bem46 backgrounds are being analyzed microscopically. We present a working model summarising our results and hypotheses, how the BEM46 protein impacts the fugus' lipid homeostasis and -as a consequence of this- influences eisosome formation and its endophytic behavior.
1. Kumar A et al. Biochem Biophys Res Commun. 2013;438(3).
2. Joshi A et al. J Biol Chem. 2018;293(44):16953–63.

3. Fiskerstrand T et al. Am J Hum Genet. 2010;87(3):410–7.
4. Parmentier ML et al. J Neurosci. 2000;15;20(14):RC84.
5. Mochizuki S et al. Plant Cell. 2005;17(2):537–47.
6. Mercker M et al. Curr Genet. 2009;55(2):151–61.
7. Kollath-Leiß K et al. Eukaryot Cell. 2014;13(8):1051–63.

P3.212 - The role of cell wall remodeling in early divergent Mucoromycotina response to bacterial and plant defenses

H. Barrett[1], *M.L. Gaspar*[1], *C. Lax*[2], *V. Garre*[2], *T. Pawlowska*[1]
[1]Cornell University, Plant Pathology and Plant Microbe Biology, Ithaca, United States, [2]Universidad de Murcia, Departamento Genética y Microbiología, Facultad de Biología, Murcia, Spain

The fungal cell wall is known to respond dynamically to environmental stress through extracellular perception and cell wall integrity monitoring, but its role in inter-organizational interactions is not well understood. Using the early divergent fungus *Rhizopus microsporus* (Mucoromycotina) and the bacterium Mycetohabitans spp. as a model, we have observed cell wall remodeling in response to bacterial antagonism. We found differential expression of genes encoding synthesis and modification of cell wall components chitin, fucose, glucan, mannose, and galactose under bacterial antagonism. We investigated the composition of the cell wall by fluorescently staining individual components, then imaging the fluorescence by microscopy and quantifying median fluorescence of each stain by flow cytometry. We also visualized the internal structure of the wall through transmission electron microscopy. We detected an increase in chitin content in the presence of bacteria, which appears to be involved in the defensive reinforcement of the cell wall. My research aims to uncover the role of extracellular perception and cell wall integrity sensors in activating this response under various biological stresses. I hypothesize that this defensive remodeling is driven by the interacting Protein Kinase A (PKA), Cell Wall Integrity (CWI), and High Osmolarity Glycerol (HOG) pathways, and will occur in response to multiple mechanisms of antagonism from plants and bacteria.
I aim to uncover the dynamics and mechanism of remodeling with the goal of developing antifungal treatments that more directly target stress tolerance mechanisms activated during infection.

P3.213 - MAT Loci in the mortierellaceae: genomic, empirical and molecular evidence

K. Mondron[1], *W. Gilmour*[1], *M. Korne*[2], *A. Sow*[2], *J. Nettnin*[3], *A. Idnurm*[4], *G. Bonito*[2,5], *J. Uehling*[1]
[1]Oregon State University, Botany and Plant Pathology, Corvallis, United States, [2]Michigan State University, Microbiology, Genetics, and Immunology, East Lansing, United States, [3]Iowa State University, Genetics, Development, and Cell Biology, Ames, United States, [4]University of Melbourne, School of BioSciences, Parkville, Australia, [5]Michigan State University, Plant, Soil and Microbial Sciences, East Lansing, United States

Fungi have a remarkably diverse repertoire of sexual strategies and spore stages that are intimately tied to their ecological success. Early studies of fungal sexuality included model systems within the Mucorales (Mucoromycotina, Mucoromycota) such as the genera *Phycomyces*, *Mucor*, and *Rhizopus*. The genetic basis of canonical Mucoralean sexual reproduction has been well described, and involves two out-crossing mating types, plus (+) and minus (-), determined by the genetic content at the mating type (MAT) locus, which encode different high-mobility group (HMG) transcription factors (TFs). MAT loci have not been identified or described in the Mortierellomycotina (Mucoromycota) although sexual spore (zygospore) production is frequently observed. In this study, we leveraged MycoCosm's MCL Cluster tool and the genomes of *Linnemannia elongata* (Mortierellomycotina) isolates of known mating types to identify two potential MAT loci for the Mortierellomycotina. These candidate MAT loci encode HMG and homeodomain (HD) TFs. We conducted intraspecies zygospore production assays by co-culturing isolates on low-nutrient media. Sexually fertile isolates were thereby assigned to compatibility groups subject to further genomic and molecular evaluation. Using the Hidden Markov Model-based computational tool Orthofisher, our *L. elongata* (Mortierellomycotina) isolate compatibility data, and PCR amplification of the HMG and HD candidate MAT loci, discussion will center on a three-pronged approach of genomic, empirical (zygospore production), and molecular evidence.

P3.214 - Fungal protein required for kinesin-1-mediated dynein localization at the microtubule plus ends

R. Qiu[1], *M. Delgado*[2], *M. Pinar*[2], *J. Zhang*[1], *P. Polonio*[2], *M.A. Peñalva*[2], *X. Xiang*[1]
[1]Uniformed Services University of the Health Sciences, Bethesda, United States, [2]Centro de Investigaciones Biológicas Margarita Salas, Consejo Superior de Investigaciones Científicas, Madrid, Spain

Cytoplasmic dynein transports cargoes toward the microtubule minus ends but the dynein-cargo interaction is facilitated by kinesin-dependent accumulation of dynein at the microtubule plus end. It is unclear how kinesin-1 transports dynein to the plus ends. In mammalian cells, the dynein-kinesin-1 interaction is mediated by kinesin light chains, but light chains were not found in filamentous fungi. Here in *Aspergillus nidulans*, we identified a kinesin-1 (KinA)-binding protein KobA needed for kinesin-1-dependent dynein-localization at the microtubule plus end. KobA is a fungal-specific protein present in some but not all species of Ascomycota. Alphafold2 prediction indicates that KobA binds to the C-terminal cargo-binding domain of KinA, which is consistent with experimental data. Loss of KobA had little effect on the general function of KinA in supporting hyphal growth via transporting secretory vesicles. Specifically, while KinA becomes almost essential for hyphal growth upon loss of myosin-V, KobA is largely dispensable. However, loss of KobA causes dynein to localize along microtubules instead of accumulating at the plus end, a phenotype similarly exhibited by the Δ*kinA* mutant. Similar to the Δ*kinA* mutant, the Δ*kobA* mutant also exhibits a defect in dynein-mediated early endosome transport. Thus, KobA seems to be specifically required for transporting dynein to the plus ends. This function is unlikely related to kinesin-1 activation because in the presence of a kinesin-1 auto-inhibition mutation that makes the motor constitutively active, KobA is still required for dynein localization

at the microtubule plus ends. Whether and how KobA may regulate the dynein-kinesin-1 interaction is being investigated.

P3.215 - Ctr9, a subunit of Paf1 complex, promotes the pathogenicity of Candida albicans by regulating methionine metabolism

S. Park[1], *J. Park*[1], *J. Kim*[1], *Y.-J. Cho*[1], *J.-S. Lee*[1]
[1]Kangwon National University, Molecular Bioscience, Chuncheon-si, Korea, Republic of

Candida albicans, a part of normal flora, is an opportunistic fungal pathogen and causes severe health issues in immunocompromised patients. Its pathogenicity is intricately linked to the transcriptional regulation of its metabolic pathways. Paf1 complex (Paf1C) is a crucial transcriptional regulator that is highly conserved in eukaryotes. The objective of this study was to explore the role of Paf1C in the metabolic pathways and how it influences the pathogenicity of *C. albicans*. Paf1C knockout mutant strains of *C. albicans* (*ctr9*Δ/Δ, *leo1*Δ/Δ, and *cdc73*Δ/Δ) were generated using the CRISPR-Cas9 system. To investigate the effect of Paf1C on pathogenicity, macrophage interaction assays and mouse survival tests were conducted. The growth patterns of the Paf1C knockout mutants were analyzed through spotting assays and growth curve measurements. Transcriptome analysis was conducted under yeast conditions (30°C without serum) and hyphal conditions (37°C with 10% FBS), to further elucidate the role of Paf1C in the pathogenicity of *C. albicans*. *CTR9* deletion resulted in the attenuation of *C. albicans* virulence, in macrophage and mouse models. Furthermore, we confirmed that the reduced virulence of the *ctr9*Δ/Δ mutant can be attributed to a decrease in *C. albicans* cell abundance. Moreover, transcriptome analysis revealed that metabolic processes required for cell proliferation are impaired in *ctr9*Δ/Δ mutant. Notably, *CTR9* deletion led to the downregulation of methionine biosynthetic genes and the cAMP-PKA signaling pathway-related hypha essential genes, which are pivotal for virulence. Our results suggest that Ctr9-regulated methionine metabolism is a crucial factor for determining *C. albicans* pathogenicity.

P3.216 - Dominant negative effect on UPR and RIDD by expression of RNase-inactive IreA in *Aspergillus oryzae*

M. Yokoyama[1], *K. Gomi*[1], *T. Shintani*[1]
[1]Tohoku University, Graduate School of Agricultural Science, Sendai, Japan

Aspergillus oryzae is a promising host for recombinant protein production due to its high protein secretion capacity, but the productivity of heterologous proteins is significantly low. This may be due to the removal of mRNAs and/or proteins by quality control mechanisms in the host during gene expression and protein secretion. Therefore, we focused on Regulated IRE1-dependent decay (RIDD), mRNA degradation mechanism in the endoplasmic reticulum (ER). Under ER stress, the ER transmembrane sensor protein IreA is activated through oligomerization and phosphorylation, and specifically degrades secretory protein mRNAs targeted to the ER membrane by its RNase activity. IreA is also involved in the splicing of *hacA* mRNA in the unfolded protein response (UPR), and its gene disruption is lethal. In this study, we introduced

mutation into the RNase domain of IreA and we analyzed the effects on *hacA* splicing and RIDD. Mutations were introduced into highly conserved amino acids in the RNase domain identified from the multiple alignments with other organisms. The resulting *ireA* mutants were expressed with its own promoter in a strain that can suppress expression of host-derived *ireA* by addition of thiamine. Mycelial growth was not complemented by expression of the *ireA* mutants in the presence of thiamine, but rather was even more inhibited. Furthermore, expression of mutant *ireA* reduced *hacA* splicing ability and RIDD of amylase mRNA under ER stress condition even when endogenous *ireA* was not suppressed. These results indicate that the expression of RNase-inactive IreA has a dominant-negative effect.

P3.217 - The alkaline protease PrtA as a model secreted protein in the intra and extracellular trafficking mediated by SltA and the retromer

I. Picazo[1], *S. Rodriguez-Pires*[1,2], *S. Abib-Ait-Bakadir*[1], *E.A Espeso*[1]
[1]Centro Investigaciones Biologicas Margarita Salas, CSIC, Cellular and Molecular Biosciences, Madrid, Spain, [2]Universidad Complutense de Madrid, Genetics, Physiology and Microbiology, Madrid, Spain

Proteases have been largely used to understand the mechanisms of intracellular trafficking in fungi. In *Saccharomyces cerevisiae* the localization of carboxypeptidase Y, a vacuolar protease, served to identify genes involved in the generation of vesicles, endosomes, vacuoles and their interactions and maturation processes. Among the vacuolar-protein-sorting mechanisms identified is the retromer. The retromer enables the recycling of proteins during maturation of early endosomes and participates in quality control of secreted proteins. Studies in *Aspergillus nidulans* using extracellular proteases allowed the identification of two members of the retromer complex. Here we extend the identification of retromer subunits and we use the alkaline protease PrtA to investigate the role of the retromer in the secretion of matured forms of this extracellular protease. We also identified a role of the transcription factor SltA in maintaining correct transcriptional and protein expression levels and in the secretion process of PrtA.

P3.218 - Differential interaction dynamics and cellular uptake of fungal Antifungal Proteins (AFPs) and engineered AFP chimeras

M. Giner-Llorca[1], *C. Ropero-Pérez*[1], *F. Gallego del Sol*[2], *D.D. Thomson*[3], *S. Garrigues*[1], *E.M. Bignell*[3], *A. Marina*[2], *P. Manzanares*[1], *J.F. Marcos*[1]
[1]Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Paterna, Spain, [2]Instituto de Biomedicina de Valencia (IBV-CSIC), Valencia, Spain, [3]University of Exeter, Exeter, United Kingdom

Antifungal proteins (AFPs), such as the highly active PeAfpA and the moderately active PeAfpB from *Penicillium expansum*, show promising antifungal activity. We have analysed their interaction with the citrus fruit pathogen *Penicillium digitatum*, along with two rationally designed chimeras that incorporate PeAfpA residues into the PeAfpB sequence. Using live-cell fluorescence microscopy with BODIPY-labelled AFPs, we observed distinct dynamics of

interaction and internalisation across different *P. digitatum* morphotypes (quiescent conidia, swollen conidia, germlings, and hyphae). PeAfpA localised at the cell wall (CW) of quiescent conidia in a punctate, non-uniform pattern, which changed to a uniform distribution with increased intensity after the transition to swollen conidia. Conidia from melanin biosynthetic gene mutants (*pksP*/*alb1* and *arp2*) did not show the punctate distribution of PeAfpA. Although PeAfpA affected germination, it remained attached to the CW of swollen conidia and germlings without entering the cell. In hyphae, PeAfpA internalised through the growing hyphal tip after CW binding, in a non-endocytic but energy-dependent process that preceded vacuolisation and cell death. Conversely, the less active PeAfpB effectively inhibited hyphal elongation but remained bound to the CW under our assay conditions, indicating mechanistic differences from PeAfpA. Interestingly, a highly active PeAfpB::PeAfpA chimera with a single residue substitution internalised like PeAfpA. Internalisation did not necessarily cause cell death, as an inactive PeAfpB::PeAfpA chimera entered hyphal cells, suggesting the existence of specific intracellular targets. This study offers valuable mechanistic insights into the antifungal action and cell internalisation of AFPs.

P3.219 - Role of kinesin-3 and of the Hook1 adaptor in early endosome and peroxisome transport in *Podospora anserina*

L. Peraza-Reyes[1], *F. Hernandez-Sanchez*[1], *S. Schroder*[1], *F. Frutis-Osorio*[1]
[1]Universidad Nacional Autonoma de Mexico, UNAM, Institute of Cellular Physiology, Mexico City, Mexico

Fungal cell growth and development depend on a precise spatiotemporal organelle distribution. In filamentous fungi, this process relies on microtubule-based transport systems. In these fungi, early endosomes are highly motile organelles that are transported by cytoplasmic dynein and kinesin-3 motors. Dynein-early endosome interaction depends on the adaptor Hook1, whereas in *Ustilago maydis* this protein is also involved in the recruitment of kinesin-3 to these organelles. Early endosomes interact with multiple organelles –such as peroxisomes, lipid droplets and the endoplasmic reticulum– and they facilitate their transport while moving along microtubules. However, the cargoes that are transported by early endosomes in fungi differ. Here we analyzed the transport system involved in early endosome and peroxisome trafficking in the model fungus *Podospora anserina*. We show that both organelles exhibit fast, long-range anterograde and retrograde movements along hyphae with similar kinetics. We found that the movement of peroxisomes in both directions depends on the kinesin-3 motor KIN2, and that these organelles co-migrate with a GFP-tagged version of this protein. We demonstrate that the early-endosome displacements in both directions also depend on KIN2, and that the directed anterograde and retrograde movements of both organelles require HOOK1. However, we found that the elimination of this protein produces different outcomes in the hyphal localization of these organelles. Moreover, we observed low frequency of peroxisome-early endosome co-transport, suggesting that most peroxisomes move independently of early endosomes. Our results suggest that the peroxisome transport system of *P. anserina* is different to that described in other fungi.

P3.220 - The lipid flippases Drs2 and Neo1 have distinct functions in Candida albicans

S. Bogliolo[1], *R.A Arkowitz*[1], *M. Bassilana*[1]
[1]University Côte d'Azur - CNRS - INSERM, Institute of Biology Valrose, Nice, France

Cell growth relies on the asymmetric distribution of lipids, both in the different cellular compartments and across lipid bilayers. In particular, flippases transport lipids across the membrane bilayer to generate and maintain such asymmetry and we have previously shown that Drs2 is critical for *Candida albicans* filamentous growth, virulence and susceptibility to the antifungal drug fluconazole, compared to Dnf1-3 (1, 2). Here we show that Neo1 is also important for invasive filamentous growth, yet deletion of the lipid transfer protein Osh4 does not recover filamentous growth in a *neo1* mutant, as it does in a *drs2* mutant (2). Furthermore, while *neo1* cells grow similar to wild-type cells in the presence of azole drugs, they are hypersensitive to echinocandins; in contrast, *drs2* cells grow similar to wild-type cells in the presence of this class of drugs. Interestingly, deletion of *OSH3* in a *drs2* mutant specifically restores growth on azole drugs, while deletion of either *OSH3, OSH4* or *OSH7* in a *neo1* mutant restores growth on echinocandins. Drs2 and Neo1 both localize to the Golgi, however overexpression of Drs2 does not recover the defects in *neo1* cells and, reciprocally, overexpression of Neo1 does not recover the defects in *drs2* cells. Together, our results indicate that these flippases regulate growth and stress responses *via* distinct processes, and we are further investigating their functions, particularly in lipid distribution and Golgi integrity, as well as their interplay with lipid transfer proteins.

1. Labbaoui *et al.* PLoS Pathog, 2017.
2. Basante-Bedoya *et al*. PLoS Genet, 2022.

P3.221 - Genetic dissection of the role of proteins associated with ER-PM contacts in *Aspergillus nidulans*: VapA is essential for growth and polarized recycling of lipid flippases

X. Georgiou[1], *S. Politi*[1], *G. Diallinas*[1,2]
[1]University of Athens, Department of Biology, Athens, Greece, [2]Foundation for Research and Technology, Institute of Molecular Biology and Biotechnology, Heraklion, Greece

Endoplasmic reticulum (ER)-plasma membrane (PM) tethering proteins play a crucial role in eukaryotic cells by anchoring the ER to the PM and other cellular membranes, establishing contact sites that serve as platforms for non-vesicular lipid transfer and lipid homeostasis. This study explores the cellular role of ER-PM tethering proteins in *Aspergillus nidulans* through reverse genetics and quantitative live-cell imaging. By systematically deleting genes involved in ER-PM contact formation, we identify VapA (single homologue of Scs2/22 in *Saccharomyces cerevisiae*) as essential for proper growth and apical distribution of specific polarized membrane cargoes, while it does not affect the steady state localization on non-polarized cargoes, such the UapA purine transporters or the major proton pump ATPase PmaA. More specifically, VapA is

required for the apical localization of lipid flippases DnfA and DnfB, as well as the R-SNARE protein SynA. Interestingly, VapA is redundant for the apical localization of chitin synthase ChsB, which is also an apically localized membrane protein. Preliminary findings further suggest that VapA is involved in the local recycling of apical markers at the subapical region necessary for polarity maintenance during hyphal growth. This work paves the way to uncover mechanistic insights into the VapA role in membrane cargo trafficking, thus broadening our understanding of ER-PM contact functions.

P3.222 - Understanding loss of thermotolerance in two *Cryptococcus gattii* VGI isolates

Q. Ma[1], *D. Tamayo*[1], *N. Helmstetter*[1], *J. Harrison*[1], *R. May*[2], *M. Fisher*[3], *R. Farrer*[1]
[1]University of Exeter, MRC Centre for Medical Mycology, Exeter, United Kingdom, [2]University of Birmingham, School of Biosciences, Birmingham, United Kingdom, [3]Imperial College London, School of Public Health, London, United Kingdom

Cryptococcosis is a life-threatening systemic mycosis caused by two etiological agents: *Cryptococcus neoformans* and *Cryptococcus gattii*. *C. gattii* causes primarily respiratory infections and can infect both immunocompetent and immunocompromised individuals. To date, *C. gattii* has been sub-categorised into 6 monophyletic lineages, VGI to VGVI. Among the 6 lineages, VGI is the 2nd most common worldwide, and has an increasing number of cases reported in Europe. To explore the genetic and phenotypic diversity of *C. gattii*, we have sequenced and screened our collection of ~60 isolates including all six lineages. Our phenotype screens revealed two VGI isolates (CBS1622 and CBS919) that are sensitive to human host temperature (37°C). Both isolates showed no signs of growth at 37°C in YPD or minimal media and reduced virulence at 37°C in the invertebrate model *Galleria mellonella*, but not at 30°C. Significantly lower fungal burden was observed in the larvae infected by these isolates at 37°C on day 5 and day 7 post infection. Surprisingly, no live CBS919 cells were recovered from the host on day 7, indicating a tremendous reduction in its *in vivo* fitness. Moreover, reduced growth of CBS1622 and CBS919 in RPMI media supplemented with 2% glucose suggests that certain ingredients of RPMI may facilitate growth at host temperature. To gain insights into the genetic mechanism, we performed genomic sequencing and analysis. No variants were identified in the HSF genes or critical genes in the calcineurin pathway, suggesting other unknown regulatory cascades are associated with this phenotype.

P3.223 - Functional analysis of maltose/isomaltose transporters using the nuclear translocation assay of the transcription factor AmyR in *Aspergillus oryzae*

S. Fujita[1], *D.M. Jeong*[1], *K. Gomi*[1]
[1]Tohoku University, Grad. Sch. Agric Sci., Sendai, Japan

Maltose uptake is crucial for amylolytic enzyme production in the industrially-important filamentous fungus *Aspergillus oryzae*. Previously, we reported that MalP acts as a major maltose

transporter in *A. oryzae*, and a *malP* disruptant exhibited a significant decrease in maltose consumption and α-amylase activity. Intracellular maltose is converted to isomaltose, which induces the nuclear translocation of the transcription factor AmyR, regulating amylolytic enzyme production. Interestingly, extracellular isomaltose also rapidly induces nuclear translocation of AmyR, although isomaltose is barely incorporated in the cell, suggesting that *A. oryzae* has an isomaltose sensor protein or significantly low-affinity transporter contributing to AmyR activation. In this study, we used the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) to examine whether an isomaltose sensor or transporter exists. Firstly, the nuclear translocation of AmyR was not observed upon the addition of maltose in the presence of CCCP, suggesting that MalP is a proton symporter. Also, the isomaltose-induced nuclear translocation of AmyR was suppressed even at low concentrations of CCCP. This result suggests that isomaltose is not recognized by a sensor but is taken up in quite small amounts by an unidentified sugar transporter(s). We also present the results of similar experiments conducted with the model fungus *Aspergillus nidulans* in which an isomaltose sensor/transporter has been identified[1)].
1) Jeong Da Min et al., The 32nd Fungal Genetics Conference, 293A (2024).

P3.224 - Microfluidic control reveals chemotropism of fungal hyphae to nutrients and pH

N. Takeshita[1]
[1]University of Tsukuba, Tsukuba, Japan

The importance of fungi in ecological systems and pathogenicity hinges on their ability to search for nutrients, substrates, and hosts. Despite this, the question of whether fungal hyphae exhibit chemotropism toward them remains largely unresolved and requires close examination at the cellular level. Here, we designed a microfluidic device to assess hyphal chemotropism of *Aspergillus nidulans* in response to carbon and nitrogen sources, as well as pH. Within this device, hyphae could determine their growth direction in a two-layer flow with distinct compositions that were adjacent but non-mixing. Under conditions with and without a carbon source, hyphae changed growth direction to remain in the presence of a carbon source, but it was still difficult to distinguish between differences in growth and chemotropism. Although nitrogen sources such as ammonia and nitrate are important for growth, the hyphae indicated negative chemotropism to avoid them depending on the specific transporters. This fungus grows equally well at the colony level in the pH range of 4 to 9, but the hyphae exhibited chemotropism to acidic pH. The proton pump PmaA is vital for the chemotropism to acid pH, while the master regulatory for pH adaptation PacC is not involved, suggesting that chemotropism and adaptive growth via gene expression regulation are distinct regulatory mechanisms. Despite various plasma membrane transporters are distributed across membranes except at the hyphal tip, the control of growth direction occurs at the tip. Finally, we explored the mechanisms linking these two phenomena, tip growth and chemotropism.

P3.225 - RNA-binding protein SsdA shows dynamic localisation and transport during hyphal growth in *Aspergillus nidulans*

D. Modaffari[1], *E.W. Wallace*[1], *K.E. Sawin*[1]
[1]University of Edinburgh, School of Biological Sciences, Edinburgh, United Kingdom

Filamentous fungi grow through highly polarized hyphal extension, requiring precise spatial coordination of vesicle fusion and cell wall material delivery at the growing tip. While mRNA transport along hyphae is well-documented in fungi, the relationship between RNA regulation and polarized growth remains poorly understood. Using *Aspergillus nidulans* as a model organism, we characterized SsdA, a putative RNA-binding translational repressor homologous to *Saccharomyces cerevisiae* Ssd1.
Bioinformatic analysis revealed that putative SsdA RNA targets are enriched for cell wall-related proteins, suggesting a role in regulating fungal cell wall synthesis. Live-cell fluorescence microscopy demonstrated that SsdA moves along microtubules in association with early endosomes, dependent on the endosomal hitchhiking-mediator PxdA. Notably, we observed that SsdA particles are absent from growing hyphal tips.
Our findings describe the dynamic localization pattern of SsdA during hyphal growth. We hypothesise SsdA might regulate tip-specific translation by repressing target mRNAs during transport and releasing this repression at hyphal tips. Ongoing work is investigating the dynamics of SsdA's RNA targets and the role of its upstream kinase CotA.

P3.226 - In Candida albicans, manganese homeostasis modulates cell wall unmasking through Smf12 and calcineurin pathway

M. Henry[1,2], *A. Sellam*[1,2]
[1]Université de Montréal, Immunologie, Microbiologie, Montréal, Canada, [2]Institut de Cardiologie de Montréal, Montréal, Canada

Due to transition metals scarcity within human hosts, fungal pathogens have evolved sophisticated mechanisms to uptake and utilize micronutrients leading to a battle at the host-pathogen nexus. This process named nutritional immunity, where iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) are sequestered by the host to limit microbial growth and virulence. We have previously shown that *SMF12*, a member of Mn Nramp transporters the opportunistic yeast *C. albicans*, plays an important role in Mn internalisation. Its inactivation led to many cellular defects such as attenuation of virulence and decrease of protein glycosylation. It is known that metals availability affects recognition by the immune system through the modulation of Pathogen-associated molecular patterns (PAMPs) exposure. However, processes required for PAMP exposure during Mn starvation haven’t been investigated yet. Initially, we performed transcriptional profiling of *smf12* mutant in *C. albicans* under Mn starvation. *smf12* profile correlated with the set of genes modulated by calcineurin and was similar to the transcriptomic signature of *C. albicans* cells exposed to the antifungal caspofungin that targets fungal cell wall. Transcripts linked to cell wall remodelling, such as chitinase (CHT2, CHT3) and glucanase (ENG1, YWP1), involved in cell wall PAMP masking, were down-regulated. In fact, during

manganese starvation, *smf12* exhibits glucan and chitin unmasking. We also observed significant morphological defects associated with cell wall stress that impaired cell internalization by macrophages. To our knowledge, this work represents the first assessment of Mn homeostasis and its contribution to cell wall unmasking.

P3.227 - Initiation of endosymbiosis in *Rhizopus microsporus* indicates a shift from antagonism to commensalism

T. Gassler[1], *A. Holderbusch*[1], *G. Giger*[1], *A. Sintsova*[1], *M. Bortfeld-Miller*[1], *B. Dehapiot*[2], *S. Sunagawa*[1], *J.A. Vorholt*[1]
[1]ETH Zurich / Institute of Microbiology, Department of Biology, Zurich, Switzerland, [2]ETH Zurich / ScopeM, Zurich, Switzerland

Endosymbioses represent intricate and dynamic relationships between organisms that may involve pathogenic phases during their emergence. Here, we use fluidic force microscopy (FluidFM) to induce cell-in-cell interactions to directly probe the early stages of endosymbiosis. We introduced the opportunistic pathogen *Ralstonia pickettii* into a non-endosymbiotic strain of *Rhizopus microsporus*, simulating the unstable early phase of endosymbiosis. This approach allowed us to explore mechanisms that might overcome initial challenges in the stabilization of such an interaction. The intracellular presence of *R. pickettii* affected fitness and induced stress responses in the novel fungal host. Adaptations were observed at the phenotypic, genetic and transcriptional levels, indicating a shift from pathogenic antagonism to commensalism as the interaction progressed. Using high-throughput microscopy and custom-trained deep learning models, we tracked individual spores and quantified fungal growth and host responses. Our work offers insights into early processes of endosymbiosis, highlighting the role of mechanisms that mitigate pathogenicity and promote compatibility in stabilizing these interactions.

P3.228 - Endocytosis in Aspergillus nidulans

M. Pinar[1], *M. Delgado*[1], *P. Polonio*[1], *S. Fandiño*[1], *E. Espeso*[1], *M.A. Penalva*[1]
[1]CSIC Centro de Investigaciones Biologicas Margarita Salas, Madrid, Spain

Endocytic recycling is crucial for the hyphal mode of life. However, the process of endocytic internalization (referred to as endocytosis), which mediates the first step in this pathway, is insufficiently understood. We report a detailed investigation of endocytosis in Aspergillus nidulans, arguably the most thorough analysis ever carried out in a filamentous fungus. Endocytosis occurs at cortical actin patches, reflecting the fact that vesicle internalization is powered by actin polymerization. We have developed an extensive set of fluorescent chimeric reporters of F-actin and endogenously FP-tagged several of the components of the so denoted, by Drubin and Kaksonen, actin polymerization module of endocytosis. We have determined, using kymographs derived from movies acquired with a time resolution of 5 fps, that the time of residence of F-actin in cortical patches is 13 seconds. We have investigated the role of Arp2/3 and the mechanism by which this complex, that gives rise to highly branched actin structures, is seeded with preformed linear actin filaments whose nucleation is formin-independent. We have

studied how actin polymerization in endocytic patches is terminated by the concerted action of capping protein and accessory factors, and how subsequent F-actin severing by cofilin and associates plays a key role in endocytosis. For each and every protein predicted to have a crucial role in endocytic internalization, we demonstrate, using heterokaryon rescue techniques, that ablation of the corresponding gene is lethal. For those genes which play a non-essential role we demonstrate that the extent of their involvement correlates with efficiency of endocytic recycling.

P3.301 - Comprehensive RNA-seq analysis of regulatory genes controlled by KERS complex linked to morphogenesis and secondary metabolism in *Aspergillus flavus*

S. Shabeer[1], *T. Tri Bui*[1], *P. Agarwal*[1], *C. Koon Ho Wong*[2], *O. Bayram*[1]
[1]Maynooth University, Department of Biology, Maynooth, Ireland, [2]University of Macau, Faculty of Health Science, Taipa, Macao, SAR of China

Secondary metabolism in fungi is a crucial process responsible for the production of both harmful and beneficial compounds. Aflatoxins, produced primarily by *Aspergillus flavus* and *Aspergillus parasiticus*, are among the most notable toxic metabolites. These aflatoxins are associated with aspergillosis and various acute and chronic diseases in humans and animals. Their biosynthesis is regulated by complex pathways involving multiple gene clusters. Recently, a novel complex termed KERS, consisting of KdmB, EcoA, RpdA, and SntB, was identified in *A. flavus*. This tetrameric complex regulates genes linked to primary and secondary metabolism. To identify KERS-dependent genes, RNA sequencing of mutants lacking KdmB, EcoA, RpdA, and SntB was performed at 20 and 48 hours, focusing on gene regulation during these metabolic phases. RNA-seq analysis revealed differential expression of 659 genes at 20 hours and 722 genes at 48 hours in the mutants compared to the wild type, with 39 and 29 genes downregulated, respectively. Genes involved in transcription and regulation that were uniquely downregulated are being deleted to investigate their role in aflatoxin production, pathogenicity, and fungal morphology. Uncovering these genes and their potential functions will enhance our understanding of the genetic mechanisms driving secondary metabolism in fungi. Current state of the project will be presented.

P3.302 - Unveiling basidiomycete biosynthetic potential for sustainable chemical production

T. Horch[1]
[1]Technical University of Denmark, The Novo Nordisk Foundation Center for Biosustainability, Lyngby, Denmark

Our heavy dependence on fossil-derived chemicals as the basis of countless products and processes essential to modern life urges the exploration of more sustainable replacements. The specialized metabolism of fungi holds promise for advancing sustainable bio-based chemical production. Cataloguing the fungal chemical and biosynthetic repertoire is crucial to leverage their full potential. Basidiomycetes, a diverse fungal division encompassing both esteemed

culinary mushrooms and notoriously toxic species, remain largely unexplored in terms of their specialized metabolism. A major challenge for genome-based efforts for specialized metabolite discovery from basidiomycete is the lack of both genomic data and efficient bioinformatic tools. Here, we present the genome-guided investigation of basidiomycete specialized metabolism. We developed a bioinformatic tool that facilitates gene-calling, ultimately improving prediction of biosynthetic genes encoded in basidiomycete genomes. This tool supported the comprehensive investigation of biosynthetic potential across a wide range of basidiomycetes. We then sequenced the genomes of select species from metabolically gifted genera and employed a semi-automated workflow to extract and analyze their metabolomes. Through integrative omics, this work offers insight into the chemical repertoire of basidiomycetes and paves the way for heterologous expression-based discovery of previously unknown chemical architectures and enzymatic functions. Ultimately, this work will support the development of sustainable methods for chemical production.

P3.303 - Combination of RNA and ChIP-seq reveals target regulatory genes controlled by the MERCK histone demethylase complex in *Aspergillus nidulans*

P. Agarwal[1], *T. Bui*[1], *S. Shabeer*[1], *K. Kavanagh*[1], *D. Fitzpatrick*[1], *C. Wong*[2], *O. Bayram*[1]
[1]Maynooth University, Biology, Maynooth, Ireland, [2]University of Macau, Health Sciences, Macau, China

Aspergillus spp. contribute significantly to annual food and feed losses through the production of mycotoxins such as aflatoxin B1 (AFB1) and Sterigmatocystin (ST). These mycotoxins, part of a broader group of secondary metabolites (SMs) produced by *Aspergillus spp*., are regulated by molecular mechanisms driven by post-translational modifications.
A five-protein complex known as the 'MERCK' complex was identified and associated with secondary metabolism in both *Aspergillus nidulans* and *Aspergillus flavus*. The absence of this complex results in defects in sexual fruit body formation, sporulation and ST production. To uncover the MERCK complex's role in the regulation of SM biosynthesis in *A. nidulans*, RNA-Seq and ChIP-Seq were used to identify key direct targets of the MERCK complex at the primary metabolism (PM) stage (20h growth) and the SM stage (48h growth) in deletion mutants and wild type (WT).
A comparison of deletion mutants with the wild type identified 214 genes commonly downregulated in at the SM stage across all mutants. The top five most differentially regulated genes with regulatory functions (e.g. transcription factors, chromatin binding proteins, kinases) were selected for further functional characterization. Knockout mutants of these genes show various defects in growth, reproduction and ST production, confirming that the MERCK complex's function is mediated through these targets. Additional data and phenotypic analysis are being carried out. The findings will shed light on the role of this novel conserved chromatin complex in secondary metabolism regulation.

P3.304 - Understanding secondary metabolite production based on biosynthetic gene cluster-specific transcription factor binding targets

L. Fan[1], *S. Guo*[1], *L. Pardeshi*[1], *R. Chen*[1], *K.H. Wong*[1]
[1]University of Macau, Macau, China

Fungi possess remarkable capabilities for synthesizing diverse secondary metabolites (SMs) with significant applications in medicine, agriculture, and industry. SM biosynthetic genes are often organized in clusters on fungal genomes. Genome sequencing has identified many novel SM biosynthetic gene clusters (BGCs) in fungi, representing a rich resource for drug discovery. However, most SM BGCs remain transcriptionally silent under standard laboratory conditions, presenting challenges for their identification. Many BGCs contain a transcription factor gene that activates the biosynthetic genes within the cluster under specific conditions. Whether cluster-specific transcription factors can bind and regulate genes outside their cluster is not clear. We hypothesize that BGC transcription factors may regulate non-BGC genes whose functions could affect the biosynthesis of the intended SM. Therefore, it may be possible to deduce the conditions affecting SM production from its cluster-specific transcription factor target information. To demonstrate this, we conducted Chromatin Immuno-Precipitation followed by Sequencing (ChIP-Seq) to map the genome-wide binding sites of AflR – the well-studied transcription factor controlling the biosynthesis of the harmful mycotoxins sterigmatocystin (ST) and aflatoxin. Our results revealed numerous AflR bindings beyond the ST BGC, exerting control over various physiological processes. More importantly, based on AflR target information, we successfully devised a condition that affects ST production. Taken together, this work provides valuable insights into the regulation of ST and aflatoxin biosynthesis and introduces a novel approach to activate cryptic SM BGCs, enabling the discovery of novel secondary metabolites.

P3.305 - Investigation of biosynthetic gene clusters associated with mycotoxin production in *Penicillium* species isolated from chestnuts

M. Garello[1], *E. Piombo*[2], *S. Prencipe*[1], *F. Buonsenso*[3], *V. Guarnaccia*[1,4], *M. Marcet-Houben*[5], *T. Gabaldon*[5,6], *D. Spadaro*[1,4]
[1]University of Turin, Department of Agricultural, Forest and Food Sciences, Grugliasco, Italy, [2]Swedish University of Agricultural Sciences, Department of Forest Mycology and Plant Pathology, Uppsala Biocenter, Uppsala, Sweden, [3]University of Florence, Department of Chemistry 'Ugo Schiff', Sesto Fiorentino, Italy, [4]University of Turin, Centre of Competence for the Innovation in the Agro-Environmental Sector—AGROINNOVA, Grugliasco, Italy, [5]Barcelona Supercomputing Center - Centro Nacional de Supercomputación, Barcelona, Spain, [6]Institute for Research in Biomedicine, Barcelona, Spain

Due to their relatively high water and starch content, chestnuts represent a favorable substrate for fungal growth. Among the fungi commonly isolated from these sources, *Penicillium* species attract a significant interest, due to their great biosynthetic potential, which also includes several mycotoxins. Enzymes associated with the synthesis of secondary metabolites are usually grouped in discreet loci, called biosynthetic gene clusters (BGCs), but the presence alone does not

guarantee metabolite production. In this work, the genomes of ten *Penicillium* spp., including the newly discovered *Penicillium taurinense*, were sequenced and gene prediction was performed. Data from these species, as well as from twenty publicly available *Penicillium* genomes was parsed to assess the presence of BGCs associated with some of the most common mycotoxins produced by *Penicillium* spp. in chestnuts, in particular verrucosidin, patulin, penitrem A, mycophenolic acid, meleagrin and andrastin A. The production of these mycotoxins was investigated by analytical methods on crude mycelium extracts, in particular by means of HPLC-MS/MS and HPLC-DAD. Results of both bioinformatic and chemical analyses confirmed the presence of BGCs associated with all the investigated metabolites in at least one of the considered *Penicillium* spp., including a previously unreported roquefortine C cluster in *P. taurinense* and *P. nalgiovense*, as well as an andrastin A cluster in *P. taurinense* and *P. flavigenum*. These results provide a better picture of the role of different *Penicillium* spp. in mycotoxin production on chestnuts, as well as the structure of the associated biosynthetic gene clusters.

P3.306 - Cross-kingdom interactions of *Ustilago* and *Pseudomonas* via secondary metabolites

P. Künzel[1], *N. Wierckx*[1]
[1]Forschungszentrum Jülich, Institute of Bio- and Geosciences Biotechnology (IBG-1), Jülich, Germany

Many fungal strains in the *Ustilaginaceae* family secrete itaconic acid (ITA) along with its derivatives, 2-hydroxyparaconate (2-HP) and itatartarate (ITT), under nitrogen limitation. While ITA has well-known antimicrobial properties, the ecological role of its derivatives remain largely unexplored. Notably, the bacterium *Pseudomonas aeruginosa* possesses a specialized ITA metabolic operon with genes essential for tolerance to ITA derivatives, suggesting a molecular arms race within their shared habitat (de Witt *et al*., 2023). Inhibition studies using *Ustilago* supernatants and purified 2-HP and ITT on non-pathogenic *Pseudomonas putida* KT2440, engineered with the ITA operon from *P. aeruginosa* PAO1, identified the uncharacterized putative ring-cleaving dioxygenase Rdo_{PA} (PA0880) as a key mediator of tolerance to the ITA derivatives. Interestingly, the ITA synthesis cluster in several fungal strains, such as *Ustilago maydis* and *Aspergillus terreus* contains an uncharacterized gene with high sequence similarity to *rdo_{PA}* (UMAG_12299, *rdo1*; ATEG_10557, *rdoA*). Complementation studies and biochemical characterization confirm that these evolutionarily distant enzymes share lactonase activity for the enantioselective conversion of (S)-2-HP to (S)-ITT. Combined with the finding that (S)-2-HP inhibits ITA degradation, this conversion clarifies Rdo's physiological role in *Pseudomonas*, while its role in fungi, for which mitochondrial localization is predicted, remains unclear. Future studies will use genetic modifications to adjust fungal production and bacterial degradation of these secondary metabolites, incorporating transporters to regulate metabolic flux. These targeted modifications aim to mimic the dynamic evolutionary interplay within the ecological niche in a synthetic co-culture, advancing our understanding about the rules of interaction.

P3.307 - Overexpression of a truncated HMG coA reductase in *Fusarium fujikuroi*

M.C. Limon[1], *I. Borrego-Serrano*[1,2], *R.M Padilla*[2]
[1]Universidad de Sevilla, Departamento de Genética, Sevilla, Spain, [2]Grupo Agrotecnología S.L., Alicante, Spain

Fusarium fujikuroi is well known for its production of gibberellins, which cause elongation of infected rice plants. Gibberellins are synthesized by the mevalonate pathway, which shares some intermediates with the pathways of other terpenoids such as ergosterol or carotenoids. The first steps of this pathway include that mediated by 3-hydroxy-3-methylglutaryl CoA (HMG coA) reductase, which converts HMG CoA to mevalonic acid (MVA). This enzyme has a regulatory domain located at the amino-terminal end, which is involved in the inhibition of protein activity. Strains overexpressing a truncated version of the *hmgR* gene (OE:*thmgR*) were obtained in the wild-type strain and in a carotenoid-overproducing strain of *F. fujikuroi* IMI 58289. Several candidates were selected based on their gibberellin production in inducing culture media. The best gibberellin producers were selected for further characterization under different culture conditions in flasks. The carotenoid content of mycelia from OE:*thmgR* transformants was not increased compared to those of the parental strains, indicating that either the additional substrate is preferentially diverted to the gibberellin pathway, or that the CarRA enzyme is limiting for carotenoid production.
Expression of the *hmgR* gene and of genes representative of the gibberellin (*cps/ks*) and carotenoid pathways (*carRA*) was analyzed by qRT-PCR in the OE:*thmgR*, OE:*thmgR carS*- and parental strains. Higher levels of *hmgR* mRNA were detected in the OE:t*hmgR* and :*thmgR carS*- strains in comparison to the parental strains while no increase was observed in *carRA* or *cps/ks* mRNAs in the OE:t*hmgR* transformants.

P3.308 - Biosynthetic landscape of Pezizomycotina and its link to fungal lifestyle

A. Pasinato[1,2], *G. Singh*[1,2]
[1]University of Padua, Biology, Padua, Italy, [2]University of Padova, Botanical Garden of Padova, Padua, Italy

Pezizomycotina are prolific producers of secondary metabolites with potential applications in the pharmaceutical, cosmetic, and agrochemical industries. These fungal metabolites are often linked to specific ecological functions, and their biochemical diversity may be shaped by the distinct lifestyles of different fungal classes. Advances in bioinformatics now allow for genome mining, gene identification, and clustering, helping to explore the genetic basis of this diversity.
In this study, we analyzed 139 fungal genomes from six Pezizomycotina classes to identify all polyketide synthase (PKS), terpene, non-ribosomal peptide synthase (NRPS), ribosomally synthesized and post-translationally modified peptide (RiPP), and hybrid biosynthetic gene clusters (BGCs), uncovering 5,647 BGCs.
Our results indicate that lichenized and endophytic fungi possess more BGCs compared to

parasitic/pathogenic and free-living fungi, with a significant enrichment in PKS gene clusters. We further examined the correlation between phylogeny and BGC richness in lichenized fungi by comparing closely related lichenized and non-lichenized fungi from Dothidiomycetes and Eurothiomycetes and found that lichenized fungi in general exhibited similar BGC enrichment to that observed in the largest lichenized fungal class, Lecanoromycetes.
This study emphasize the impact of lifestyle as well as evolutionary lineage on BGC richnessand provide evidence establishing lichens as promising targets for future biotechnological exploration of their secondary metabolite potential.

P3.309 - Biocontrol fungi: pest antagonists or bioactive secondary metabolite factories?

L.V. Lopez Llorca[1]
[1]University of Alicante, Department of Marine Sciences and Applied Biology, Plant Pathology Laboratory, Alicante, Spain

All main fungal taxa include pest antagonists. Nematophagous (NF) and Entomopathogenic Fungi (EF) are good examples. Nematophagous Fungi, for instance, have a key role in nematode biomanagement, especially with parasitic species of agronomic and health importance. In the course of the coevolution with their hosts NF and EF have gained the capacity to synthesize a wide array of secondary metabolites. The NF *Pochonia chlamydosporia* (*Pc)* (Clavicipitaceae) is the main cause of suppressivity of soils to plant parasitic nematodes worldwide. GC/MS-SPME identified a total of 97 VOCs from two EF (*Beauveria bassiana* and *Metarhizium robertsii*) and *Pc*. *Pc* was the largest volatile producer with 2/3 of the VOCs found. Pc VOCs repel plant pests. They block pest attraction to their pheromones or crop hosts under field conditions. Consequently they cause electrophysiological and transcriptomical resposes in their insect targets. Chitosan is a modulator of VOCs production by *Pc*. Fungal age, culture conditions and time of exposure to chitosan influence VOCs production. We have investigated chitosan, as substrate for biofuel production by fungi. In a comparative study, *Pc* was the largest ethanol producer from chitosan. Our studies are a starting point to develop chitin-chitosan based biofuels. Pc is also an antagonist of plant pathogenic fungi. Metabolomics and transcriptomics show the role of secondary metabolites in Pc fungicidal activities. Pc metabolites also have activity vs. infectious diseases, tumors or as anti-inflammatory drugs. *Omics* and Synthetic Biology can help us to exploit Biocontrol Fungi as cell factories for Agro, Health and other Biotechnological applications.

P3.310 - The ecology of penicillin production in *Penicillium chrysogenum*

A. Hatmaker[1], *N. Keller*[2], *B. Wolfe*[3], *N. Gunde-Cimerman*[4], *M. Drott*[1]
[1]USDA-ARS, Cereal Disease Laboratory, Saint Paul, United States, [2]University of Wisconsin, Medical Microbiology & Immunology, Madison, United States, [3]Tufts University, Biology, Medford, United States, [4]University of Ljubljana, Biology, Ljubljana, Slovenia

The filamentous fungus *Penicillium chrysogenum* has impacted human life in tremendous ways through the industrial production of penicillin. However, the natural ecology of the species has not yet been thoroughly studied. To explore the evolution of the penicillin gene cluster and its possible links to the population structure of *P. chrysogenum*, we collected isolates from a diverse array of ecological niches, including both natural and built environments. We generated whole genome sequencing for 80 isolates and downloaded publicly available data for an additional 25 isolates. Some isolates identified as *P. chrysogenum* were instead found to be closely related species such as *Penicillium rubens* upon whole genome sequencing. We assembled genomes and predicted biosynthetic gene clusters of all isolates and identified genetic clusters within *P. chrysogenum* to explore differences in secondary metabolism among genetic populations. We also examined the secondary metabolites produced by a subset of isolates of both *P. chrysogenum* and *P. rubens*. Our work sheds light on the impact of ecology on the evolution of the penicillin gene cluster and production of this important group of antibiotics in natural populations of *P. chrysogenum* and related species.

P3.311 - Saintopin biosynthesis implies a non-canonical polyketide cyclization mechanism

C. Wieder[1,2], *S. Galwas*[2], *R. Wiechert*[3], *K. Seipp*[3], *K. Andresen*[2], *A. Schüffler*[1], *T. Opatz*[3], *E. Thines*[1,2]
[1]Institut für Biotechnologie und Wirkstoff-Forschung gGmBH, Mainz, Germany, [2]Johannes Gutenberg-University, Microbiology and Biotechnology, Mainz, Germany, [3]Johannes Gutenberg-University, Department of Chemistry, Mainz, Germany

In ongoing effort to discover new natural products, we isolated the fungal strain IBWF 003-21, which exhibits vibrant purple-pigmented conidia. We isolated the compound responsible for the coloration and identified it as the tetracyclic polyketide saintopin, a topoisomerase I/II inhibitor first reported in 1990, that has not been investigated much since. The chemical structure of saintopin differs from that of other fungal napthacenediones, implying a divergent biosynthetic mechanism. Genome mining identified six non-reducing polyketide synthases (nrPKS), that were introduced into the heterologous host *Aspergillus oryzae* OP12 for product analysis. Expression of *stpA* led to the production of a tetracyclic pyrone similar to saintopin, making it the prime candidate for saintopin biosynthesis. Deletion of *stpA* in the native producer abolished the production of saintopin, confirming its involvement in saintopin biosynthesis. A metallo-β-lactamase-like thioesterase (*stpB*) and a flavin-dependent monooxygenase (*stpC*), which are canonically required for biosynthesis of fungal napthacenediones did not cluster with *stpA* but were found elsewhere in the genome. Coexpression of the accessory genes led to heterologous production of saintopin in *A. oryzae*, elucidating the biosynthesis. While the exact mechanism for the cyclization of saintopin remains elusive, we provide first evidence of a new cyclization mechanism divergent from that of other fungal napthacenediones.

P3.312 - Combination of RNA and ChIP-seq reveals regulatory genes of fungal morphogenesis and secondary metabolism controlled by the chromatin binding KERS complex in *Aspergillus nidulans*

T.-T. Bui[1], *S. Shabeer*[1], *P. Agarwal*[1], *C.K.H. Wong*[2], *Ö. Bayram*[1]
[1]Maynooth University, Biology, Maynooth, Ireland, [2]University of Macau, Faculty of Health Sciences, Taipa, Macao, SAR of China

The KERS complex is crucial for epigenetic regulation in Aspergillus nidulans, affecting chromatin remodeling, transcription, and secondary metabolite production. It consists of KdmB (a histone demethylase), EcoA (a histone acetyltransferase), RpdA (a histone deacetylase), and SntB (a chromatin-associated protein). To identify genes dependent on the KERS complex, RNA sequencing (RNA-seq) was performed on deletion mutants of kdmB, ecoA, rpdA, and sntB compared to the wild-type strain at 20 hours (primary metabolism, PM) and 48 hours (secondary metabolism, SM) of incubation. Analysis revealed significant changes, with 500 to over 4,000 genes differentially expressed in the mutants. Notably, 204 common genes were differentially regulated during PM, and 545 during SM. Of these, 25 genes were downregulated across all mutants at 20 hours, and 104 were downregulated at 48 hours, highlighting the shared regulatory roles of KERS components in activating secondary metabolism, including mycotoxin Sterigmatocystin production. The top five downregulated regulatory genes, bound by the KERS complex, are being deleted and studied for their roles in development and metabolism. These findings improve our understanding of how the KERS complex coordinates fungal development with secondary metabolite production in *A. nidulans*. Current state of the project will be presented.

P3.313 - Identification of a biosynthetic gene cluster involved in the production of red pigments in *Pseudogymnoascus verrucosus* of Antarctic origin

I. Vaca[1], *D. Palma*[1], *V. Oliva*[1], *V. Ergas*[1], *M. Montanares*[1], *D. Travisany*[2], *P. Villanueva*[3], *R. Chávez*[4]
[1]Universidad de Chile, Facultad de Ciencias, Santiago, Chile, [2]Universidad de Las Américas, Núcleo de Investigación en Data Science, Santiago, Chile, [3]Universidad Andrés Bello, Center for Bioinformatics and Integrative Biology, Santiago, Chile, [4]Universidad de Santiago de Chile USACH, Departamento de Biología, Santiago, Chile

The fungus *Pseudogymnoascus verrucosus* is known for producing red pigments under specific culture conditions, though the chemical nature and biosynthetic origin of these pigments remain unknown. In our laboratory, we have a collection of *P. verrucosus* strains of Antarctic origin which exhibits red pigmentation. This study aimed to identify the biosynthetic origin of these pigments. The genome of a strain of *P. verrucosus* namely FAE27 was sequenced and assembled. The analysis of this genome revealed a biosynthetic gene cluster (BGC) similar to the ankaflavin and azanigerone BGCs from *Monascus pilosus* and *Aspergillus niger*, respectively, which produce red azaphilone-type pigments. To evaluate whether this BCG is responsible for red

pigmentation in *P. verrucosus* FAE27, a gene within this BGC that encodes a non-reducing polyketide synthase, named *azpA*, was inactivated using CRISPR/Cas9 technique. Transformants disrupted in the *azpA* gene exhibited an albino phenotype. Furthermore, chemical analysis of these transformants confirmed the absence of compounds absorbing at 530 nm. These results suggest that the *azpA* gene is essential for red pigment production in *P. verrucosus*. We further confirmed the presence of *azpA* in three additional *P. verrucosus* strains that also exhibit intense red pigmentation by PCR. Notably, RT-PCR analyses showed that *azpA* expression occurred exclusively under pigment-producing conditions in all these strains. In conclusion, our study supports that the identified BGC in *P. verrucosus* FAE27, closely related *to* ankaflavin and azanigerone BGCs, is responsible for the biosynthesis of red pigments in *P. verrucosus*.
This work was funded by FONDECYT project 1211830.

P3.314 - Discovery and biosynthesis of antifungal polyhydroxy-polyketide acrophialocinol

C. Wieder[1,2], *M. Künzer*[1,2], *R. Wiechert*[3], *K. Seipp*[3], *K. Andresen*[2], *A. Schüffler*[1], *T. Opatz*[3], *E. Thines*[1,2]
[1]Institut für Biotechnologie und Wirkstoff-Forschung gGmBH, Mainz, Germany, [2]Johannes Gutenberg-University, Microbiology and Biotechnology, Mainz, Germany, [3]Johannes Gutenberg-University, Department of Chemistry, Mainz, Germany

During ongoing screening efforts for antifungal compounds, *Acrophialophora levis* IBWF 127-08 garnered attention due to the potent antifungal activity of its extracts against a variety of phytopathogenic fungi. Bioactivity-guided isolation led to identification of the main active components as two novel PMA-clade polyhydroxy-polyketides we termed acrophialocinol and its dehydroxylated precursor acrophialocin. Mining the *de novo* sequenced genome of *A. levis* IBWF 127-08 identified a candidate biosynthetic gene cluster *acr*, whose genes were then (co-)expressed in *Aspergillus oryzae* OP12 to heterologously reconstitute the biosynthetic pathway. Metabolite analyses of the transformants revealed that biosynthesis of acrophialocinol may occur via either of the direct precursors acrophialocin or malaysic acid, and involves the hrPKS AcrA, which in turn relies on the *trans*-ER AcrB for alkenyl reduction as well as the truncated NRPS AcrC for hydrolytic product release. While the cytochrome p450 monooxygenase AcrE is required for hydroxylation of a terminal methyl group, α-hydroxylation of the precursors pre-acrophialocin and malaysic acid is catalyzed by the α-ketoglutarate-dependent dioxygenase AcrF, representing an unprecedented reaction in polyhydroxy-polyketide biosynthesis. Furthermore, heterologous expression of the RTA1-like protein coding gene *acrD*, which is conserved across PMA-clade biosynthetic gene clusters, in *A. oryzae* RIB40 conferred resistance to acrophialocin, therefore likely contributing to polyhydroxy-polyketide autoresistance in the native producer.

P3.315 - Characterization of key elements in ochratoxin A biosynthesis in *Aspergillus steynii* through heterologous expression in *Aspergillus nidulans*

S. Rodriguez-Pires[1,2], L. Minguela-Rodriguez[1], B. Patiño[2], J. Gil-Serna[2], E.A Espeso[1]
[1]Centro Investigaciones Biologicas Margarita Salas, CSIC, Cellular and Molecular Biosciences, Madrid, Spain, [2]Universidad Complutense de Madrid, Genetics, Physiology and Microbiology, Madrid, Spain

Aspergillus steynii is one of the main producers of ochratoxin A (OTA), a mycotoxin with significant implications for food safety and causes economic losses worldwide. Although the OTA biosynthesis pathway has been partially elucidated, and despite advances in molecular tools, its regulatory mechanisms remain unknown, limiting the development of effective control strategies. The genes responsible for OTA synthesis are organized in a cluster and are likely regulated by a bZIP-type transcription factor. In this study, two key elements of the OTA biosynthesis cluster in *A. steynii* were investigated using *Aspergillus nidulans* as a model for heterologous protein expression.
Gene replacement at the InuA (inulinase) locus of *A. nidulans* was achieved using PEG/$CaCl_2$-mediated transformation with OTAhal or OTAbZIP from *A. steynii*, and successful insertion was confirmed through PCR genotyping and immunodetection. Additionally, both proteins fused with GFP were visualized via epifluorescence, showing subcellular localization of OTAhal::GFP in the cytoplasm and the OTAbZIP::GFP transcription factor in the nucleus. Furthermore, the ability of the OTA bZIP transcription factor to recognize the native promoter of the *A. steynii* halogenase gene was evaluated. Immunodetection results showed an increase in OTAhal::HA_{3x} when the OTA bZIP factor is present, demonstrating the direct role of the *A. steynii* OTA bZIP transcription factor in the regulation of at least one essential element of the OTA biosynthetic pathway.

P3.316 - Analysis of the ophiobolin biosynthetic gene cluster in Bipolaris maydis

S. Kanzaki[1], K. Yabuta[2], H. Terao[2], K. Onoe[2], K. Tsuji[2], R. Nakamichi[2], Y. Yoshimoto[2], D. Cecilia Ruiz-Nava[3], A. Yoshimi[2,3], M. Miyashita[2], H. Miyagawa[2], M. Kawauchi[2], Y. Honda[2], C. Tanaka[2,3]
[1]Kyoto University, Agriculture, Kyoto, Japan, [2]Kyoto University, Graduate School of Agriculture, Kyoto, Japan, [3]Kyoto University, Graduate School of Global Environmental Studies, Kyoto, Japan

We have previously discovered that a gene-disrupted mutant strain of the plant pathogenic fungus *Bipolaris maydis*, Δ*crz1*, exhibited reduced hyphal growth associated with the premature secretion of ophiobolin A (OA). Crz1 is a transcription factor involved in the calcineurin signaling pathway, which helps regulate stress responses in fungi. Furthermore, through genetic and biochemical analyses using the Δ*crz1* strain, we identified a putative ophiobolin biosynthetic gene cluster (OBGC) and revealed that two genes within the OBGC, *OPH1* (terpene synthase gene) and *OPH2* (cytochrome P450 gene), are essential for OA biosynthesis. However, the

complete OA biosynthetic pathway has not yet been elucidated, and the functions of several genes within the OBGC remain to be fully understood. In this study, we investigated how the other genes within the OBGC (*OPH3–OPH7*) are involved in OA biosynthesis. First, we compared the expression levels of all genes within the OBGC and found that all of them significantly increased in the Δ*crz1* strain compared to the wild-type strain. Next, we attempted to generate double-disrupted mutants for each gene using the Δ*crz1* strain as a parental strain. As a result, we successfully generated double-disrupted mutants of all genes except for the transcription factor-encoding gene *OPH3*. Moreover, the expression levels of all genes within the OBGC, except *OPH3*, decreased in the Δ*crz1*Δ*oph4* mutant strain, suggesting that Oph4 is involved in the regulation of gene expression within the OBGC. We are currently performing quantitative analyses of ophiobolins in each double-disrupted mutant using LC/MS.

P3.317 - LaeA is a positive regulator of the biosynthesis of relevant specialized metabolites in *Penicillium roqueforti*

R. Chávez[1], *Y. Marcano*[1], *M. Montanares*[2], *C. Gil-Durán*[1], *K. González*[1], *I. Vaca*[2]
[1]Universidad de Santiago de Chile (USACH), Facultad de Química y Biología, Santiago, Chile,
[2]Universidad de Chile, Facultad de Ciencias, Santiago, Chile

Penicillium roqueforti is an important producer of specialized metabolites with potential biotechnological applications, as well as mycotoxins, some of which are present in cheeses ripened with this fungus. In recent years, significant progress has been made in elucidating the genetic basis of specialized metabolism in *P. roqueforti*. However, key aspects, such as the regulatory mechanisms governing the biosynthesis of these metabolites, remain not fully understood. In this study, we investigated the role of *laeA*, a gene encoding a methyltransferase known to be a global regulator of fungal specialized metabolism, in the biosynthesis of relevant metabolites in *P. roqueforti*. To achieve this, we used a CRISPR/Cas9 system to generate *laeA*-disrupted mutants, and, additionally, produced mutants overexpressing *laeA*. We then evaluated the impact of *laeA* disruption and overexpression on the production of important metabolites in *P. roqueforti*, specifically roquefortine C, mycophenolic acid, and andrastin A. Our results indicated that *laeA* disruption caused a significant reduction in the production of these metabolites, while *laeA* overexpression led to enhanced biosynthesis compared to the wild-type strain. These results were supported by the analysis of relative transcription levels of key genes within the biosynthetic gene clusters responsible for the production of roquefortine C, mycophenolic acid, and andrastin A. In conclusion, LaeA acts as a positive regulator of the biosynthesis of roquefortine C, mycophenolic acid, and andrastin A in *P. roqueforti*.
This research was funded by grants ANID—FONDECYT N°1211832, DICYT “código 022343CR_Ayudante” from Vicerrectoría de Investigación, Desarrollo e Innovación, Universidad de Santiago de Chile.

P3.318 - Activation of cryptic biosynthetic gene clusters through genetic manipulation of global regulator mcrA in Penicillium expansum

A. LaCorte[1], *J. Shyong*[1], *S. Dziedzic*[1], *C. Wang*[1,2]
[1]University of Southern California, Department of Pharmacology and Pharmaceutical Sciences, Los Angeles, United States, [2]University of Southern California, Department of Chemistry, Los Angeles, United States

Fungal secondary metabolites (SMs) are organic compounds produced by fungi that are not essential for survival, but provide ecological advantages against other microbes. Due to the potency of these SMs used for defense and the complexity of the compound structures, many metabolites often have biological activities beneficial to humans. Previous studies have demonstrated that SMs are produced by groups of genes working together, forming biosynthetic gene clusters (BGCs); however, because many BGCs are silent under normal laboratory conditions, additional SMs have yet to be discovered and tested for potential bioactivity. Targeting a negative global regulator of fungal secondary metabolism, multicluster regulator A (mcrA), will allow for the upregulation of SMs and the possibility for discovery of new natural products.
Using a combinational approach of one-strain-many-compounds (OSMAC) and genetic manipulation via the CRISPR Cas9 genome editing system, we aimed to knock out mcrA in Penicillium expansum (IMV00074). Successful transformation of the strain resulted in the activation of multiple silent BGCs in various culture conditions as shown in our liquid chromatography-mass spectroscopy (LC-MS) analysis. This demonstrated the emergence of new compounds and the upregulation of others in the mcrAΔ strain, creating a natural product library, on which we will perform high throughput screening for novel bioactive properties.

P3.319 - Activation of a sorbicillinoid pathway through deletion of mcrA in Penicillium rubens YAP001

S. Dziedzic[1,2], *J. Shyong*[1], *T.Q.D. Huynh*[3], *C.-K. Lee*[3,4], *C. Kenkel*[5], *C. Wang*[1,2]
[1]University of Southern California, Department of Pharmacology and Pharmaceutical Sciences, Los Angeles, United States, [2]University of Southern California, Department of Chemistry, Los Angeles, United States, [3]Taipei Medical University, School of Pharmacy, Taipei, Taiwan, China , [4]Taipei Medical University, Ph. D Program in Clinical Drug Development of Herbal Medicine, Taipei, Taiwan, China , [5]University of Southern California, Department of Biological Sciences, Los Angeles, United States

Fungal secondary metabolites (SMs) are complex organic compounds comprising a variety of biological activities essential in medicine. Due to the immense potential of SMs to be solutions to currently incurable diseases, it is essential to explore new avenues for upregulation of SMs known to provide useful bioactive properties. In particular, the discovery of sorbicillinoids has led to the detection of compounds containing various bioactivities against human pathogens as well as identification of cytotoxic effects providing potential as an anticancer agent. However,

due to the low yield of the production of fungal SMs, we are in pursuit of novel approaches to upregulate the pathways of these valuable metabolites.
Analysis of the fungal genome has established that genes responsible for producing SMs are clustered together, forming biosynthetic gene clusters (BGCs). One strategy for activating BGCs with the objective of upregulation in SM production is through the deletion of the negative global regulator of secondary metabolism, *mcrA*. Previous literature has shown that deletion of *mcrA* in various fungal strains has resulted in stunningly different metabolic profiles with compounds upregulated in the mutated strain. Upon generating a *mcrAΔ* strain in *Penicillium rubens* (YAP001), we found that sorbicillin was not only upregulated, but the mutant strain also produced the dimeric product, trichodimerol, which often exhibits stronger biological activities compared to sorbicillin. This suggests that genetic manipulation of global regulators in filamentous fungi is an effective technique in activating pathways of interest for the purpose of drug discovery.

P3.320 - Identification of metabolites from a bacterium isolated from sugar beet leaves that inhibits the fungus *Cercospora beticola*

D. Back[1], *M. Christenson*[2], *M. Natwick*[2], *M. Bolton*[1]
[1]USDA, Sugar Beet Research Unit, Fargo, United States, [2]North Dakota State University, Plant Pathology, Fargo, United States

Burkholderia is a genus of bacteria that has gained popularity for its potential uses in agriculture due to its production of a variety of biologically potent secondary metabolites. In this study, we have isolated and identified a strain of *Burkholderia contaminans* from sugar beet leaves that inhibits the fungus *Cercospora beticola*, the causal agent of cercospora leaf spot on sugar beet leaves. The goal of this study is to identify small molecule secondary metabolites from *B. contaminans* that play a role in the inhibitory effects of *B. contaminans* against *C. beticola*. Utilizing HPLC and LCMS analyses, we have identified secondary metabolites, such as the pyochelins, from the culture extract of *B. contaminans*. Additionally, we noticed that the inhibitory effects of *B. contaminans* is increased when *B. contaminans* is grown as a co-culture with *C. beticola*. Results from our chemical analyses show that the production of the pyochelins is upregulated when grown as a co-culture making these compounds candidates for the observed inhibition of *B. contaminans* against *C. beticola*.

P3.321 - Genetic manipulation of global regulator mcrA in Penicillium rubens to generate a natural product library for high throughput bioactivity screening

T. Millam[1], *C. Wang*[1], *J. Shyong*[1]
[1]University of Southern California, Pharmacology, Los Angeles, United States

Fungal secondary metabolites (SMs) are diverse organic compounds often associated with medicinal properties that have changed the landscape of medicine. These SMs include the antibiotic, penicillin, and the cholesterol-lowering agent, lovastatin. Through genome sequencing

and analysis, it has been shown that natural products are encoded by biosynthetic gene clusters (BGCs) in the fungal genome. Because most BGCs are silent under normal laboratory conditions, it is crucial to find new ways to activate them. Doing so will allow for the discovery of compounds with bioactivity with the potential to have anticancer, antibacterial, and antifungal effects.
Our strain of focus, Penicillium rubens (IMV00188), is part of the Institute of Microbiology Virology collection collected from the Chernobyl nuclear power plant and the surrounding areas. Using the CRISPR-Cas9 genome editing system, we knocked out the negative global regulator, mcrA, which typically suppresses multiple biosynthetic pathways. We confirmed the deletion of mcrA by designing sequencing primers to perform diagnostic PCR and comparing the growth of wild-type and mcrA knockout strains under various culture conditions. Upon analysis of SM production with liquid chromatography-mass spectroscopy (LC-MS) procedures, we found that our mutant strain activates various pathways otherwise not seen in the wild-type strain. Ultimately, we aim to generate a natural product library and use high throughput screening to investigate the bioactive properties of these upregulated and newly produced compounds.

P3.322 - Enhancing secondary metabolite production in Penicillium camemberi through genetic manipulation of global regulator mcrA for high-throughput screening

A. Shih[1], *J. Shyong*[1], *S. Dziedzic*[1], *C. Wang*[2,1]
[1]Alfred E. Mann School of Pharmacy and Pharmaceutical Sciences, University of Southern California, Pharmacology and Pharmaceutical Sciences, Los Angeles, United States, [2]Dornsife College of Letters, Arts, and Sciences, University of Southern California, Chemistry, Los Angeles, United States

Fungal secondary metabolites (SMs) are complex organic compounds of ranging biological activity with potential applications in medicine, industry, and agriculture[1]. Prime examples of these compounds include penicillin, aflatoxin, and lovastatin. These secondary metabolites are encoded by biosynthetic gene clusters (BGCs) most of which are silent under normal laboratory conditions. We are constantly exploring new biological methods to activate silent BGCs because SMs have great potential for application in drug development due to their various bioactive characteristics. For instance, Penicillium has been known to produce a wide array of SM compounds including mycotoxins, immunosuppressants, and cholesterol reducing agents[2]. The Wang lab has previously shown that deletion of mcrA, a negative global regulator of secondary metabolism, can activate normally silenced BGCs [3]. The focus of this project was to knockout mcrA in Penicillium camemberti (IMV00769) and generate a new metabolic profile for discovery of new compounds. We used the CRISPR-Cas9 genome editing system to knockout mcrA and confirmed the knockout with diagnostic PCR and growth on selective plates. Then, we grew the wild-type (WT) and mcrA knockout (mcrAΔ) strains in various conditions and extracted them using ethyl acetate. These extracts were analyzed with Liquid Chromatography– Mass Spectrometry (LCMS) to generate metabolic profiles for the WT and mcrAΔ strains. The mcrAΔ strain produced vastly different metabolic profiles compared to the WT in multiple tested conditions. Ultimately, the different metabolites produced by the mcrAΔ strain will be further isolated and tested for potential pharmacological and industrial application.

P3.323 - Extraction and derivatization of chaetoglobosin A from *Chaetomium globosum* for anti-cancer therapy

P.F. Chiu[1], *S. Long*[1], *C.C. Wang*[1,2]
[1]University of Southern California, Pharmacology & Pharmaceutical Sciences, Los Angeles, United States, [2]University of Southern California, Chemistry, Los Angeles, United States

Chaetoglobosin A (Ch A), which consists of (indol-3-yl)methyl, perhydroisoindolone, and macrocyclic ring moieties, is a secondary metabolite derived from *Chaetomium globosum* (*C. globosum*). Ch A is known for inhibiting actin polymerization and disrupting cell division. It has been shown to inhibit the growth of blood cancer cells, PC-3, HCT116, and MDA-MB-231 cell lines, highlighting its potential for clinical application. However, Ch A lacks selectivity, leading to acute toxicity in the spleen and thymus, as well as liver and kidney damage. To address the issue of poor selectivity, conjugating Ch A with a targeting moiety offers a promising solution. In this study, we optimized the production of Ch A by culturing wild-type *C. globosum* in various media. The yield of Ch A increased from 2 mg/L in potato dextrose agar plates to 54 mg/L in oatmeal agar plates when cultured at 25°C for 12 days in the dark. Moving forward, we plan to conjugate Ch A with an antibody using biodegradable or stable linkers to form an antibody-drug conjugate, with the aim of improving Ch A's safety profile and enhancing its clinical potential.

P3.324 - RIPPing it out! Whole-genome investigation uncovers RIPP hidden diversity in lichens

A. Pasinato[1,2], *G. Singh*[1,2]
[1]University of Padova, Department of Biology, Padua, Italy, [2]University of Padova, Botanical Garden of Padova, Padua, Italy

The lichen biosynthetic landscape, like that of other non-symbiotic fungi, is primarily composed of polyketide, non-ribosomal peptide, and terpene biosynthetic pathways. Recent studies show that ribosomally synthesized and post-translationally modified peptides (RiPPs) make up a significant portion (10-14%) of this biosynthetic space in non-lichenized fungi. While RiPP biosynthetic pathways are better understood in model fungi, the diversity and distribution of these BGCs in lichenized fungi remains unknown. RiPPs stand out for their promising bioactive properties and potential pharmaceutical applications.
In this study, we conducted the first systematic identification and comparison of RiPP BGCs in lichenized fungi using 111 genomes. We combined BGC clustering and phylogenetic analyses to trace the evolution of homologous RiPP clusters across different taxa. We found that RiPPs account for approximately 15% of lichenized fungal BGCs, consistent with non-lichenized fungi. Notably, we identified two conserved RiPP clans within the Parmeliaceae family, with UstYa/Yb homologs as core genes, suggesting their link to fungal dikaritins, which are predominantly mycotoxins. Our results indicate that taxon-specific RiPP BGCs are common in lichenized fungi, and that their putative products differ from those in non-lichenized fungi, potentially encoding for novel bioactive compounds.

This study lays the groundwork for further exploration of these biologically intriguing yet understudied compounds by mapping their biosynthetic clusters and evolutionary relationships.

P3.325 - A role for melanin and perylene quinones for abiotic and biotic stress tolerance

J. Gao[1], *R. Fischer*[2]
[1]Karlsruhe Institute of Technology, Institute for Applied Biosciences, Department of Microbiology, Karlsruhe, Germany, [2]Karlsruhe Institute of Technology, Institute for Applied Biosciences, Department of Microbiology, Karlsruhe Institute of Technology, Germany

The black mold *Alternaria alternata* is one of the most widespread contaminants of food and feed, and a weak plant pathogen. It produces a large diversity of secondary metabolites with the phyto- and mycotoxins perylene quinones (PQs) as prominent examples. We discovered recently that the PQ altertoxin (ATX) biosynthesis shares most enzymes with the DHN-melanin pathway[1]. However, melanin is formed in aerial hyphae and spores, but ATXs are synthesized in substrate hyphae. Furthermore, we proved that 1,8-DHN is the last common intermediate required for DHN-melanin and ATXs formation. The enzyme dimerizing 1,8-DHN to ATXs remained unknown. To identify the dimerization enzyme encoding gene, we performed genome-wide expression analyses with different mutant strains producing much more or much less ATXs as compared to the wild type. A small gene cluster was discovered where the expression in the different mutants correlated well with the amount of ATXs formed. The secondary metabolite profiles of gene knock-out mutants generated by CRISPR/Cas9 suggested a key role of this gene cluster in ATXs biosynthesis. HRMS and MS/HRMS analyses to reveal how 1,8-DHN is dimerized to form ATXs are on the way. Furthermore, co-incubation of *A. alternata* strains with *Aspergillus nidulans* and *Penicillium species*, proved that ATXs help *A. alternata* to compete with other fungi. In summary, this study revealed that *A. alternata* uses two gene clusters plus several genes scattered in the genome to produce DHN-melanin and PQs and that both products are important to cope with the environmental stress UV light and competing fungi.

P3.326 - What are the real products of BGCs? Activation of silent fungal biosynthetic gene yields different metabolites than heterologous expression

I. Burger[1], *K. Peikert*[1], *S. Leonhartsberger*[1], *P. Atanasova*[1], *L. Fourtis*[1], *R. Fried*[1], *M. Schmal*[1], *C. Suster*[1], *C. Stanetty*[1], *D. Schnalzer*[1], *R. Birner-Gruenberger*[1], *R.L. Mach*[1], *A.R. Mach-Aigner*[1], *F. Rudroff*[1], *M. Schittmayer*[1], *C. Zimmermann*[1]
[1]TU Wien, Vienna, Austria

Secondary metabolites are derived from biosynthetic gene clusters (BGCs), many of which remain silent under standard laboratory conditions. Two common strategies are used to study these silent BGCs: heterologous expression in a host or activation within the native fungus. We activated the silent BGCs responsible for ilicicolin H and dichlorodiaporthin in the industrially important ascomycete *Trichoderma reesei*, both of which had previously been studied using

heterologous expression.
Upon comparing our results with the literature, we identified significant differences. For ilicicolin H, we discovered a novel derivative, ilicicolin K, which shows antifungal activity against *Candida auris*. Regarding the dichlorodiaporthin BGC, we identified the true final product, which had not been detected in earlier heterologous expression studies, and revealed that an enzyme plays a different role in the native host compared to heterologous expression. Additionally, we observed that many side-products reported in the literature are shunt products, likely arising from host enzyme activity or imbalanced enzyme levels.
Heterologous expression is generally regarded as a more reliable method for linking metabolites to their respective BGCs. However, our findings suggest that heterologous expression provides only part of the picture. To fully understand a BGC, we recommend employing both heterologous expression and native activation strategies in parallel.

P3.327 - Metabolic engineering of *Aspergillus violaceofuscus*

C. Wieder[1,2], *C. Laubenstein*[1,2], *L. Fischer*[2], *R. Wiechert*[3], *K. Seipp*[3], *A. Schüffler*[1], *T. Opatz*[3], *E. Thines*[1,2]
[1]Institut für Biotechnologie und Wirkstoff-Forschung gGmbH, Mainz, Germany, [2]Johannes Gutenberg-University, Microbiology and Biotechnology, Mainz, Germany, [3]Johannes Gutenberg-University, Department of Chemistry, Mainz, Germany

Fungal secondary metabolites (SMs) are a rich source for bioactive compounds with various applications in agriculture, biotechnology and medicine. However, discovering novel compounds with desirable bioactivities remains challenging as numerous gene clusters involved in their synthesis remain silent under laboratory conditions. *Aspergillus violaceofuscus* is a fungus with significant biosynthetic potential, containing over 90 predicted biosynthetic gene clusters (BGCs), albeit only few metabolites are produced under standard culturing conditions. In this study, we aimed to engineer the secondary metabolism of *A. violaceofuscus* by creating genetic dereplication strains with reduced metabolic background and overexpressing cryptic biosynthetic genes. We were able to establish an efficient Cas9-mediated microhomology-directed repair (MHDR) protocol, achieving integration rates of up to 90 % and successfully deleted the core genes encoding for the biosynthesis of the major metabolites eupenoxide and himeic acid A. Homologous overexpression of a cryptic NRPS-like coding gene led to the production of a new compound we termed violafuranone A. In conclusion, we demonstrate metabolic manipulation of *A. violaceofuscus* and the potential of this species as a source for novel SMs. These findings pave the way for further investigations into the secondary metabolism of *A. violaceofuscus*.

P3.328 - An environmental isolate of *Pseudomonas*, 20EI1, reduces *Aspergillus flavus* growth in an iron-dependent manner and alters secondary metabolism

A.M Calvo[1], *E.M Wyman*[2], *S. Grayburn*[1], *M.K Gilbert*[2], *M.D Lebar*[2], *J.M Lohmar*[2], *J.W Cary*[2], *T.J. Sauters*[3], *A. Rokas*[3]
[1]Northern Illinois University, Dekalb, United States, [2]USDA-ARS, New Orleans, United States, [3]Vanderbilt University, Nashville, United States

Aspergillus flavus is an opportunistic pathogenic fungus that infects oilseed crops worldwide. When colonizing the plants, it produces mycotoxins, including carcinogenic compounds such as aflatoxins. Mycotoxin contamination results in an important economic and health impact. The design of new strategies to control *A. flavus* colonization and mycotoxin contamination is paramount. In this study, we identified a promising new isolate of *Pseudomonas* spp., 20EI1, and observed that it is able to reduce the growth of *A. flavus*. Furthermore, we determined that this growth inhibition is iron-dependent. To further elucidate the nature of this bacterial-fungus interaction, we performed chemical and transcriptomics analyses. In the present study, *Pseudomonas* 20EI1 reduced or blocked the production of aflatoxin, as well as cyclopiazonic acid and kojic acid. Expression of iron-related genes was altered in the presence of the bacteria and genes involved in the production of aflatoxin were down-regulated. Iron supplementation partially reestablished their expression. Expression of other secondary metabolite (SM) genes was also reduced by the bacteria, including genes of clusters involved in cyclopiazonic acid, kojic acid and imizoquin biosynthesis, while genes of the cluster corresponding to aspergillicin, a siderophore, were upregulated. Interestingly, the global SM regulatory gene *mtfA* was significantly upregulated by 20EI1, which could have contributed to the observed alterations in SM. Our results suggest that *Pseudomonas* 20EI1 is a promising biocontrol against *A. flavus*, and provide further insight into this bacterial-fungal interaction affecting the expression of numerous genes, among them those involved in SM.

P3.401 - Three [2Fe-2S] cluster-binding regions regulate the functional transitions of the *Aspergillus fumigatus* iron regulator HapX for adaptation to iron starvation, sufficiency and excess

S. Oberegger[1], *M. Misslinger*[1], *H. Haas*[1]
[1]Institute of Molecular Biology, Medical University Innsbruck, Biocenter, Innsbruck, Austria

Accurate sensing of cellular iron levels is vital, as this metal is essential but toxic in excess. The iron-sensing transcription factor HapX is crucial for virulence of *Aspergillus fumigatus*, the predominant human mold pathogen. Its absence impairs growth under iron limitation and excess, but not under moderate iron availability, suggesting that HapX switches between three states to adapt to varying iron availability. Previous studies have shown that HapX senses iron excess by [2Fe-2S] cluster binding to three phylogenetically conserved cysteine-rich regions (CRRs), termed CRR-A, -B, and -C.
This study suggests that the HapX state transitions are regulated by the different propensities of the CRRs to coordinate [2Fe-2S] clusters resulting in cumulative occupancies that depend on iron availability. In the iron starvation state, CRR-B and -C lack [2Fe-2S] clusters, the iron sufficiency/neutral state features clusters in CRR-B and/or -C and the iron excess state has clusters in all CRRs. Combinatorial mutation of CRR-B and -C caused synthetic lethality by locking HapX in the iron starvation state, leading to uncontrolled iron uptake, iron accumulation, repression of iron-consuming pathways and impaired iron detoxification. Loss of the C-terminal 27-amino acid region of HapX, which is crucial for the iron starvation state and was found to contain an F-box protein Fbx22-binding degron, rescued the synthetic lethality.

P3.402 - A Fusarium graminearum protein interaction network of trichothecene biosynthesis pathways

G. Subramaniam[1]
[1]Agriculture Canada, Ottawa, Canada

Activation of biosynthetic gene clusters (BGCs) in fungi requires coordination and integration of many environmental cues. As an example, *Fusarium graminearum*, the main causal agent of the Fusarium head blight disease (FHB) in wheat produces an array of secondary metabolites including the mycotoxin deoxynivalenol (DON) that are regulated by carbon and nitrogen sources and affected by the physiological status of the cell. To understand the contribution of many input sources that regulate BGC in *F. graminearum*, we undertook a protein interaction network analysis in yeast of ~ 300 *F. graminearum* proteins involved directly or indirectly in the regulation of DON. The resulting network named Fusarium Network of Trichothecene Associated Proteins (FuNTAP) exhibited properties of a small-world network with protein hubs that regulated biosynthetic pathways other than DON. Corroborative evidence was obtained from genetic analysis and metabolomic profiling of major hubs. Finally, the expression of one of the major hubs tagged with TurboID in the fungus validated our supposition that major hubs exert their control by interacting with various proteins, temporally and spatially.

P3.403 - Redox regulation of proteins through reversible cysteine oxidation in *Neurospora crassa* during cellulose degradation and anaerobiosis

L. Bidondo[1], *J.-C. Gaillard*[2], *E. Drula*[1], *J. Armengaud*[2], *J.-G. Berrin*[1], *M.-N. Rosso*[3], *L. Tarrago*[1]
[1]Aix-Marseille University/Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, Fungal Biodiversity and Biotechnology, Marseille, France, [2]Université Paris-Saclay/Commissariat à l'Énergie Atomique et aux Énergies Alternatives, Département Médicaments et Technologies pour la Santé, Bagnols-sur-Cèze, France, [3]Aix-Marseille University/Institut National de recherche pour l'Agriculture, l'Alimentation et l'Environnement, Fungal Biodiversity and Biotechnology, Marseille, France

Reactive oxygen species (ROS) can act as signaling molecules by oxidizing cysteine residues to disulfide bonds. Redox signaling was shown to alter enzyme activity, control protein localization, or regulate transcription factors in many organisms. However, little is known about the role of cysteine oxidation in fungi. Fungi obtain carbon by degrading plant cell walls in a process involving ROS production. Moreover, environmental constraints can also induce variations in ROS levels, such as exposure to anaerobiosis. Our research aims to identify proteins in *Neurospora crassa* that undergo reversible cysteine oxidation during cellulose degradation and under varying environmental conditions, to determine their role in fungal adaptation. Thioredoxins (Trx) are thiol oxidoreductases that play crucial roles in ROS response by reducing disulfide bonds in redox-regulated proteins in many organisms. Their mechanism involves a transient intermolecular disulfide bond with their targets. We exploited this mechanism by combining Trx affinity chromatography with label-free shotgun proteomics to identify potential

redox-regulated proteins from *N. crassa* grown on cellulose. We identified 1,938 proteins, including key metabolic enzymes and proteins potentially involved in signaling like kinase and transcription factors. Notably, two enzymes involved in heme synthesis, the 5-aminolevulinate synthase and the coproporphyrinogen-III oxidase, emerged as top candidates. Interestingly, these two enzymes were also highly abundant during exposure to anaerobiosis as we showed by comparative proteomics. These results suggest that these enzymes may be redox-regulated by ROS induced during environmental constraints. We are currently investigating the role of Cys-oxidation on the activity of these enzymes.

P3.404 - The role of HFB1 in the mate recognition and sexual reproduction initiation in *Trichoderma reesei*

F. Yi[1], *M. Ding*[2], *G. Pang*[2], *I. Druzhinina*[3], *F. Cai*[1]
[1]Sun Yat-sen University, School of Ecology, Shenzhen, China, [2]Nanjing Agricultural University, Jiangsu Provincial Key Lab for Solid Organic Waste Utilization, Nanjing, China, [3]Royal Botanic Gardens, Kew, Richmond, United Kingdom

Sexual reproduction is a main driving force for genetic diversity. *Trichoderma reesei*, which belongs to one of the most well-studied model filamentous fungi for its significant industrial value in enzyme production, represents a typical bipolar heterothallic ascomycete. However, despite its significance, numerous unresolved mysteries persist regarding its molecular mechanism of sexual reproduction. Our preliminary results identified a signaling molecule, HFB1, from the extracellular proteins of *T. reesei*. This molecule is essential for initiating sexual reproduction and the "female fertility" of MAT1-1 mating type. Phenotypic profile of gene deletion mutants indicated a crucial role of HFB1 in maintaining the female fertility of the MAT1-1 mating type. Results from *in situ* fluorescently tagged strains and RNA-seq analysis demonstrated that HFB1 is involved in the early process of mating partner recognition and is primarily secreted by the MAT1-1 partner and sensed by the MAT1-2 type. Importantly, we also observed that, without the HFB1 signal provided by the MAT1-1 type, the transcription of the "sexual determination gene" *mat1-2-1* is abolished, resulting in an insufficient response such as significantly reduced pheromone secretion from the opposite mating type. Therefore, HFB1 is suggested a novel cell communication factor upstream of the pheromone signaling system, in which the intracellular signal transduction cascades and key regulatory elements await discovery.

P3.405 - EOP-1: a key protein for cell-cell interaction with an AIM24-like domain and an extended IDR that might drive droplet formation

L. Strzelczyk[1], *A. Oostlander*[1], *J. Grzegowski*[1], *M. Schumann*[1], *U. Brandt*[1], *A. Fleißner*[1]
[1]Technische Universität Braunschweig, Institute of Genetics, Braunschweig, Germany

Cell fusion is essential for eukaryotic development, yet its molecular mechanisms remain poorly understood. Our research focuses on *Neurospora crassa*, a well-established model organism for studying this process.
During colony formation, genetically identical germinating spores undergo chemotropic

interactions and fuse to establish a mycelial colony. The proteins SO and MAK-2 exhibit alternating and opposing membrane recruitment patterns in the interacting germlings, suggesting a dialogue-like dynamic, though the underlying mechanisms driving this recruitment remain unclear.
We have identified EOP-1 as an interaction partner of SO. Notably, *eop-1* gene deletion mutants are unable to undergo chemotropic interactions. Furthermore, EOP-1 dynamically colocalizes with SO at the plasma membrane of germling tips. Protein structure predictions suggest that EOP-1 contains an unstructured domain as well as an AIM24-like domain similar to the *Streptococcus pyogenes* protein SPYM3_0169 (RMSD 1.627 Å).
To study the function of the AIM24-like domain, we generated EOP-1Δα-helix mutants that lack the C-terminal α-helix. This truncation results in an altered EOP-1 localization along the entire plasma membrane. Although EOP-1Δα-helix localizes to tips upon germling contact, fusion was unsuccessful, leading to enlarged contact points, mirroring phenotypes of the ergosterol biosynthesis mutant Δ*erg-2*. Our ongoing research aims to compare changes in contact recognition in both EOP-1Δα-helix and Δ*erg-2* mutants, including SO and MAK-1 recruitment and activation.
We propose that EOP-1's unstructured domain facilitates droplet formation, which may explain its rapid recruitment to the plasma membrane during cell-cell interactions. Preliminary data support this droplet-promoting function and further studies will investigate its role in cell-cell interactions.

P3.406 - Acidification by germlings initiates inter- and intraspecies conidial anastomosis tube formation

A. Rudolph[1], *N. Rotermund*[2], *A. Fleissner*[2], *D. Nordzieke*[1]
[1]Georg-August University Göttingen, Genetics of Eukaryotic Microorganisms, Göttingen, Germany, [2]University Braunschweig, Fungal Genetics, Braunschweig, Germany

Conidial anastomosis tube (CAT) fusion is a conserved feature in many ascomycete species. There are indications that network formation by CATs increases the fitness of the young forming colony. In addition, the induction of pathogenicity programs and CAT fusions are interwoven processes. The fungus *Colletotrichum graminicola* produces two types of asexual spores, falcate and oval shaped conidia, of which only oval conidia can undergo CAT fusion. Although there is increasing evidence that conserved signals mediates fungal germling attraction also in interspecies interactions, the responsible attractants are unknown.
For the identification of the germling attractant signals, we analyzed secretome harvested during *C. graminicola* germling fusion. Comparison of samples generated by fusion-competent oval and fusion-incompetent falcate conidia shows that pH values of oval conidia samples are more acidic. Germling fusion assays indeed showed that efficient network formation is dependent on pH. By the application of a 3D printed device for the analysis of chemotropic growth, we further identified distinct pH values as chemoattractants for *C. graminicola* germlings whose perception is mediated by the G-protein coupled receptor (GPCR) CgSte3. Moreover, we showed that signal transduction requires the cell wall integrity (CWI) pathway scaffold CgSo, which homologs are essential for germling fusion in other fungi. Similar to *C. graminicola*, *B. cinerea* and *N. crassa*

intraspecies interactions are pH-dependent. Together our findings suggest that acidification by germlings is a conserved prerequisite for CAT fusion in different fungal species.

P3.407 - Role of the red-light and blue-light photoreceptors in the photoadaptation of transcription in *Aspergillus nidulans*

A. Franco-Cano[1], *G. Gutiérrez*[1], *L.M. Corrochano*[1], *D. Cánovas*[1]
[1]University of Seville, Department of Genetics, Seville, Spain

Fungal responses to light include the regulation of the developmental programs and the regulation of secondary metabolism, among others. The responses to light are mediated by induction of gene expression. However, this induction is transient, and gene expression returns to dark levels in a process known as photoadaptation. *In Aspergillus nidulans*, light sensing requires both the red photoreceptor, the phytochrome FphA, the blue photoreceptors, LreA and LreB, and the UV/blue photoreceptor CryA. Although the *Aspergillus* genomes lack a homolog of *vvd*, the gene responsible for photoadaptation in *Neurospora crassa*, photoadaptation is still observed in *A. nidulans*, suggesting the existence of an alternative regulatory mechanism. To study photoadaptation in A. *nidulans*, the wild-type and photoreceptor mutant strains were exposed to light for different times ranging from 15 to 240 min. RNAseq experiments show that light regulates over 20% of the genome. Clustering analysis of light-regulated genes resulted in six regulatory profiles, which were similar in the wild type and the Δ*lreA* Δ*lreB* mutant, but not in the Δ*fphA* strain. The deletion of *fphA* led to a reduction in the number of light-regulated genes (from 2596 to 1680) with a transcriptional pattern very different to that observed in the wild type. Gene onthology revealed a sequential rewiring of gene expression: metabolic changes come first, possibly due to mitochondrial stress, followed by nucleic acid modification and repair. Based on the expression profiles, our results suggest the existence of novel regulators that could be responsible of photoadaptation in *Aspergillus*.

P3.408 - N-acetylglucosamine sensing in Trichoderma reesei

S.F. Ullah[1], *M. Oreb*[2], *E. Boles*[2], *V. Srivastava*[1], *V. Seidl-Seiboth*[3], *B. Seiboth*[3], *L. Kappel*[1]
[1]KTH Royal Institute of Technology, Division of Glycoscience, Department of Chemistry, Stockholm, Sweden, [2]Goethe University, Institute of Molecular Biosciences, Faculty of Biological Sciences, Frankfurt, Germany, [3]TU Wien, Research Division Biochemical Technology, Institute of Chemical, Environmental and Bioscience Engineering, Vienna, Austria

N-acetylglucosamine (GlcNAc), the monomeric subunit of chitin and glucosaminoglycans, is involved in diverse signalling pathways in dimorphic yeasts and bacteria, where it is related to morphogenetic switching, mating, stress, virulence and cell death. GlcNAc has further been shown to promote plant growth by shaping the bacterial soil community. Here we propose a regulatory network for GlcNAc sensing in soil fungi. Using *Trichoderma reesei* as a model organism we showed that GlcNAc impacts expression of around 2,100 genes. Apart from primary metabolism, secondary metabolism was strongly affected. Two key regulators of GlcNAc catabolism, the NDT80-like transcriptional regulator, RON1, and a GlcNAc sensor, the

GCN5-related histone acetyltransferase NGS1, are differential regulators of two-thirds of these genes. Finally, we characterized the third regulator of GlcNAc sensing in *T. reesei*, which is the highly specific GlcNAc transporter NGT1. Interestingly, while internal GlcNAc activates GlcNAc catabolic gene expression, in contrast to dimorphic yeasts, the pathways for defense and pathogenicity seem to be induced in *T. reesei* by external GlcNAc. Given the ancestral role of *Trichoderma* spp. in the fungal kingdom and the highly conserved GlcNAc catabolism cluster including their regulators in many species of fungi, we propose that GlcNAc signaling in filamentous soil fungi might induce several signalling cascades related to metabolic processes, stress, and defence reactions. Our findings contribute to understanding sugar metabolism and sensing in filamentous soil fungi, which in turn might impact microbiome composition and thus soil fertility.

P3.409 - Characterization of Regulation of Ace2 and Morphogenesis (RAM) network components in Aspergillus fumigatus carbon source-mediated morphogenesis

D.N.J. Bale[1], *A. Martin-Vicente*[1], *H. Thorn*[1,2], *J. Xie*[1,2], *A. Nandi*[1], *U. Perez Cuesta*[1], *J. Fortwendel*[1,3]
[1]University of Tennessee Health Science Center, Clinical Pharmacy and Translational Science, Memphis, United States, [2]University of Tennessee Health Science Center, PhD Program in Pharmaceutical Sciences, Memphis, United States, [3]University of Tennessee Health Science Center, Microbiology, Immunology and Biochemistry, Memphis, United States

Aspergillus fumigatus CotA is a conserved protein kinase of the RAM network that we recently identified as essential for morphogenesis and virulence dependent on host niche-relevant carbon sources. In yeast, the RAM network controls cell proliferation, cell wall integrity, and stress responses and is composed of multiple proteins, including the CotA ortholog, Cbk1, and the additional associated proteins, Mob2, Tao3, Hym1, Sog2, and Kic1 which have not been previously characterized in *A. fumigatus*. To test if any of these RAM components are involved in the carbon source- and morphogenesis-related phenotypes uncovered in our CotA mutant, we first identified single copy orthologs for *hym1*, *kic1*, *sog2*, *tao3*, and *mob2* in the *A. fumigatus* genome through BLAST analyses. Then, using CRISPR/Cas9, we successfully deleted *hym1*, *kic1*, and *tao3* orthologs. After multiple unsuccessful attempts to delete *sog2* and *mob2* orthologs, indicating essentiality, we generated doxycycline-inducible conditional mutants. We then analyzed each strain for growth and morphogenesis in media containing glucose or non-preferred carbon sources (acetate, casamino acids, and ethanol) as the sole source of carbon. Although the deletion of *hym1* and repression of either *sog2* or *mob2* ortholog inhibited hyphal morphogenesis under all carbon sources, no significant differences were observed between the mutant and wild type strains on non-preferred carbon sources, suggesting that the role of CotA in carbon source-dependent morphogenesis is not controlled by any of these regulatory proteins. Further protein-protein interaction studies will determine how these proteins interact and if their function in regulating CotA activity is conserved in *A. fumigatus*.

P3.410 - Structural and functional characterization of endogenous TORC1 and TORC2 fungi-specific subunits

L. Bonadei[1], *N. Yakobov*[1], *A. Gillet*[2,1], *R. Loewith*[1]
[1]Université de Genève, Department of Cellular and Molecular Biology, Geneva, Switzerland,
[2]Centre Médical Universitaire de Genève, Geneva, Switzerland

The Target Of Rapamycin (TOR) protein kinase assembles into two complexes, TORC1 and TORC2, that maintain cell physiology in response to environmental changes [1,2]. Specifically, TORC1 resides on vacuolar/lysosomal membranes where it is activated through direct binding with Rag (EgoComplex) and Rheb GTPases in response to nutrient availability. In turn, TORC1 signals control protein synthesis and degradation (autophagy) to regulate cell mass homeostasis. TORC2 resides on the plasma membrane where it is regulated by upstream membrane stressors such as osmotic shocks or temperature changes. TORC2 signals subsequently control turgor pressure and lipid production and trafficking to regulate cell surface homeostasis. Perhaps unsurprising, TORC1 and TORC2 signaling has been implicated in fungal pathogenicity [3,4]. These observations suggest that these signaling pathways, especially fungal-specific elements of these pathways, represent interesting drug targets. Building on this hypothesis, we have recently extended our studies of TOR signaling from Saccharomyces cerevisiae to Magnaporthe oryzae, the causative agent of the devastating rice blast disease. Cryogenic Electron Microscopy (Cryo-EM) structures of M. oryzae TORC1 and TORC2 highlight the existence of fungal specific components and reveal additional attributes of these complexes that were missing from previous structures. This work both broadens our global understanding of TOR signaling and begins to reveal, at near atomic resolution, druggable nodes for agriculture and potentially clinical applications.

P3.411 - Non-Dikarya fungi share the TORC1 pathway with animals, not with yeasts

D. Barua[1], *M. Płecha*[1], *A. Muszewska*[1]
[1]Institute of Biochemistry and Biophysics Polish Academy of Sciences, Laboratory of Fungal Bioinformatics, Warsaw, Poland

Target of rapamycin (TOR) discovered in the budding yeast Saccharomyces cerevisiae, is a highly conserved serine/threonine kinase acting as a regulatory hub between cell and its environment. In fungi, as in mammals, the TORC1 pathway is essential for coordinating cell growth in response to nutrient availability. The activation of TOR complex 1 (TORC1) is similar in yeast and mammals, while its inhibition is more complex in the latter. This divergence of TORC1 regulation opens the question of how common are the yeast and mammalian pathway variants in the fungal tree of life. In this work, we trace the evolutionary history of TORC1 components throughout the fungal kingdom. Our findings indicate similarities in TORC1 inhibition between mammals and non-Dikarya fungi. These fungi contain the mammalian-specific KICSTOR complex required for GATOR1-mediated inhibition of TORC1. They also possess orthologs of serine, arginine and methionine sensors of TORC1 pathway that orchestrate

the response to nutrient starvation in mammals. The Rheb-TSC mediated activation of mammalian TORC1 that was lost in Saccharomycotina was also conserved in non-Dikarya fungi. Moreover, upregulation of the TORC1 inhibitory genes in response to growth in anaerobic conditions in Mucoromycotina species suggests the functional role of these genes similar to mammals. Overall, our findings indicate that the TORC1 pathway in non-Dikarya fungi resembles mammalian TORC1, while Saccharomycotina have lost many of the inhibitory components and evolved alternate regulatory mechanisms. Furthermore, our work highlights the limitations of using S. cerevisiae as a fungal model while putting forward other fungi as possible research models.

P3.412 - Genetic dissection of the chemical defense of ink cap mushroom *Coprinopsis cinerea* against fungivorous nematodes

S. Mai[1], *D. Croll*[2], *M. Künzler*[1]
[1]ETH Zürich, D-BIOL, Zürich, Switzerland, [2]Université de Neuchâtel, Institute of Biology, Neuchâtel, Switzerland

Fungivorous nematodes are ecologically relevant predators of fungal mycelia. The chemical defense of the model agaricomycete *Coprinopsis cinerea* against these predators includes both a constitutive (autonomous) and an inducible layer. The inducible layer is manifested by the production of a series of nematotoxic intracellular proteins in the vegetative mycelium of this fungus upon attack by the stylet-feeding fungivorous nematode *Aphelenchus avenae*. Interestingly, this inducible antinematode defense can propagate along specific hyphae acropetally and basipetally. The signalling pathways responsible for triggering this inducible anti-nematode defense response and the nature of signals mediating its propagation remain unclear. Moreover, the physiological relevance of the chemical defense of *C. cinerea* against fungivorous nematodes has not been assessed.
Recently, we tested the vegetative mycelia of several monokaryotic and dikaryotic *C. cinerea* strains for their susceptibility to *A. avenae* grazing. We found that the nematode populations thrived rapidly on some strains, while struggling to propagate on others. To unravel the genetic basis of this difference, we crossed a permissive and a prohibitive strain and isolated 123 F1 progenies. Phenotyping revealed that 84 strains of the progenies were prohibitive, while the rest were permissive. Bulk segregant analysis (BSA) of prohibitive and permissive pools of F1 progenies identified several loci associated with the permissive phenotype, including a previously characterized AB-like toxin gene, *cctx2*, which harbors two deleterious mutations that may lead to its loss-of-function in the permissive strain. We will use CRISPR-Cas9 to create a knock-out strain to validate the role of *cctx2* in conferring the permissive phenotype.

P3.413 - Damage-inducing compounds elicit an antibacterial defense response in the model mushroom *Coprinopsis cinerea*

E. Alessandri[1], *J. Welman*[2], *M. Künzler*[1]
[1]ETH Zürich, Institut für Mikrobiologie, Zürich, Switzerland, [2]University of Zürich, Department of Biochemistry, Zürich, Switzerland

Fungi and bacteria often share the same ecological niches, which can lead to competition for limited resources such as space and nutrients. The saprophytic mushroom *Coprinopsis cinerea* responds to antagonistic bacteria with secretion of antibacterial molecules, such as the lysozyme LYS1, which targets peptidoglycan in the bacterial cell wall. This specific response is also induced by the bacterial cell-free supernatant, suggesting the existence of soluble elicitors derived from either bacterial cell lysis or active secretion.
To efficiently detect the induction of antibacterial defense in *C. cinerea*, we generated a bioluminescent reporter strain by fusing the promoter of the *lys1* gene to the coding region of a secreted variant of the luciferase Nanoluc (cNluc).
Preliminary data generated with this reporter strain show that induction of antibacterial defense is multifactorial and caused by compounds that compromise cell integrity. Inducing compounds include hydrolytic enzymes and lipopeptides, which disrupt cell wall constituents and membrane lipids, respectively.
Interestingly, the *lys1* gene is also strongly induced by dithiothreitol (DTT), a reducing agent and inhibitor of protein folding in the endoplasmic reticulum (ER). DTT is a well-established elicitor of the unfolded protein response (UPR), a central regulatory pathway required for ER homeostasis that is conserved across eukaryotes. Our findings suggest that the antibacterial defense response in *C. cinerea* might be functionally connected to the UPR.
Our future work will aim at elucidating the molecular connection between antibacterial defense, cell wall and membrane damage, and the UPR in *C. cinerea*.

P3.414 - Photoadaptation in the absence of VVD: regulation of transcription by light in *Phycomyces blakesleeanus*

M. Corrochano Luque[1], *G. Gutierrez*[2], *D. Canovas*[2], *L.M Corrochano*[2]
[1]University of Seville, Genetics, Seville, Spain, [2]University of Seville, Seville, Spain

The responses to light in *Neurospora crassa* require the WCC, a photoreceptor and transcription factor complex composed of WC-1 and WC-2 that binds to the promoters of light-regulated genes. Binding and activation by the WCC is transient, leading to the temporal activation of transcription, photoadaptation. This process requires the blue-light photoreceptor VVD that negatively regulates the WCC. The genome of the early-divergent fungus *Phycomyces blakesleeanus* encodes three *wc-1* genes, and four *wc-2* genes, but no *vvd* gene. Light-dependent transcription requires MadA and MadB, homologs of WC-1 and WC-2, respectively. Photoadaptation has been reported for several genes but it is not known how photoadaptation takes place in the absence of a *vvd* homolog.
We have characterized photoadaptation in *Phycomyces* by RNAseq using mycelia exposed to light from 15 to 240 min. Light-regulated genes were classified in six clusters, based on the transcription patterns. 541 genes were induced, while 105 genes were repressed. Most of the light-inducible genes showed photoadaptation. We identified three waves of light-dependent transcription with maxima at 15, 30 and 60 minutes. The light-regulated *wc* genes were included in the 15 min cluster, an indication of their role in the early response. We have identified two genes with fast and high light-dependent transcription that resemble the transcriptional pattern of *vvd* in *N. crassa*: *wctD*, a *wc-2* gene, and the heat-shock gene *hspA*. These proteins could disrupt

the activity of the Mad complex to regulate transcription, and are candidates to regulate photoadaptation in *Phycomyces*.

P3.415 - A cryptochrome-like photolyase regulates the oxidative stress response in Aspergillus nidulans

A. Landmark[1], *R. Fischer*[1]
[1]Karlsruhe Institute of Technology, Karlsruhe, Germany

Fungi sense changes in temperature and light conditions, which indicate shifts of various stress signals such as osmotic pressure or reactive oxygen species (ROS). To cope with these stimuli, fungi use a variety of sensor proteins such as the red/far-red light receptor phytochrome and the corresponding stress response, the conserved high-osmolarity glycerol (HOG) pathway. This pathway relies on a two-component phosphorelay system that results in the activation of stress-related gene expression. Although a variety of stress conditions act as input stimuli, the signal cascade is always linked to the HOG pathway with similar downstream targets. Therefore, fungi need additional systems to respond and fine-tune the response to specific stress factors.
Here, we show a novel way in which the fungal photolyase/cryptochrome CryA is involved in the oxidative stress response (OSR) of *Aspergillus nidulans*. CryA interacts in the nucleus with the heme oxygenase HoxB, a homologue of the Hox1 protein that protects against ROS in mammals. Exposure to extracellular ROS results in the shuttle of CryA and HoxB to the mitochondria *in vivo*. This ROS-dependent shuttle is linked to the N-terminal extension of CryA. Quantitative PCR revealed that HoxB acts as a positive regulator and CryA as a negative regulator of OSR-related gene expression. This stress-sensing role of heme oxygenases and cryptochromes is comparable in *Alternaria alternata* and possibly other fungis. Taken together, these results suggest a novel role for fungal cryptochromes and heme oxygenases in sensing and regulating environmental stress signals.

P3.416 - Investigation of signalling pathways controlling gasdermin cell death in *Podospora anserina*

K. Subban[1], *A. Daskalov*[1]
[1]ImmunoConcEpT, CNRS, UMR 5164, University of Bordeaux, Bordeaux, France

Gasdermins are pore-forming, membrane-targeting proteins with key roles in mammalian regulated cell death (RCD) and immunity. Gasdermins are also conserved in prokaryotes and integral to anti-phage defense systems in bacteria. In fungi, two different gasdermin homologs, from *Neurospora crassa* and *Podospora anserina*, have been revealed to control RCD in the context of heterokaryon incompatibility (HI). HI occurs in the chimeric heterokaryotic cells resulting from the anastomosis of genetically distinct fungal individuals and serves as prophylactic cell death against parasitism. Gasdermin proteins are generally activated by proteolytic cleavage, including in fungi. Remarkably, most fungal gasdermins (fGSDMs) are genomically clustered with a protease-encoding gene. These proteases are often multi-domain proteins carrying a serine protease of the S8 clade or a caspase-like (CHAT) protease domain.

Based on their protein architectures, we have hypothesized that the fGSDM-controlling proteases are molecular sensors controlling immunity-related cell death in fungi. However, the precise molecular mechanisms of action, specific signals and regulation of these proteases remain unelucidated, similarly to the biological roles of the fGSDM pathways.
Here, we explore these questions for two fGSDM-controlling proteases from *P. anserina*. First, we have undertaken to define the interactome for each of the two putative molecular sensors using next-generation yeast two-hybrid screens. This approach led to the identification of numerous interacting partners for each of the two proteases. Among those, several proteins with putative roles in cell death. Next, we will explore the protein-protein interaction networks in *P. anserina* to elucidate the regulation and signaling mechanisms of the two fGSDM-controlling proteases.

P3.417 - Are1 implication in iron metabolism

C. Baldin[1], *D. Flatschacher*[1], *S. Pierson*[1], *U. Schreiner*[1], *M. Alilou*[2], *L. Atanasova*[3], *S. Zeilinger*[1]
[1]University of Innsbruck, Department of Microbiology, Innsbruck, Austria, [2]University of Innsbruck, Institute of Pharmacy/Pharmacognosy, Innsbruck, Austria, [3]BOKU University, Institute of Food Technology, Vienna, Austria

Aspergillus AreA GATA-like transcription factor AreA is widely regarded as a key regulator in overcoming nitrogen catabolite repression (NCR). Its role in activating genes repressed by NCR is a critical adaptive feature in numerous species, facilitating the utilization of non-preferred nitrogen sources when favored ones, such as ammonium or glutamine, are scarce. *Trichoderma*, a genus of filamentous ascomycetes commonly found in the rhizosphere, is globally distributed and thrives in various ecological niches. Its relevance to sustainable agriculture lies in the potential of certain species to act as biological control agents, particularly due to their ability to target a wide range of economically important fungal phytopathogens through mycoparasitism. Despite its importance, our understanding of NCR and its regulatory mechanisms in *Trichoderma* species remains limited, leaving significant gaps in our knowledge of how these fungi adapt to diverse environments. Characterization of the AreA ortholog in the mycoparasitic fungus *T. atroviride*, named Are1, not only confirmed previously identified roles in nutrients adaptation and NCR relief, but also led to the discovery of previously unknown connections between this transcription factor and many other cellular processes, including iron metabolism and siderophore biosynthesis.

P3.418 - Biotin capture of the proxiome of the SmSTRIPAK complex in *Sordaria macrospora* identifies a greenbeard gene as a putative interactor

L.S. Hollstein[1], *K. Schmitt*[2], *O. Valerius*[2], *S. Pöggeler*[1]
[1]Georg-August-University of Göttingen, Department of Genetics of Eukaryotic Microorganisms, Göttingen, Germany, [2]Georg-August-University of Göttingen, Department of Molecular Microbiology and Genetics, Göttingen, Germany

For more than two decades, the filamentous ascomycete *Sordaria macrospora* (Sm) has served as a model organism to investigate the striatin-interacting phosphatase and kinase (SmSTRIPAK) complex. This multiprotein phosphatase assembly plays a crucial role in regulating vegetative growth, hyphal fusion, and the development of fruiting bodies.
To identify potential target proteins, we screened for protein-protein interactions within the cellular microenvironment of the SmSTRIPAK complex utilizing the Biotin Identification (BioID) method. This technique relies on the *in vivo* labeling of proximal proteins by a promiscuous biotin ligase which is fused to the protein of interest. Biotinylated proteins are then specifically enriched by biotin affinity capture and identified through mass spectrometry. Using one component of the complex, the SIKE-like SmSTRIPAK complex interactor 1 (SCI1), we captured already known SmSTRIPAK components as well as candidate interactors. One of these candidates, determinant of communication 2 (SmDOC2), exhibited significant enrichment across multiple SCI1-BioID experiments, utilizing various control setups.
The communication genes *doc-1*, *doc-2* and *doc-3* were initially characterized in the closely related ascomycete *Neurospora crassa*. Here, the *doc* genes were shown to be involved in the pre-contact communication between two hyphae. They prevent the fusion of genetically dissimilar hyphae by suppressing the oscillation of MAK-2, which is required for communication and chemotropic interactions.
We have generated deletion strains of *Smdoc1* and *Smdoc2* in *S. macrospora* and are performing BioID experiments using SmDOC1 and SmDOC2 as bait to explore the underlying molecular mechanism.

P3.419 - Molecular characterization of Dic3 in Bipolaris maydis and its role in resistance to dicarboximide and phenylpyrrole fungicides and osmotic stress

D.C. Ruiz-Nava[1], *Y. Saigyo*[2], *M. Shimomura*[2], *K. Tsuji*[2], *A. Yoshimi*[1,2], *M. Kawauchi*[2], *Y. Honda*[2], *C. Tanaka*[1,2]
[1]Kyoto University, Graduate School of Global Environmental Studies, Kyoto, Japan, [2]Kyoto University, Graduate School of Agriculture, Kyoto, Japan

The mode of action of the broad-range dicarboximide and phenylpyrrole fungicides on filamentous fungi is not fully understood, but it is thought to be similar, involving two-component signaling systems regulating osmotic responses. As previous studies that have focused on the resistance mechanisms of the fungal pathogen *Bipolaris maydis*, the present study sheds light on the function of *Dic3*, a gene whose function remained unknown which confers mutant *B. maydis* strains resistance to the aforementioned fungicides. A genome-comparison approach using sibling strains of the wild type and the *Dic3* mutant strains suggested a single point mutation in the gene was responsible for the drug-resistant phenotype. Transformation of the *Dic3* mutant with the wild-type allele restored drug sensitivity, confirming the mutation's role in resistance. Furthermore, based on unsuccessful disruption attempts, *Dic3* is suggested to be an essential gene in *B. maydis*. Sequence analysis revealed this gene to be a homolog of the human *SART3* which is involved in RNA splicing. The point mutation responsible for drug resistance occurred within HAT sequence repeats, a conserved motif found in SART3 homologs, with the exception of yeasts. Yeast two-hybrid analysis showed that Dic3 interacts with the response

regulator Skn7, with the mutant Dic3 protein exhibiting significantly weaker binding. These results suggest that Skn7, through its interaction with Dic3, mediates the response to both osmotic stress and fungicide action, and that the HAT repeat domain of Dic3 is crucial for this interaction.

P3.501 - From symbiont to pathogen: the effects of plant lipid metabolism in the lifestyle of *Colletotrichum tofieldiae*

J.L. Hernández Ayala[1]
[1]University of Cologne, Cologne, Germany

Fungal symbiosis plays a pivotal role in plant health and nutrient acquisition. *Colletotrichum tofieldiae* (Ct), acts as a beneficial symbiont for *Arabidopsis thaliana (A.t.)* in phosphate deprived conditions. New research suggests that *Ct* has a growth suppressing effect in plants whose de-novo lipid synthesis becomes compromised. This PhD project aims to investigate the transition of *Ct* from symbiosis to pathogenesis, focusing on lipid transport and pathogenicity-related genetic pathways. We aim to understand how early-stage colonization of roots is influenced by these molecular pathways and explore recovery methods the beneficial phenotype.
Gene knockouts will be used to target pathogenicity-related genes in both *Ct* and the closely related pathogen *Colletotrichum incanum*. Co-cultivation experiments with *A.t.*, including transgenic lines featuring genes derived from *Lotus japonicus (L.j.)* involved in the establishment of arbuscular mycorrhizal symbiosis (AMS) Lj*Ram2*, Lj*Str1*, Lj*Str2*, will assess whether the symbiotic relationship can be restored. Root architecture will be analyzed alongside fungal hyphal morphology, and RNAseq will be used to investigate regulatory profiles during the early stages of colonization.
This research aims to understand the role of lipids in regulating fungal behavior and its implications in the fungal lifestyle. We expect to observe a restoration of the beneficial symbiotic phenotype in *A.t.* lines expressing mycorrhizal genes. We hypothesize that by complementing lipid-biosynthesis compromised *A.t.* mutants with AMS-related genes derived from *L.j.* will lead to a restoration of the plant-growth promoting effect of *C.t.* in co-cultivation with *A.t.* The insights gained will contribute to our understanding of plant-fungal interactions symbiosis and pathogenicity.

P3.502 - Comparative analysis of the transcriptome of the beneficial endophyte *Colletotrichum tofieldiae* in maize and *Arabidopsis thaliana*

E. Torres[1], *S. Díaz-González*[1], *S. González-Bodí*[1], *C. González-Sanz*[1], *S. Sacristán*[1]
[1]Universidad Politécnica de Madrid, Centro de Biotecnología y Genómica de Plantas, Madrid, Spain

Colletrotrichum tofieldiae is an endophytic fungus originally isolated from *Arabidopsis thaliana*. Different studies have shown the ability of this fungus to colonise the roots of both *A. thaliana* and other agronomically valuable hosts, providing several fitness advantages to the plants.
In the present study, an RNA-Seq experiment was conducted to obtain transcriptomic data of the

fungus interacting with maize at two early time points of the interaction. The results were used to perform a differential gene expression analysis. Functional categories within the differentially expressed genes were examined and functional enrichment analyses using Gene Ontology (GO) terms were performed. The same pipeline was applied to previously published raw transcriptomic data of *C. tofieldiae* in the interaction with *A. thaliana*, allowing the comparison between the findings obtained in both hosts.
The comparative analyses of *Ct*0861 transcriptomes in both host plants and at different timepoints has revealed distinct temporal dynamics, suggesting divergent strategies for the colonisation and establishment of trophic interactions with different hosts. Common trends in the interaction with both hosts are the high induction of genes encoding secreted proteins and transporters during host colonisation, while genes involved in secondary metabolite biosynthesis exhibit low expression levels *in planta*.

P3.503 - Analysis of arbuscular mycorrhizal fungi and *Candidatus* Moeniiplasma glomeromycotorum levels in the grassland roots in response to drought

Z. Niu[1], *E. Stewart*[1], *S. Carrasco Bonet*[1], *E. Haskins*[1], *B. Aynalem*[1], *T. Caruso*[1], *O. Lastovetsky*[1]
[1]University College Dublin, Dublin, Ireland

Arbuscular mycorrhizal fungi (AMF) form symbioses with most plants and benefit plant growth. AMF also harbor endobacteria, *Candidatus* Glomeribacter gigasporarum (*Ca*Gg) was detected in several species of the family AMF and their functions in AMF biology is considered to be a mutualistthe. The *Candidatus* Moeniiplasma glomeromycotorum(*Ca*Mg) are distributed in the cytoplasm of the AMF spores and hyphae, the role of *Ca*Mg in AMF biology remains uncharacterized experimentally. In this study we aimed at assessing the effect of environmental parameters (drought among other) on the level of AMF and *Ca*Mg abundance in plant roots. Drought and control plots were established at two farms in Ireland, where root samples of Yorkshire fog (*Holcus lanatus*), creeping buttercup (*Ranunculus repens*), and white clover (*Trifolium repens*) were harvested. . Two methods for assessing AMF root colonization will be utilized: the traditional root staining and microscopy method, alongside a novel qPCR method with AMF-specific primers. In addition, *Ca*Mg levels will be quantified using specific primers. The development of high-throughput methods for evaluating the abundance of AMF and endobacteria in plants has the potential to enhance our understanding of endobacteria-AMF-plant interactions in an agricultural setting.

P3.504 - Exploring plant-fungal interactions: insights from *Neurospora crassa* and *Brachypodium distachyon*

H. Winter[1], *K. Kolláth-Leiss*[1], *F. Kempken*[1]
[1]Christian-Albrechts-University, Molecularbiology and Genetics, Kiel, Germany

Fungi play a dynamic role in complex relationships with plants. There is an abundance of variability shown in these linkages, including mutualistic and pathogenic interactions. Pathogenic

fungi pursue biotrophic and hemibiotrophic lifestyles, damaging their host counterpart in the prosses to utilize their nutrients. On the contrary, mutualistic relationships like mycorrhiza (MR) result in mutually beneficial outcomes for both fungus and plants. These associations appear when filamentous fungi invade plant root systems in order to create a stable and mutually beneficial coexistence.
Utilizing two well-known model organisms – the ascomycete *Neurospora crassa* and the grass *Brachypodium distachyon* – this research explores plant-fungal interactions in an intriguing and reliable way, allowing the execution of thorough investigations in cell biology and genetics.
Despite being a saprophytic fungus with an abundance of studies, not much is known about the ecological attributes of *N. crassa*. On the other hand, *B. distachyon*, a sweet grass closely related to important crops, is remarkably flexible in the environment and engages in a range of fungal interactions, including mutualistic and damaging relationships.
Using electron, fluorescence and confocal laser scanning microscopy characterizing this interaction. Our findings suggest that most plant root tissue is unimpaired by fungal invasion, though single hyphae show growth in the apoplastic space, root cortex cells and the vascular cylinder. The infected cells were often surrounded by non-infected, living neighbor cells.
Employing the *Neurospora* knock-out collection we present specific mutants with hampered root colonization.

P3.505 - Interactions between grazing, abiotic factors, and fungal communities: insights from reanalysis using virtual taxons

E. Olsen[1]
[1]University of the Faroe Islands, Faculty of Education, Tórshavn, Faroe Islands, Denmark

In several field studies investigating arbuscular mycorrhizal (AM) symbioses and their responses to abiotic factors like temperature and nutrient availability, grazing has consistently emerged as a significant factor influencing the results.
The fungal species identified include both AM fungi and dark septate endophytes (DSE). Both groups have demonstrated responses not only to grazing but also to various abiotic factors.
The techniques for identifying fungal species have varied, ranging from sequencing selected clones to pyrosequencing. Despite using AM-specific primers, the latter method identified multiple fungal species, only 25% of which were glomalean. Among the glomalean fungi, the most prevalent species was one that has a worldwide distribution and has consistently shown a response to grazing in our studies.
In DSE, a correlation was observed between the percentage of root length colonized and water-soluble soil phosphate content. Due to variations in the primer sets used across laboratories, a reanalysis of fungal sequences is required to better compare findings with other studies on abiotic factors. This poster presents a reanalysis of fungal sequences using the MaarjAM database and explores similarities in fungal responses to grazing and abiotic factors based on the fungal virtual taxons identified.

P3.506 - Exploring endophytic fungal diversity in wheat by metabarcoding and *in vitro* selection

M. Beccaccioli[1], *L. Lascala*[1], *L. Faino*[1], *M. Reverberi*[1]
[1]Sapienza University of Rome, Department of Environmental Biology, Rome, Italy

Endophytic fungi are ubiquitous endosymbionts in plants and important components of their development. To become endophytes, microorganisms should colonize the plant endosphere using specific properties as motility, attachment, plant–polymer degradation and the evasion of plant defences; the diversity of endophytic community depends on plant- and environment-specific factors.
Proposing investigation aims to select and characterize fungal endophytes from seeds of several durum and soft wheat varieties harvested in Italian peninsula in 2023. Since endophytic fungi are highly diversified, two parallel protocols for fungal endophyte identification have been developed, one by metabarcoding, using the MinION sequencing platform (ONT), and one exploiting the classical *in vitro* cultivation and identification.
The two methodologies have allowed to generate a map of the distribution of fungal endophytes along the Italian peninsula, also considering the different climatic conditions, and cultivation strategies. The screening results showed an emerging pathogen *Fusarium boothi* not previously reported in the Italian territory, and beneficial fungal endophytes as *Chaetomium globosum*, *Epicoccum* spp., and *Alternaria destruens* already known for their activities. Once potential beneficial endophytes have been identified, an *in vitro* screening on their biological activity against the main wheat fungal pathogens was performed.
Wheat production is greatly challenged by phytopathogenic fungi outbreaks that are evolving and spreading to new geographical areas, posing a significant threat for global food security and environmental sustainability. The findings of this work provide new insights for screening fungal endophytes and developing biocontrol strategies as an alternative to disease mitigation in wheat.

P3.507 - Isolation and functional analysis of an endophytic fludioxonil-resistant *Fusarium solani m*utant for integrated tomato crop protection

A. Malandrakis[1,2], *O. Tsiouri*[3], *M. Kissandraki*[1], *K. Papadopoulou*[3], *N. Kavroulakis*[1]
[1]ElGO-DIMITRA, Institute of Olive Tree, Subtropical Plants and Viticulture, Chania, Greece,
[2]Technical University of Crete, School of Environmental Engineering, Chania, Greece,
[3]University of Thessaly, Department of Biochemistry and Biotechnology, Larissa, Greece

A spontaneous *Fusarium solani* mutant strain (FsK-F) resistant to the fungicide fludioxonil was characterized. The stability of resistance of FsK-F was confirmed by fungitoxicity tests, which revealed no significant reduction in resistance levels after five subcultures in fludioxonil-free media. Cross-resistance experiments revealed that FsK-F was also resistant to iprodione but remained sensitive to fungicides with different modes of action, including the benzimidazole carbendazim, triazoles prochloraz and difenoconazole, and the QoI pyraclostrobin. Fitness bioassays demonstrated that FsK-F was more sensitive to osmotic pressure but showed no

significant difference from the parental strain (FsK) in terms of mycelial growth, spore production, and heat sensitivity. Importantly, FsK-F retained its ability to colonize tomato roots and protect plants against both abiotic and biotic stresses, indicating that resistance mutation(s) did not affect the strain's beneficial traits. Genetic analysis revealed a deletion in the OS-1 gene, which encodes a histidine kinase involved in osmotic regulation, as the probable cause of FsK-F's fludioxonil resistance. Its stable, high-level resistance to fludioxonil and iprodione, coupled with the beneficial properties of the FsK-F strain make it a promising candidate for use in IPM programs, potentially reducing the need for chemical fungicides utilizing the strain's plant-protective capabilities.

This study was co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Rural Development Program (RDP/ΠΑΑ) 2014 – 2020, under the call "Cooperation for environmental projects, environmental practices and actions for climate change" (project code: M16SYN2- 00254).

P3.508 - Investigating the symbiosis between *Candidatus* Moeniiplasma glomeromycotorum endobacteria in arbuscular mycorrhizal fungi

Y. Guan[1]
[1]University College Dublin, Dublin 14, Ireland

Arbuscular mycorrhizal fungi (AMF, Glomeromycotina) are essential plant symbionts, providing mineral nutrients in exchange for carbon and gaining attention in sustainable agriculture. AMFcan host intracellular bacteria (endobacteria) and two of these have been described: *Candidatus* Glomeribacter gigasporarum (*Ca*Gg) and *Candidatus* Moeniiplasma glomeromycotorum (*Ca*Mg). *Ca*Gg have been shown to affect fungal energy metabolism, sporulation, and hyphal proliferation, but its environmental prevalence in AMF spores is low (2%). *Ca*Mg, on the other hand, are very common in field AMF spores (80%), but their role in AMF has not yet been characterised experimentally.Despite this , it has been hypothesized that CaMg act as conditional mutualists of AMF.

To begin investigating the role of CaMg, AMF trap cultures were set up using soil from three different sites (a dune, an agricultural grassland and an apple orchard). AMF spores were isolated from a portion of the trap culture soil and the remaining soil was used to set up a second trap culture "round". AMF spores isolated from each "round" were surface-sterilised and screened for presence of *Ca*Mg using specific primers. Our results suggest that during the continuous passage of AMF in pot cultures, the incidence level of *Ca*Mg in AMF spores declines. This observation implies that *Ca*Mg might be eliminated from AMF population under trap culture conditions indicating that the potential benefit associating with hosting *Ca*Mg is lower under these controlled conditions. Future work is aimed at exploring the effect of CaMg endobacteria on the AMF physiology and further investigating the symbiosis between them.

P3.509 - Sterol metabolism of ophiostomatalean fungi associated with the spruce bark beetle Ips typographus (Coleoptera, scolytinae)

C. Vandekerkhove[1], *E. Gelhaye*[1], *M. Morel-Rouhier*[1], *S. Darnet*[1]
[1]University of Lorraine, UMR IAM Tree Microbe Interactions, Vandoeuvre les Nancy, France

Bark beetles are insects responsible for forest pest crisis, mainly colonizing Norway spruce (*Picea abies*). These insects are associated with ophiostomatalean fungi. The nature of the interaction between *Ophiostoma piceae* and the beetle *Ips typographus* could be an exosymbiosis mediated by monoterpene chemical signals, but it is not yet well understood. In insects, lipids also play important roles for growth, metamorphosis and reproduction, however insects are sterol auxotrophic organisms. The main objective of this study is to characterize the sterol metabolism of ophiostomatalean fungi associated with bark beetles. The genome analysis reveals that all genes for sterol biosynthesis are identified but a putative 24-methylene lophenol-methyltransferase (SMT2), a plant-restricted enzyme, is present in *O. piceae* and no ortholog was found for the sterone ketoreductase (ERG27) and acyl-CoA sterol acyl transferase 1 (ARE). The sterol determination of *O. piceae* reveals three conjugate forms and accumulation of biosynthetic intermediates of the ergosterol pathway, but no sterol with a lateral chain with 2 carbons at C24 position, possible product of the putative SMT2-like enzyme. Characterizing the sterol metabolism could lead to understand the fungal contribution for the insect lipid nutrition and its reproduction in tree bark.

P3.510 - What makes a host a host: how basal fungi perceive and respond to endosymbiotic bacteria

E. Scales[1], *O. Lastovetsky*[2], *M.L. Gaspar*[1], *T. Pawlowska*[1]
[1]Cornell University, Plant Pathology and Plant-Microbe Biology, Ithaca, United States,
[2]University College Dublin, Dublin, Ireland

Beneficial endohyphal bacteria are widely found in basal fungi, including arbuscular mycorrhizal fungi, which support the growth of nearly all terrestrial plants. Nonetheless, the process of endosymbiont accommodation remains poorly understood in fungi. Endosymbiont accommodation challenges hosts' immunity, yet the relationship ultimately arrives at a state of mutual benefit. The model system our lab group has pioneered for study of fungi and their endohyphal bacteria utilizes a host strain of the early-diverging mold *Rhizopus microsporus* (Rm), its endosymbiont *Mycetohabitans* sp. B13 (B13), and a non-host strain of Rm. While the host strain reliably engages in a stable endosymbiosis with B13, the non-host strain exhibits hallmarks of antagonism upon exposure to B13. This work utilizes the analysis of RNA-seq data from both host and non-host strains to describe the host strain's unique transcriptional response to B13 during endosymbiosis establishment and identify putative signaling and regulatory networks driving the distinction between mutualistic and antagonistic interactions in this system. These analyses direct our efforts in uncovering the mechanistic underpinnings of a potential endosymbiont accommodation regimen in fungi.

P3.511 - Dark matter of an orchid: metagenome associated with the rhizosphere of *Dactylorhiza traunsteineri*

G.A. Vignolle[1,2], L. Zehetner[1], C. Zimmerman[1], D.F. Savio[1], O. Paun[3], R.L. Mach[1], A.R. Mach-Aigner[1], J. Charest[1]
[1]Institute of Chemical, Environmental and Bioscience Engineering, TU Wien, Vienna, Austria, [2]Center for Health & Bioresources, Competence Unit Molecular Diagnostics, AIT Austrian Institute of Technology GmbH, Vienna, Austria, [3]Department of Botany and Biodiversity Research, University of Vienna, Vienna, Austria

Large multicellular organisms, such as plants, engage in intricate interactions with a diverse array of microorganisms in their surrounding environment. These microbial communities, collectively known as the plant microbiota, play an essential role in promoting plant growth, facilitating nutrient acquisition, and bolstering resistance against pathogens, thereby contributing to overall plant health in natural ecosystems. *Dactylorhiza traunsteineri*, a rare orchid species native to marshlands and alpine regions of the eastern hemisphere, is particularly sensitive to environmental changes. Its endangered status in central Europe, combined with its notorious difficulty to cultivate *ex situ*, underscores the urgency of understanding the symbiotic relationships crucial for its survival. In our study, we employ metagenomic shotgun sequencing and genome assembly to investigate the fungal communities associated with *D. traunsteineri*. By unraveling the unexplored secondary metabolite pathways within these fungal genomes, we aim to discover novel bioactive compounds that could hold potential for ecological and pharmaceutical applications.

P3.512 - Carbon-for-nutrient exchange dynamics and mutualist reciprocity in *Arabidopsis thaliana* and *Colletotrichum tofieldiae* interactions

J. Gaertner[1], M. Bucher[1]
[1]University of Cologne, Institute of Plant Science, Köln, Germany

Arbuscular mycorrhizal symbiosis (AMS) is one of the oldest extant plant-microbe associations. Notwithstanding the benefits of nutrient exchange, AMS requires the plant to release carbon assimilates to its fungal symbiont. It is probably this energy cost to the host associated with AM fungi (AMF) that has led to the loss of AMS in some plant species during evolution. *Brassicaceae* are AMF non-hosts and so is the most commonly used model plant, *Arabidopsis thaliana*. Furthermore, many AMF are difficult to cultivate because of their obligate biotrophy. *A. thaliana* being a non-host for AMF, is a significant complication for research into the molecular mechanisms underlying AMS. *A. thaliana*, however, is known to form a mutualistic relationship with *Colletotrichum tofieldiae*, a facultatively symbiotic free-living ascomycete from a well-studied genus that includes many plant pathogens. In addition, *C. tofieldiae* is readily cultivable and transformable and therefore represents a viable system for investigating the molecular and genetic basis of root endophytism in *A. thaliana*.
In my project, the carbon-for-nutrients dynamic underpinning the plant-fungal nutrient exchange

is investigated using stable- and radioisotope labelling of WT and mutant lines of both *A.thaliana* and *C.tofieldiae.* The immediate question to be answered is whether the bidirectional exchange of inorganic phosphate from the fungus and fatty acids from the plant occurs in reciprocity. By strengthening the synthetic symbiotic partnership established in this project, it will be possible to identify both plant and fungal traits that will enable us to construct specific mutualistic symbioses that improve host plant growth and fitness.

P3.513 - The mycobiont of the lichen *Xanthoria parietina* produces the siderophore ferrichrome in axenic culture and as mycobiont

I. Happacher[1], *G. Pichler*[2], *M. Kraihammer*[3], *K. Faserl*[4], *B. Sarg*[4], *C. Decristoforo*[3], *K. Haselwandter*[5], *G. Winkelmann*[6], *M. Brock*[7], *I. Kranner*[2], *H. Haas*[1]
[1]Institute of Molecular Biology, Biocenter, Medical University Innsbruck, Innsbruck, Austria, [2]Department of Botany, University of Innsbruck, Innsbruck, Austria, [3]Department of Nuclear Medicine, Medical University Innsbruck, Innsbruck, Austria, [4]Institute of Medical Biochemistry, Protein Core Facility, Biocenter, Medical University Innsbruck, Innsbruck, Austria, [5]Department of Microbiology, University of Innsbruck, Innsbruck, Austria, [6]Department of Microbiology & Biotechnology, University of Tübingen, Tübingen, Germany, [7]Fungal Biology Group, School of Life Sciences, University of Nottingham, Nottingham, United Kingdom

Iron is an essential micronutrient for virtually all living organisms but is toxic in excess. Despite its high abundance, the availability of this metal in aerobic environments is low. As a result, microorganisms have evolved strategies for both high-affinity acquisition and detoxification of iron. There are still significant gaps in our knowledge regarding iron homeostasis-maintaining mechanisms in symbiotic fungi such as lichens.
Employing a bioassay based on *Aspergillus fumigatus* mutant strains with different defects in iron acquisition and biochemical analyses, the current study demonstrates that the mycobiont of the globally distributed lichen *Xanthoria parietina* synthesizes the siderophore ferrichrome both in the natural lichen thallus and in axenic culture. Genome mining characterized the genetic make-up of the siderophore-mediated iron assimilation and revealed the potential for reductive iron assimilation in this mutualistic organism. The *X. parietina* non-ribosomal peptide synthetase mediating ferrichrome biosynthesis, here termed XpSidC, was functionally characterized by heterologous expression in an *Aspergillus fumigatus* mutant lacking production of ferrichrome-type siderophores. Remarkably, XpSidC displays a novel compact domain architecture for ferrichrome synthetases that is highly conserved in other lichen mycobiont species.

P3.514 - Establishing *Paxillus involutus* as a genetically tractable model for studying ectomycorrhizal symbiosis

V. Kreszies[1], *S. Herzog*[1], *E. Friedrichs*[1], *B. Harders*[1], *E. Megalonidou*[1], *T. Mosig*[1], *H. Reichelt*[1], *S. Tasnim*[1], *H. Wege*[1], *V. Woge*[1], *J. Muhr*[2], *I. Teichert*[1]
[1]University of Goettingen, Forest Botany and Tree Physiology, Goettingen, Germany, [2]University of Goettingen, Laboratory for Radioisotopes, Forest Botany and Tree Physiology, Goettingen, Germany

Mycorrhizal symbiosis is a mutualistic interaction between plants and fungi. Ectomycorrhizal fungi (ECM) are common in temperate and boreal forests. They form a mantle around the root tips, extraradical hyphae reaching into the soil and the Hartig net between the root cortex cells. The trees benefit from this symbiosis by improved access to water and mineral nutrients and in return the fungi acquire carbohydrates from the trees. Furthermore, ECM protect their hosts from abiotic stresses as well as from pathogens. Many studies have investigated ectomycorrhiza, and transcriptomic analyses have identified a huge number of differentially regulated genes possibly important for symbiosis. Yet, molecular analyses of mycorrhizal interaction remain scarce. Here, we aim to establish a mycorrhizal model system with *Paxillus involutus*, a basidiomycete that undergoes symbiosis with diverse tree species, including poplar. We study the morphology and physiology of various *P. involutus* strains to define growth conditions suitable for downstream experiments. These experiments include mycorrhization assays of poplar plants as well as phosphorus-uptake experiments in changing physiological conditions. For genetic analyses we focus on developing a protocol for protoplast-mediated and *Agrobacterium*-mediated transformation. We will show data regarding several selection markers and strategies for generating suitable vector systems allowing the testing of different promoters for gene expression in *P. involutus*. With a robust transformation system for *P. involutus*, in combination with genetically modified poplar *Populus x canescens*, we will be able to unravel the molecular mechanisms underlying ectomycorrhizal symbiosis, which is of major importance for understanding temperate and boreal forest ecosystems.

P3.515 - Investigating wild Scottish grasses for endophyte use towards adapted resilience in barley and other grass crops

L.I. Rangel[1], *S.E. Light*[2], *V. Buswell*[3], *B. Keillor*[1], *D. Bienkowski*[3]
[1]The James Hutton Institute, Cell & Molecular Sciences, Dundee, United Kingdom, [2]University of California Cooperative Extension, Yuba City, United States, [3]The James Hutton Institute, Information & Computational Sciences, Dundee, United Kingdom

Scottish barley remains a dominant crop in terms of planted area and product-related export yet annual reduced yields due to biotic and abiotic stresses continue. Many microorganisms can reside between and within living plant cells and exist as mutualistic symbionts. These endophytic bacteria and fungi are an underexplored biocontrol source in the UK, although endophyte-plant combinations are commercially available in other countries, such as New Zealand and the USA. Specifically, fungal *Epichloë* endophytes have been shown to confer intrinsic protection from pests and pathogens in grasses as well as offering plant growth promoting properties. Wild barley has been surveyed for *Epichloë* endophytes in a range of countries where it was found that *Epichloë* isolates taxonomically cluster based on geographical location rather than host, suggesting the importance of local adaptation. Knowing this, we collected Scottish wild barley nation-wide and characterised bacterial and fungal endophytes with an emphasis on local *Epichloë* species. Seed was collected from wild barley species for endophyte community analysis and a repository culture collection. Endophyte presence in the seed suggests vertical transmission within the plant, a desired trait for isolates used in plant protection for containment purposes and genetic maintenance through asexual reproduction only. Additionally, culture-independent methods have provided information regarding endophyte species occurrence as well as

geographical or cultivar association among endophyte communities. Currently no inventory of endophytes in native UK wild barley relatives exists. This study will underpin ongoing research to test domestic barley resilience to abiotic and biotic stresses when containing endophytic microorganisms.

P3.516 - Genomic characterization of fungal endophytes with biopesticide potential

J. Rojas[1,2], *C.E. Díaz*[3], *M.F. Andrés Yeves*[1], *A. Gónzalez-Coloma*[1], *J. Imperial*[4]
[1]Instituto de Ciencias Agrarias (ICA, CSIC), Madrid, Spain, [2]Universidad Politécnica de Madrid, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Madrid, Spain, [3]Instituto de Productos Naturales y Agrobiología (IPNA, CSIC), La Laguna, Spain, [4]Centro de Biotecnología y Genética de Plantas (UPM-INIA/CSIC), Pozuelo de Alarcón, Spain

There is an urgent demand for the development of greener and more sustainable pesticides due to the environmental and health concerns associated to the use of conventional synthetic pesticides. A promising approach is the exploration of biopesticides derived from endophytic fungi. Fungi are well-known sources of secondary metabolites (e.g., polyketides, terpenes, alkaloids), many of which have important applications in medicine, industry, and agriculture, and it is believed that production of such metabolites by endophytic fungi could play a role in protecting the host plant against biotic stresses, thus justifying their existence as symbionts. Despite its potential, much of the metabolic and genomic diversity of endophytic fungi remains undiscovered. We have undertaken characterization of the biopesticide potential of endophytic fungi isolated from plants already known to produce active biopesticides: two Macaronesian endemic plants, *Persea indica* and *Bethencourtia palmensis,* and the common wormwood (*Artemisia absinthium*). In this study, we present the annotated genomes of six endophytic fungal isolates selected for their activity against common plant pests, including insects, parasitic nematodes, bacterial pathogens, and fungal pathogens. These isolates include *Stemphylium* sp. from *A. absinthium*, *Phyllosticta* sp. from *P. indica*, and *Stemphylium* sp., *Alternaria* sp., and *Epicoccum* sp. from *B. palmensis*. Their potential for biopesticide production has been evaluated through the identification of their biosynthetic gene clusters involved in secondary metabolite production. This genomic and biosynthetic information provides a valuable resource for further investigations into the molecular pathways and potential biotechnological applications of these endophytic fungi.

P3.601 - Relationships between detoxification enzymes of white-rot fungi and their chemical environment

E. Gelhaye[1], *M. Morel-Rouhier*[2]
[1]INRAE, Interactions Arbres/Microorganismes, Nancy, France, [2]Universite de Lorraine, Interactions Arbres/Microorganismes, Nancy, France

White rot fungi possess the ability to degrade and mineralize all organic components of wood and have been widely studied for this property. Phylogenomic analyses suggest that the evolution of these fungi is linked to the presence of gene families encoding enzymes for the decomposition of

polymers present in wood such as peroxidases but also enzymes involved in import and detoxification pathways such as glutathione transferases.
Since several years, we have developed a " Functional Reverse Chemical Ecology" approach using these enzymes. A high-throughput method based on the measurement of the thermostability of the studied enzymes allowed to quantify the interactions between GSTs from the white rot fungus *Trametes versicolor* and extracts from different wood species from temperate and tropical forests. Quantification of these interactions showed a positive correlation between the antifungal properties of these extracts and their interactions with these GSTs of fungal origin. This approach, coupled with a targeted fractionation approach, has made it possible to identify molecules of interest but also to better understand and model the natural sustainability of tropical tree species. Overall, these data strongly support the hypothesis that detoxification systems and in particular GSTs can be effectively used as targets in "Reverse Chemical Ecology" approaches. This " Functional Reverse Chemical Ecology" should allow to characterize (i) the influence of a "chemical ecosystem" on the structural and functional evolution of multigenic families involved in detoxification within organisms and inversely, from the same experimental data, (ii) functionally the specialized metabolites from this "chemical ecosystem"

P3.602 - Exploring the HOG pathway mediated stress response in Aureobasidium pullulans

Z.A. Qayyum[1], *R. Mach*[1], *A. Mach-Aigner*[1], *C. Zimmermann*[1]
[1]TU Wien, Institute of Chemical, Environmental and Bioscience Engineering, Wien, Austria

The High Osmolarity Glycerol (HOG) pathway plays a crucial role in osmotic stress response and is particularly significant in dimorphic fungi that inhabit fluctuating, extreme environments. In *Saccharomyces cerevisiae*, this pathway is primarily activated through two sensory branches namely SLN1 and SHO1, while filamentous fungi typically utilize one of the two branches. Although the HOG pathway is well-characterized in model organisms, its role in non-model fungi, such as *Aureobasidium pullulans*, remains underexplored. Our research focuses on *A. pullulans*, a dimorphic, black, yeast-like fungus, known for its ability to thrive in a wide range of environments, including high osmolarity conditions. We observed that an impaired SLN1 branch increases stress sensitivity and locks the fungus in a yeast-like morphology, highlighting the critical role of the SLN1 branch in stress tolerance and morphological plasticity. We aim to uncover how this branch impairment affects downstream HOG pathway components, compatible solute production, melanin biosynthesis, and overall stress response in *A. pullulans* using quantitative real-time PCR (qRT-PCR) and transcriptomic analysis (RNA-seq). Preliminary results suggest significant differences in gene expression patterns between mutant and wild-type strains, particularly in glycerol synthesis, compatible solute production, and cell wall remodelling genes. Our findings provide novel insights into the molecular mechanisms underlying the remarkable stress adaptation and morphological plasticity in *Aureobasidium pullulans*.

P3.603 - Elucidating the role of *N*-deglycosylating enzymes in *Neurospora crassa* physiology

A. Samaras[1], *M. Karlsson*[1], *G. Tzelepis*[1]
[1]Swedish University of Agricultural Sciences, Forest Mycology and Plant Pathology, Uppsala, Sweden

N-deglycosylation, the removal of the glycan chain from N-glycoproteins, is critical for the further degradation of misfolded glycoproteins. Two key enzymes are involved in this process: peptide N-glycosidases (PNGases) and endo-β-N-acetylglucosaminidases (ENGases). In this study, we investigated the role of PNGases and ENGases in the physiology of the model species *Neurospora crassa*. Its genome contains two putative PNGases and two ENGases. We focused on the acidic and cytosolic PNGase (NCU04643) and ENGase (NCU01393) as previous data showed a secretion defect of the cytosolic ENGase in ascomycetes, while the role of the acidic PNGase is unknown. Our transcriptomic data showed that the *NCU04643* gene was induced under endoplasmic reticulum (ER) stress conditions, while no induction was observed for *NCU01393*. Furthermore, phenotypic analysis revealed that the ENGase deletion strain higher growth rate under ER, oxidative and hypoxic stress conditions compared to the wildtype strain. In contrast, the growth of the PNGase deletion strain was severely impaired by these conditions, suggesting the involvement of the deglycosylation process in fungal stress tolerance. Further experiments are planned to elucidate the precise role of these enzymes in fungal biology, such as RNAseq analysis, construction of double deletion mutants, enzymatic characterization of these proteins, and confocal microscopy. In conclusion, these preliminary results provide new insights into the biological significance of N-deglycosylation in fungal stress responses and contribute to a broader understanding of glycoprotein homeostasis, contributing to the discovery of new targets for future antifungal agents.

P3.604 - Stress-responsive Afu4g10610 gene plays a role in cell wall maintenance and osmotic regulation in *Aspergillus fumigatus*

E. Pelegri-Martinez[1], *U. Perez-Cuesta*[2], *S. Cendon-Sanchez*[1], *A. Ramirez-Garcia*[1], *X. Guruceaga*[3], *A. Rementeria*[1]
[1]University of the Basque Country (UPV/EHU), Department of Immunology, Microbiology and Parasitology, Leioa, Spain, [2]University of Tennessee Health Science Center (UTHSC), Department of Clinical Pharmacy and Translational Science, Memphis, TN, United States, [3]Institute for Multidisciplinary Research in Applied Biology (IMAB), Pamplona, Spain

Aspergillus fumigatus is the most pathogenic species among the fungi of the genus *Aspergillus* and has a high incidence and mortality in immunocompromised patients. Therefore, two distinct *in vitro* infection models of *A. fumigatus*, using murine macrophages (RAW 264.7) and human lung epithelial cells (A549), were employed to identify important genes for fungal adaptation during infection. Transcriptomic analyses of co-incubated *A. fumigatus* uncovered 140 fungal genes up-regulated in common between both models. Furthermore, these results were compared with a transcriptomic study of *in vivo* murine infection model previously published by our group,

13 consistently up-regulated genes were identified in all three infection models. Among them, we investigated the Afu4g10610 gene through the deletion mutant strain (*Δ10610*) generated by CRISPR-Cas9 gene-editing technique. This gene encodes a dimeric A/B barrel domain that is potentially involved in stress response. Phenotypic analysis showed increased sensitivity to cell wall stressors and enhanced resistance to osmotic compounds compared to the wild-type strain Af293. Furthermore, RT-qPCR expression analysis showed a significant imbalance in key mediators of the Cell Wall Integrity (CWI) and High Osmolarity Glycerol (HOG) pathways. Although the precise role and interactions of this gene remain to be elucidated, this dimeric protein seems to be involved in these pathways.

P3.605 - The oxylipin-responsive transcription factor ZfpA mediates tolerance against fungicidal tip lysis by β-1,3-glucan synthase targeting antifungals in *A. fumigatus*

D. Calise[1], *B. Chadwick*[2,3], *C. Hull*[1,2], *J. Coon*[2,3], *N. Keller*[1,4]
[1]University of Wisconsin - Madison, Medical Microbiology & Immunology, Madison, United States, [2]University of Wisconsin - Madison, Biomolecular Chemistry, Madison, United States, [3]Morgridge Institute for Research, Madison, United States, [4]University of Wisconsin - Madison, Plant Pathology, Madison, United States

The saprophytic fungus *Aspergillus fumigatus* is responsible for nearly all cases of invasive aspergillosis (IA) in addition to allergic and chronic pulmonary diseases. Treatment of IA is limited to only three approved classes of antifungals with echinocandins serving as an important salvage therapy when azoles fail. Echinocandins, such as caspofungin, are semisynthetic derivatives of natural lipopeptides produced by soil-dwelling Leotiomycetes and Eurotiomycetes fungi. These drugs act by inhibiting the enzyme β-1,3-glucan synthase to impair cell wall biogenesis. Although considered fungistatic against *A. fumigatus*, echinocandins exert both fungistatic inhibition of hyphal growth and fungicidal lysis of apical tip compartments. We found the transcription factor ZfpA mediates protection by the fungal oxylipin 5,8-diHODE against the fungicidal activity of echinocandins. ZfpA also contributes to tolerance of enfumafungin—a novel and structurally dissimilar β-1,3-glucan synthase inhibitor with similar antifungal activity. Interestingly, ZfpA does not protect against the cell wall stressor Congo Red indicating a specific role in β-1,3-glucan targeting antifungal tolerance. To identify ZfpA regulated proteins that prevent apical tip lysis, we performed proteomic analysis of WT, Δ*zfpA*, and OE::*zfpA* strains in response to caspofungin treatment. Candidate proteins were subsequently deleted and mutants assessed for increased susceptibly to caspofungin induced tip lysis.

P3.606 - Functional characterization of sugar transporters from *Neurospora crassa* and their involvement in coping with stress conditions

E. Tamayo[1], *L.M. Schaffer*[1], *B. Nada*[1], *I. Hafermann*[1], *J.P. Benz*[1]
[1]Technical University of Munich, Fungal Biotechnology in Wood Science, Holzforschung München, Freising, Germany

Filamentous fungi like *Neurospora crassa* can take up and metabolize various sugars from the environment, such as those found in agricultural wastes, which can be exploited in biotechnology. However, although fungi possess a variety of sugar transporter genes in their genome, most of them have not yet been characterized in their specific function. For example, to date, no transporters for fructose and sucrose have been identified in *N. crassa*, although this would be useful for the development of tools for the characterization of sugar transporters from other organisms. Furthermore, the involvement of sugar transport in the adaptability of fungi under abiotic stress experienced both in nature and during biotechnological fermentations has not yet been well studied. To identify high-probability candidates, uptake profiles of sugars of interest were recorded after subjecting *N. crassa* to a series of relevant induction conditions and correlated with the expression levels of all sugar transporter genes. By comparing this functional profile with the phylogenetic relationships of sugar transporters across several fungal species, we could identify transporters candidates for specific sugars. In this work, transporters for fructose and sucrose were identified and their function tested. Moreover, the influence of different stress conditions on sugar transport in *N. crassa* was explored, which led us to identify a novel monosaccharide transporter involved in osmotic stress in *Neurospora* sp.
The new knowledge could be used to better understand the mechanisms of sugar regulation in particular in response to abiotic stresses in fungi and improve fungal nutritional values under stress conditions.

P3.607 - Gene family expansions contribute to oxidative stress resistance across diverse yeasts

L. Horianopoulos[1], *K. Aranguiz*[1], *Q. Handy*[1], *L. Elkin*[1,2], *K. Segura Abá*[3], *R. Wrobel*[1], *S.-H. Shiu*[3,4], *A. Rokas*[5], *C.T. Hittinger*[1]
[1]University of Wisconsin-Madison, DOE Great Lakes Bioenergy Research Center, Wisconsin Energy Institute, Madison, United States, [2]Medical College of Wisconsin, Cell Biology, Neurobiology, and Anatomy, Milwaukee, United States, [3]Michigan State University, DOE Great Lakes Bioenergy Research Center, East Lansing, United States, [4]Michigan State University, Plant Biology, East Lansing, United States, [5]Vanderbilt University, Biological Sciences and Evolutionary Studies Initiative, Nashville, United States

Reactive oxygen species (ROS) are encountered by yeasts during routine metabolism, as well as during interactions with other organisms. The nature and frequency of these interactions as well as the balance between fermentative and respiratory metabolism may have prompted the evolution of a range of ROS resistance levels according to each species' life history strategy. We characterized the variation of ROS resistance across the yeast subphylum *Saccharomycotina* and identified gene family expansions underlying this trait (preprint: https://doi.org/10.1101/2024.08.14.607963). By measuring the relative growth of yeast species in the presence of tert-butyl hydroperoxide, which generates ROS, we classified yeasts as resistant or sensitive to ROS. We employed a machine learning model using a random forest algorithm to predict species' classifications and to identify gene families which were predictive of ROS resistance. The most predictive gene families included reductases and, interestingly, were

enriched in gene families related to cell wall organization. We functionally confirmed that the overexpression of the old yellow enzyme reductase resulted in increased resistance to ROS and that mutants lacking multiple mannosyltransferases were hypersensitive to ROS. The predictions of the machine learning model also revealed that several species were consistently misclassified, including the important pathogen, *Candida auris*, suggesting that there may be regulatory rewiring that prevents the full expression of ROS-mitigating genes or yet-uncharacterized mechanisms of resistance. Altogether, this work highlights gene family expansion as an important mechanism for ROS resistance and identifies new gene families related to ROS resistance that may be fruitful targets for antifungal therapeutics.

P3.608 - Transcriptome analysis of *Aspergillus oryzae* under exposure to fungistatic compounds of lignocellulosic side streams

M. Korpioja[1], *A. Kovalchuk*[1]
[1]VTT Technical Research Centre of Finland, Espoo, Finland

Carbohydrate-rich side streams of different industries (e.g., pulp and paper) are considered suitable carbon sources for cultivation of filamentous fungi. However, these streams often contain compounds that inhibit fungal growth. *Aspergillus oryzae* is a biotechnologically important filamentous fungus used in the production of variety of endogenous and recombinant proteins. Notably, the *A. oryzae* genome contains one of the highest numbers of predicted ATP-binding cassette (ABC) transporters among filamentous fungi, which often function as efflux transporters, exporting xenobiotics and antifungals.
This project aimed to better understand *A. oryzae*'s response to chemical stress induced by selected side stream components and identify potential genes involved in xenobiotic tolerance and detoxification. A transcriptomic study was conducted by growing *A. oryzae* with and without five common fungistatic compounds found in pulp and paper industry side streams. RNA sequencing data were generated from samples collected at five time points to explore adaptation mechanisms.
The study identified several upregulated genes involved in phases I–III of detoxification, including alcohol dehydrogenases, cytochrome P450 monooxygenases, dioxygenases, and MFS and ABC transporters. The induction of numerous redox enzymes may help fungal cells overcome oxidative stress caused by fungistatic compounds, contributing to their chemical transformation and metabolization. Upregulation of transporter-encoding genes likely allows the cells to keep intracellular concentrations of fungistatic compounds below the toxic levels by excreting them or sequestering them in the vacuole. This study complements existing data and provides new insights into the roles of oxidoreductases and transporters in *A. oryzae*'s response to fungistatic stress.

P3.609 - Set1-mediated H3K4 methylation is required for Candida albicans virulence by regulating intracellular level of reactive oxygen species

J. Kim[1], *S. Choi*[2], *E.-J. Lee*[2], *J.-S. Lee*[1]
[1]Kangwon National University, Department of Molecular Bioscience College of Biomedical Science, Chuncheon, Korea, Republic of, [2]Korea University, Department of Life Sciences, School of Life Sciences and Biotechnology, Seoul, Korea, Republic of

Candida albicans is an opportunistic human fungal pathogen that exists in normal flora but can cause infection in immunocompromised individuals. The transition to pathogenic *C. albicans* requires a change of various gene expressions. Because histone-modifying enzymes can regulate gene expression, they are thought to control the virulence of *C. albicans*. Indeed, the absence of H3 lysine 4 (H3K4) methyltransferase Set1 has been shown to reduce the virulence of *C. albicans*; however, Set1-regulated genes responsible for this attenuated virulence phenotype remain unknown. Here, we demonstrated that Set1 positively regulates the expression of mitochondrial protein genes by methylating H3K4. In particular, levels of cellular mitochondrial reactive oxygen species (ROS) were higher in Δ*set1* than in the wild-type due to the defect of those genes' expression. Set1 deletion also increases H_2O_2 sensitivity and prevents proper colony formation when interacting with macrophage *in vitro*, consistent with its attenuated virulence *in vivo*. Together, these findings suggest that Set1 is required to regulate proper cellular ROS production by positively regulating the expression of mitochondrial protein genes and subsequently sustaining mitochondrial membrane integrity. Consequently, *C. albicans* maintains proper ROS levels via Set1-mediated transcriptional regulation, thus establishing a rapid defense against external ROS generated by the host.

P3.610 - Transcriptional profiling reveals the role of *Candida albicans* Rap1 in oxidative stress response

H.-Y. Chen[1], *W.-H. Wang*[1], *S.-Y. Chen*[1], *C.-Y. Lan*[1,2]
[1]National Tsing Hua University / Institute of Molecular and Cellular Biology, Hsinchu, Taiwan, China , [2]National Tsing Hua University / School of Medicine, Hsinchu, Taiwan, China

Candida albicans is a commensal fungus in humans but can cause opportunistic infections, including life-threatening invasive candidiasis, particularly in immunocompromised patients. The ability of *C. albicans* to adapt to diverse environmental stress conditions is a key to its commensalism and virulence within the host. Rap1 is a conserved DNA-binding protein in yeasts, protozoa, and mammalian cells, playing multiple functions, including telomere regulation. Intriguingly, our previous study showed that Rap1 is also involved in cell wall integrity, biofilm formation, and virulence in *C. albicans*. In this work, using RNA-seq analysis and other approaches, the role of *C. albicans* Rap1 in oxidative stress response was further revealed. Additionally, the relationship between Rap1-mediated oxidative stress response and the mitogen-activated protein kinase (MAPK) Hog1, the transcription factor Cap1, and the TOR signaling was

determined. Together, these findings expand our understanding of the complex signaling and transcriptional mechanisms regulating stress responses in *C. albicans*.

P3.611 - Neurospora crassa through the lenses of stress adaptation – the role of transcription factor CCG8 in vitro as well as in Galleria melonella larvae

J. Víglaš[1], *K. Hollá*[2], *P. Gajdoš*[3], *S. Miček*[1], *P. Farkaš*[4], *P. Olejníková*[1]
[1]Institute of Biochemistry and Microbiology, Slovak University of Technology in Bratislava, Bratislava, Slovakia, [2]Institute of Food Science and Nutrition, Slovak University of Technology in Bratislava, Department of Nutrition and Food Quality Assessment, Bratislava, Slovakia, [3]Institute of Biotechnology, Slovak University of Technology in Bratislava, Bratislava, Slovakia, [4]Institute of Chemistry, Center for Glycomics, Slovak Academy of Scienses, Bratislava, Slovakia

The scientific field of antifungal resistance and tolerance is currently expanding its reach and with it, there is a need to have a strong foundation for substantiation of gathered knowledge. While new knowledge in (pathogenic) yeasts may be compared with information in the *Saccharomyces cerevisiae*, filamentous fungi lack such reference microorganisms. In this work, we subjected *Neurospora crassa* into such a role. While being non-pathogenic, we discovered that *N. crassa* still seem to have machinery to cope with stress conditions, including the response to antifungal agents. Transcription factor *CCG8* appeared to be involved in various stress conditions, e.g., stress to plasma membrane (including azoles) and cell wall (congo red). *CCG8* regulates numerous genes (doi: 10.3389/fmicb.2018.00027). Gene enrichment analysis of this dataset revealed *CCG8* to be indeed regulating genes involved in cell wall organization and lipid metabolism. HPLC-FID chromatography proved *N. crassa* Δccg8 to contain more linolenic acid, while in wild-type *N. crassa*, linoleic acid dominated. ATR-FTIR spectroscopy suggested *N. crassa* Δccg8 to have a higher relative ratio of proteins to sugars in the cell wall of the fungus. Finally, *in vivo* clearance of conidia of *N. crassa* in *Galleria mellonella* showed that the immune system of larvae reacted faster to the presence of *N. crassa* Δccg8 conidia (melanization). The hemocyte levels varied considerably, probably depending on physiological state of larvae, rather than conidia, though the rate of clearance of larvae seems faster with *N. crassa* Δccg8 conidia.
This work was supported by research grants No. VEGA 1/0388/22 and 09I03-03-V04-00659.

P3.612 - Exploring glycerol transport and function in *Neurospora crassa*

B. Nada[1], *E. Tamayo*[1], *J.P. Benz*[1]
[1]Technical University of Munich, Fungal Biotechnology in Wood Science, TUM School of Life Sciences, Freising, Germany

Under varying environmental conditions, fungi rely on glycerol as a central molecule for osmoregulation playing an essential role in maintaining cellular stability. Besides simple metabolism, the transport of glycerol is playing a significant role for stress adaptation. Despite glycerol's crucial role in cellular homeostasis, the mechanisms governing its transport across

plasma membranes is still unclear. Our study used *Neurospora crassa* as a model organism to investigate the mechanisms underlying glycerol transport in filamentous fungi. We focused on two putative glycerol transporters: NCU08052, a member of the aquaporin family, and the putative glycerol: H^+-symporter NCU02591. The primary investigation was performed using growth and uptake assays of glycerol, in which single mutant deletion strains of the target genes were compared to the wild type strain. Results showed a significant reduction in glycerol uptake, confirmed by growth defects on glycerol as the sole carbon source. To elucidate the function of these candidate proteins, complementary strains are being generated using the overexpression promoter p*CCG-1* and the native system. In addition, GFP tagging is being used to determine the cellular localization. To better define their function, we are expressing these genes in a heterologous system (*Saccharomyces cerevisiae* EBY.VW4000) that lacks the endogenous glycerol transporter Stl1. These findings will help to better understand glycerol transport in fungi and their adaptation to environmental stress.

P3.613 - Growth response of *Aspergillus oryzae* to soil component humic acid and elucidation of its mechanism

K. Sakai[1], *L. Liu*[1], *K.-I. Kusumoto*[1]
[1]Osaka University, Engineering, Suita, Japan

Aspergillus oryzae is a filamentous fungi used in the production of traditional fermented food products such as sake, soy sauce, and miso for hundreds of years. *A. oryzae* was thought to be domesticated from saprophytic ancestor strain by humans to adapt for fermentation and brewing after isolated from environment. In recent years, several reports of *A. oryzae* isolation from environmental samples have suggested that *A. oryzae* may possesses mechanisms to respond to and avoid various environmental stresses. We used humic acid (HA) as one of the environmental components and found that HA affected the growth of *A. oryzae* differently depending on the strain (growth promotion, growth inhibition, or no change) [Liu *et al*., JGAM (2023)]. Since growth responses and genetic classification were somewhat correlated, genetic changes during domestication may contributed to HA responses. To elucidate the mechanisms of HA response, genome sequences comparison was performed between strains that showed different HA responses and RNA-seq analysis under the cultivation condition with or without HA. Several candidate genes thought to be involved in HA response were selected, especially growth promotion, and constructed disruption strains. Among the disruptants, some with weakened HA responses were obtained, and we are investigating these strains in detail.

P3.614 - The oxidative stress response of *Neurospora crassa* in the presence of green tea catechins

K. Hollá[1], *J. Víglaš*[2], *L. Bírošová*[1], *P. Olejníková*[2]
[1]Institute of Food Science and Nutrition, Slovak University of Technology, Department of Nutrition and Food Quality Assessment, Bratislava, Slovakia, [2]Institute of Biochemistry and Microbiology, Slovak University of Technology in Bratislava, Bratislava, Slovakia

Catechins, natural flavonoids present in green tea, exhibit multiple biological activities beneficial for human health. These compounds are recognized as effective scavengers of reactive oxygen species (ROS); however, they can act as prooxidants in elevated concentrations. In this work, we investigated the antifungal efficacy of catechin-hydrate and epicatechin on the model organism *Neurospora crassa*, phytopathogen *Alternaria alternata*, and human pathogen *Aspergillus fumigatus*. For *N. crassa* we further examined the inhibitory potential of these catechins in combination with azole compounds in different concentrations. In experiments with catechin-hydrate, an additive effect was observed when combined with voriconazole and amphotericin B, while epicatechin demonstrated an additive effect in combination with voriconazole and micafungin. It is known that the generation of ROS is one mechanism of action of catechins against bacterial cells. However, there is a lack of information about the effects of catechins on the oxidative stress response in filamentous fungi. Therefore, we analyzed the expression of genes encoding antioxidant enzymes (*sod1*, *cat1*, *mrp*, and *pcat*) in *N. crassa* following treatment with varying concentrations of catechin-hydrate and epicatechin. We observed that catechin exposure significantly upregulates the expression of *cat1* which aligns with a corresponding increase in catalase relative specific activity. Additionally, we monitored the production of ROS in the mycelium of *N. crassa* using dihydrofluorescein diacetate probe, finding that ROS generation began early after catechin exposure (2 hours).
This work was supported by research grants No. VEGA 1/0388/22 and No. VEGA 1/0464/21.

P3.615 - Effects of the *Cryptococcus neoformans* high Cu stress response on fungal pathogenesis

C. Probst[1], *M. du Plooy*[2], *C. Denning-Jannace*[3], *K. Franz*[3], *J.A. Alspaugh*[1,2]
[1]Duke University, School of Medicine, Medicine, Durham, United States, [2]Duke University, School of Medicine, Molecular Biology and Microbiology, Durham, United States, [3]Duke University, Chemistry, Durham, United States

The ability to sense, import but also detoxify Cu is crucial for microbial pathogens during the course of an infection. Previous studies conducted with the human fungal pathogen *Cryptococcus neoformans* (*Cn*) revealed two extreme Cu environments encountered during infection: A high, toxic Cu environment during lung infection and a Cu limiting environment within the brain. The transcription factor (TF) *Cn* Cuf1 regulates both high- and low-Cu stress responses, suggesting a unique role for this Cu-responsive TF for gene regulation during Cu stress. To investigate possible mechanisms of high Cu sensing by Cuf1, we mutated a conserved region of cysteine residues within a predicted high Cu-responsive Cys-rich region in this transcription factor. Subsequent analysis of Cuf1 transcriptional activity demonstrated that this site mutation desensitizes Cuf1 to high Cu stress and is sufficient to uncouple Cuf1 driven high Cu stress responses from low Cu stress responses.
To assess the effects of blunted high Cu stress responses on pathogenesis, we performed an inhalational mouse model of infection. No significant difference in lung fungal burden was observed, however, we noted distinct macroscopic differences in the lungs of mice infected with WT compared to lungs infected with strains with a blunted high Cu stress response. Histopathology analysis confirmed altered immune responses between these two groups. Based on these findings, we hypothesize that Cuf1-driven high Cu responses are not required for

colonization of the mouse lung but modulate immune recognition and inflammation at the site of infection.

P3.616 - Responding to cell surface damage in *Candida albicans*

L. Chevalier[1], *M. Bassilana*[1], *R.A. Arkowitz*[1]
[1]University Côte d'Azur - CNRS - INSERM, Institute of Biology Valrose, Nice, France

Fungal virulence depends on the ability to sense and respond to dynamic external stimuli, including chemical and mechanical cues encountered at infection sites. Mechanosensors—proteins located on the fungal cell surface—play a critical role in detecting these mechanical cues and converting them into intracellular signals. These sensors are essential for processes such as cell wall remodeling, morphogenesis, and infection. The extensively studied mechanosensor, Wsc1 has been shown to mediate a range of cell wall stress responses in model yeasts.
In the opportunistic fungal pathogen *Candida albicans*, virulence is associated with the transition from a yeast-like ellipsoid form to an invasive filamentous form. Mechanical constraints are known to alter filament morphology and growth, but how *C. albicans* senses and responds to these physical cues remains poorly understood. This work aims to unravel the processes of mechanosensing and surface repair during *C. albicans* filamentous growth, with a focus on key proteins, including Wsc1.
Using single-cell time-lapse fluorescence imaging, we monitored the localization of Wsc1 and other proteins involved in cell wall remodeling in response to UV laser-induced surface damage. While Wsc1 was not recruited to the damage site, critical proteins such as active Rho1 GTPase, along with protein kinase C (Pkc1) and chitin synthase (Chs3) transiently accumulated at the wound, similar to findings in *S. cerevisiae*. Notably, the recruitment of these proteins was faster and to a greater extent in hyphal cells compared to budding cells, suggesting an enhanced capacity for surface repair in the *C. albicans* filamentous cells.

P3.617 - Characterization of a stress-induced circadian clock in *Alternaria alternata*

J. Groß[1], *A. Goaty*[2], *L. Larrondo*[2], *R. Fischer*[1]
[1]Karlsruhe Institute of Technology, Baden-Württemberg, Germany, [2]Universidad Cathólica de Chile, Santiago, Chile

Anticipating environmental changes during the course of a day is advantageous for many organisms and depends on an endogenous circadian clock. Circadian clocks are conserved from cyanobacteria to human and rely at the molecular level on positive and negative transcription-translation feed-back loops (Larrondo, 2025). In fungi the circadian clock has been analyzed in great detail in *Neurospora crassa* and was shown to also work in some other fungi. However, in some fungi, the clock is conditional and strongly depends on nutritional cues, or crucial components of the *N. crassa* clock are absent in fungi like *Aspergillus nidulans*. Here we studied *Alternaria alternata*, which harbors the Frq protein FrqA and the blue-light photoreceptor LreA as central clock components from *N. crassa*. Using the clock-controlled *frq* promoter we

constructed a luciferase-based *A. alternata* strain and were able to induce rhythmic expression of luciferase with white- or blue light or with temperature cycles for entrainment. However, a *free-run* of the clock under constant conditions was hardly visible. In order to decipher possible links with other signaling pathways, we analyzed the HOG pathway and found that in *hog* mutants the circadian clock showed robust *free-run* cycles. This interrelationship was explained by physical interaction of FrqA with the HOG pathway transcription factor AtfA. We present evidence that the circadian clock in *A. alternata* is conditional and only works under extreme stressful conditions. Furthermore, we show that FrqA plays a role in fungal interactions possibly through the control of secondary metabolite production.

Workshop Oral Presentations

Asperfest

WS1.01 - Co-evolution of biological processes as revealed by whole genus genome sequencing of *Aspergillus*

R.P. de Vries[1], *I.V. Grigoriev*[2], *M.R. Andersen*[3], *S.E. Baker*[4]
[1]Westerdijk Fungal Biodiversity Institute, Fungal Physiology, Utrecht, Netherlands, [2]DOE Joint Genome Institute, Berkeley, United States, [3]Technical University of Denmark, Technical University of Denmark, Lyngby, Denmark, [4]Pacific Northwest National Laboratory, Environmental Molecular Sciences Division, Richland, United States

The rapid expansion of the set of fungal genomes has provided many new insights in the biology of these organisms and their diversity. *Aspergillus* is one of the best studied fungal genera, due to its relevance as an opportunistic pathogen of humans and animals, a spoilage organism of many foods and its wide application in biotechnological processes. In recent years we have generated genome sequences for nearly 300 *Aspergillus* species, providing an unprecedented dataset of a fungal genus.
This dataset has now been used to generate an evolutionary roadmap of biological processes to identify at which level each of these processes has gone through major changes, focusing on the genus, subgenus, section and species level. Rather than studying the conservation of individual genes or gene groups, we analyzed the evolutionary pattern at the level of process covering the following topics: carbon utilization (CAZy-genes, primary carbon metabolism, sugar transporters), nitrogen utilization (nitrogen and amino acid metabolism), secondary metabolism, stress response, development and propagation (mating, sexual/asexual development, conidiation). Subsequently, the evolutionary patterns of these processes have been compared to reveal which processes co-evolve and which occur at different taxonomic levels. This has provided new insights into fungal evolution, which will also be a template for other whole genus genome projects, such as those of *Penicillium* and *Trichoderma*.

WS1.02 - Evolutionary transcriptomics to understand conidial development and germination of pathogenic and non-pathogenic Aspergillus species

P. Wu[1], *K.H. Wong*[1]
[1]University of Macau, Macao, China

Fungal conidia are the main infectious propagules of many human fungal pathogens. Inhalation of conidia by the host is a major entry route for pathogenic fungi, such as *Aspergillus fumigatus*, that cause lung and systemic infections. In order to cause lung infection, the inhaled conidia have

to germinate and grow within the lung of a host. Conidial size and germination are believed to be important attributes of pathogenic species. Therefore, understanding conidia development and germination has important medical implications. In this study, we performed transcription profiling to study conidial development and germination in the human pathogen *Aspergillus fumigatus* and the non-pathogenic research model *Aspergillus nidulans*. Comparative and evolutionary transcriptomic analysis revealed that the transcriptomes of the two species during conidial germination are highly conserved, including numerous ancient genes with a pattern reminiscent of the "Hourglass" evolution model. In contrast, the conidiation process involved many modern genes, conforming to a "Reverse Funnel-like Model" of evolution. These findings suggest that the pathogenic features of *A. fumigatus* may have evolved, at least in part, from conidiation rather than germination. Evolutionary transcriptomic analysis of additional non-pathogenic and pathogenic species will provide further insights into the evolution of fungal pathogenicity. These findings have important implications for combating fungal infections and developing novel antifungal strategies.

WS1.03 - High recombination rates in *Aspergillus fumigatus* allows for bulk quantitative trait locus (QTL) mapping of known and novel azole resistance and fitness traits

C. Valero[1], *M. Bottery*[2], *B. Auxier*[3], *F. Stanford*[4], *P.S Dyer*[4], *M. Bromley*[1]
[1]The University of Manchester, Manchester Fungal Infection Group, Manchester, United Kingdom, [2]The University of Manchester, Manchester Fungal Ingection Group, Manchester, United Kingdom, [3]Wageningen University, Wageningen, Netherlands, [4]University of Nottingham, Nottingham, United Kingdom

Aspergillus fumigatus is an environmental fungus that can cause life-threatening or debilitating lung diseases. The number of *A. fumigatus* human infections resistant to the first-line treatment azole drugs have increased over last years which has been linked to the widespread use of azoles fungicides in agriculture. Azole resistance is primarily caused by variants in the gene coding the azole target Cyp51A, however alternative and complementary non-target variants are increasingly recognized as the cause for antifungal resistance which are potentially coupled with variants associated with increased fitness. Recently, it has been demonstrated that *A. fumigatus* harbours the highest known rate of meiotic crossovers during sexual reproduction generating a highly recombinant progeny which allows for fine mapping of traits of interest. Here, we have developed a high-throughput bulk QTL mapping approach to identify variants causing azole resistance in *A. fumigatus*. An azole sensitive strain was crossed with an environmental strain with known mechanism of azole resistance (*cyp51A*$^{TR34/L98H}$) and pooled F1 progeny was exposed to voriconazole (0.5µg/ml). Using a custom QTL bioinformatic pipeline we were able to identify not only the variant conferring azole resistance (*cyp51A*$^{TR34/L98H}$) but also complementary variants contributing to general fitness. This technique offers a great potential for identifying the underlying mechanism of complex polygenic traits such as antifungal resistance and fitness.

WS1.04 - Distinct trafficking routes of polarized and non-polarized membrane cargoes in *Aspergillus nidulans*

G.M. Sagia[1], X. Georgiou[1], G. Chamilos[2,3], S. Dimou[1], G. Diallinas[1,3]
[1]University of Athens, Department of Biology, Athens, Greece, [2]University of Crete, School of Medicine, Heraklion, Greece, [3]Foundation for Research and Technology, Institute of Molecular Biology and Biotechnology, Heraklion, Greece

Membrane proteins are sorted to the plasma membrane (PM) via Golgi-dependent trafficking. However, our recent studies challenged the essentiality of Golgi in the biogenesis of specific transporters. Here, we investigate the trafficking mechanisms of membrane proteins by following the localization of the polarized R-SNARE SynA versus the non-polarized transporter UapA, synchronously co-expressed in wild-type or isogenic genetic backgrounds repressible for conventional cargo secretion. In wild-type, the two cargoes dynamically label distinct secretory compartments, highlighted by the finding that, unlike SynA, UapA does not colocalize with the late-Golgi. In line with early partitioning into distinct secretory carriers, the two cargoes collapse in distinct ERES in a sec31ts background. Trafficking via distinct cargo-specific carriers is further supported by showing that repression of proteins essential for conventional cargo secretion does not affect UapA trafficking, while blocking SynA secretion. Overall, this work establishes the existence of distinct, cargo-dependent, trafficking mechanisms, initiating at ERES and being differently dependent on Golgi and SNARE interactions.

WS1.13 - Tracking hyphal fusion in highly diverse *Aspergillus fumigatus* populations to identify the formation of multi-drug resistance heterokaryon compatibility groups

M. Bottery[1], E. Tancredi[1], A. Sarkar[2], S. Gupta[3], N. Osherov[2], M. Bromley[1]
[1]University of Manchester, Division of Evolution, Infection and Genomics, Manchester, United Kingdom, [2]Tel Aviv University, Tel Aviv, Israel, [3]Trinity College Dublin, Dublin, Ireland

Aspergillus fumigatus is a significant human pathogen capable of causing severe pulmonary infections. Heterokaryon formation, achieved through hyphal fusion, promotes the horizontal transfer of ecologically important traits among related individuals. This is particularly important within long-term chronic infections where local genetic diversity, generated through mutation, can be disseminated within mycelial networks. Stressors such as antifungal treatment can induce hyphal fusion, potentially stimulating the accumulation of multiple resistance alleles within single individuals. However, hyphal fusion is tightly regulated to limit non-self-fusion through a multigenic incompatibility system controlled by heterokaryon incompatibility (*het*) loci, which likely evolved to prevent the spread of selfish genetic elements such as viruses and transposable elements. To investigate hyphal fusion dynamics and identify novel *het* loci, we developed a high-throughput method to track hyphal fusion events between *A. fumigatus* isolates with distinct resistance profiles within large, diverse populations. Our results demonstrate that fusion preferentially occurs between isogenic partners but can also take place with genetically distinct but phylogenetically closely related individuals, suggesting the existence of fusion compatibility

groups. By screening genetic loci for high divergence between groups and low divergence within groups, we identified putative novel *het* loci. These results highlight that cell fusion is not restricted to isogenic strains and can promote the sharing of genetically distinct nuclei harbouring different resistance alleles between individuals. This mechanism could contribute to the rapid spread of multiple resistances within *A. fumigatus* populations.

WS1.14 - Dominant negative effect on UPR and RIDD by expression of RNase-inactive IreA in *Aspergillus oryzae*

M. Yokoyama[1], *K. Gomi*[1], *T. Shintani*[1]
[1]Tohoku University, Graduate School of Agricultural Science, Sendai, Japan

Aspergillus oryzae is a promising host for recombinant protein production due to its high protein secretion capacity, but the productivity of heterologous proteins is significantly low. This may be due to the removal of mRNAs and/or proteins by quality control mechanisms in the host during gene expression and protein secretion. Therefore, we focused on Regulated IRE1-dependent decay (RIDD), mRNA degradation mechanism in the endoplasmic reticulum (ER). Under ER stress, the ER transmembrane sensor protein IreA is activated through oligomerization and phosphorylation, and specifically degrades secretory protein mRNAs targeted to the ER membrane by its RNase activity. IreA is also involved in the splicing of *hacA* mRNA in the unfolded protein response (UPR), and its gene disruption is lethal. In this study, we introduced mutation into the RNase domain of IreA and we analyzed the effects on *hacA* splicing and RIDD. Mutations were introduced into highly conserved amino acids in the RNase domain identified from the multiple alignments with other organisms. The resulting *ireA* mutants were expressed with its own promoter in a strain that can suppress expression of host-derived *ireA* by addition of thiamine. Mycelial growth was not complemented by expression of the *ireA* mutants in the presence of thiamine, but rather was even more inhibited. Furthermore, expression of mutant *ireA* reduced *hacA* splicing ability and RIDD of amylase mRNA under ER stress condition even when endogenous *ireA* was not suppressed. These results indicate that the expression of RNase-inactive IreA has a dominant-negative effect.

WS1.15 - Discovery and characterization of the first fungal granulin in *Aspergillus fumigatus*

U. Perez-Cuesta[1], *X. Guruceaga*[2], *S. Cendon-Sanchez*[3], *E. Pelegri-Martinez*[3], *A. Martin-Vicente*[1], *H. Thorn*[1,4], *J. Xie*[1,4], *A. Nandi*[1], *D. Bale*[1,4], *A. Ramirez-Garcia*[3], *J. Fortwendel*[1,5], *A. Abad-Diaz-de-Cerio*[3], *A. Rementeria*[3]
[1]University of Tennessee Health Science Center, Clinical Pharmacy and Translational Science, Memphis, United States, [2]Institute for Multidisciplinary Research in Applied Biology, Public University of Navarre, Pamplona, Spain, [3]University of the Basque Country, Department of Immunology, Microbiology and Parasitology, Leioa, Spain, [4]University of Tennessee Health Science Center, PhD Program in Pharmaceutical Sciences, Memphis, United States, [5]University of Tennessee Health Science Center, Deparment of Microbiology, Immunology and Biochemistry, Memphis, United States

Granulin is a secreted growth factor conserved among eukaryotic organisms. In humans, it is related to neuronal, autoimmune, and cancer diseases because it has a role in survival, growth modulation, migration, inflammation, and wound repair. It was thought that fungi had lost this type of domain since there is no sequence homolog, but in this work, we described a conserved protein in filamentous fungi with the same 3D structure. Different approaches using *Aspergillus fumigatus* mutant strains were employed to demonstrate that human and fungal proteins have similar functions and localization. Phenotypic characterization of the deletion strain revealed that the fungal protein is implicated in cell proliferation, polarization, conidiation, morphology, septation, stress resistance, and cell wall integrity. The absence of the gene produced a significantly lower expression of the cell wall integrity pathway and microtubule and cell end markers-related genes. The protein was found in the secretome being one of the first described extracellular polarization determinants of *A. fumigatus*. Therefore, the protein was localized in the cell membrane during germination and in the external hyphae of solid colonies. Finally, genetic replacement of the fungal protein with human Granulin A confirmed the homology between both proteins since this mutant strain almost phenocopied wild-type strain rescuing the defects observed in the deletion strain.

WS1.16 - The multipurpose cell factory *Aspergillus niger* can be engineered to produce hydroxylated collagen

T. Morris[1], *T.C. Cairns*[1], *V. Meyer*[1]
[1]Technische Universität Berlin, Berlin, Germany

Advances in tissue printing and wound healing necessitate a continuous global supply of collagen. Microbial systems are highly desirable to meet these demands as recombinant collagenous proteins can be guaranteed as free from animal viruses. The filamentous cell factory *Aspergillus niger* has been instrumental for decades in the production of organic acids, enzymes and proteins, yet this fungus has not been explored for recombinant collagen production. In this study, we conducted extensive genetic engineering and fermentation optimization to provide proof of principle that *A. niger* can produce hydroxylated collagen.
We used a modular cloning system to generate a suite of cassettes encoding numerous n-terminal secretion signals, biodesigned/native collagen genes and, additionally, various prolyl-4-hydroxylases (P4H) for protein hydroxylation. These were expressed in a previously constructed *A. niger* isolate which is capable of producing the crucial P4H cofactor ascorbic acid. We conducted a wide range of media optimization studies to increase collagen production and hydroxylation levels. Additionally, we deleted an endopeptidase encoding gene, which was likely responsible for degrading secreted collagen. These studies generated an isolate capable of secreting partially hydroxylated collagen to titres of approximately $5mgL^{-1}$. Comparative transcriptomic analyses are currently ongoing to identify further candidate genes for genetic and metabolic engineering approaches.

WS1.18 - RNA-binding protein SsdA shows dynamic localisation and transport during hyphal growth in *Aspergillus nidulans*

D. Modaffari[1], *E.W. Wallace*[1], *K.E. Sawin*[1]
[1]University of Edinburgh, School of Biological Sciences, Edinburgh, United Kingdom

Filamentous fungi grow through highly polarized hyphal extension, requiring precise spatial coordination of vesicle fusion and cell wall material delivery at the growing tip. While mRNA transport along hyphae is well-documented in fungi, the relationship between RNA regulation and polarized growth remains poorly understood. Using *Aspergillus nidulans* as a model organism, we characterized SsdA, a putative RNA-binding translational repressor homologous to *Saccharomyces cerevisiae* Ssd1.
Bioinformatic analysis revealed that putative SsdA RNA targets are enriched for cell wall-related proteins, suggesting a role in regulating fungal cell wall synthesis. Live-cell fluorescence microscopy demonstrated that SsdA moves along microtubules in association with early endosomes, dependent on the endosomal hitchhiking-mediator PxdA. Notably, we observed that SsdA particles are absent from growing hyphal tips.
Our findings describe the dynamic localization pattern of SsdA during hyphal growth. We hypothesise SsdA might regulate tip-specific translation by repressing target mRNAs during transport and releasing this repression at hyphal tips. Ongoing work is investigating the dynamics of SsdA's RNA targets and the role of its upstream kinase CotA.

WS1.19 - Reference pangenomes improve ‘omics analysis of fungi by capturing their genetic diversity: a demonstration from *Aspergillus fumigatus*

M. Perrier[1,2], *C. Puerner*[3], *A.A. Golicz*[4], *J.E. Stajich*[5], *R.A. Cramer*[3], *A.E. Barber*[1,2]
[1]Friedrich Schiller University, Institute of Microbiology, Jena, Germany, [2]Friedrich Schiller University, Cluster Balance of the Microverse, Jena, Germany, [3]Dartmouth College, Geisel School of Medicine, Department of Microbiology and Immunology, Hanover, United States, [4]Justus Liebig University, Department of Agrobioinformatics, IFZ Research Center for Biosystems, Land Use and Nutrition, Giessen, Germany, [5]University of California-Riverside, Department of Microbiology and Plant Pathology, Riverside, United States

Fungi harbour a tremendous amount of genomic diversity, including marked differences in gene content even within the same species. A prominent example is *Aspergillus fumigatus*, a ubiquitous environmental mould responsible for an estimated 1.5 million deaths annually. Only 69% of the total genes of the species are conserved in all isolates, with a large number showing presence-absence variation. Due to their absence in the reference strains, the role of these accessory genes in stress resistance, metabolism, and virulence remains unknown. To create a tool that captures species’ diversity with the ultimate goal of understanding the function of these accessory genes, we used 26 near-chromosomal level genome assemblies to create a pangenome reference for *A. fumigatus*. This reference has a length of 38 Mbp, 30% longer than the current Af293 reference, and encodes 2,260 ORFs absent in Af293. This novel tool can be used for the

unbiased but computationally straightforward analysis of genomic and transcriptomic data from diverse strains. As a demonstration that the graph pangenome better captures *A. fumigatus*' diversity, alignment of genomic and transcriptomic data resulted in notably more reads aligned than the linear reference. Ongoing work uses this new reference for the high-resolution quantification of the genomic adaptations that occur during chronic infection and to understand the role of the accessory genome in the virulence of *A. fumigatus* using a large transcriptomic dataset. This work highlights the value of reference pangenomes for improving our understanding of strain heterogeneity and how it contributes to diverse biological processes.

WS1.20 - Elucidating the antifungal modes of action of G-quadruplex-stabilising ligands in A. fumigatus

I. Storer[1], *G. Middleton*[1], *E. Barclay*[2], *J. McColl*[3], *B. Lezé*[1], *Z. Waller*[4], *S. Bidula*[1]
[1]University of East Anglia, School of Chemistry, Pharmacy and Pharmacology, Norwich, United Kingdom, [2]John Innes Centre, Bioimaging Facility, Norwich, United Kingdom, [3]University of East Anglia, Henry Wellcome Laboratory for Cell Imaging, Norwich, United Kingdom, [4]University College London, School of Pharmacy, London, United Kingdom

Invasive aspergillosis, caused by fungal pathogens in the genus *Aspergillus*, causes almost 2 million deaths each year. Due to the emergence of antifungal resistance, the drugs we use to treat fungal infections are becoming increasingly ineffective. Therefore, new drugs with novel mechanisms of action are urgently needed. We have shown that ligands that stabilise G-quadruplexes (G4s), four-stranded secondary structures found in DNA and RNA, prevent the growth of *A. fumigatus*, *Candida* spp., and dermatophytes. Here, we show that the G4-stabilising ligand, PhenDC3, increased the number of stable G4s in A. fumigatus RNA. Notably, spores treated with PhenDC3 became swollen, but did not germinate. This increase in spore size was associated with a significant increase in the thickness of the cell wall. Similarly, another G4-stabiliser, pyridostatin, prevented germination and spore swelling. TEM imaging indicated that PhenDC3 could significantly impact organelle organisation. These impacts were explored further by imaging the nuclei, mitochondria, cell membrane, peroxisomes, and vacuoles. Finally, we investigated transcription using RNAseq and uncovered differential expression of genes associated with primary metabolism upon PhenDC3 treatment. These genes are predicted to contain G4s by prediction software, suggesting G4 sequences in these genes were stabilised by PhenDC3, preventing transcription. This work describes the first steps in identifying the target or targets of G4-stabilising ligands PhenDC3 and pyridostatin to guide the design of fungal-specific DNA/RNA-binding antifungal agents.

WS1.21 - Multi-omics analysis of a fungal cell factory producing recombinant enzyme controlled by a constitutive or inducible promoter

J.V. Bernardi[1,2], *E.P. Antoniel*[1], *M.F. Cavalheiro*[2], *L.O.S. Silva*[1], *M.M. Brandão*[2], *A. Damasio*[1]
[1]UNICAMP, Biochemistry and Tissue Biology, Campinas, Brazil, [2]UNICAMP, Center for Molecular Biology and Genetic Engineering, Campinas, Brazil

Aspergilli are filamentous fungi known for their high secretion capacity, making them effective cell factories for industrial enzyme production. However, some challenges can limit enzyme titers, so optimizing fungal strains is crucial to enhance enzyme production. This study compares two *Aspergillus nidulans* strains expressing α-L-arabinofuranosidase (AbfA) from *Aspergillus fumigatus*, controlled by either a constitutive (glyceraldehyde-3-phosphate dehydrogenase promoter, pgpdA, from *A. nidulans*) or an inducible (glucoamylase promoter, pglaA, from *Aspergillus niger*) promoter. The strain with the constitutive promoter showed 0.71-fold higher AbfA secretion and 4.11-fold increased enzyme activity. However, higher *abfA* mRNA expression (5.49-fold) was detected by qPCR in the strain with the inducible promoter. To further understand why the strain with higher mRNA levels secretes the lowest amount of enzyme, we performed RNA-seq and proteomic. Transcriptomic confirmed higher *abfA* levels in the pglaA::abfA strain (2.65-fold). Gene ontology enrichment revealed that oxidative stress was overrepresented under the control of the pglaA, while amino acid biosynthesis was highlighted for the pgpdA. Transcript levels of unfolded protein response genes were similar in both strains, suggesting that misfolded proteins may not limit AbfA secretion in the pglaA::abfA strain. Co-expression network identified genes whose expression regulation was directly associated with AbfA production, and their roles in fungal cell factories are under investigation. Proteomic data showed differential expression of proteins involved in signaling, DNA packaging, and protein synthesis. AbfA was not found in the intracellular proteome, indicating effective secretion. The transcriptomic-proteomic correlations will contribute to unraveling potential bottlenecks that limit AbfA control by the inducible promoter.

WS1.22 - FungiDB: a bioinformatics resource for facilitating data exploration, analysis, and integration for fungal and oomycete species

E. Basenko[1], *O. Harb*[2], *D. Roos*[2], *A. Jones*[1]
[1]University of Liverpool, Institute of Systems, Molecular and Integrative Biology, Liverpool, United Kingdom, [2]University of Pennsylvania, Department of Biology, Philadelphia, United States

FungiDB, a component of the VEuPathDB project, provides a one-stop shop for omics data exploration and analysis for over 300 fungal and oomycete species, including organisms on the WHO fungal priority list. FungiDB integrates diverse data types and enables researchers to interrogate the data using various tools such as the search strategy system, a genome browser, and comprehensive gene pages. Integrated data types include genomic, transcriptomic, proteomic, metabolomic, population-level and phenotypic studies and more. Built-in orthology enables cross-species inferences and enhances the reusability of datasets across the integrated organisms. Key features of FungiDB include:
- Extensive information on genomes, including gene record pages and automated and expert-curated annotations.
- Tools for conducting comparative genomics, analyzing protein structures, exploring gene regulatory networks, and investigating pathogen-host interactions.
- Integration of publicly available datasets, enabling researchers to conduct in silico experiments and explore data enrichment analyses in the context of existing data.
- Ongoing curation and community-driven enhancements to genomes in the genome editor

Apollo to ensure the capture of up-to-date, high-quality annotations by leveraging community expertise.
Future advancements are aimed at integrating AI-driven tools to enhance literature curation and metadata annotation and incorporating tools for new data types.

WS2.02 - Using the CRISPR/Cas9 system for GFP-tagging of endogenous genes in *Neurospora crassa*

S. Grüttner[1]
[1]CAU Kiel/Botanical Institute, Kiel, Germany

Neurospora crassa is a widely used eukaryotic model organism due to its diverse biology and rapid growth. Traditional genetic manipulation methods, such as homologous recombination (HR), are time-consuming and labor-intensive[1]. Conversely, the RNA-guided CRISPR/Cas9 system provides high efficiency, is easy to handle, and allows precisely targeted mutagenesis. This system has already been successfully applied in some filamentous fungi[2]. We have developed a user-friendly CRIPSR/Cas9 system for *N. crassa* by integrating the *cas9* sequence into the fungal genome and introducing guide RNA (gRNA) as naked RNA via electroporation into the cell[3]. This method was utilized to generate loss-of-function mutants of *N. crassa* and to simultaneously edit two genes. Here we show, that our system is also suitable for GFP-tagging of endogenous genes, and it effectively increases the efficiency of HR compared to the HR efficiency without the Cas9-induced dsDNA break. Compared to traditional methods, the user-friendly and effective system provides a rapid and efficient method for generating loss-of-function and knock-in mutants in *N. crassa*.

References:

1. Wang, S. *et al.* Molecular tools for gene manipulation in filamentous fungi. *Appl Microbiol Biotechnol* **101**, 8063–8075 (2017).
2. Schuster, M. & Kahmann, R. CRISPR-Cas9 genome editing approaches in filamentous fungi and oomycetes. *Fungal Genetics and Biology* vol. 130 43–53 Preprint at https://doi.org/10.1016/j.fgb.2019.04.016 (2019).
3. Grüttner, S. & Kempken, F. A user-friendly CRISPR/Cas9 system for mutagenesis of *Neurospora crassa*. *Sci Rep* **14**, (2024).

WS2.03 - Exploring plant-fungal interactions: insights from *Neurospora crassa* and *Brachypodium distachyon*

H. Winter[1], *K. Kolláth-Leiss*[1], *F. Kempken*[1]
[1]Christian-Albrechts-University, Molecularbiology and Genetics, Kiel, Germany

Fungi play a dynamic role in complex relationships with plants. There is an abundance of variability shown in these linkages, including mutualistic and pathogenic interactions. Pathogenic fungi pursue biotrophic and hemibiotrophic lifestyles, damaging their host counterpart in the prosses to utilize their nutrients. On the contrary, mutualistic relationships like mycorrhiza (MR) result in mutually beneficial outcomes for both fungus and plants. These associations appear when filamentous fungi invade plant root systems in order to create a stable and mutually beneficial coexistence.

Utilizing two well-known model organisms – the ascomycete *Neurospora crassa* and the grass *Brachypodium distachyon* – this research explores plant-fungal interactions in an intriguing and reliable way, allowing the execution of thorough investigations in cell biology and genetics. Despite being a saprophytic fungus with an abundance of studies, not much is known about the ecological attributes of *N. crassa*. On the other hand, *B. distachyon*, a sweet grass closely related to important crops, is remarkably flexible in the environment and engages in a range of fungal interactions, including mutualistic and damaging relationships.
Using electron, fluorescence and confocal laser scanning microscopy characterizing this interaction. Our findings suggest that most plant root tissue is unimpaired by fungal invasion, though single hyphae show growth in the apoplastic space, root cortex cells and the vascular cylinder. The infected cells were often surrounded by non-infected, living neighbor cells. Employing the *Neurospora* knock-out collection we present specific mutants with hampered root colonization.

WS2.04 - The BEM46 protein at the crossroads of polarity maintenance, auxin biosynthesis and endophytic growth of *Neurospora crassa*: a working model

K. Kollath-Leiss[1], *H. Winter*[1], *F. Kempken*[1]
[1]CAU zu Kiel, Botanical Genetics and Molecularbiology, Kiel, Germany

The BEM46 protein is conserved across eukaryotes and shares homology with human ABHD12, a lipase linked to neurological disorders [1–3]. Investigations in *Drosophila melanogaster* [4], *Arabidopsis thaliana* [5], and our research in *N. crassa* suggest an elusive function of the protein in polarity maintenance. In *N. crassa*, BEM46 affects ascospore and conidiospore germination and is localized to the endoplasmic reticulum (ER) and eisosomes [6]. The protein interacts with the tryptophan synthase and influences the fungus' complex auxin biosynthesis [7]. Whole transcriptome and *in silico* analyses suggest BEM46's potential lipase activity, which is being investigated *in vitro*. Additionally, eisosome formation and the endophytic behavior of *N. crassa* in different bem46 backgrounds are being analyzed microscopically. We present a working model summarising our results and hypotheses, how the BEM46 protein impacts the fugus' lipid homeostasis and -as a consequence of this- influences eisosome formation and its endophytic behavior.
1. Kumar A et al. Biochem Biophys Res Commun. 2013;438(3).
2. Joshi A et al. J Biol Chem. 2018;293(44):16953–63.
3. Fiskerstrand T et al. Am J Hum Genet. 2010;87(3):410–7.
4. Parmentier ML et al. J Neurosci. 2000;15;20(14):RC84.
5. Mochizuki S et al. Plant Cell. 2005;17(2):537–47.
6. Mercker M et al. Curr Genet. 2009;55(2):151–61.
7. Kollath-Leiß K et al. Eukaryot Cell. 2014;13(8):1051–63.

WS2.05 - Molecular interactions and functional role of the essential protein TEA-5 in polarized growth of *Neurospora crassa*

R.R. Mouriño-Pérez[1], *O.A. Callejas-Negrete*[1], *A.N. Espino-Vazquez*[2], *P.A. Lopez-García*[1]
[1]Centro de Investigación Científica y de Educación Superior de Ensenada, Department of Micorbiology, Ensenada, Mexico, [2]Universidad Autónoma de Baja California, Instituto de Investigaciones Oceanológicas, Ensenada, Mexico

This study examines the roles and molecular interactions of three proteins in the TEA complex of *Neurospora crassa*: TEA-1, TEA-4, and TEA-5, homologous to Tea1/TeaA, Tea4/TeaC, and Mod5/TeaR in *S. pombe*/*A. nidulans*., with emphasis in TEA-5. This complex is crucial for interactions between microtubules and the actin cytoskeleton at growth sites. While TEA-1 and TEA-4 mutants showed no impact on fungal growth or morphogenesis, the deletion of *tea-5* proved lethal at the ascospore stage, calling for the study of a heterokaryotic Δ*tea-5*^{Het} mutant. This strain displayed significant reductions in elongation rate (43%), biomass (57%), and conidia production (68%), alongside a two-fold increase in branching rate compared to the wild type. The Δ*tea-5*^{Het} mutant also had a disorganized cytoskeleton and a small, unstable Spitzenkörper. To further investigate TEA protein interactions, Co-Immunoprecipitation (Co-IP) experiments were performed with GFP-Trap and analyzed through LC-MS/MS. Results confirmed physical interactions among TEA-1, TEA-4, and PP1 (homologous to Dis2 in *S. pombe*) and an association between TEA-1 and microtubules. Additional novel interactions of TEA-4 and TEA-5 with proteins linked to cell wall biogenesis and vesicle trafficking were also identified. These findings underscore TEA-5 essential role in *N. crassa*'s polarized growth, with interactions among TEA proteins and regulatory molecules like PP1 being vital for microtubule-actin dynamics.

4o

WS2.06 - Exploring selective autophagy in the filamentous fungus *Sordaria macrospora*: identifying interactors of the pexophagy receptor SmNBR1 via BioID

S. Groth[1], *A. Werner*[2], *K. Schmitt*[3], *O. Valerius*[3], *S. Pöggeler*[1]
[1]Georg-August-University of Göttingen, Department of Genetics of Eukaryotic Microorganisms, Göttingen, Germany, [2]Georg-August-University of Göttingen, Department for Molecular Structural Biology, Göttingen, Germany, [3]Georg-August-University of Göttingen, Department of Molecular Microbiology and Genetics, Göttingen, Germany

Selective autophagy is a conserved subcellular process that uses receptors to target specific cargo for degradation in the vacuole. In the filamentous ascomycete *Sordaria macrospora, neighbour of BRCA1 (SmNBR1) was identified as receptor for the* selective autophagy of peroxisomes (pexophagy). However, little is known about further players in the pexophagy of *S. macrospora*. Thus, we aimed to identify SmNBR1-interactors by employing the Biotin Identification (BioID) method. Hereby, SmNBR1 is fused to a promiscuous biotin-ligase, which labels neighbouring

proteins with biotin. Biotin-labelled proteins are then enriched by affinity purification and identified by liquid chromatography coupled to mass spectrometry (LC-MS). In these experiments, we identified several proteins to be putative SmNBR1 interactors. Among them are the core autophagy protein SmATG11 and a coupling of ubiquitin to ER degradation (CUE) domain-containing protein, which we termed SmCUE3. Since ATG11 homologs in other organisms are scaffold proteins crucial for various selective autophagy processes, SmATG11 is a reasonable candidate, and we are currently characterizing its role in the life cycle of *S. macrospora*. The enrichment of the ubiquitin-binding protein SmCUE3, however, could explain a previously unexplored difference between mammalian/plant NBR1 homologs and fungal NBR1 homologs. While the former exhibit an ubiquitin-associated (UBA) domain, this sequence motif is lacking in fungal homologs. Therefore, we speculate that SmCUE3 might serve as an adaptor between SmNBR1 and its ubiquitinated cargo by replacing the missing UBA domain. To test this hypothesis, we are currently verifying the interaction of SmNBR1 with SmCUE3 and its function in selective autophagy.

WS2.07 - Genome wide exploring the autophagy-related genes in filamentous fungi and the functional characterization of *atg1* and *atg5* in *Neurospora crassa*

M. Yuan[1], *Q. Liu*[1], *J. Li*[1], *C. Tian*[1]
[1]Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, China

Autophagy is a membrane-dependent degradation process that is well understood in yeast and animal cells, but the molecular basis of autophagy in filamentous fungi including the genes network and detail functions are still poorly understood. We will try understanding the autophagy pathway from genome wide exploring the genes those are involved in filamentous fungi using comparative genomics and functional genomics. The transcriptome data show that the genes for ribosome biogenesis, amino acid synthesis and DNA replication is significantly suppressed and that the expression of genes related to DNA repair is significantly induced in macroautophagy-related genes *atg1* and *atg5* deficient *Neurospora crassa*. Macroautophagy deficient cells display accumulation of stacked ring-like membrane structures, impaired mycelium viability and sporulation ability, as well as significantly changed hyphae morphology. These results indicate that autophagy genes are deeply involved in cell development and hypha growth in filamentous fungi, and could be the good targets to engineer for industrial filamentous fungi in future.

WS2.08 - Biotin capture of the proxiome of the SmSTRIPAK complex in *Sordaria macrospora* identifies a greenbeard gene as a putative interactor

L.S. Hollstein[1], *K. Schmitt*[2], *O. Valerius*[2], *S. Pöggeler*[1]
[1]Georg-August-University of Göttingen, Department of Genetics of Eukaryotic Microorganisms, Göttingen, Germany, [2]Georg-August-University of Göttingen, Department of Molecular Microbiology and Genetics, Göttingen, Germany

For more than two decades, the filamentous ascomycete *Sordaria macrospora* (Sm) has served as a model organism to investigate the striatin-interacting phosphatase and kinase (SmSTRIPAK) complex. This multiprotein phosphatase assembly plays a crucial role in regulating vegetative growth, hyphal fusion, and the development of fruiting bodies.
To identify potential target proteins, we screened for protein-protein interactions within the cellular microenvironment of the SmSTRIPAK complex utilizing the Biotin Identification (BioID) method. This technique relies on the *in vivo* labeling of proximal proteins by a promiscuous biotin ligase which is fused to the protein of interest. Biotinylated proteins are then specifically enriched by biotin affinity capture and identified through mass spectrometry. Using one component of the complex, the SIKE-like SmSTRIPAK complex interactor 1 (SCI1), we captured already known SmSTRIPAK components as well as candidate interactors. One of these candidates, determinant of communication 2 (SmDOC2), exhibited significant enrichment across multiple SCI1-BioID experiments, utilizing various control setups.
The communication genes *doc-1*, *doc-2* and *doc-3* were initially characterized in the closely related ascomycete *Neurospora crassa*. Here, the *doc* genes were shown to be involved in the pre-contact communication between two hyphae. They prevent the fusion of genetically dissimilar hyphae by suppressing the oscillation of MAK-2, which is required for communication and chemotropic interactions.
We have generated deletion strains of *Smdoc1* and *Smdoc2* in *S. macrospora* and are performing BioID experiments using SmDOC1 and SmDOC2 as bait to explore the underlying molecular mechanism.

WS2.10 - EOP-1: a key protein for cell-cell interaction with an AIM24-like domain and an extended IDR that might drive droplet formation

L. Strzelczyk[1], *A. Oostlander*[1], *J. Grzegowski*[1], *M. Schumann*[1], *U. Brandt*[1], *A. Fleißner*[1]
[1]Technische Universität Braunschweig, Institute of Genetics, Braunschweig, Germany

Cell fusion is essential for eukaryotic development, yet its molecular mechanisms remain poorly understood. Our research focuses on *Neurospora crassa*, a well-established model organism for studying this process.
During colony formation, genetically identical germinating spores undergo chemotropic interactions and fuse to establish a mycelial colony. The proteins SO and MAK-2 exhibit alternating and opposing membrane recruitment patterns in the interacting germlings, suggesting a dialogue-like dynamic, though the underlying mechanisms driving this recruitment remain unclear.
We have identified EOP-1 as an interaction partner of SO. Notably, *eop-1* gene deletion mutants are unable to undergo chemotropic interactions. Furthermore, EOP-1 dynamically colocalizes with SO at the plasma membrane of germling tips. Protein structure predictions suggest that EOP-1 contains an unstructured domain as well as an AIM24-like domain similar to the *Streptococcus pyogenes* protein SPYM3_0169 (RMSD 1.627 Å).
To study the function of the AIM24-like domain, we generated EOP-1Δα-helix mutants that lack the C-terminal α-helix. This truncation results in an altered EOP-1 localization along the entire plasma membrane. Although EOP-1Δα-helix localizes to tips upon germling contact, fusion was unsuccessful, leading to enlarged contact points, mirroring phenotypes of the ergosterol

biosynthesis mutant Δ*erg-2*. Our ongoing research aims to compare changes in contact recognition in both EOP-1Δα-helix and Δ*erg-2* mutants, including SO and MAK-1 recruitment and activation.
We propose that EOP-1's unstructured domain facilitates droplet formation, which may explain its rapid recruitment to the plasma membrane during cell-cell interactions. Preliminary data support this droplet-promoting function and further studies will investigate its role in cell-cell interactions.

WS2.11 - Role of checkpoint kinases in regulating *Neurospora* circadian clock under DNA damage stress

Y. Yang[1], *Z. Duan*[1], *X. Liu*[1]
[1]Institute of Microbiology, Chinese Academy of Sciences, Beijing, China

Circadian rhythms allow organisms to adjust to daily environmental fluctuations. The interplay between the circadian clock, the cell cycle, and DNA repair has been extensively documented, yet the epigenetic control of the circadian clock by the DNA damage response remains relatively unexplored. Here, we showed that checkpoint kinases CHK1 and CHK2 regulate chromatin structure under DNA damage stress in *Neurospora crassa* to maintain robust circadian rhythms. Under DNA damage stress, deletion of *chk1* and *chk2* disrupted the rhythmic transcription of the clock gene *frq* by suppressing the rhythmic binding of the transcription activator WCC at the *frq* promoter, as the chromatin structure remained condensed. Mechanistically, CHK1 and CHK2 interacted and bound at the *frq* promoter to phosphorylate H3T11, promoting H3 acetylation, especially H3K56 acetylation, to counteract the histone variant H2A.Z deposition, establishing a suitable chromatin state to maintain the robust circadian rhythm despite DNA damage. Additionally, a genome-wide correlation was discovered between H3T11 phosphorylation and H3K56 acetylation, showing a specific function at the *frq* promoter that is dependent on CHK1 and CHK2. Furthermore, transcriptome analysis revealed that CHK1 and CHK2 are responsible for robust rhythmic transcription of metabolic and DNA repair genes under DNA damage stress. These findings highlight the essential role of checkpoint kinases in maintaining robust circadian rhythms under DNA damage stress.

WS2.12 - Cross-species comparison of AlphaFold-derived G protein-coupled receptor structures reveals novel melatonin-related receptor inNeurospora crassa

C. Maienza[1], *G. Lamoureux*[1,2], *K. Lee*[1,3]
[1]Rutgers University - Camden, Center for Computational and Integrative Biology, Camden, United States, [2]Rutgers University - Camden, Department of Chemistry, Camden, United States, [3]Rutgers University - Camden, Department of Biology, Camden, United States

Melatonin, a molecule with diverse biological functions, is ubiquitously present in living organisms. There is significant interest in understanding melatonin signal transduction pathways in humans, particularly due to its critical role in regulating the sleep-wake cycle. However, a

knowledge gap remains in fully elucidating the mechanisms by which melatonin influences circadian regulation. To bridge this gap, there is a growing need for a model system to study the role of melatonin in circadian clocks, with *Neurospora crassa* being a promising candidate. As a first step in this investigation, we focused on identifying melatonin receptors in *N. crassa*. Given the lack of sequence similarity between potential receptors in this fungus and known human melatonin receptors, we utilized structural similarity analysis through AlphaFold2. This approach led to the identification of a strong candidate gene, *gpr-3*, which shares structural similarities with human melatonin receptors. Experimental validation confirmed that the removal of GPR-3 from cells results in the absence of melatonin signaling. This proof-of-concept study underscores the potential of *N. crassa* as a model organism for circadian research and demonstrates the broader applicability of using AlphaFold2, especially when sequence similarity does not lead to candidate genes, for identifying novel receptors across different species.

WS2.13 - Gene expression dynamics associated with the developmental processes of conidial germination and polar growth in filamentous fungi

V. Halder[1], *W. Zheng*[1], *C. Dong*[2], *F. Trail*[3], *O. Yarden*[4], *J.P. Townsend*[1]
[1]Yale University, New Haven, United States, [2]Chinese Academy of Sciences., Beijing, China, [3]Michigan State University, East Lansing, United States, [4]Hebrew University of Jerusalem, Rehovot, Israel

Polar growth, the dominant form of growth in filamentous fungi, plays a crucial role in their exploring the environment, interacting with each other, and establishing infection in hosts. Unraveling the molecular mechanisms underlying polar growth is critical in advancing our knowledge of fungal biology, from their initial response to the environment to the development of pathogenicity. To better understand the genetic basis of asexual spore germination, we aimed to identify the genes associated with this developmental process and their divergences of function in different fungal species.
We performed a comparative genomic analysis to identify conserved and species-specific mechanisms regulating polar growth in filamentous fungi. This analysis examined gene expression during conidial germination extending from isotropic to polarized growth to the first hyphal branch, in several fungal species grown in a common medium representing saprobic and pathogenic lifestyles. Additionally, we examined gene expression patterns in pathogenic fungi grown in media representative of host environments to investigate how polar growth is modulated during the initiation of fungal infection.
The model fungus *Neurospora crassa*, with its intricate branching and hyphae fusion, represents an ideal non-pathogenic reference of polar growth. By leveraging the *Neurospora crassa* knockout library, we delved into the cognate functional roles of the genes highlighted in our comparative analyses. This approach validates the significance of specific genes in polar growth dynamics and offers insights into both fungal development and pathogenicity, enriching our understanding of these complex biological processes.

WS2.14 - Developmental gene expression and genome evolution in the shift from asexual propagation to sexual resistance in *Neurospora crassa*

J.R. Wang[1], *Y.-W. Wang*[1], *V. Halder*[1], *Z. Wang*[1], *J.P. Townsend*[1]
[1]Yale School of Public Health, Biostatistics, New Haven, United States

The switch from asexual to sexual reproduction is critical for the success of filamentous fungi in responding to diverse environmental challenges. However, standard culturie protocols for the model filamentous fungus *Neurospora crassa* use synthetic agars promoting either the asexual or sexual cycle—leaving the natural switch understudied. To characterize this key developmental process and provide insights into how genomic evolution has shaped it, we sampled RNA from cultures of *N. crassa* (*mat-A*, FGSC2489) on carrot agar, which supports development from the appearance of aerial hyphae through growth of hyphal knots, asexual conidiophores, and formation to maturation of unfertilized sexual protoperithecia. Genes with known functions in asexual and sexual reproduction, such as *ccg-4* and *matA-2* were expressed as expected, upregulated during asexual conidiation and growth of young sexual protoperithecia. Genes involved in DNA methylation and chromatin remodeling showed minimal change across development. Astoundingly, of 312 previously identified transcription factors, expression increased in 92% of genes during asexual maturation and 84% of genes during sexual maturation. In contrast, expression decreased for 44% of genes during early sexual development. This intervening period of downregulation suggests cellular deprogramming during the organism's transition to sexual development. Additionally, compared to genes with phylogenetically older homologs, genes with phylogenetically younger homologs (present only in species diverging more recently from *N. crassa*) showed larger changes in expression across development, especially in late sexual maturation. Our findings provide both confirmatory results and novel explanations of developmental gene regulation and evolution during the asexual-sexual switch in *N. crassa*.

WS2.15 - Polycomb repressive complex II balances vegetative growth and perithecial development in *Neurospora crassa*

A. Deaven[1], *A. Ameri*[1], *M. Lane*[1], *A. Abduljalil*[2], *Z. Lewis*[1]
[1]University of Georgia, Microbiology, Athens, United States, [2]University of Nevada, Reno, Microbiology & Immunology, Reno, United States

In most branches of the fungal kingdom, sexual development marks a dramatic shift from simple multicellular hyphae to large fruiting bodies composed of multiple layers of unique tissue types. It is essential to tightly regulate and coordinate signals to prevent unnecessary energy investment into building these structures. In *Neurospora crassa*, we have found that Polycomb Repressive Complex II (PRC2) establishes domains of repressive chromatin across genes that are uniquely expressed during sexual development. PRC2 is a highly conserved regulator of multicellular development that represses genes by establishing domains of histone H3 lysine 27 tri-methylation (H3K27me3) across conditionally activated genes. Loss of PRC2 activity results in the

precocious formation of perithecia-like structures in the absence of a compatible mating-type partner. However, bulk ChIP-seq has revealed that only subtle changes to the distribution of H3K27me3 occur in perithecia, even at genes that are strongly induced during sexual development. In contrast, we identified one intergenic region adjacent to the coding sequence of a predicted forkhead transcription factor, *vsd-1*, that loses enrichment of H3K27me3. *vsd-1* is essential for female development and suppresses the development of false perithecia in an H3K27me3-deficient background. Together, we have found that histone methylation is a key mediator of signals that promote sexual development and prevents aberrant perithecia development. We propose that upregulation of sexual development genes occurs via subtle, tissue-specific modifications to the epigenome as well as coordinated activity of specific transcription factors.

WS2.16 - Transcriptional adaptation is conserved in the filamentous fungus *Neurospora crassa* and plays a considerable role in fungal fitness

H. Haj Hammadeh[1], *D. Stainier*[1]
[1]Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany

Transcriptional adaptation (TA) is a cellular response to certain genetic perturbations, where mRNA destabilizing mutations in one gene trigger the transcriptional modulation of other genes, known as adapting genes. At the molecular level, the mutant mRNA, rather than the loss of protein, leads to the transcriptional changes in the adapting gene(s).
Despite the significance and growing interest in genetic compensation, transcriptional adaptation has only been investigated in higher eukaryotes. Consequently, it remains unclear whether this phenomenon occurs in basal eukaryotes as well.
Here, we report transcriptional adaptation in the filamentous fungus *Neurospora crassa* and find that this process requires factors involved in mutant mRNA decay, similar to findings in *C. elegans* and zebrafish as well as in mouse cells in culture. Specifically, premature termination codon (PTC) but not full-locus deletion (FLD), alleles of *camk-1* display upregulation of *vsd-8*. A PTC allele in the gene *camk-1* exhibits a milder phenotype than the FLD allele. Notably, knocking out the adapting gene *vsd-8* in the *camk-1* PTC allele leads to a phenotype similar to that observed in the *camk-1* FLD allele. In addition, knocking out the nonsense-mediated decay (NMD) factors *upf-3* and, *upf-2* in the *camK-1* PTC allele also leads to a phenotype similar to that observed in the *camk-1* FLD allele.
Altogether, these results provide evidence for transcriptional adaptation in *N. crassa*, establishing it as a powerful model for further investigation into the underlying molecular mechanisms.

WS2.17 - Conserved DNA methylation within the Neurospora genus

J. Svedberg[1], *J. Reimegård*[2], *H. Johannesson*[1]
[1]Stockholm University, Department of Ecology, Environment and Plant Sciences, Stockholm, Sweden, [2]Science for Life Laboratory, ScilifeLab, Department of Cell and Molecular Biology, National Bioinformatics Infrastructure Sweden, Uppsala, Sweden

5-methylcytosine DNA methylation is mostly found in repetitive regions in *Neurospora*, where repeat induced point-mutations lead to the formation of H3K9me3 mediated heterochromatin which in turn induces DNA methylation. There has been little evidence of methylation of genic sequences and of control of gene expression through dynamic methylation throughout the life cycle. Most previous studies on methylation in *Neurospora* have focused on *N. crassa*, and we have gained significant knowledge of the molecular underpinnings of DNA methylation based on work done in this species, but little has been known of DNA methylation in the rest of the genus. In a previous study, we investigated DNA methylation patterns by performing bisulfite sequencing of 10 strains from 5 different *Neurospora* species. Here we use this dataset to investigate patterns of conservation of methylation between these species, and identify an excess of sites where methylation is highly conserved in all species. Unlike most methylated sites, which are found in repeats, these are instead mostly located within genes and they are also associated with a specific G-rich sequence motif. Using transcriptomic data we investigate the association between methylation and gene expression in several different tissues and under several different growth conditions and we also investigate the causes of methylation at conserved sites and to what extent it differs from methylation caused by the canonical pathway described in *N. crassa* through the use of deletion mutants.

WS2.18 - Towards understanding mechanisms of de novo epigenetic silencing

F. Carlier[1]
[1]Institut Pasteur, Department of Mycology, Paris, France

In eukaryotes, repetitive DNA can become silenced de novo, transcriptionally or post-transcriptionally, by processes that appear independent of strong sequence-specific cues. The mechanistic nature of such processes remains poorly understood. We found that in the fungus *Neurospora crassa*, de novo initiation of both transcriptional and post-transcriptional silencing was linked to perturbed chromatin induced experimentally at the tetO array. In this system, transcriptional silencing was mediated by classical constitutive heterochromatin. On the other hand, post-transcriptional silencing resembled repeat-induced quelling, but occurred normally when homologous recombination was inactivated. Therefore, this process was named 'recombination-independent quelling' (RIQ). We also found that all silencing of the perturbed tetO array required SAD-6, a fungal ortholog of the conserved SWI/SNF chromatin remodeler ATRX, which was further required to maintain nucleosome occupancy in the face of perturbation.These and other results suggested a model in which the de novo initiation of transcriptional and post-transcriptional silencing is coupled to the remodeling of perturbed chromatin [1]. To better understand the mechanism of this newly described phenomenon, we conducted a forward-genetics screen to identify additional required factors. Among several conserved candidates, our effort pinpointed a variant of histone H4 (hH4v) that was absolutely essential for the initiation of both heterochromatin and RIQ by the remodeling-dependent pathway. Our preliminary analysis indicated that hH4v plays additional roles in regulating the state of chromatin in response to stress throughout the genome.
[1] Carlier et al. (2024). Remodeling of perturbed chromatin can initiate de novo transcriptional and post-transcriptional silencing. PNAS 121, e2402944121.

WS2.19 - Effects of the polar growth-related genes on the growth and the regulation of cellulase expression in Neurospora crassa

L. Qin[1], *Y. Chen*[1], *X. Jiang*[1]
[1]Fujian Normal University, Fuzhou, China

Saprophytic filamentous fungi are the primary decomposers of lignocellulose in nature, possessing strong protein secretion capabilities that are often utilized for the production of enzymes and recombinant proteins. The protein secretion in filamentous fungi occurs mainly through the hyphal tips. Therefore, it is generally agreed that restricting the polar growth and increasing branching of hyphae is an effective method to enhance protein secretion. Our laboratory screened a large number of mutants related to polar growth and discovered that the deletion strains of *pla-7*, *kin-1* and *ede-1* encoding phospholipase D, kinesin, and endocytic protein in *Neurospora crassa*, respectively, exhibited significantly increase in hyphal branching and biomass accumulation under sucrose carbon sources. Although deletion of these genes hindered cellulase induction on cellulose in shake flasks, mis-expressing *clr-2* restored cellulase production in Δ*pla-7*, Δ*kin-1* and Δ*ede-1* strains, and we surprisedly found that these strains constitutively expressing *clr-2* demonstrated higher protein secretion levels under both sucrose and cellulose carbon sources. We further utilized global transcriptional profiling combined with genetic and physiological analyses to investigate how these genes affected the induction and secretion of cellulases. *pla-7*, *kin-1*, and *ede-1* are localized to the plasma membrane, and these genes not only affected the transcription of *clr-2* at the transcriptional level, but also participated in vesicle formation and trafficking as well as vesicle fusion to the plasma membrane. Our data provide the possibility to develop the model organism *N. crassa* suitable for bioreactor fermentation to produce secreted proteins.

WS2.20 - Functional characterization of sugar transporters from *Neurospora crassa* and their involvement in coping with stress conditions

E. Tamayo[1], *L.M. Schaffer*[1], *B. Nada*[1], *I. Hafermann*[1], *J.P. Benz*[1]
[1]Technical University of Munich, Fungal Biotechnology in Wood Science, Holzforschung München, Freising, Germany

Filamentous fungi like *Neurospora crassa* can take up and metabolize various sugars from the environment, such as those found in agricultural wastes, which can be exploited in biotechnology. However, although fungi possess a variety of sugar transporter genes in their genome, most of them have not yet been characterized in their specific function. For example, to date, no transporters for fructose and sucrose have been identified in *N. crassa*, although this would be useful for the development of tools for the characterization of sugar transporters from other organisms. Furthermore, the involvement of sugar transport in the adaptability of fungi under abiotic stress experienced both in nature and during biotechnological fermentations has not yet been well studied. To identify high-probability candidates, uptake profiles of sugars of interest were recorded after subjecting *N. crassa* to a series of relevant induction conditions and

correlated with the expression levels of all sugar transporter genes. By comparing this functional profile with the phylogenetic relationships of sugar transporters across several fungal species, we could identify transporters candidates for specific sugars. In this work, transporters for fructose and sucrose were identified and their function tested. Moreover, the influence of different stress conditions on sugar transport in *N. crassa* was explored, which led us to identify a novel monosaccharide transporter involved in osmotic stress in *Neurospora* sp.
The new knowledge could be used to better understand the mechanisms of sugar regulation in particular in response to abiotic stresses in fungi and improve fungal nutritional values under stress conditions.

WS2.21 - Redox regulation of proteins through reversible cysteine oxidation in *Neurospora crassa* during cellulose degradation and anaerobiosis

L. Bidondo[1], *J.-C. Gaillard*[2], *E. Drula*[1], *J. Armengaud*[2], *J.-G. Berrin*[1], *M.-N. Rosso*[3], *L. Tarrago*[1]
[1]Aix-Marseille University/Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, Fungal Biodiversity and Biotechnology, Marseille, France, [2]Université Paris-Saclay/Commissariat à l'Énergie Atomique et aux Énergies Alternatives, Département Médicaments et Technologies pour la Santé, Bagnols-sur-Cèze, France, [3]Aix-Marseille University/Institut National de recherche pour l'Agriculture, l'Alimentation et l'Environnement, Fungal Biodiversity and Biotechnology, Marseille, France

Reactive oxygen species (ROS) can act as signaling molecules by oxidizing cysteine residues to disulfide bonds. Redox signaling was shown to alter enzyme activity, control protein localization, or regulate transcription factors in many organisms. However, little is known about the role of cysteine oxidation in fungi. Fungi obtain carbon by degrading plant cell walls in a process involving ROS production. Moreover, environmental constraints can also induce variations in ROS levels, such as exposure to anaerobiosis. Our research aims to identify proteins in *Neurospora crassa* that undergo reversible cysteine oxidation during cellulose degradation and under varying environmental conditions, to determine their role in fungal adaptation.
Thioredoxins (Trx) are thiol oxidoreductases that play crucial roles in ROS response by reducing disulfide bonds in redox-regulated proteins in many organisms. Their mechanism involves a transient intermolecular disulfide bond with their targets. We exploited this mechanism by combining Trx affinity chromatography with label-free shotgun proteomics to identify potential redox-regulated proteins from *N. crassa* grown on cellulose. We identified 1,938 proteins, including key metabolic enzymes and proteins potentially involved in signaling like kinase and transcription factors. Notably, two enzymes involved in heme synthesis, the 5-aminolevulinate synthase and the coproporphyrinogen-III oxidase, emerged as top candidates. Interestingly, these two enzymes were also highly abundant during exposure to anaerobiosis as we showed by comparative proteomics. These results suggest that these enzymes may be redox-regulated by ROS induced during environmental constraints. We are currently investigating the role of Cys-oxidation on the activity of these enzymes.

WS2.22 - Roles for heterotrimeric G-proteins and adenylyl cyclase in transcriptional control of cellulase gene expression in Neurospora crassa

K. Borkovich[1], *Y. Oza*[1], *L. Collier*[1], *M. Quinn*[1], *A. Carrillo*[1]
[1]University of California, Riverside, Microbiology and Plant Pathology, Riverside, United States

Our previous work demonstrated roles for several heterotrimeric G protein subunits and adenylyl cyclase in the complete conversion of Avicel (cellulose) to glucose (Avicelase activity) in *Neurospora crassa*. We obtained evidence for transcriptional regulation of 5-6 cellulase genes in some mutants using qRT-PCR. Both the Avicelase activity and cellulase gene expression defects were rescued by exogenous cAMP in several mutants. In this study, we performed mRNAseq to identify global patterns of gene expression in wild type and three mutants–the Gα mutants Δ*gna-1* and Δ*gna-3* and the adenylyl cyclase mutant Δ*cr-1*–after overnight growth on glucose followed by transfer to glucose or cellulose. We identified more than 2000 genes that were upregulated in the wild-type strain on cellulose as compared to glucose. Predicted cellulases were among the top up-regulated genes in this group in wild type. Expression of most cellulases and several transcription factors previously implicated in regulation of cellulase gene expression were downregulated in the three mutants, with Δ*cr-1* displaying the greatest defects. Overexpression of the transcription factor *clr-2* restored Avicelase activity in the mutants. Our results demonstrate that heterotrimeric G protein and cAMP signaling strongly impact transcriptional control of cellulase activity, through regulation of the downstream CLR-2 transcription factor in *N. crassa*.

WS3.02 - Elucidation of pathogenicity mechanisms of the opportunistic human pathogen *Fusarium oxysporum*

N. Mavani[1], F. Schmidt[2], I. Heineking[2,3], E. Garbe[1], O. Kurzai[4,5], A. Brakhage[2], G. Walther[5], S. Janevska[1]
[1]Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (Leibniz-HKI), (Epi-)Genetic Regulation of Fungal Virulence, Jena, Germany, [2]Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (Leibniz-HKI), Molecular and Applied Microbiology, Jena, Germany, [3]Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (Leibniz-HKI), Phagosome Biology and Engineering, Jena, Germany, [4]University of Würzburg, Institute for Hygiene and Microbiology, Medical Microbiology and Mycology, Würzburg, Germany, [5]Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (Leibniz-HKI), National Reference Center for Invasive Fungal Infections, Jena, Germany

Fungi of the genus *Fusarium* can cause life-threatening infections in immunocompromised patients and serious, poorly treatable infections of the cornea, called keratitis, which primarily affect otherwise healthy contact lens wearers. *Fusarium* species are extraordinary trans-kingdom pathogens, which can infect multiple hosts, *i.e.*, plants, animals and humans. Little is known about how *Fusarium* is able to proliferate in the mammalian host and which virulence factors contribute to this. In our laboratory, we use *Fusarium oxysporum* as a model. Remarkable host specificity has been demonstrated for plant pathogenic isolates of *F. oxysporum*, with each so-called host-specific form (*forma specialis*) being able to infect one to several hosts. This host-pathogen interaction is mediated by specific virulence genes encoding effectors, which are found on accessory and mobile pathogenicity chromosomes.
Recent genomic studies on two human isolates of *F. oxysporum* (*Fo*), *Fo* NRRL 32931 (invasive fusariosis isolate) and *Fo* NRRL 47514 (keratitis isolate) revealed unique accessory regions absent in so far characterized plant-pathogenic isolates. *Fo* NRRL 32931 contains four different accessory chromosomes with putative virulence genes that could provide an advantage in the mammalian host. We are investigating this by generating derived strains of *Fo* NRRL 32931 lacking the accessory chromosomes 12-15, and by transferring these chromosomes to the endophytic strain, *Fo* 47, without pathogenicity chromosome. We are establishing *in vitro* cell culture experiments to evaluate the contribution of accessory chromosomes 12-15 to the virulence of *F. oxysporum*, which can offer starting points for the development of therapeutic approaches in the future.

WS3.03 - Increased copper-resistance in the *Fusarium oxysporum* species complex

R. Palos-Fernández[1], E. Jiménez-Jiménez[1], J. Dijkstra[2], A. van Westerhoven[3,2], M.F Seidl[3], G.HJ Kema[2], A. Di Pietro[1], M. Sánchez López-Berges[1]
[1]Universidad de Córdoba, Genética, Córdoba, Spain, [2]Wageningen University & Research, Laboratory of Phytopathology, Wageningen, Netherlands, [3]Utrecht University, Theoretical Biology and Bioinformatics, Department of Biology, Utrecht, Netherlands

Fungi colonize different niches, from soil and plants to humans, obtaining nutrients from diverse sources while implementing regulatory mechanisms to rapidly respond to limiting or excessive conditions. Copper is an essential micronutrient but toxic at high concentrations. It is used as an agricultural fungicide and deployed by macrophages during anti-fungal immune bombardment. Thus, fungi face selective pressure for increased copper-tolerance both in agricultural and clinical settings.
Copper-excess is sensed by the conserved transcription factor Ace1, which transcriptionally activates the copper-exporting ATPase Crp1. Loss of Ace1 or Crp1 leads to severe copper-sensitivity and attenuation of virulence in human pathogens. Here we surveyed copper-tolerance in different isolates of *Fusarium oxysporum* (*Fo*), a fungal pathogen that causes vascular wilt disease in many important crops and opportunistic infections in humans. Most of the plant and clinical *Fo* isolates were highly copper-tolerant compared to other plant or human pathogens such as *F. graminearum*, *F. verticillioides* or *Aspergillus fumigatus*. Interestingly, increased copper-tolerance in *Fo* appears to correlate with the presence of a cluster of two copper-related genes located on accessory regions, one of them encoding a transcription factor with homology to Ace1. The role of this cluster is supported by the finding that, unlike in other fungi, inactivation of Ace1 in *Fo* f. sp *lycopersici 4287* does not affect copper-tolerance and *crp1* expression. Furthermore, *Fo* f. sp. *cubense* mutants that have lost an accessory chromosome harboring the cluster become highly copper-sensitive. Our results suggest that accessory genes mediate copper-tolerance in *Fo* and may impact fungal pathogenesis.

WS3.04 - Impact of *Fusarium* species composition and incidence on onion basal rot in Northeastern Israel

O. Degani[1,2], E. Dimant[1], E. Margalit[3]
[1]MIGAL—Galilee Research Institute, Plant Sciences, Kiryat Shmona, Israel, [2]Tel-Hai College, Faculty of Sciences, Tel-Hai, Israel, [3]Israel Ministry of Agriculture and Rural Development, Extension Service, Bayit Dagan, Israel

Fusarium basal rot (FBR) places a significant limitation on onion (*Allium cepa*) production worldwide. The disease can be observed throughout the entire crop cycle. This research explored the composition and incidence of *Fusarium* species involved in FBR outbursts in two onion fields in northeast Israel, one in Galilee (Hula Valley) and the second in the Golan Heights, where the disease incidences reached 8%. Using colony morphology, microscopic taxonomic keys, and molecular methods, a new, unreported *Neocosmospora* (previously *Fusarium solani*) species

complex (SC, mostly *N. falciformis*) was discovered as a wildly spread member of the *Fusarium* pathobiome community. This SC was also less virulent in seed germination (42–52% higher sprout biomass, $p < 0.05$) and bulb pathogenicity tests (41–45% less necrotic) than *Fusarium acutatum*. Whereas the Galilee yellow Orlando (Riverside) onion cultivar bulbs sampled were colonized by *Neocosmospora* SC (70%) and two other, less abundant species, *F. oxysporum* f. sp. *cepae* and *F. acutatum* (15% each), the Golan Heights field's *Fusarium* community showed host specificity. In the Golan Heights field, *F. oxysporum* f. sp. *cepae* inhabited the red Ha2 onion cultivar bulbs, whereas *F. acutatum* colonized the yellow Ha1 cultivar (40% and 50% prevalence along with *Neocosmospora* SC). A better understanding of this disease complexity, affected by different *Fusarium* species and with a divergence in host susceptibility and virulence, is critical for developing disease management strategies. Since each *Fusarium* species reacts differently to pest control treatments, changes in the species composition may require specifically adapted management solutions.

WS3.05 - Plant stress perception alters inter-kingdom communication in the rhizosphere

S. Vitale[1], *M. Papaianni*[1], *M. Renesi*[1], *F. Salzano*[1], *I. Dilelio*[1], *F. Pennacchio*[1], *D. Turrà*[1], *S.L. Woo*[1], *G. Diretto*[2], *M. Lorito*[1], *N. Lombardi*[1]
[1]University of Naples "Federico II", Portici, Italy, [2]Italian National Agency for New Technologies, Energy and Sustainable Development, Portici, Italy

Root exudates (REs) released by plants are pivotal in mediating multitrophic interactions within the rhizosphere, acting as primary signaling blends that influence microbial community composition and function. Despite their importance, the mechanisms by which stressed plants alter RE chemical profiles and subsequently affect rhizospheric microbial dynamics remain poorly understood. Here we examined the impact of abiotic (mechanical wounding) and biotic stresses (infection by *Botrytis cinerea*, chewing by *Spodoptera littoralis*, and aphid infestation by *Macrosiphum euphorbiae*) on tomato plants. The resultant REs were subjected to chemotropic assays to assess their effects on the biological control agents (BCAs) *Trichoderma afroharzianum* and *Beauveria bassiana*, as well as the phytopathogen *Fusarium oxysporum*. All REs demonstrated inhibitory and repellent effects on the conidia and germ tubes of *Fusarium oxysporum*, while attractiveness of BCA germ tubes. Notably, REs from insect-stressed plants exhibited the highest chemotropic activity on BCAs and the strongest repellence to fungal pathogens. To elucidate the molecular underpinnings of this inter-kingdom signaling, we analyzed the root-secreted metabolome and conducted activity-guided fractionation of selected REs. Our findings suggest that low molecular weight proteins are implicated in the repellent activity against pathogens. This study reveals that both conserved and unique molecular signatures are involved in plant stress responses and their recognition by soil microbial communities. These insights advance our understanding of plant-microbe interactions and highlight the potential for developing stress-resilient crops with enhanced pathogen resistance.

WS3.06 - Developing RNAi-based fungicides to control fusarium head blight

M. Djavaheri[1], T. Bender[1], K. Horner[1], M.-A. Henriquez[2], O. Molina[2], S. Berraies[3], S. Rosa[4], R. Kutcher[5], B. Blackwell[6], S. Sharpe[1], S. Clarke[7], G. Subramaniam[6], H. Borhan[1], S. Robinson[1]
[1]Agriculture Canada, Saskatoon, Canada, [2]Agriculture Canada, Morden, Canada, [3]Samia Berraies, Swift Current, Canada, [4]Cerom, Saint-Mathieu-de-Beloeil, Canada, [5]University of Saskatchewan, Plant Science, Sask, Canada, [6]Agriculture Canada, Ottawa, Canada, [7]National Research Council, Saskatoon, Canada

Fusarium head blight (FHB) is a serious fungal disease of cereal crops including wheat, durum, barley, oats and corn. FHB is caused by several species from the genus Fusarium with *F. graminearum* causing the most concern. FHB affects kernel development, reducing both yield and grade. Furthermore, the disease contaminates grain with mycotoxins which can render it worthless should toxins exceed strict threshold limits.
Genetic resistance to FHB is limited and where identified, it exhibits polygenic inheritance complicating breeding strategies. FHB management relies on timely fungicide application to suppress disease outbreaks. Chemical pesticides are subject to increasing scrutiny by regulatory bodies over health and environmental concerns, accelerating the need to develop alternative solutions for pest management. RNAi is a promising approach for crop disease management where dsRNA designed to key pathogen gene targets can trigger silencing, leading to fungal cell death. The sequence specificity encoded in RNA restricts fungicide control to the pathogen of concern, leaving beneficial species unharmed. Since dsRNA can be applied as a foliar spray, the application of these Next-Generation Fungicides avoids the need for transgenic crop production, maintaining access to lucrative export markets.
Here, we present the potential of RNAi-based gene silencing to control FHB in wheat. The strategy used to rapidly identify effective dsRNA trigger sequences from greenhouse-based disease assays will be presented. We will discuss the challenges of evaluating efficacious dsRNA trigger sequences in replicated field trials which include meeting regulatory compliance, scaling dsRNA production and dsRNA application.

WS3.08 - Role of phenotypic switching in the regulation of *Fusarium oxysporum* development and pathogenicity

P. Gutierrez-Escribano[1], A. Di Pietro[1]
[1]Universidad de Córdoba, Genetics, Córdoba, Spain

Phenotypic plasticity ensures rapid adaptation to ever-changing environments. Non-genetic phenotypic heterogeneity is widely observed in various biological systems and poses a significant challenge in cancer therapy. In fungal pathogens, epigenetically determined phenotypic switching has been linked to differentiation, host colonisation and the emergence of drug resistance. *Fusarium oxysporum* is a large species complex (FOSC) of ascomycete lineages that causes vascular wilt disease in over one hundred different plant hosts and is also an important emerging human pathogen. We report that the tomato wilt isolate *F. oxysporum* f. sp. *lycopersici* Fol4287

as well as the human pathogenic isolate MRL8996, can stochastically activate heritable and reversible switches between different phenotypic states, referred to as “white” and “pink” based on the colour of the colonies when grown in the presence of vital stains. These switch phenotypes differ in their morphology, physiology and pathogenicity traits. We investigated the role of various environmental signals in triggering the phenotypic transition as well as the transcriptional circuits involved in the establishment and maintenance of phenotypic variants. Our data suggest that phenotypic switching plays an important role in the adaptive response of *F. oxysporum* and is critical for understanding fungal microevolution within the host-pathogen context.

WS3.09 - X-ray microtomography and MAP kinase signaling reveal distinct stages of *Fusarium oxysporum* root invasion and colonization patterns

V. Guastaferro[1], S. Vitale[1], L. D’Amico[2], E. Longo[2], G. Tromba[2], N. Minc[3], M. Delarue[4], L. Di Costanzo[1], D. Turra[1]
[1]University of Naples “Federico II”, Department of Agricultural Sciences, Portici, Italy, [2]Elettra – Sincrotrone Trieste S.C.p.A., AREA Science Park, Basovizza, Italy, [3]Institut Jacques Monod, Paris, France, [4]LAAS-CNRS, Université de Toulouse, CNRS, Toulouse, France

Soil-borne fungal vascular pathogens are major agricultural threats, yet their precise root invasion mechanisms remain poorly understood. Here, we employed fluorescence microscopy, X-ray micro-computed tomography (micro-CT), nanofabrication, and reverse genetics approaches to investigate how *Fusarium oxysporum* invades and colonizes plant roots. Using chemically inert biomimetic platforms, we demonstrate that fungal hyphae can both follow surface ridges and adapt to confined spaces through thigmodifferentiation, forming extremely thin filaments similar to those observed growing between plant epidermal cells. This invasion process is regulated by distinct MAP kinase signaling cascades: the Fmk1 pathway controls initial root penetration, while the High-Osmolarity Glycerol (HOG) pathway facilitates apoplastic colonization, likely in response to varying osmotic conditions across root tissues. We show that hyphae can penetrate and extend through sub-micrometric pores characteristic of the plant apoplast, ultimately accessing xylem vessels. Notably, X-ray microtomography enabled non-destructive visualization of intricate fungal colonization patterns typically challenging to observe through conventional imaging methods. These findings advance our understanding of the physical and molecular mechanisms governing soil-borne pathogen-root interactions and may inform targeted disease management strategies.

WS3.10 - Conidia not identical as you may think

S. Covo[1], D. Mukherjee[1]
[1]Hebrew University, Rehovot, Israel

Microconidia are oval mostly G1 asexual spores of *Fusarium oxysporum* that start the fungus disease cycle. We found that conidia isolated from solid media are smaller and shifted more towards G1 population than conidia isolated from liquid culture. In agreement. liquid based

conidia are more metabolically active and germinate faster. Interestingly, liquid based conidia repair UV damage faster, survive UV damage better with no increase in the rate of mutagenesis. We then asked are all the spores of liquid-based conidia respond the same way to UV? Surprisingly, 5-10% of all spores do not germinate in response to UV even after 24 hours, some don't germinate even after 48 hours. We were able to isolate this arrested population based on its size and characterize it. This arrested population shows decreased plating efficiency and increased mutagenesis. Surprisingly even the mutation spectrum of this population is different from the entire irradiated conidia. Unlike our expectation, the amount of DNA damage in this population is relatively low and just slightly higher than the filaments that are able to recover from the irradiation. To sum up we will present how metabolic and genetic components affect mechanisms of mutagenesis that drive the evolution of a fungal plant pathogen.

WS3.11 - Effect of volatile organic compounds from bacterial biocontrol agents against the tomato vascular wilt pathogen Fusarium oxysporum

C. Ruiz Roldán[1], *C. Navarro Laguna*[2], *M.J. Cardador Dueñas*[3], *L. Arce Jiménez*[3], *A. Di Pietro*[2]
[1]Univeristy of Cordoba, Faculty of Sciences, Genetics, Cordoba, Spain, [2]University of Cordoba, Faculty of Sciences, Genetics, Cordoba, Spain, [3]University of Cordoba, Faculty of Sciences, Analytical Chemistry, Cordoba, Spain

Chemical fungicides play an important role in the prevention of plant diseases caused by fungal pathogens, but their use entails important disadvantages. Currently, biological control with bacterial biocontrol agents (BCAs) is one of the most promising alternatives. Recent research suggests that volatile organic compounds (VOCs) produced by rhizosphere microorganisms play important roles in signaling and biocontrol. In this study, we examined the effect of VOCs from two rhizosphere isolates of the genus *Pseudomonas* (PICF6 and PICF7) on *Fusarium oxysporum* (Fo), a fungal phytopathogen that causes vascular wilt on a wide variety of crops. Using a sandwich plate assay, we detected a significant inhibition of microconidia germination by VOCs emitted by PICF6 and PICF7. Some of the emitted VOCs were identified by gas chromatography coupled to ion mobility spectrometry (GC-IMS) and mass spectrometry (GC-MS). Germination inhibition tests with some of the pure identified compounds confirmed their inhibitory effect on spore germination. Currently, we are testing the effectiveness of single VOCs in preventing vascular wilt disease caused by Fo. Our results suggest the existence of VOC-mediated molecular communication mechanisms between *Pseudomonas* and *Fusarium* that could be of interest for application in biological control.

WS3.12 - A Fusarium graminearum protein interaction network of trichothecene biosynthesis pathways

G. Subramaniam[1]
[1]Agriculture Canada, Ottawa, Canada

Activation of biosynthetic gene clusters (BGCs) in fungi requires coordination and integration of many environmental cues. As an example, *Fusarium graminearum*, the main causal agent of the Fusarium head blight disease (FHB) in wheat produces an array of secondary metabolites including the mycotoxin deoxynivalenol (DON) that are regulated by carbon and nitrogen sources and affected by the physiological status of the cell. To understand the contribution of many input sources that regulate BGC in *F. graminearum*, we undertook a protein interaction network analysis in yeast of ~ 300 *F. graminearum* proteins involved directly or indirectly in the regulation of DON. The resulting network named Fusarium Network of Trichothecene Associated Proteins (FuNTAP) exhibited properties of a small-world network with protein hubs that regulated biosynthetic pathways other than DON. Corroborative evidence was obtained from genetic analysis and metabolomic profiling of major hubs. Finally, the expression of one of the major hubs tagged with TurboID in the fungus validated our supposition that major hubs exert their control by interacting with various proteins, temporally and spatially.

WS3.13 - Characterization of the antifungal effect of *Bacillus amyloliquefaciens* against *Fusarium oxysporum*

A. Yáñez-Vilches[1], *F.M. Laddaga*[1], *C. Ruiz-Roldán*[1], *A. Di Pietro*[1]
[1]Universidad de Córdoba, Genetics, Córdoba, Spain

The soil-borne ascomycete *Fusarium oxysporum* comprises a large number of clonal isolates that cause devastating vascular wilt disease on more than 150 different crops. A promising alternative to chemical control is the use of biocontrol agents (BCAs) that inhibit the pathogen or improve plant defence. The bacterial BCA strain *Bacillus amyloliquefaciens* BO7 secretes an antifungal lipopeptide (LP) with a strong inhibitory effect against *F. oxysporum* both in vitro and in the rhizosphere of tomato plants (Romano et al. 2011, J Nat Prod). The aim of this study is to understand the inhibitory mechanism of the LP for its use in biocontrol and the development of new control strategies against *Fusarium* wilt. For that purpose, **t**he morphogenetic effect of BO7 LP on *Fusarium* was analyzed by light microscopy and through a variety of phenotypic assays such as drop tests or cellophane penetration assay. We found that the LP triggers a rapid response in *Fusarium* and exhibit a synergistic effect when applied in combination with cell wall destabilizing agents and affects β-tubulin. The morphological and developmental changes elicited by the LP negatively affect the invasive ability of the fungus, causing reduced pathogenicity (Vitullo et al. 2012, Plant Pathol.). In conclusion, *B. amyloliquefaciens* BO7 produces lipopeptides that reduce the pathogenicity of *F. oxysporum* making it a highly promising BCA.

WS3.14 - The emerging threat of Fusarium Head Blight in Ethiopia: emerging pathogens, mixed mycotoxins, and interspecies interactions

L. Degenring[1], *A. Gemechu*[2], *V. Rafiei*[3], *G. Regasa*[2], *K. Munsamy*[4], *N. Yilmaz*[4], *Y. Dong*[5], *M. Rouse*[6], *M. Drott*[7], *A. Hatmaker*[8]
[1]University of Minnesota, Twin Cities, Agronomy, Saint Paul, United States, [2]Ethiopian Institute of Agricultural Research, Addis Ababa, Ethiopia, [3]Swedish University of Agricultural Sciences, Department of Forest Mycology and Plant Pathology, Uppsala, Sweden, [4]University of Pretoria, Forestry and Agricultural Biotechnology Institute, Pretoria, South Africa, [5]University of

Minnesota, Twin Cities, Plant Pathology, Saint Paul, United States, [6]USDA-ARS, Sugarcane Field Station, Canal Point, United States, [7]USDA-ARS, Cereal Disease Laboratory, St Paul, United States, [8]USDA-ARS, Cereal Disease Laboratory, Saint Paul, United States

As Ethiopia pushes towards self-sufficiency in wheat production, it has escaped large outbreaks of fusarium head blight (FHB), a disease that threatens wheat production globally. However, in 2022, FHB incidences in Ethiopia rose to 80%, with some areas experiencing 100% disease severity. Here we provide insights into the etiology of this disease outbreak and point towards future directions to mitigate the emerging threat of FHB on a global scale. While most wheat samples from 2022 exhibited low trichothecene levels, 26% exceeded recommended thresholds and several contained multiple trichothecene variants. We obtained 64 isolates from the outbreak and identified diverse members of the Fusarium graminearum species complex (FGSC) and many Epicoccum species. Our findings reveal that while Epicoccum alone causes minimal disease on wheat, its presence can have a small but synergistic impact on disease symptoms when F. graminearum has already infected. The FGSC species contributing to the outbreak are rare on a global scale. Genomic analyses reveal that Fusarium aethiopicum has persisted in Ethiopia for decades and shares ancient ancestry with a newly emerged novel species in the FGSC that we formally described as a new species. SNP-based analyses suggest high clonal fraction among FGSC isolates in Ethiopia, raising questions about a recent population expansion. The unique diversity and species composition of the 2022 Ethiopian outbreak underscores the importance of addressing emerging threats in a globalized agricultural economy to secure food safety, food security, and global food equity amid a changing climate.

WS3.15 - Exploring the association between *Fusarium* mycotoxins (T2 and HT-2) and grain microbiome in Irish oats

N. Subramani[1], *D. Bucur*[2], *M. Di Rocca*[3], *N. McKenna*[4], *B. Greer*[4], *S. Kildea*[2], *M. Danahar*[3], *L. Black*[5], *F. Doohan*[1]
[1]School of Biology and Environmental Science, Earth Institute, University College Dublin, Belfield, Dublin, Ireland, [2]Department of Crop Science, Teagasc Crops, Environment and Land Use Programme, Carlow, Ireland, [3]Teagasc Food Research Centre, Ashtown, Dublin, Ireland, [4]Institute for Global Food Security, National Measurement Laboratory: Centre of Excellence in Agriculture and Food Integrity, Queen's University, Belfast, United Kingdom, [5]Agri-Food and Biosciences Institute, Crossnacreevy, United Kingdom

Oats are a nutrient-rich, gluten-free cereal crop valued in plant-based diets for their versatility in a wide range of products. However, *Fusarium* contamination in oats and the associated mycotoxins, particularly T2 and HT-2 toxins, pose significant health risks. With the 2024 EU regulation increasing the maximum limits for these toxins in oats, there is a growing need for comprehensive food safety strategies, focusing on enhanced monitoring and control in oat production. Despite progress in mycotoxin research, the relationship between *Fusarium* contamination, mycotoxin accumulation, and the oat grain microbiome remains underexplored. This study hypothesizes that the grain microbiome composition is linked to specific *Fusarium* species and may influence the accumulation of T2 and HT-2 toxins. In 2022 and 2023, we

collected 141 preharvest oat samples from across Ireland, along with agronomy and weather data. All samples tested positive for the presence of trichothecene-producing *Fusarium* species using qPCR. Further testing with species-specific primers revealed that *F. langsethiae* was the most prevalent species, followed by *F. poae*. LC-MS analysis detected varying levels of T2 and HT-2 toxins, with 105 samples below the limit of detection (10 µg/kg) and 7 samples exceeding the EU limit of 1250 µg/kg. Based on toxin occurrence, all 141 samples were selected for 16S rRNA and ITS sequencing to assess microbial diversity. Ongoing sequencing and bioinformatics analyses aim to reveal microbial interactions that influence T2 and HT-2 toxin accumulation, potentially identifying beneficial microbes for biocontrol. This could aid in the early detection of mycotoxin risks, optimize storage conditions, and promote oat varieties that resist harmful fungi, advancing sustainable agriculture and reducing chemical inputs.

WS3.16 - Overexpression of a truncated HMG coA reductase in *Fusarium fujikuroi*

M.C. Limon[1], *I. Borrego-Serrano*[1,2], *R.M Padilla*[2]
[1]Universidad de Sevilla, Departamento de Genética, Sevilla, Spain, [2]Grupo Agrotecnología S.L., Alicante, Spain

Fusarium fujikuroi is well known for its production of gibberellins, which cause elongation of infected rice plants. Gibberellins are synthesized by the mevalonate pathway, which shares some intermediates with the pathways of other terpenoids such as ergosterol or carotenoids. The first steps of this pathway include that mediated by 3-hydroxy-3-methylglutaryl CoA (HMG coA) reductase, which converts HMG CoA to mevalonic acid (MVA). This enzyme has a regulatory domain located at the amino-terminal end, which is involved in the inhibition of protein activity. Strains overexpressing a truncated version of the *hmgR* gene (OE:*thmgR*) were obtained in the wild-type strain and in a carotenoid-overproducing strain of *F. fujikuroi* IMI 58289. Several candidates were selected based on their gibberellin production in inducing culture media. The best gibberellin producers were selected for further characterization under different culture conditions in flasks. The carotenoid content of mycelia from OE:*thmgR* transformants was not increased compared to those of the parental strains, indicating that either the additional substrate is preferentially diverted to the gibberellin pathway, or that the CarRA enzyme is limiting for carotenoid production.
Expression of the *hmgR* gene and of genes representative of the gibberellin (*cps/ks*) and carotenoid pathways (*carRA*) was analyzed by qRT-PCR in the OE:*thmgR*, OE:*thmgR carS*- and parental strains. Higher levels of *hmgR* mRNA were detected in the OE:t*hmgR* and :*thmgR carS*- strains in comparison to the parental strains while no increase was observed in *carRA* or *cps/ks* mRNAs in the OE:t*hmgR* transformants.

WS3.17 - A secondary metabolite from *Beauveria bassiana* reprograms root exudate recognition and virulence in *Fusarium oxysporum*

M. Ranesi[1], *S. Vitale*[1], *A. Staropoli*[1], *L. Di Costanzo*[1], *M. Lorito*[1], *S.L. Woo*[2], *F. Vinale*[3], *D. Turrà*[1]
[1]Università degli Studi di Napoli Federico II (Napoli), Department of Agricultural Sciences, Portici, Italy, [2]Università degli Studi di Napoli Federico II (Napoli), Department of Pharmacy, Naples, Italy, [3]Università degli Studi di Napoli Federico II (Napoli), Department of Veterinary Medicine and Animal Production, Naples, Italy

Several soil-borne microorganisms secrete organic acids (OAs) that serve multiple ecological functions including pH modulation, ion availability regulation, mineral acquisition facilitation, and inter-species communication. However, how such molecules regulate tritrophic interactions among pathogens, plants, and biocontrol agents remains largely unknown. Here, we characterized OAs production among different isolates of the endophytic biocontrol fungus *Beauveria bassiana* (*Bb*) and identified a novel metabolite, Bb1993, with dual functionality: reducing environmental pH at high concentrations and inhibiting germ tube formation and growth of *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) at low concentrations. Root treatment with either *Bb* spores or Bb1993 altered Root Exudates (REs), making them repellent to *Fol* germ tubes despite increased peroxidase accumulation, a major fungal chemoattractant. Dual confrontation assays between *Fol* and *Bb* colonies, and direct Bb1993 application, enhanced *Fol*'s invasive growth on cellophane membranes. Our results demonstrate that Bb1993 exerts both direct and plant-mediated effects on *Fol* by modulating spore germination, plant recognition, and penetration. Ongoing experiments aim to identify the biologically active molecules from REs of Bb1993-treated tomato plants that confer repellent activity against *Fol*. These findings provide new insights into the chemical dialogue mediating plant-microbe interactions and suggest novel strategies for metabolite-based biological control.

WS3.18 - The enigmatic role of mini-chromosomes in *Fusarium verticillioides*

M. Reverberi[1], *A. Grottoli*[2], *V. Scala*[2], *M. Beccaccioli*[1], *M.C. Ferrara*[1], *A. Doddi*[1], *L. Faino*[1]
[1]University of Rome, Department of Environmental Biology, Roma, Italy, [2]Council for Agricultural Research and Economics, Research Centre for Plant Protection and Certification, Rome, Italy

Fusarium verticillioides (*Fv*) is a maize pathogen that mycotoxins like fumonisins. To better understand *Fv* virulence, the genome of *Fv*10027 was sequenced, assembled and compared to the genome of *Fv*7600. Comparative genomics between *Fv*7600 and *Fv*10027 showed a difference in genome size of about 1.4 Mb despite 99% nucleotide identity. Additionally, the genome of *Fv*10027 add two mini-chromosomes of about 1 Mb and 750 Kb not present in *Fv*7600. To determine the presence of these two mini-chromosomes in the Italian *Fv* population, 24 strains were sequenced, and presence/absence analysis showed that only three *Fv* strains had those

additional chromosomes. The analysis of *Fv*10027 dispensable chromosomes showed enrichment of repetitive elements but we were not able to detect any enrichment on secreted proteins and or highly induced genes after infection. Additionally, we found that the 1Mb chromosome does not have any virulent effect on the Fv10027 infecting mays. Intriguingly, BLAST analysis on the *Fv*10027 proteins codified on mini-chromosomes have the best identity with *F. oxysporum* proteins located at dispensable chromosome 3 and 6 rather than *Fv*7600. Moreover, synonyms substitution analysis suggests that mini-chromosomes of *F. verticillioides* were probably not acquired through a horizontal chromosomal transfer from *F. oxysporum* but rather originated before the split of the two species. Currently, we are testing whether additional chromosomes of *F. verticillioides* can be useful in the interaction with other organisms like humans. Now, we are testing 20 *F. verticillioides* strains isolated from human infection of mays to understand whether there is any host specificity.

WS3.19 - A selfish Spore Killer element contributes to mitotic stability of a dispensable fungal chromosome

G. Puebla Planas[1], *D. Hazal Ayhan*[2], *P. Gutierrez Escrivano*[1], *L. Gomez Gil*[1], *C. Lopez Díaz*[1], *S. Janevska*[3], *M. Sanchez Lopez Berges*[1], *L.J. Ma*[2], *A. Di Pietro*[1]
[1]University of Cordoba, Cordoba, Spain, [2]University of Massachusetts Amherst, Massachusetts, United States, [3]Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Jena, Germany

Fungi carry dispensable genomic regions, including entire chromosomes, which are not essential for survival and can be lost at high frequency under certain environmental conditions. One unanswered question is how such dispensable regions persist in the population despite their mitotic instability. Here we studied the mechanisms promoting stability of a dispensable chromosome in the fungal pathogen *Fusarium oxysporum* (*Fo*), which causes vascular wilt disease in more than a hundred different crop species and opportunistic infections in humans. We found that the conserved core chromosome 12 (chr12) is frequently lost during serial passaging on plates in the clinical keratitis *Fo* isolate MRL8996, but not in the tomato pathogenic isolate Fol4287. Sequence analysis revealed that chr12 of MRL8996 lacks a 90 kb region present in Fol4287, which harbors a single copy of Spore Killer (Spok). Spoks are a class of genetic elements that act as meiotic drivers by killing neighboring cells lacking the element. Strikingly, transfer of the single Spok element from chr12 of Fol4287 to fluorescently labelled chr12 of MRL8996 led to a significant reduction in spontaneous chromosome loss events, as determined by flow cytometry. Importantly, no stabilizing effect was observed upon transfer of a Spok allele carrying a point mutation previously shown to abolish the killer activity of Spok *in Podospora anserina*. Our results suggest that selfish meiotic drivers such as Spoks contribute to mitotic stability of dispensable chromosomes in fungi.

WS3.20 - A taxonomic revision of the *Fusarium sambucinum* species complex

M. Sandoval-Denis[1], *M. Costa*[1], *R. Schumacher*[2], *A. Kermode*[3], *A. Buddie*[3], *M. Ryan*[3], *Y. Baker*[4], *W. Meyer*[5], *J. Groenewald*[1], *P. Crous*[1]
[1]Westerdijk Fungal Biodiversity Institute, Evolutionary Phytopathology, Utrecht, Netherlands, [2]retired, Fürstenwalde/Spree, Germany, [3]CABI, Egham, Surrey, United Kingdom, [4]Julius Kühn Institute Institute, Epidemiology and Pathogen Diagnostics, Braunschweig, Germany, [5]Julius Kühn Institute, Epidemiology and Pathogen Diagnostics, Braunschweig, Germany

Devastating plant pathogens, endophytes, rare human opportunistic pathogens and saprotrophs, are some known lifestyles of the *Fusarium sambucinum* species complex (FSAMSC). The FSAMSC represents a clade of morphologically diverse, globally distributed, mycotoxigenic fungi. It is of central relevance for *Fusarium* taxonomy as it includes the conserved generic type of *Fusarium* (*F. sambucinum*), and that of the sexually typified *Gibberella* (*G. pulicaris*). We tested recent phylogenetic circumscriptions in FSAMSC using multigene phylogenetics, traditional cultural and morphological analyses, and coalescent-based species tree estimations methods using a large set of strains from four culture collections (*i.e.*, BBA, CBS, IMI and NRRL) plus recent isolations from diverse substrates. Seventy-five species are resolved in FSAMSC making it one of the most speciose groups in *Fusarium*. However, only 40 phylospecies are currently linked to types and Latin binomials. Thirty-four novel species are described, and illustrations are provided for all the species studied. Differences between coalescent-based methods and genealogical concordance results challenge the current species delimitations within *F. graminearum sensu lato* (*F. graminearum* species complex, a species aggregate within FSAMSC), and lesser-studied species like *F. armeniacum*, *F. longipes*, *F. sibiricum*, and *F. sporotrichioides*. In line with these results, narrower species circumscriptions are proposed, which correlate with distinctive morphological features. Fresh material of *F. sambucinum* was collected from its original substrate and location (*Sambucus nigra*, Germany), and its identity confirmed by morphological and mating experiments. An epitype is designated, thus finally stabilising the taxonomy of this important taxon, now circumscribed within a genetically and morphologically well-characterised phylogenetic clade.

WS3.21 - Population genomic analyses reveal geographic structure in *Fusarium verticillioides*

S. Harding[1], *C. Andorf*[2], *R. Proctor*[1], *H. Kim*[1]
[1]U.S. Department of Agriculture, Peoria, United States, [2]U.S. Department of Agriculture, Ames, United States

Fusarium is among the fungi with the greatest negative impact on agriculture because many species cause severe diseases on diverse crops and/or contaminate crops with mycotoxins. *F. verticillioides* causes maize ear rot and is the primary cause of fumonisin contamination in maize, but isolates can differ markedly in levels of fumonisins they produce. Studies of collections of isolates from individual countries, regions and/or hosts have provided insight into genetic bases

for these differences. However, it is not known whether there is substantial variation within *F. verticillioides* related to geographic, climactic and/or host origin. Such information can aid development of broadly effective strategies to reduce *F. verticillioides*-incited fumonisin contamination and ear rot in maize. To address this knowledge gap, we generated genome-wide single nucleotide polymorphisms (SNPs) from 113 *F. verticillioides* isolates collected from 12 countries and representing five continents. We then used the SNP data to estimate intraspecific genetic variation and population structure. We found evidence that the isolates constitute four distinct populations, that admixture of populations has occurred, and that population structure is geographically partitioned. These data provide critical insight into how genetic variation is distributed and shared across continental boundaries, which can potentially inform region-specific control strategies and thus, broadly increase the effectiveness of efforts to control mycotoxin contamination in maize.

WS3.22 - Analysis of the diversity in the *Fusarium oxysporum* and *Fusarium solani* species complexes in indoor environments and clinical isolates

E. Garbe[1], *U. Farooq*[1], *A. Ullah*[1], *S. Janevska*[1], *G. Walther*[2]
[1]Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, (Epi-)Genetic Regulation of Fungal Virulence, Jena, Germany, [2]Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, National Reference Center for Invasive Fungal Infections, Jena, Germany

Fusarium is a highly diverse and ubiquitously distributed fungal genus. In humans it can cause devastating systemic infections in severely sick patients but also superficial infections, such as of the cornea, in otherwise healthy individuals. In Germany, the species causing these infections mainly belong to the *F. solani* (FSSC) and *F. oxysporum* (FOSC) species complexes. Yet, only little is known about the specific ecology and infection mechanisms of these opportunistic species. Interestingly, they are frequently encountered in indoor habitats like bathrooms. Furthermore, previous work found a dominance of specific sequence types (STs) in indoor *Fusarium* isolates, and the presence of potentially virulence-associated accessory chromosomes (ACs) in two genome-sequenced clinical FOSC isolates. Given these aspects, we now investigated: (I) Does FOSC and FSSC species diversity differ in clinical and environmental samples? (II) Do specific STs dominate in those groups? (III) How diverse are ACs in FOSC isolates and are they linked to specific environments? Therefore, we collected clinical and indoor *Fusarium* isolates, performed molecular species identification and sequence typing, screened for known AC sequences in FOSC isolates and used whole-genome sequencing for identification of suspected, hitherto unknown ACs. Overall, we found with few exceptions the same species in both, clinical and indoor samples and dominating STs for *F. veterinarium* (FOSC) and *F. petroliphilum* (FSSC), while other species were more diverse. Also, whole-genome sequencing revealed great variability of AC sequences in FOSC isolates, thereby creating starting points for subsequent investigation of their putative role in virulence and adaptation to specific environments.

WS3.23 - Genomics approach in analysis of *Fusarium* apple fruit rot

M. Petres[1], *D.H. Ayhan*[2], *M. Grahovac*[1], *D. Budakov*[1], *M. Loc*[1], *T. Dudas*[1], *L.-J. Ma*[2]
[1]University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia, [2]University of Massachusetts Amherst, Department of Biochemistry and Molecular Biology, Amherst, MA, United States

A well-known phytopathogen of cereals and maize *Fusarium graminearum* has recently been detected, along with *F. avenaceum*, as a causal agent of stored apple fruit rot. The significance of these species is evident not only in the yield loss of stored apples, but also in the increased risk of mycotoxin contamination in infected fruits.
In our previous research, we assembled *F. graminearum* genome (strain TaB10 from Serbia), and compared it with the reference genome of the strain PH-1, and the global genome comparison showed high percentage of synteny. Despite the high similarity of two genomes a total of 67 unique genes of the TaB10 genome were identified by combining different approaches. Further analyses revealed enrichment of uniquely found genes in effector proteins, particularly in apoplastic effectors, which may be significant for pathogenicity in apples.
These results emphasize the crucial role of genomic research in revealing the pathogenic processes of *F. graminearum*. Future research will focus on identification of genes underpinning pathogenic interaction between *Fusarium* and apple fruit utilizing RNA sequencing and gene knockout using CRISPR/Cas9, evaluating eco-friendly control methods including bioagents and physical measures, and exploring advanced molecular strategies like RNAi for effective pathogen management.
This work was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Project No. 451-03-66/2024-03/200117) and by the United States Fulbright Scholar Program.

WS4.02 - Anaerobic fungi as treasure trove for biomass degradation machinery for exploitation in renewables-based biotechnology

S. Shen[1], J. Matthews[1], M. Bowden[2], M. Blow[3], K. LaButti[3], V. Ng[3], C. Nicora[2], S. Leichty[2], S. Purvine[2], I. Grigoriev[3], J. van Munster[1]
[1]SRUC, Department of Plant and Soil Sciences, Edinburgh, United Kingdom, [2]Pacific Northwest National Laboratory, Richland, United States, [3]U.S Department of Energy Joint Genome Institute, Berkeley, United States

Lignocellulosic biomass is a promising feedstock for renewables-based biotechnology, but complete degradation of this material remains challenging. We investigate anaerobic gut fungi (AGF, phylum *Neocallimastigomycota*) to provide potential solutions. These fungi have distinct degradation mechanisms compared to the aerobic fungi usually employed as enzyme producers in biotechnology: they employ plant-biomass penetrating hyphae and leverage an arsenal of enzyme complexes that are unique among fungi. Despite lacking oxygen dependent lignin-degrading machinery or LPMO's, these AGF are highly effective in degrading raw lignocellulose.
Here we demonstrate how the degradative activity of three AGF isolates from genera *Neocallimastix, Caecomyces and Piromyces*, has distinct effects on plant biomass composition and architecture, via integration of distinct lignocellulose characterization techniques. Growth experiments indicated the fungi also had distinct capacities for metabolism of simple sugars that are derived from lignocellulose. These findings suggest that each species has unique roles and degradation capabilities. To further explore this, we are now using RNA-seq and untargeted proteomics to compare how our *Neocallimastix* and *Caecomyces* isolates leverage their degradative machinery during wheat straw fermentation.

WS4.03 - Exploring mucoromycota and endosymbiont diversity and biology using whole genome based life identification numbers

H. Johnson[1], K. Belay[2], R. Mazloom[2], L. Heath[2], B. Vinatzer[2], D. Haak[2], N. Grunwald[1], J. Uehling[1]
[1]Oregon State University, Corvallis, United States, [2]Virginia Tech, Blacksburg, United States

Advancements in high-throughput sequencing technology have increased the availability of fungal whole genome sequences (WGSs); however, the ever-increasing number and length of sequences in DNA databases represents a computational challenge. Additionally, only a fraction of kingdom fungi has been described and new taxa often disrupt current naming conventions. To meet these challenges, we propose adapting the life identification number (LIN) concept, a concept designed for prokaryotes that assigns labels known as LINs to individual genomes reflecting genomic similarity, for the analysis of fungi. The LIN workflow combines efficient k-mer and hashing algorithms for initial database-wide pairwise comparisons followed by a single average nucleotide identity computation which, in conjunction with the LIN concept, permits rapid, sensitive, and scalable WGS-based identification. This research focuses on the use of LINs

to understand taxonomic relationships and elucidate biology of the Mucoromyota as well as the diversity and evolution of their endohyphal bacteria, and taxonomy determined via the LIN system will be compared to orthologous gene-based phylogeny. We expect LIN-based classifications will resolve Mucoromycota from the genus to intraspecific variants including variation in accessory or repetitive chromosomes. Further, we expect LIN-based endosymbiont phylogeny and its comparison to free-living relative and host Mucoromycota phylogenies will reveal insights into the evolutionary history of this symbiosis. We anticipate this approach of LIN-based Mucoromycota identification will be extensible to other fungal phyla, permitting strain level identification of isolates with no need for conventional Linnean classification and allowing rapid inference of phenotypes like fungicide resistance and virulence of pathogenic fungi.

WS4.04 - Genome-based phylogeny of the Mucoraceae and related families reveals temperature adaptation as the main driver of evolution

G. Walther[1], *J. Stajich*[2], *L. Wagner*[1], *O. Kurzai*[3,1]
[1]Leibniz Institute for Natural Product Research and Infection Biology Hans Knöll Institute (Leibniz-HKI), NRZMyk, Jena, Germany, [2]University of California Riverside, Microbiology & Plant Pathology Dept, Riverside, United States, [3]University of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany

The basal fungal family Mucoraceae (Mucorales, Mucoromycota) comprises fungi with highly diverse ecology and morphology including soil and dung inhabiting saprotrops as well as parasites of plants, animals and fungi. Based on the limited data available, we recognize the whole range from cosmopolitans to endemic species. Several *Mucor* species and *Actinomucor elegans* can cause human infections (mucormycosis).
Our previous studies on the evolution of Mucoraceae and closely related families were based on multi-locus phylogenies and revealed three lineages of species with *Mucor* morphology which would require a split of the genus *Mucor*. However, not all nodes of the backbone were supported. To clarify the existence of 3 *Mucor* lineages we performed a genome based phylogenetic analysis including more than 120 genomes, of which one half was generated as part of the Zygolife project and the other half for this study. The genome analyses are not yet complete because position of few taxa still vary depending on the number of genes and the outgroup used. Nevertheless, our genome analyses indicate so far that *Mucor* is paraphyletic forming a single lineage the includes the Mycotyphaceae and the Choanephoraceae. Morphological traits of zygospores and sporangiophores are only characteristic on the subclade level. Adaption to temperatures seem to be a main driver of evolution in this lineage because *Mucor* species with low maximal growth temperatures form a clade and species with higher maximal growth temperatures form another clade together with the thermotolerant Mycotyphaceae and Choanephoraceae.

WS4.05 - Genomic hints to Mortierellomycota resilience and adaptability

A. Muszewska[1], *D. Barua*[1], *M. Orłowska*[2], *B. Sokołowska*[1], *M. Ciach*[3], *S. Piłsyk*[1], *A. Okrasińska*[2], *J. Pawłowska*[2]
[1]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland, [2]University of Warsaw, Faculty of Biology, Biological and Chemical Research Centre, Warsaw, Poland, [3]University of Warsaw, Faculty of Mathematics, Informatics and Mechanics, Warsaw, Poland

Mortierellomycota encompasses ubiquitous soil- and plant-associated fungi, potentially the descendants of the first colonizers of land. Current ecological and biological knowledge of Mortierellomycota representatives is mostly related to their ability to produce valuable fatty acids. Mortierellomycota is related to both Mucoromycota and Glomeromycota, with which they share the predisposition to harbor endohyphal and associated bacteria. We performed genomic analyses for Mortierellomycota genomes deposited in GenBank database. Their genomes are compact with moderate amounts of genes and mobile elements. However, they display several unique traits, including potent metabolic capabilities, often reflected in gene duplications. They are also particularly adapted to aquatic environments and their lipid composition seems to be analogous to Blastocladiomycota. Mortierellomycota lost genes involved in ergosterol synthesis, and seem to have no diacylglycerol kinase. Instead, they have an expanded repertoire of lipid peroxidation, lipid degrading and sterol-binding proteins. Based on gene presence, they likely have sphingomyelin, like the Opisthokonta ancestors, animals and current Umbelopsidales. Mortierellomycota have the most complex fucose metabolism among fungi with two fucose synthesis pathways. Compared to Mucoromycotina, they possess several expansions of peptidases and oligopeptide transporters. They have duplicated genes enabling the usage of purines as secondary nitrogen sources in nitrogen-limiting conditions. Their proteomes are shaped by both vertical inheritance and horizontal gene transfer. Likewise Glomeromycota, they acquired NOD-like receptors, and like Basidiobolus, they acquired NRPS metabolic clusters from associated bacteria. Taken together, Mortierellomycota displays a list of unique properties among Fungi. Here, we summarize puzzling features we identified previously, together with new findings.

WS4.06 - Analysis of potential DNA 6mA readers in early-diverging fungi

P. Carrillo-Marín[1], *C. Lax*[1], *E. Navarro*[1], *F.E. Nicolás*[1], *V. Garre*[1]
[1]Universidad de Murcia, Departamento de Genética y Microbiología, Facultad de Biología, Murcia, Spain

DNA methylation is a key epigenetic modification, with 5-methylcytosine (5mC) and N6-methyladenine (6mA) being the most notable. Recent studies have revealed the importance of 6mA in certain eukaryotes, such as green algae, ciliates, and Early-diverging fungi. In particular, in the basal fungus *Rhizopus microsporus*, approximately 1.5% of adenines have been observed

to be methylated. The identification of 6mA in promoters of transcriptionally active genes has raised interest in its potential function as a regulator of gene expression.
While readers of 6mA in RNA (m6A) have been characterized, with the YTH domain being the most common, only two readers of 6mA in DNA have been characterized: Jumu in *Drosophila melanogaste*r and SSBP1 in humans. In this study, a unique YTH and SSBP1 ortholog, YTHDC1 and SSBP1 respectively, have been identified in R. microsporus. YTHDC1 is particularly relevant as it is the only member of the YTH family localized in the nucleus, and the inability to detect m6A in Mucorales RNA suggests that it may have a distinct functional role in this organism. Additionally, orthologs of YTHDC1 and SSBP1 have been found in other Mucorales, indicating a possible evolutionary conservation of their function.
The generation of *ythdc1* and *ssbp1* mutants could be key to describing how this epigenetic modification regulates gene expression. Furthermore, EMSA assays will demonstrate whether YTHDC1 and SSBP1 specifically bind to DNA probes containing 6mA, providing evidence for the 6mA-based regulatory mechanisms of gene expression.

WS4.07 - Coordinated adaptation of gene families has shaped the genome of dimorphic fungi

G. Tahiri Zainane[1], *C. Lax*[1], *T. Gabaldón*[2], *V. Garre*[1], *F.E. Nicolás Molina*[1]
[1]University of Murcia, Genetics and Microbiology, Murcia, Spain, [2]Barcelona Supercomputing Center, Life Sciences - Comparative Genomics, Barcelona, Spain

Dimorphism is a critical virulence factor in fungi, enabling pathogens to transition between yeast and the invasive mycelium form, which helps them evade the host immune response. Through comprehensive transcriptomic analysis of the yeast and mycelial forms of the dimorphic fungus *Mucor lusitanicus*, we identified that approximately 30% of its protein families are dimorphic, characterized by paralog genes that are expressed in yeast and those specifically expressed in mycelium. These dimorphic families are randomly distributed along genome and are involved in multiple biological processes.
Notably, a thorough genomic analysis revealed that *M. lusitanicus* utilizes head-to-head (H2H) genes to coordinately regulate genes from dimorphic families associated with related functions. These conserved genetic structures across the Mucorales control around 78% of the H2H genes co-expressed within the same morphology. This indicates that these genetic structures could serve as predictors of the morphology in which a particular paralog is expressed, as orthologs expressed in mycelium cluster together in phylogenetic analyses.
Moreover, comparative genomic analysis between dimorphic and non-dimorphic fungi showed that dimorphic fungi have an expanded set of genes within dimorphic families, whereas non-dimorphic fungi possess an expanded array of genes predominantly expressed in the mycelial form.
Altogether, the integration of transcriptomic, genomic, and phylogenetic analyses enables the development of a predictive model to distinguish between dimorphic and non-dimorphic fungi. This model enhances our understanding of their evolutionary mechanisms and potential strategies for evading the immune response.

WS4.08 - Coupling genome-wide positive selection analysis with transcriptomics to infer molecular bases of the *Rhizopus microsporus* – *Mycetohabitans* symbiosis

M. Branine[1], *E. Scales*[2], *S. Mondo*[3,4], *T. Pawlowska*[2]
[1]Cornell University, Department of Microbiology, Ithaca, United States, [2]Cornell University, School of Integrative Plant Science, Ithaca, United States, [3]U.S. Department of Energy Joint Genome Institute, Berkley, United States, [4]Colorado State University, Department of Agricultural Biology, Fort Collins, United States

The mucoromycete *Rhizopus microsporus* (*Rm*) and its bacterial endosymbiont *Mycetohabitans* spp. are an emerging model system for symbioses of early-diverging fungi and bacterial endosymbionts due to its experimental tractability and genomic resources. In this symbiosis, *Mycetohabitans* provides its host with secondary metabolite toxins, controls fungal asexual and sexual propagation, and alters fungal lipid metabolism. We hypothesize that the strong influence of *Mycetohabitans* on *Rm* host biology has significant consequences for the evolutionary trajectory of host fungi. Importantly, *Rm* isolates naturally free of endosymbionts (nonhosts) permit comparative analyses into if and how endosymbiotic bacteria influence the evolution of fungal hosts. To begin addressing this question, we implemented a genome-wide positive selection analysis in host and nonhost strains of *Rm* by calculating the non-synonymous to synonymous substitution rate ratio (dN/dS) of all single-copy orthologs. Under a branch model, we identified 28 genes putatively under positive selection in host *Rm* strains (dN/dS>1). To provide further evidence for the role of these genes in symbiosis, we leveraged transcriptomic data comparing two *Rm* hosts cured of their endosymbionts to their WT counterparts. While several genes from our positive selection analysis were differentially expressed (DE) in either host, only two were DE in both strains. One of these genes encodes a SRF-like protein with orthology to the transcription factor RlmA, a regulator of asexual differentiation in ascomycetes. Given the reproductive control exerted by *Mycetohabitans*, we hypothesize this transcription factor is playing a similar role in mucoromycete fungi, which we will test in future functional studies.

WS4.09 - The lifestyle of mucoromycotina 'fine root endophytes' through the genomic lens

A. Wanke[1], *A. Williams*[2], *V.R. Morelos*[3], *A. Dallaire*[4,5], *S. Au*[5], *S. Pressel*[3], *K. Field*[2], *S. Schornack*[1]
[1]Sainsbury Laboratory Cambridge University, Cambridge, United Kingdom, [2]University of Sheffield, Sheffield, United Kingdom, [3]Natural History Museum, London, United Kingdom, [4]Royal Botanic Gardens Kew, Richmond, United Kingdom, [5]University of Cambridge, Cambridge, United Kingdom

'Fine Root Endophytes' from the Mucoromycotina clade (MFRE) play a crucial role in terrestrial nutrient cycling due to their dual saprotrophic and mycorrhizal traits. Over the last decade, microscopic and physiological studies have provided valuable insights into the mycorrhizal

relationship between MFRE and plants, highlighting intracellular colonisation and nutrient-for-carbon exchange. However, the molecular mechanisms underlying the saprotrophic and mycorrhizal lifestyles of MFRE remain poorly understood. In this study, we employ genomic, transcriptomic and molecular genetic approaches on two MFRE isolates to explore symbiotic and saprotrophic strategies in this mycorrhizal lineage. We generated the first genome assemblies for MFRE and their bacterial endosymbionts, utilising both short-read (Illumina) and long-read (Nanopore) sequencing technologies. Through comparative genomic and transcriptomic analyses, we identified distinct signatures for transporters and secreted proteins (such as hydrolases and effector candidates) under *in vitro* and *in planta* conditions. Our findings highlight a unique molecular blueprint that distinguishes the saprotrophic and mycorrhizal lifestyles of MFRE from other mycorrhizal fungi.

WS4.11 - Structure of Mucoromycota fungal communities and their associated endosymbiotic bacteria across two different biomes in the U.S. and Israel

N. Reynolds[1], *K. Amses*[2], *J. Uehling*[3], *R. Adeleke*[4], *M. Sternberg*[5], *M. Branine*[6], *T. Pawlowska*[7]
[1]California Polytechnic University, Pomona, Biological Sciences, Pomona, United States, [2]University of Pennsylvania, Perelman School of Medicine, Philadelphia, United States, [3]Oregon State University, Department of Botany and Plant Pathology, Corvallis, United States, [4]North-West University, Unit for Environmental Sciences and Management, Potchefstroom, South Africa, [5]Tel Aviv University, School of Plant Sciences and Food Security, Tel Aviv, Israel, [6]Cornell University, Graduate Field of Microbiology, Ithaca, United States, [7]Cornell University, School of Integrative Plant Science, Ithaca, United States

Despite the ecological importance of Mucoromycota fungi as mycorrhizal symbionts, opportunistic human and plant pathogens, and post-harvest spoilage agents, they remain understudied compared to Dikarya. Fundamental aspects such as geographical distribution, dispersal patterns, and community structure remain unclear. The endosymbiotic bacteria (EB) that many Mucoromycota species harbor have generated new questions regarding their effects on fungal host evolution. EB have varying effects on the host fungi depending on the species, influencing asexual and sexual reproduction and metabolic functioning. To investigate communities, we collected rhizosphere soils from four sites in California and two in Israel representing two biomes (Desert and Mediterranean scrub). Metabarcoding data were generated using bacterial (16S rDNA) and fungal (28S rDNA) primers and show several OTUs unique to each habitat. A nested PCR approach was designed for 16S to enrich samples for known EB groups. Both biotic filtering and dispersal filtering significantly affected fungal and bacterial communities; however, dispersal filtering was only significant over larger distances (km scale). Desert samples had a higher proportion of fungal OTUs assigned to opportunistic human pathogenic species not detected from the coast and had higher proportions of Zoopagomycota. On the other hand, coastal samples had greater diversity of mycorrhizal OTUs (Glomerales and Endogonales). Mediterranean scrub samples showed higher proportions of *Mycoplasma*-related EB, corresponding to the greater diversity of mycorrhizal OTUs. Desert samples showed the opposite, with higher abundance of *Burkholderia*-related EB. Overall, our results indicate co-

occurrence of EB communities with their fungal hosts, and desert environments are likely reservoirs for opportunistic pathogens.

WS4.12 - Peculiarities in the genome maintenance of Arbuscular Mycorrhizal Fungi

D. Barua[1], *A. Muszewska*[1]
[1]Institute of Biochemistry and Biophysics Polish Academy of Sciences, Laboratory of Fungal Bioinformatics, Warsaw, Poland

Arbuscular Mycorrhizal Fungi (AMF) are a division of early diverging fungi (Glomeromycota) that establish symbiotic relationships with 71% of land plants. AMF absorb phosphorus and nitrogen from the soil and transfer them to host plants, in exchange for carbon sources. Recent studies have shown how nutrient transport occurs between the fungus and the host plant. Glomeromycota possess multinucleate cells with non-identical genetic material in individual nuclei. This leads to their nuclei behaving like a population rather than an individual in every species. The complexity of laboratory handling of symbiotic Glomeromycota representatives hinders molecular studies. Despite the economic and ecological significance, the genetic basis of many biological processes in this phylum are not known. In this study, we attempt to expand the search for nucleic acid processing components in AMF to explain the plausible mechanisms of genome maintenance and DNA repair in this lineage using an in-silico approach. Our findings reveal several gene losses, including the Rad9-Hus1-Rad1 (9-1-1) clamp complex, GINS 1, 3, and 4 along with Mcm10 of the CMGE helicase complex, RecQ and SecA helicases, FAN1 nuclease acting downstream of the Fanconi Anemia pathway, all of which are key cell cycle and DNA repair proteins conserved across eukaryotes. We also find losses of mitochondrial biogenesis (Pet127) and centromere (CENPM, CBF3) proteins essential for metabolism and DNA binding respectively. These recurrent loss of genome maintenance processes during the course of evolution points to the presence of alternative mechanisms that enable AMF to evolve and proliferate.

WS4.13 - Initiation of endosymbiosis in *Rhizopus microsporus* indicates a shift from antagonism to commensalism

T. Gassler[1], *A. Holderbusch*[1], *G. Giger*[1], *A. Sintsova*[1], *M. Bortfeld-Miller*[1], *B. Dehapiot*[2], *S. Sunagawa*[1], *J.A. Vorholt*[1]
[1]ETH Zurich / Institute of Microbiology, Department of Biology, Zurich, Switzerland, [2]ETH Zurich / ScopeM, Zurich, Switzerland

Endosymbioses represent intricate and dynamic relationships between organisms that may involve pathogenic phases during their emergence. Here, we use fluidic force microscopy (FluidFM) to induce cell-in-cell interactions to directly probe the early stages of endosymbiosis. We introduced the opportunistic pathogen *Ralstonia pickettii* into a non-endosymbiotic strain of *Rhizopus microsporus*, simulating the unstable early phase of endosymbiosis. This approach allowed us to explore mechanisms that might overcome initial challenges in the stabilization of

such an interaction. The intracellular presence of *R. pickettii* affected fitness and induced stress responses in the novel fungal host. Adaptations were observed at the phenotypic, genetic and transcriptional levels, indicating a shift from pathogenic antagonism to commensalism as the interaction progressed. Using high-throughput microscopy and custom-trained deep learning models, we tracked individual spores and quantified fungal growth and host responses. Our work offers insights into early processes of endosymbiosis, highlighting the role of mechanisms that mitigate pathogenicity and promote compatibility in stabilizing these interactions.

WS4.14 - Understanding *Rhizopus v*irulence in mucormycosis

A. Guerrero[1], *D. Stark*[2], *D. E. Corzo-León*[3], *J. King*[2], *E. R. Ballou*[3], *J. K. Uehling*[1]
[1]Oregon State University, Botany and Plant Pathology, Corvallis, United States, [2]University of Sheffield, Biosciences, Sheffield, United Kingdom, [3]University of Exeter, Exeter, United Kingdom

Mucormycosis is a dangerous emerging infectious disease for which there are few effective treatments. *Rhizopus microsporus* (Mucorales, Mucoromycota) is among the fungi that cause mucormycosis. This fungus and its pathogenic relatives frequently harbor Burkholderiaceae-related endobacteria (BRE). It has been demonstrated that BRE maintain the genetic and functional capacity to produce secondary metabolites. These products have the potential to manipulate host immune defenses, facilitate fungal invasion and pathogenesis. Some evidence suggests that endosymbionts of clinical *Rhizopus* strains may impart similar benefits by weakening human immune systems. However, not all clinical *Rhizopus* strains contain endosymbionts. Thus, it is unclear how and to what degree endobacteria contribute to fungal pathogenicity in humans. Our goal is to understand how bacterial – fungal dynamics relate to virulence. We will leverage a library of dozens of clinically and environmentally derived *Rhizopus* strains to meet this goal. The proven ability to mix and match endosymbionts, recent developments in CRISPR genome editing, and the application of robust virulence assays will allow us to test specific fungal genes and bacteria for contributions to pathogenicity. We expect this research to provide insights into the role of endobacterial and fungal virulence factors in the development of mucormycosis, ultimately improving our biological understanding of a deadly and increasingly common disease.

WS4.15 - The role of rhizoferrin in growth and virulence of *Rhizopus microsporus*

U. Binder[1], *J. Scheler*[2], *C. Lax*[3], *B. Sarg*[2], *F. Nicolas*[3], *T. Telser*[2], *B. Zelger*[2], *I. Bauer*[2], *K. Faserl*[2], *C. lass-Flörl*[2], *V. Garre*[3]
[1]Medical University Innsbruck/Institute of Hygiene and Medical Microbiology, Innsbruck, Austria, [2]Medical University Innsbruck, Innsbruck, Austria, [3]Universidad de Murcia, Murcia, Spain

Mucormycosis, an invasive fungal infection caused by Mucorales fungi, presents a significant threat particularly for patients with conditions such as uncontrolled diabetes, hematological

malignancies, and COVID-19 co-infections. Iron acquisition is vital for Mucorales pathogenicity, with elevated serum free iron levels intensifying their virulence. Investigating virulence factors to potentially find new drug targets is urgently needed for this group of fungi.
Fungi secrete siderophores to enable chelation and uptake of ferric iron. For clinically relevant mucormycetes it has been shown that a polycarboxylate siderophore, rhizoferrin, is secreted. This study aims to elucidate the role of the rhizoferrin synthetase encoding gene (rfs) and its product rhizoferrin, in the growth and virulence potential of Rhizopus microsporus.
Assessment of rhizoferrin production via chrome azurol S (CAS)-assays and High-performance liquid chromatography (HPLC), growth assays under varying iron or (xeno)siderophore availabilities were conducted for both *R. microsporus* wild type and rfs-deletion mutants. Galleria mellonella larvae were utilized to study virulence potential of wt versus deletion strains with or without addition of (xeno)siderophores or co-infection with Pseudomonas aeruginosa.
Rfs deletion resulted in non-detectable amounts of rhizoferrin and significantly reduced virulence potential in the *Galleria* mellonella infection model. Further, rfs deletion strains were unable to form hyphae within Galleria larvae and growth reduction/unability under low iron conditions was evident. Currently investigations are carried out to determine if virulence and germination can be restored in the presence of iron or (xeno)siderophores, applied directly or vi co-incubation with Pseudomonas.

WS4.16 - The development of a Tet-off system in Mucorales unveils the crucial role of DNA 6mA in pathogenesis

C. Lax[1], *M. Sanchís*[2], *Y. Ahmiane*[2], *E. Navarro*[1], *J. Capilla*[2], *F.E. Nicolás-Molina*[1], *V. Garre*[1]
[1]University of Murcia, Genetics and Microbiology, Murcia, Spain, [2]Universitat Roviria i Virgili, Ciències Mèdiques Bàsiques, Reus, Spain

The scarcity of available molecular tools in early-diverging fungi (EDF) has hindered the study of crucial aspects of their biology compared to higher fungi (or Dikarya). Some species of Mucorales are opportunistic human pathogens causing a lethal fungal disease known as mucormycosis. To unravel the molecular mechanisms underlying this virulence, tools are needed to precisely characterize the involvement of specific genes in the infection process. In this study, we adapted a tetracycline-inducible (Tet-off) expression system to characterize genes in in vivo infection models. The development of this system was made possible by the previous development of genetic modification tools in the fungus *Rhizopus microsporus*. Only the precise adaptation of the components of this system to the particularities of this fungus enabled their correct expression and function. To validate the developed system, we used the *mta1* gene encoding an adenine methyltransferase responsible for 6mA deposition, an essential epigenetic modification in the DNA of this fungus. In vitro assays demonstrated the effect of tetracycline in regulating this gene under the control of this system. In vivo murine infection studies further demonstrated the functionality of the system and the crucial role of adenine methylation in the infection process. The tool developed here can be a key platform for characterizing genes that are determinants in the pathogenesis of Mucorales.

WS4.17 - Experimental preclinical imaging-compatible animal models of mucormycosis

U. Binder[1], *A. Resendiz-Sharpe*[2], *J. Scheler*[1], *C. Kandlbauer*[1], *M. Navarro*[3], *F. Nicolas*[3], *I. Bauer*[1], *C. Lass-Flörl*[1], *V. Garre*[3], *G. Vande Felde*[2]
[1]Medical University Innsbruck, Innsbruck, Austria, [2]KU Leuven, Leuven, Belgium, [3]Universidad de Murcia, Murcia, Spain

Infections by mucormycetes are a serious threat in the clinical setting mainly due to their fast progression and limited treatment options.
We aimed to generate luciferase expressing Mucorales strains to be used for non-invasive monitoring of the infection in different animal models of mucormycosis – especially to establish multimodal, imaging-compatible preclinical mouse models - and as an alternative tool for *in vitro* drug testing.
Codon-optimized firefly luciferase without the peroxisomal target sequence, under the control of two different promoters was cloned into auxotrophic *M. lusitanicus* recipient strains. Positive transformants were checked for gene integration. Growth pattern and light emission under various conditions was determined by luminometer. Selected strains were used in Galleria infection assays and a neutropenic mouse model to determine infection by BLI imaging.
Firefly luciferase, with a single integration was successfully expressed in *M. lusitanicus.*, Light emission could be measured by luminometer and visualized in animal models. High light signal was obtained in infected *Galleria* larvae 48h after infection but decreased at 96h in those still alive. Similar results were obtained in mice and results correlated with the status of immunosuppression and weight loss. Overall, strains are usable for real-time, non-invasive infection monitoring and could also be used in the testing of antifungal efficacy by means other than survival.
The successful visualization of *M. lusitanicus* infection by a non-invasive method in insect and murine models, offers new ways to study mucormycosis and by extending this method to other species, will give valuable new insights in the pathogenesis of mucormycosis.

WS4.18 - Host brain environment triggers RNAi epimutation in the human pathogen *Mucor circinelloides*

M. Navarro-Mendoza[1], *C. Pérez-Arques*[1], *E. Cao*[1], *Z. Xu*[1], *Y. Choi*[1], *Z. Chang*[1], *Y.-S. Bahn*[2], *A. Moseman*[3], *J. Heitman*[1]
[1]Duke University, Department of Molecular Genetics and Microbiology, Durham, United States, [2]Yonsei University, Department of Biotechnology, Seoul, Korea, Republic of, [3]Duke University, Department of Integrative Immunobiology, Durham, United States

Mucorales are basal fungi causing severe human infections known as mucormycoses, particularly in immunocompromised and COVID-19 patients. Despite aggressive treatments, mortality can reach 90% in disseminated infections, mainly due to poor diagnosis and antimicrobial drug resistance. *Mucor* species can develop transient antimicrobial drug resistance through RNAi-based epimutations, adapting to different stresses through a reversible epigenetic mechanism.

These epimutations exhibit organ-specific induction, being more prone to arise after central nervous system (CNS) infections. Immunofluorescence cryosections of brain tissue infected with *Mucor* fluorescent strains revealed a specific strong inflammation and granuloma formation compared to other organs. We identified seven loci targeted by RNAi epimutations during brain invasion in mice, named *bep* for brain epimutations (*bepA* to *bepG*). *bepA* encodes a serine-threonine kinase related to Mpk1, involved in cell wall stress responses. *M. circinelloides bepA* mutants were resistant to calcineurin inhibitors FK506 and cyclosporine A, showing mycelial growth in the presence of these inhibitors. On the other hand, *bepA* overexpression reversed this phenotype. This suggests BepA is an antagonist to the phosphatase calcineurin that promotes the yeast-to-hyphal transition. To understand BepA roles in host adaptation, we studied *Mucor* interactions with brain endothelial cells and crossing the blood-brain barrier (BBB). We also monitored infections in immunosuppressed mice, analyzing cryosections of infected organs with *bepA* mutant fluorescent strains. Preliminary data indicate that *bepA* mutants cause more severe BBB damage compared to wild type. Ongoing research will clarify how *bepA* silencing is triggered in the CNS.

WS4.19 - RNAi drives heritability of epimutational antimicrobial resistance

C. Pérez-Arques[1], *M.I. Navarro-Mendoza*[1], *Z. Xu*[1], *G. Walther*[2], *J. Heitman*[1]
[1]Duke University, Department of Molecular Genetics and Microbiology, Durham, United States, [2]Hans Knöll Institute, National Reference Center for Invasive Fungal Infections, Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany

Epigenetic modifications that alter gene expression without changing the DNA sequence –known as epimutations– are a widespread phenomenon in eukaryotic organisms. Epimutations may arise from RNA interference, DNA methylation, and/or chromatin modifications, contributing to antifungal drug resistance and affecting virulence traits in fungal pathogens. Our research identifies RNAi epimutations in fertile *Mucor* phylogenetic species within the *Mucor circinelloides* species complex, a neglected group of human pathogens. These RNAi epimutations arise spontaneously upon an external stress and can be transmitted to the next generation in the absence of the initial insult. The inheritance pattern observed for these epimutations is DNA sequence-independent and non-Mendelian.
The absence of other repressive chromatin marks typically associated with epigenetic inheritance highlights that small RNA (sRNA) molecules act as the sole determinants of inheritance. This conclusion is further supported by unique sRNA signature patterns shared between epimutant parents and their progeny. Our findings demonstrate that epimutations are broadly present across the *Mucor* species complex and act exclusively through posttranscriptional gene silencing to control gene expression, advancing the understanding of genetic and epigenetic inheritance mechanisms in eukaryotes. Although epimutations are stable through both mitosis and meiosis, their detection may pose a challenge in typical culture methods employed in clinical diagnostics given that these frequently involve growth in the absence of drug selective pressure. Understanding how epimutations arise and modify gene expression may enable their detection in clinical settings and provide solutions for the challenges posed by rising antimicrobial drug resistance.

WS4.20 - Understanding of primary resistance to echinocandin in *Mucor*

A. Rendon[1], *A. Garcia*[1], *E.Y. Huh*[2], *A. Ko*[1], *S.C. Lee*[2]
[1]University of Texas at San Antonio, San Antonio, United States, [2]Texas Tech University Health Science Center, Immunology and Molecular Microbiology, LUbbock, United States

Mucormycosis, which is caused by Mucorales fungi, is poses a challenge to immunocompromised patients. As the susceptible cohort increases, the incidence of mucormycosis is rising. The mortality rate for mucormycosis reaches 90% in cases of disseminated infection. However, there are limited options in the current armamentarium for mucormycosis due to the high intrinsic resistance of Mucorales to available antifungal drugs. Echinocandins are the newest antifungal drugs that are highly efficacious in the treatment of many fungal infections. Mucorales, however, exhibit primary resistance to these drugs. Echinocandins inhibit the *fks* genes that encode beta 1,3-glucan synthases, and the model Mucorales *Mucor* genome harbors three copies of *fks* genes. To understand the primary resistance mechanism, we looked at the amino acid sequence of the gene products and found that the three *fks* genes in *Mucor* encode intrinsically altered amino acids in the Hotspot 1 region. Nonsynonymous mutations in Hotspot 1 in *Candida* species have been known for major resistance mechanisms to echinocandin. To elucidate if these inherited changes in Hotspot 1 of the *Mucor* Fks's are involved in echinocandin resistance, we expressed *Mucor fks* genes and measure drug resistance and susceptibility in *Candida albicans*. The expression and function of *Mucor* Fks were confirmed. Interestingly, the engineered *C. albicans* strains expressing *Mucor fks ge*nes are similarly susceptible to echinocandin. These results demonstrate that the *Mucor* beta 1,3-glucan synthases themselves, despites the inherited changes in Hotspot1, are also susceptible for echinocandin and other unknown mechanism(s) is involved in the intrinsic resistance.

WS4.21 - The role of cell wall remodeling in early divergent Mucoromycotina response to bacterial and plant defenses

H. Barrett[1], *M.L. Gaspar*[1], *C. Lax*[2], *V. Garre*[2], *T. Pawlowska*[1]
[1]Cornell University, Plant Pathology and Plant Microbe Biology, Ithaca, United States, [2]Universidad de Murcia, Departamento Genética y Microbiología, Facultad de Biología, Murcia, Spain

The fungal cell wall is known to respond dynamically to environmental stress through extracellular perception and cell wall integrity monitoring, but its role in inter-organizational interactions is not well understood. Using the early divergent fungus *Rhizopus microsporus* (Mucoromycotina) and the bacterium Mycetohabitans spp. as a model, we have observed cell wall remodeling in response to bacterial antagonism. We found differential expression of genes encoding synthesis and modification of cell wall components chitin, fucose, glucan, mannose, and galactose under bacterial antagonism. We investigated the composition of the cell wall by fluorescently staining individual components, then imaging the fluorescence by microscopy and quantifying median fluorescence of each stain by flow cytometry. We also visualized the internal

structure of the wall through transmission electron microscopy. We detected an increase in chitin content in the presence of bacteria, which appears to be involved in the defensive reinforcement of the cell wall. My research aims to uncover the role of extracellular perception and cell wall integrity sensors in activating this response under various biological stresses. I hypothesize that this defensive remodeling is driven by the interacting Protein Kinase A (PKA), Cell Wall Integrity (CWI), and High Osmolarity Glycerol (HOG) pathways, and will occur in response to multiple mechanisms of antagonism from plants and bacteria.
I aim to uncover the dynamics and mechanism of remodeling with the goal of developing antifungal treatments that more directly target stress tolerance mechanisms activated during infection.

WS4.22 - Identification of two new genes controlling dimorphism in Mucorales

G. Navarro Del Saz[1], *G. Tahiri*[1], *E. Navarro*[1], *V. Garre*[1], *F.E. Nicolas*[1]
[1]Universidad de Murcia, Facultad de Biología, Genética y Microbiología, Murcia, Spain

Mucormycosis is a fungal infection caused by species of the Mucorales order, primarily affecting immunocompromised individuals and showing high mortality rates. Some species, like *Mucor lusitanicus*, exhibit dimorphic development, alternating between yeast and mycelial forms, with the latter being virulent. Genomic studies have identified gene families that are differentially regulated in each form. Notably, two gene families involved in iron uptake were found: ferroxidases (*fet3a*, *fet3b*, *fet3c*) and permeases (*ftr1m* and *ftr1y*), with differential expression depending on the dimorphic form. It was observed that *fet3a* and *ftr1y*, expressed in yeast, share a bidirectional promoter, while *fet3b* and *ftr1m*, expressed in mycelium, are similarly clustered. To investigate gene regulation, a "DNA Pull-Down" assay was performed using protein extracts from both vegetative forms. Candidate proteins were identified, and mutants for two gene coding for an *F-box* and a *kinase protein* (*Mucci31471074* and *Mucci31468915*, respectively) were generated, showing altered yeast development. Phenotypic assays revealed no differences in media with varying iron availability. However, RT-qPCR gene expression analysis of *fet3a*, *fet3b*, *ftr1m*, and *ftr1y* was performed, alongside transcriptomic analyses of both mutants. To confirm the relationship between the *f-box* and *kinase* genes and the observed phenotypes, and to study the subcellular localization of the F-box and kinase proteins, complementation studies with recombinant wild-type genes fused to Cherry were conducted. These findings could be key to developing new treatments for mucormycosis.

WS4.23 - Mucoromycete germlings undergo regulated cell death mediated by adenylyl cyclases in the innate immune response to bacterial perception

D. Tota[1], *M.L. Gaspar*[2], *C. Lax*[3], *V. Garre*[3], *L. Esipov*[2], *T. Pawlowska*[2]
[1]Cornell University, Graduate Field of Microbiology, Ithaca, United States, [2]Cornell University, Plant Pathology & Plant-Microbiology, Ithaca, United States, [3]Universidad de Murcia, Departamento de Genética y Microbiología, Murcia, Spain

Microorganisms' survival is dependent on the ability to perceive and respond to the biotic environment. Innate immunity enables cells to sense the non-self, regulate interactions with potential pathogens, and maintain organismal integrity. Despite fungi being known to inhabit virtually all environments and interact with multiple kingdoms of life, the understanding of fungal defenses, especially that of early-divergent fungi, is in its infancy. Our work using wildtype and adenylyl cyclase mutants has revealed that germlings of *Rhizopus microsporus*, an emerging model for Mucoromycete-bacterial interactions, antagonistically respond to the bacteria *Mycetohabitans* sp. B13 and *Ralstonia pickettii* through adenylyl cyclase-mediated signaling. Response by germlings includes lipid peroxidation and regulated cell death, as characterized by fluorescence and transmission electron microscopy and flow cytometry. Our study system also includes *Mucor lusitanicus*, a model for Mucoromycete genetic manipulation. Growth inhibition assays of wildtype and adenylyl cyclase mutant strains of *R. microsporus* and *M. lusitanicus* exposed to various isolated bacterial MAMPs (microbe-associated molecular patterns) suggest that Mucoromycetes exhibit a generic stress response to the perception of bacteria, regardless of bacterial identity or activity. We hypothesize that Mucoromycotina fungi initiate a generalized innate immune response that is functionally similar to plant and animal innate immunity. As regulated cell death is a novel innate immune response in fungi, we plan to characterize the form of regulated cell death initiated by *R. microsporus* and *M. lusitanicus* in response to bacterial perception by quantifying the molecular hallmarks of regulated cell death using bioassays, fluorescence and transmission electron microscopy, and flow cytometry.

WS4.24 - MAT Loci in the mortierellaceae: genomic, empirical and molecular evidence

K. Mondron[1], *W. Gilmour*[1], *M. Korne*[2], *A. Sow*[2], *J. Nettnin*[3], *A. Idnurm*[4], *G. Bonito*[2,5], *J. Uehling*[1]
[1]Oregon State University, Botany and Plant Pathology, Corvallis, United States, [2]Michigan State University, Microbiology, Genetics, and Immunology, East Lansing, United States, [3]Iowa State University, Genetics, Development, and Cell Biology, Ames, United States, [4]University of Melbourne, School of BioSciences, Parkville, Australia, [5]Michigan State University, Plant, Soil and Microbial Sciences, East Lansing, United States

Fungi have a remarkably diverse repertoire of sexual strategies and spore stages that are intimately tied to their ecological success. Early studies of fungal sexuality included model systems within the Mucorales (Mucoromycotina, Mucoromycota) such as the genera *Phycomyces*, *Mucor*, and *Rhizopus*. The genetic basis of canonical Mucoralean sexual reproduction has been well described, and involves two out-crossing mating types, plus (+) and minus (-), determined by the genetic content at the mating type (MAT) locus, which encode different high-mobility group (HMG) transcription factors (TFs). MAT loci have not been identified or described in the Mortierellomycotina (Mucoromycota) although sexual spore (zygospore) production is frequently observed. In this study, we leveraged MycoCosm's MCL Cluster tool and the genomes of *Linnemannia elongata* (Mortierellomycotina) isolates of known mating types to identify two potential MAT loci for the Mortierellomycotina. These candidate MAT loci encode HMG and homeodomain (HD) TFs. We conducted intraspecies zygospore production assays by co-culturing isolates on low-nutrient media. Sexually fertile isolates were thereby assigned to compatibility groups subject to further genomic and molecular evaluation.

Using the Hidden Markov Model-based computational tool Orthofisher, our *L. elongata* (Mortierellomycotina) isolate compatibility data, and PCR amplification of the HMG and HD candidate MAT loci, discussion will center on a three-pronged approach of genomic, empirical (zygospore production), and molecular evidence.

WS5.01 - Evolution of the interaction between Trichoderma and land plants

I. Monte[1]
[1]University of Tuebingen ZMBP, Tübingen, Germany

Plants colonized land around 500 Mya likely aided by successful mechanisms to interact with surrounding microbes. The characterization of the interaction between extant flowering plants and microbes revealed that the fungus *Trichoderma* promotes plant growth, defense and stress tolerance in angiosperms. However, we discovered that phylogenetically diverse *Trichoderma* strains are pathogenic for non-seed plants, including model species belonging to the three bryophyte lineages and one fern. Thus, *Trichoderma* constitutes the first described microbe that establishes opposite interactions with non-seed and flowering plants. We hypothesized that the pathogenic interactions between *Trichoderma* and non-seed plants might be due to the differential behavior of *Trichoderma* towards different hosts and/or to evolutionary differences in the immune system of land plants. We are exploring these hypotheses using a combination of cell biology, omics, GWAS and reverse genetics on our main model plant, the bryophyte *Marchantia polymorpha*, and additional non-seed model plants. We ultimately aim to identify the molecular determinants responsible for the evolutionary transition of *Trichoderma*-plant interactions from pathogenic to beneficial in land plants and pinpoint the emergence of mutualistic interactions between plants and *Trichoderma*.

WS5.02 - Trichoderma afroharzianum: A Emerging Maize Pathogen in Europe

A. Pfordt[1], *A. Tiedemann*[1]
[1]Goerg-August-University of Goettingen, Department of Plant Pathology and Crop Protection, Goettingen, Germany

Trichoderma afroharzianum is a ubiquitous species occurring worldwide. Due to its mycoparasitic and endophytic properties, certain Trichoderma isolates are used in agriculture as biological plant protection and biocontrol agents. However, in 2018, a massive occurrence of *T. afroharzianum* on maize cob was observed for the first time in Germany. Since then, Trichoderma ear rot has been observed at several locations in Germany, France, and Italy, especially after dry and hot vegetations periods. Symptoms of Trichoderma ear rot are shown as massive production of green to gray-green conidia on the kernels and husk leaves. Moreover, affected crops are soft rotten characterized by excessive moisture and low starch content due to the production of alpha-amylase by the fungi. Interestingly, it was observed that most *T. afroharzianum* strains were pathogenic, while some strains of the same species showed no pathogenicity at all. In addition, certain Trichoderma strains from approved biological fungicides and soil additives have demonstrated pathogenicity after artificial inoculation. Out of the 14 tested products, three strains of biocontrol products exhibited medium to high pathogenicity.

These findings suggest that certain strains commonly used in agricultural products for their beneficial properties may also possess pathogenic characteristics.

WS5.03 - The new frontier of biocontrol: breeding for biologicals and microbiome resilience

B. Dotson[1,2], C.B. Andersen[1], Å. Lankinen[1], A. Rasmusson[2], L. Grenville-Briggs Didymus[1]
[1]Swedish University of Agricultural Sciences, Department of Plant Protection Biology, Alnarp, Sweden, [2]University of Lund, Department of Biology, Lund, Sweden

In a one health context where we consider the plant as a holobiont, traits encoded for by symbiotic microbes and the microbiome are important contributors to overall plant health. We are studying the impact of fungal (*Trichoderma afroharzianum* T22) and oomycete (*Pythium oligandrum*) biocontrol agents on sugar beet and potato health. *Trichoderma*- and *P. oligandrum* induced growth and disease protection have been shown in many cultivated plants. However, variable efficiencies have been seen, and a lack of genetic understanding of the interaction likely limits the present agricultural benefits. Studies on crop plant rhizosphere microbiomes have focused mainly on spatio-and-temporal dynamics between plant growth stages, genotypes and cropping systems. Few studies have investigated manipulation of the plant rhizosphere microbiome with the amendment of a Biological Control Agent (BCA), which is of importance for our understanding of the function of BCAs in the environment and their impact on soil/plant health. We show that *P. oligandrum* has a biostimulatory effect in a cultivar-dependent manner in potato and that it induces changes in the rhizosphere microbiome. In sugar beet we observed significant variation in the biocontrol of damping off and in growth promotion by T22 within sugar beet elite breeding lines. Results indicate that plant genotype and development are the major factors explaining the observed variation. yet different Trichoderma species/strains will differentially affect the growth outcome of each sugar beet line. This implies that efficient technological use of these BCAs will demand a tight interaction between microbiological optimisation and plant breeding.

WS5.04 - Integrating *Trichoderma gamsii* T6085 and *Clonostachys rosea* IK726 to enhance Fusarium Head Blight control in wheat

A. Petrucci[1], M. Cesarini[1], I. Vicente[2], L. Merani[3], B. Jensen[4], D.B. Collinge[4], S. Sarrocco[1]
[1]University of Pisa, Department of Agriculture, Food and Environment (DAFE), Pisa, Italy, [2]University of Salamanca, Department of Microbiology and Genetics, Institute for Agribiotechnology Research (CIALE), Salamanca, Spain, [3]University of Bologna, Department of Agricultural and Food Sciences (DISTAL), Bologna, Italy, [4]University of Copenhagen, Department of Plant and Environmental Science (PLEN), Copenhagen, Denmark

Wheat is the third staple food globally, and Fusarium Head Blight (FHB) is an important disease caused by several *Fusarium* spp., with *F. graminearum* (*Fg*) as the most relevant in Europe. The major concern of FHB is the production of mycotoxins, thereby food safety.
Biocontrol is a sustainable approach, with the biocontrol agents (BCAs) *Trichoderma gamsii*

T6085 (*Tg*) and *Clonostachys rosea* IK726 (*Cr*) proving effective individually. This study aims to improve efficiency and stability in field by combining *Tg* and *Cr*.
Compatibility was assessed *in vitro* and *in planta*. On solid agar, growth inhibition due to diffusible compounds was absent but *Tg* volatile compounds slightly inhibited *Cr* growth. In liquid cultures, *Cr* cultural filtrates inhibited *Tg* spore germination, and *Cr* inhibited *Tg* growth. Mutual growth inhibition did not occur on wheat spikes or on straw.
Following root application, no evidence of systemic modulation of defence-related (DR) genes occurred in leaves but in spikes inoculated with *Cr* (alone and combined) *Pal1*, *PR1* and *Lox1* were up-regulated 96hpi (hours post-inoculation). In spikes subsequently inoculated with *Fg*, *PR1* was slightly up-regulated by *Cr* at 24hpi. *Tg* highly up-regulated DR genes at 72hpi. BCAs co-inoculation up-regulated *Lox1* and *Pgip2* to the highest level at 96hpi without *Fg* and at 24hpi with *Fg*, respectively. Disease incidence and pathogen inoculum for the next cropping season were reduced by >90% and 99%, respectively, across treatments.
Compatibility on spikes, upregulation of DR genes and reduction of FHB symptoms suggest that combining *Tg* and *Cr* potentially enhance FHB management.

WS5.05 - Plant developmental status determines the outcome of *Trichoderma harzianum* disease control

Y. Elad[1], *N. Lindner*[1], *M. Leibman-Markus*[1], *R. Gupta*[1], *M. Bar*[1,2]
[1]ARO, Volcani Institute, Rishon LeZion, Israel, [2]Ben-Gurion University of the Negev, Department of Life Sciences, Beer-Sheva, Israel

Plant disease resistance is influenced by numerous factors, including plant age, however, the role of plant development in disease resistance is underinvestigated. Our results indicate that *Trichoderma*-based disease control is affected by plant developmental status. In tomato, leaves at different developmental stages exhibit distinct morphological and physiological characteristics that may influence their ability to resist pathogens. We examined the ability of the well-known *Trichoderma harzianum* isolate "T39", previously employed worldwide in the control of several fungal diseases, to control gray mold disease incited by *B. cinerea* in tomato plants and leaves of different ages and developmental states. Finding different levels of biocontrol activity exerted by *T. harzianum* in leaves at different developmental stages, we investigated whether this phenomenon could relate to differences in leaf microstructure or hormonal content between developing and mature leaves. Our results indicate that within a certain age window, developing leaves respond better to *T. harzianum* biocontrol than mature leaves, and that this is likely a result of both the leaf hormonal content and microstructure. In the continuous search for factors that will improve the performance of green disease control agents, we suggest that the developmental state of the plants being treated should be taken into account.

WS5.06 - Taxogenomic analysis reveals the reticulate evolution of the genus *Trichoderma*

F. Cai[1], *A. Steindorff*[2], *F. Yi*[1], *M. Ding*[3], *R. de Vries*[4], *I.V. Grigoriev*[2], *I. Druzhinina*[5]
[1]Sun Yat-sen University, School of Ecology, Shenzhen, China, [2]Joint Genome Institute (JGI), Lawrence Berkeley National Laboratory, Berkeley, United States, [3]Nanjing Agricultural University, Jiangsu Provincial Key Lab for Solid Organic Waste Utilization, Nanjing, China, [4]Westerdijk Fungal Biodiversity Institute, Fungal Physiology, Utrecht, Netherlands, [5]Royal Botanic Gardens, Kew, Richmond, United Kingdom

The increasing importance of *Trichoderma* (Hypocreales, Ascomycota) for human well-being underscores the need for a solid taxonomy and deep understanding of its biology. Recent studies have challenged the hypothesis of *Trichoderma* primary association with plant roots or soil, instead highlighting its mycoparasitism and environmental opportunism. The parasitism of closely related fungi (adelphoparasitism) likely caused the massive transfer of plant cell wall degrading enzymes from plant-pathogenic fungi to ancestral *Trichoderma* lineages, along with some reciprocal transfers of other genes. The early molecular phylogeny has shown significant variations in evolutionary rates across different clades and identified taxonomic ambiguities, such as infrageneric "taxonomic clouds" or putative metaspecies (e.g. *T. harzianum* s.l.), where numerous phylospecies remained undefined due to the lack of genealogical concordance.
The taxogenomic analysis of 66 *de novo Trichoderma* genome assemblies revealed four consistent phylogenetic clades. However, we also observed frequent gene tree incongruences, discordances between gene trees and the species tree, and inconsistent placement of individual lineages. Results from D Statistics and PhyloNet analyses were unanimous in indicating ancient gene flows within each clade (such as from an unsampled lineage or an extincted one to the ancestor of *T. effusum*) and putative hybrid speciation between certain *Longibrachiatum* species. Our findings suggest a complex history of introgression and reticulate evolution in some infrageneric groups of *Trichoderma* and the "conventional" speciation in the others. In this presentation, we'll propose a species concept accommodating the formation of metaspecies and discuss the importance of taxonomic stability in evaluating the safety of *Trichoderma*-based bioeffectors.

WS5.07 - Community Efforts to Transform Taxonomic Challenges into Advancements in Trichoderma Biology

I. Druzhinina[1]
[1]Royal Botanic Gardens Kew, Accelerated Taxonomy, Richmond, United Kingdom

Is the pathogenicity to maize a unique trait of the well-known T22 strain, a property of the *T. afroharzianum* species, or a characteristic shared by all members of the *T. harzianum* sensu lato? Could this trait be linked to the *Harzianum* Clade or even to the broader Section *Pachybasium*? These critical questions highlight the importance of precise and accurate taxonomic dissection, particularly for fungi with significant applications and biosecurity implications.
The genus *Trichoderma* has seen a dramatic increase in species diversity, with over 50 new

species described annually, adding to the nearly 500 currently recognized taxa. While this growth reflects remarkable progress in understanding *Trichoderma* diversity and evolution, it also reveals substantial geographic biases. Most species are described from North America, Europe, and China, while biodiversity hotspots in regions like the Amazon, equatorial Africa, South East Asia, and vast areas of Eurasia remain underexplored. It suggests that many newly described species are recognized within known taxa rather than from the discovery of novel forms in unexplored habitats.
Although this refinement of taxonomic resolution contributes to a better understanding of *Trichoderma* biology, it also poses significant identification challenges. Despite the availability of advanced molecular techniques, current species concepts and taxonomic frameworks are often inconsistent across the genus. This has led to the proliferation of new species with insufficient diagnostic clarity and a lack of publicly accessible type materials, hampering reproducibility and comparative analyses. Addressing these challenges requires a unified, community-driven effort to establish a widely accepted, pragmatic species concept for *Trichoderma*. The International Commission on *Trichoderma* Taxonomy (ICTT) (www.trichoderma.info), a part of the International Committee for the Taxonomy of Fungi (ICTF) (www.fungaltaxonomy.info) and affiliated with the International Union of Microbiological Societies (IUMS), seeks to fill this critical role. The ICTT aims to foster a collaborative platform where experts can discuss the current state of *Trichoderma* taxonomy, evaluate species concepts, and promote best taxonomic practices. By offering guidance to authors and editors on new species proposals and clades, the ICTT strives to enhance consistency and reproducibility in *Trichoderma* taxonomy.
This presentation will provide an overview of *Trichoderma* diversity, highlight current taxonomic challenges, and explore potential future directions. It will also encourage active participation from the *Trichoderma* research community in ICTT initiatives, aiming to transform taxonomic challenges into scientific advancements in *Trichoderma* biology.

WS5.08 - Homothallic or heterothallic: a genomic investigation into the sexual capabilities of *Clonostachys rosea*

M. Karlsson[1], *A. Wilson*[2], *E. Piombo*[1], *M. Brandström Durling*[1], *S. Chaudhary*[1], *H. De Fine Licht*[2], *M. Dubey*[1]
[1]Swedish University of Agricultural Sciences, Forest Mycology and Plant Pathology, Uppsala, Sweden, [2]University of Copenhagen, Plant and Environmental Sciences, Copenhagen, Denmark

Modes of reproduction and sexual strategies strongly influence the genetic diversity and evolutionary potential of a species. The ascomycete fungus *Clonostachys rosea* is reported to be homothallic (sexually self-fertile), although a rapid decay of genome-wide linkage disequilibrium is also reported, which is not in line with an obligate homothallic mode of reproduction. To investigate this phenomenon, we identified the mating-type (*mat*) locus in 63 genome-sequenced *C. rosea* strains under the hypothesis that each strain contains genes from both *mat* idiomorphs. Eleven strains indeed contained both *mat1-1* and *mat1-2* genes, suggesting homothallism. However, most strains harboured either *mat1-1* or *mat1-2* genes and co-existed in North America, Europe and China, suggesting heterothallism. The *mat* locus of heterothallic strains was highly conserved, and the linkage disequilibrium half decay distance was 625 bp, suggesting sexual outcrossing. The presence of conserved *mat1-1* or *mat1-2* idiomorphs in strains of other

Clonostachys species shows that heterothallism is the ancestral state. A phylogenetic analysis of 2800 single-copy orthologues revealed that homothallic and heterothallic strains clustered in two separate, well-supported clades, indicating a single lineage of homothallic *C. rosea*, followed by inter-continental dispersal. We discuss the evolutionary, genomic and applied consequences of this unique mixed-mode type of sexual reproduction.

WS5.09 - Polyphasic approach revealed the genomic and phenotypic variation across the supreme plant biomass degradation genus *Trichoderma*

P. Chen[1,2], *N. Martinez Reyes*[2,3], *M. Peng*[2], *H. Senoglu*[2], *S.K. Reddy*[2,4], *B. Henrissat*[5,6], *A. Steindorff*[7], *I.V. Grigoriev*[7], *R. Berka*[8], *I.S. Druzhinina*[9], *R.P. de Vries*[2]
[1]Nanjing Agricultural University, College of Resources and Environmental Sciences, Nanjing, China, [2]Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands, [3]Universidad de León, León, Spain, [4]Wageningen University & Research, Wageningen, Netherlands, [5]Technical University of Denmark, Department of Biotechnology and Bioengineering, Lyngby, Denmark, [6]King Abdulaziz University, Jeddah, Saudi Arabia, [7]Joint Genome Institute, Berkeley, United States, [8]Archer Daniels Midland Company, Davis, United States, [9]Royal Botanic Gardens, Kew, London, United Kingdom

The genus *Trichoderma* (Hypocreales, Ascomycota), encompassing over 400 species, is ubiquitous and thrives in diverse environments, including dead wood, soil, litter, and even on fungi. *Trichoderma* spp. exhibit a broad spectrum of nutritional strategies, engaging in saprotrophic and biotrophic interactions with bacteria, fungi, plants, animals, and other organisms. *Trichoderma* has been intensively studied for over seven decades due to its extensive applications in cellulolytic enzyme production and biocontrol against plant pathogens, numerous genetic and genomic studies have been conducted on fungi within this genus, unveiling their genomic traits and evolutionary history.
This study specifically focuses on the genomic and phenotypic diversity of *Trichoderma* species. With the inclusion of *de novo* sequenced genomes and published ones, a comprehensive genome compendium of the genus was assembled. Our phylogenomic analysis identified at least three main clades/sections. Subsequently, we predicted sugar metabolism genes and secondary metabolite genes, with the conserved sugar metabolism genes exhibiting a high correlation with the phylogenomic tree. Furthermore, a comparative analysis of the *Trichoderma* CAZome with those of other fungi revealed that, despite similar numbers of CAZyme families in *Trichoderma* genomes, only a quarter of these genes are conserved across all analyzed species. This finding suggests that recent gene deletions, likely driven by environmental pressures, have significantly shaped the current plant biomass degradation CAZyme profile within the *Trichoderma* genus. Our genotype-phenotype profiling contributes to a deeper understanding of the ecology, speciation, and biotechnological potential of *Trichoderma*.

WS5.10 - Unraveling the chromosomal landmarks of the filamentous fungus *Trichoderma reesei*

K. Métivier[1], *H. Mathis*[1], *F. Bidard*[1], *E. Jourdier*[1]
[1]IFP Energies Nouvelles, Rueil-Malmaison, France

Filamentous fungi represent a primary source of enzymes and metabolites employed in a range of industrial sectors. Of these, *Trichoderma reesei* is commonly used to produce second-generation ethanol, where it is employed to generate the cellulase enzymes that are essential for the hydrolysis stage. To enhance the long-term capabilities of fungi, the utilization of genetic tools could prove beneficial. Among them, the creation of an artificial chromosome would facilitate the simultaneous testing of multiple genes, reduce the use of selection markers, and enhance the regulation of gene expression and gene landscape control. However, there is currently no available artificial chromosome for *T. reesei*. To facilitate the construction of an artificial chromosome, it is essential to characterize the components that contribute to chromosome stability, in particular centromeres and origins of replication (ORI).
The aim of this study is to ascertain the precise localization and size of the centromeres and to identify any potential ORI in *T. reesei*. Chromatin immunoprecipitation of eGFP-tagged CenH3, the centromere-specific histone, has enabled the precise description of the centromeres inside the AT- and repeat-rich regions previously known. To identify the ORI, several techniques previously used in other species have been adapted and implemented on *T. reesei*. Loci with the potential to act as ORI have been identified and will require further investigation. The identification of the centromere and the ORI in *T. reesei* will facilitate the construction of an artificial chromosome for this species.

WS5.11 - Unveiling the diversity of *Trichoderma reesei*: new paths for biotechnological advances

T. Benocci[1,2], *C. Fiorenzani*[2], *F. Baltar*[1], *R. Baroncelli*[2]
[1]University of Vienna, Functional and Evolutionary Ecology, Vienna, Austria, [2]University of Bologna, Department of Agricultural and Food Sciences (DISTAL), Bologna, Italy

Trichoderma reesei is a well-studied saprotrophic fungus from tropical rainforests, known for its exceptional enzyme secretion. This trait has made it a cornerstone of industrial biotechnology, particularly in the production of lignocellulolytic enzymes (e.g. cellulases) used to convert plant biomass into sustainable fuels and chemicals. Despite significant advances in developing high-performance industrial strains, most are derived from a narrow genetic base, primarily the QM6a strain, leaving much of *T. reesei*'s genetic diversity unexplored.
In this study, we investigate the genetic and phenotypic diversity of nine natural *T. reesei* strains, including the reference strain QM6a, collected from diverse environments worldwide, with one rare strain originated from a marine habitat.
Through comparative genomics and phenotypic screening, we explore the genetic diversity and adaptive mechanisms these strains employ to thrive in their unique environments. Our analysis focuses on nutrient recycling, biomass degradation, Carbohydrate-Active Enzymes (CAZymes)

repertoire, regulatory pathways, and salinity tolerance.
This dataset offers valuable insights into the evolution and adaptation of *T. reesei*, uncovering potential for strain improvement in industrial biotechnology. Our findings identify promising leads for developing optimized enzyme cocktails for more efficient biomass conversion, underscoring the importance of exploring natural genetic diversity to fully unlock *T. reesei*'s potential for sustainable industrial applications.

WS5.12 - The role of HFB1 in the mate recognition and sexual reproduction initiation in *Trichoderma reesei*

F. Yi[1], *M. Ding*[2], *G. Pang*[2], *I. Druzhinina*[3], *F. Cai*[1]
[1]Sun Yat-sen University, School of Ecology, Shenzhen, China, [2]Nanjing Agricultural University, Jiangsu Provincial Key Lab for Solid Organic Waste Utilization, Nanjing, China, [3]Royal Botanic Gardens, Kew, Richmond, United Kingdom

Sexual reproduction is a main driving force for genetic diversity. *Trichoderma reesei*, which belongs to one of the most well-studied model filamentous fungi for its significant industrial value in enzyme production, represents a typical bipolar heterothallic ascomycete. However, despite its significance, numerous unresolved mysteries persist regarding its molecular mechanism of sexual reproduction. Our preliminary results identified a signaling molecule, HFB1, from the extracellular proteins of *T. reesei*. This molecule is essential for initiating sexual reproduction and the "female fertility" of MAT1-1 mating type. Phenotypic profile of gene deletion mutants indicated a crucial role of HFB1 in maintaining the female fertility of the MAT1-1 mating type. Results from *in situ* fluorescently tagged strains and RNA-seq analysis demonstrated that HFB1 is involved in the early process of mating partner recognition and is primarily secreted by the MAT1-1 partner and sensed by the MAT1-2 type. Importantly, we also observed that, without the HFB1 signal provided by the MAT1-1 type, the transcription of the "sexual determination gene" *mat1-2-1* is abolished, resulting in an insufficient response such as significantly reduced pheromone secretion from the opposite mating type. Therefore, HFB1 is suggested a novel cell communication factor upstream of the pheromone signaling system, in which the intracellular signal transduction cascades and key regulatory elements await discovery.

WS5.13 - Unveiling the H3K4me3 dynamics in cellulase induction across wild type and hyperproducer strains of *Trichoderma reesei*

S. Fajon[1], *F. Malagnac*[2], *E. Jourdier*[1], *A. Margeot*[1], *A. Pirayre*[1], *F. Bidard*[1]
[1]IFP Energies Nouvelles, Rueil-Malmaison, France, [2]Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France

The filamentous fungus *Trichoderma reesei* can secrete high levels of cellulolytic enzymes, which are used on an industrial scale to produce fermentable sugars from lignocellulosic biomass. Studies suggest that cellulase production is regulated at the chromatin level. However, genome-scale chromatin dynamics during cellulase production remain unexplored.
The aim of this work is to gain insight into the epigenetic mechanisms that occur during enzyme

production in the QM6a wild-type and RutC30 hyperproducer strains, and to assess whether RutC30 mutations affect the epigenetic landscape. To this end, H3K4me3 marks were mapped in these two strains during induction (cellulase production) and a non-induction state (no cellulase production), and this dataset was complemented by a transcriptomic study.
The results of the ChIP-Seq analysis revealed two key findings common to both strains: 1/ there is a correlation between H3K4me3 marks and gene expression levels and 2/ a limited but targeted redistribution of H3K4me3 marks towards some of the genes involved in cellulase production takes place during the induction shift. In addition, comparison of H3K4me3 patterns between the two strains revealed that RutC30 has 'constitutively' open chromatin at the key cellulase regulator genes, in contrast to the QM6a strain where these regions are only open during the induction state. This difference highlights the unique epigenetic landscape of the RutC30 strain, which may confer advantages for cellulase production. This understanding is crucial for the development of strategies to enhance enzyme production.

WS5.14 - N-acetylglucosamine sensing in Trichoderma reesei

S.F. Ullah[1], M. Oreb[2], E. Boles[2], V. Srivastava[1], V. Seidl-Seiboth[3], B. Seiboth[3], L. Kappel[1]
[1]KTH Royal Institute of Technology, Division of Glycoscience, Department of Chemistry, Stockholm, Sweden, [2]Goethe University, Institute of Molecular Biosciences, Faculty of Biological Sciences, Frankfurt, Germany, [3]TU Wien, Research Division Biochemical Technology, Institute of Chemical, Environmental and Bioscience Engineering, Vienna, Austria

N-acetylglucosamine (GlcNAc), the monomeric subunit of chitin and glucosaminoglycans, is involved in diverse signalling pathways in dimorphic yeasts and bacteria, where it is related to morphogenetic switching, mating, stress, virulence and cell death. GlcNAc has further been shown to promote plant growth by shaping the bacterial soil community. Here we propose a regulatory network for GlcNAc sensing in soil fungi. Using *Trichoderma reesei* as a model organism we showed that GlcNAc impacts expression of around 2,100 genes. Apart from primary metabolism, secondary metabolism was strongly affected. Two key regulators of GlcNAc catabolism, the NDT80-like transcriptional regulator, RON1, and a GlcNAc sensor, the GCN5-related histone acetyltransferase NGS1, are differential regulators of two-thirds of these genes. Finally, we characterized the third regulator of GlcNAc sensing in *T. reesei*, which is the highly specific GlcNAc transporter NGT1. Interestingly, while internal GlcNAc activates GlcNAc catabolic gene expression, in contrast to dimorphic yeasts, the pathways for defense and pathogenicity seem to be induced in *T. reesei* by external GlcNAc. Given the ancestral role of *Trichoderma* spp. in the fungal kingdom and the highly conserved GlcNAc catabolism cluster including their regulators in many species of fungi, we propose that GlcNAc signaling in filamentous soil fungi might induce several signalling cascades related to metabolic processes, stress, and defence reactions. Our findings contribute to understanding sugar metabolism and sensing in filamentous soil fungi, which in turn might impact microbiome composition and thus soil fertility.

WS5.15 - Are1 implication in iron metabolism

C. Baldin[1], *D. Flatschacher*[1], *S. Pierson*[1], *U. Schreiner*[1], *M. Alilou*[2], *L. Atanasova*[3], *S. Zeilinger*[1]
[1]University of Innsbruck, Department of Microbiology, Innsbruck, Austria, [2]University of Innsbruck, Institute of Pharmacy/Pharmacognosy, Innsbruck, Austria, [3]BOKU University, Institute of Food Technology, Vienna, Austria

Aspergillus AreA GATA-like transcription factor AreA is widely regarded as a key regulator in overcoming nitrogen catabolite repression (NCR). Its role in activating genes repressed by NCR is a critical adaptive feature in numerous species, facilitating the utilization of non-preferred nitrogen sources when favored ones, such as ammonium or glutamine, are scarce. *Trichoderma*, a genus of filamentous ascomycetes commonly found in the rhizosphere, is globally distributed and thrives in various ecological niches. Its relevance to sustainable agriculture lies in the potential of certain species to act as biological control agents, particularly due to their ability to target a wide range of economically important fungal phytopathogens through mycoparasitism. Despite its importance, our understanding of NCR and its regulatory mechanisms in *Trichoderma* species remains limited, leaving significant gaps in our knowledge of how these fungi adapt to diverse environments. Characterization of the AreA ortholog in the mycoparasitic fungus *T. atroviride*, named Are1, not only confirmed previously identified roles in nutrients adaptation and NCR relief, but also led to the discovery of previously unknown connections between this transcription factor and many other cellular processes, including iron metabolism and siderophore biosynthesis.

WS5.16 - Activation of the Ilicicolin BGC in *Trichoderma reesei* results in high-yield production of Ilicicolin H and a novel antifungal substance, Ilicicolin K

I. Burger[1], *M. Schmal*[1], *K. Peikert*[1], *L. Fourtis*[1], *C. Suster*[1], *C. Stanetty*[1], *D. Schnalzer*[1], *B. Hufnagel*[2], *T. Boettcher*[2], *R. Birner-Gruenberger*[1], *R.L. Mach*[1], *A. Mach-Aigner*[1], *M. Schittmayer*[1], *C. Zimmermann*[1]
[1]TU Wien, Vienna, Austria, [2]University of Vienna, Vienna, Austria

Ilicicolin H is a potent antifungal compound with a very low minimal inhibitory concentration (MIC) below 1 µg/ml. It specifically targets fungal cytochrome bc1 reductase without affecting the mammalian homolog in *in vitro* experiments. The biosynthetic gene cluster (BGC) for ilicicolin H was first identified by two independent groups in 2019. They heterologously expressed the BGC from *Talaromyces variabile* and *Neonectria* sp. DH2, respectively, in *Aspergillus nidulans*. Later, another group expressed the BGC from *Trichoderma reesei* in *Aspergillus oryzae*. All three studies reached similar conclusions regarding the biosynthesis pathway, except for the final step. Two groups suggested that the epimerization of 8-epi-ilicicolin H to ilicicolin H occurs spontaneously, while one group demonstrated that the epimerase IliE is necessary for this conversion.
We activated the ilicicolin H BGC in *T. reesei* by overexpressing its BGC-specific transcription factor, which was confirmed by RT-qPCR and proteomics. Additionally, we deleted the genes

encoding the core enzyme, *triliA* (a fusion PKS-NRPS), and the epimerase, *triliE*. Our *in vivo* data suggest that the epimerase is indeed essential for the final step of ilicicolin H biosynthesis. During our metabolome analysis, using a networking approach, we discovered a novel compound, which we named ilicicolin K. Ilicicolin K differs from ilicicolin H by a hydroxylation and the formation of an additional ring via ether bridge formation at the pyridone moiety. Ilicicolin K exhibited antifungal activity with a minimal inhibitory concentration of approximately 30 µg/ml against *Candida auris*.

WS5.17 - Small RNAs-mediated fungal interactions relevant to biocontrol

E. Piombo[1], *N.C. Konakalla*[2], *P. Kalyandurg*[2], *P. Sundararajan*[2], *D. Funck Jensen*[1], *M. Karlsson*[1], *R. Raju Vetukuri*[2], *M. Dubey*[1]
[1]Swedish University of Agricultural Sciences, Uppsala, Sweden, [2]Swedish University of Agricultural Sciences, Alnarp, Sweden

Small RNA (sRNAs)- mediated RNA silencing is emerging as a critical player in host-microbe interactions. However, its role in fungal interactions relevant to biocontrol is yet to be fully explored. Our recent research explored small RNA (sRNA)-mediated interactions between the biocontrol fungus *C. rosea* and its fungal and plant hosts. We showed that deletion of the DCL2 *gene*, which acts upstream in RNA silencing pathways, in *C. rosea* resulted in mutants with reduced specialized metabolite production, mycoparasitism and biocontrol ability. However, its root colonization ability was increased. Dual-RNA seq analysis revealed the downregulation of defense response-related genes in wheat during interactions with Δ*dcl2* strains compared to the WT, which aligns with the increased root colonization ability of DCL2 deletion strains. Furthermore, our sRNA sequencing identified 18 wheat miRNAs responsive to *C. rosea*, with three predicted to target the *C. rosea* polyketide synthase gene *pks29*, known for its role in fungal antagonism and biocontrol. Two of these miRNAs were shown to enter *C. rosea* from wheat roots with fluorescence analyses and silenced the expression of a polyketide synthase gene *pks29*, previously shown for its role in fungal antagonism and biocontrol. This provides compelling evidence for cross-kingdom RNA silencing of the *C. rosea* gene by wheat miRNAs. Our work provides insights into the mechanisms underlying biocontrol fungi-plant interactions and holds promise for future studies on sRNA-mediated RNA silencing in fungal biocontrol.

WS6.02 - Unraveling the evolutionary history of genetically diverged lineages of *Colletotrichum graminicola*

F. Rogério[1], S. De Mita[2], C. Van Oosterhout[3], B. Cuevas-Fernández[1], P. García-Rodríguez[1], S. Becerra[1], S. Gutiérrez-Sánchez[1], J. Dambolena[4], I. Buhiniček[5], S. Sukno[1], M. Thon[1]
[1]University of Salamanca, Salamanca, Spain, [2]Univ Montpellier, Montpellier, France, [3]University of East Anglia, Norwich, United Kingdom, [4]Universidad Nacional de Córdoba, Córdoba, Argentina, [5]BC Institute for Breeding and Production of Field Crops, Dugo Selo, Croatia

Maize anthracnose, caused by the ascomycete fungus Colletotrichum graminicola, is an important crop disease worldwide. The disease can result in significant yield losses and is also an important model for genetic studies. The evolutionary history of crop pathogens is shaped by a complex interaction of natural and anthropogenic factors. We investigated the evolutionary origin of genetically diverged lineages of *C. graminicola*, using a collection of 212 field isolates from 17 countries including recently collected strains from Galicia, Spain. Genomic analyses supported the existence of three geographically isolated lineages, with a significant pattern of isolation by distance. We identified two distinct gene flow patterns, driven by short and long-distance dispersion, likely resulting from the natural spread of the pathogen and the exchange of contaminated seeds. Demographic modeling indicated that North America is an intermediate between Brazil, Europe and an ancestral, unsampled source population, hypothesized to be Mesoamerican. Our analyses revealed that the global genomic structure of C. graminicola is shaped by geographic differentiation driven by long-distance migration and a long history of recombination and introgression. We show historical relationships among these lineages, identifying a potential route for fungal spread, with the North American population emerging ancestrally, followed sequentially by the Brazilian and European populations. Our research indicates that the European lineage is more virulent, which has implications for the potential emergence of new outbreaks of maize anthracnose in Europe.

WS6.03 - Comparative genomics reveals sources of genetic variability in the asexual fungal plant pathogen *Colletotrichum lupini*

J. Alkemade[1,2], P Hohmann[3], M.M. Messmer[4], T.G. Barraclough[1,2]
[1]University of Oxford, Department of Biology, Oxford, United Kingdom, [2]Magdalen College, Calleva Research Centre for Evolution and Human Science, Oxford, United Kingdom, [3]Universitat de Barcelona, Department of Biology, Healthcare and the Environment, Faculty of Pharmacy and Food Sciences, Barcelona, Spain, [4]Research Institute of Organic Agriculture (FiBL), Department of Crop Sciences, Frick, Switzerland

Fungal plant pathogens are a major cause of global crop losses, with many exhibiting compartmentalized genomes comprising core and accessory regions that facilitate rapid adaptation. The host-specific fungus *Colletotrichum lupini*significantly affects lupin (*Lupinus* spp.) cultivation and belongs to clade 1 of the *C. acutatum* species complex. This pathogen

consists of four genetically uniform clonal lineages (I–IV), yet notable variation in virulence and morphology exists within these lineages. To uncover potential sources of genetic variability in this asexual species, we analyzed the genomes of 16 *C. lupini* strains alongside 17 related *Colletotrichum* species. Phylogenomic analysis reaffirmed the presence of four distinct lineages but revealed that lineage II could be further divided into two subgroups, II-A and II-B, based on differences in genome size, gene content, transposable elements (TEs), and deletions. Variation in TE content strongly correlated with genome size, implicating TEs in driving genome expansion. Pangenome analysis highlighted a highly dynamic accessory genome, including a mini-chromosome present in lineages II, III, and IV, but absent in lineage I. Accessory genes and putative effectors were often clustered near TEs, and their presence/absence patterns were lineage-specific, indicating a role in shaping host specificity. Interestingly, no effectors were identified on the TE-rich mini-chromosome. These findings reveal mechanisms of genetic diversification in this asexual fungal pathogen and provide insights that could inform strategies for future disease management.

WS6.04 - A survey conducted to the Madeira Island (Portugal) reveals the presence of 21 species of *Colletotrichum* occurring on nature and on agricultural and ornamental plants

P. Talhinhas[1], *A. Cabral*[1], *T. Nascimento*[1], *A.P. Ramos*[1], *I. Martino*[2], *A. Prodi*[3], *D. Spadaro*[2], *R. Baroncelli*[3], *V. Guarnaccia*[2]
[1]Instituto Superior de Agronomia, Universidade de Lisboa, Lisbon, Portugal, [2]University of Torino, Grugliasco, Italy, [3]University of Bologna, Department of Agri-Food Science and Technology, Bologna, Italy

There are ca. 400 species of *Colletotrichum*, most of which known from few locations and hosts. Some, however, are ubiquitous and polyphagous. The knowledge of the diversity of modern-term *Colletotrichum* species in Europe is lagging behind that from other continents. This study reports on a survey on *Colletotrichum* species occurring on nature and on agricultural and ornamental plants in Madeira island (Portugal). Madeira has a mild climate though conditioned by altitude and topography and its flora presents many endemisms. The climatic conditions of Madeira have long encouraged the cultivation of exotic sub-tropical agricultural crops and ornamental plants, a situation further incited by tourism and by the links to the Madeiran diaspora in different parts of Africa and America. In this study 21 Colletotrichum species were identified, seven in the gloeosporioides complex, six in the acutatum complex, four in the boninense complex, three in the spaethianum complex and one in the trichellum complex. *Colletotrichum* was detected in 45 host species, with fungi from the gloeosporioides complex mostly occurring on exotic agricultural plants (e.g., mango, banana or heliconia), whereas fungi from the acutatum complex were more common on plants from nature and with *C. fioriniae* appearing typically associated to endemic plants. However, the most common fungus was *C. karsti* (boninense complex), occurring both in nature and on cultivated plants. Most of the fungus-host combinations reported here are new records. This study thus represents an advancement on the understanding of the geographical distribution and host range of *Colletotrichum* in the world.

WS6.05 - Identification of *Colletotrichum* species: unravelling species boundaries with curated databases and user-friendly tools

I. Martino[1], *V. Guarnaccia*[1,2], *A. Prodi*[3], *D. Spadaro*[1,2], *P. Talhinhas*[4], *R. Baroncelli*[3,5]
[1]University of Torino, Department of Agricultural, Forest and Food Sciences (DISAFA), Grugliasco, Italy, [2]Agroinnova Interdepartmental Centre for Innovation in the Agro-Environmental Sector, Grugliasco, Italy, [3]University of Bologna, Department of Agricultural and Food Sciences (DISTAL), Bologna, Italy, [4]Universidade de Lisboa, LEAF-Linking Landscape, Environment, Agriculture and Food Res. Centre and Terra Associate Lab., Instituto Superior de Agronomia, Lisbon, Portugal, [5]Università di Napoli Federico II, Center for Studies on Bioinspired Agro-Enviromental Technology, Portici, Italy

The *Colletotrichum* genus is among the ten most important plant pathogens worldwide, but recent taxonomic re-arrangements have raised uncertainties about past classifications. BLAST analyses have further complicated new records due to a high rate of misassigned sequences. Over 37% of sequences in NCBI are misclassified at the species complex (SC) level, indicating an even higher misidentification rate at the species level. Accurate species delineation is crucial for understanding biodiversity, evolution, and for adopting effective management strategies. While multi-locus phylogenetic analyses have been essential for *Colletotrichum* identification, whole-genome sequencing (WGS) offers unparalleled genetic resolution, allowing for precise species differentiation and improved taxonomic clarity. The CLARITY project, funded by EFSA, aims to address these challenges by developing a comprehensive database. This will include: a) type specimens with associated genetic data for all accepted species; 2) fully characterized strains from global culture collections with associated genetic data; 3) strains isolated by the consortium, with genomes sequenced within the project. Phylogenetic analyses, including Bayesian concordance analysis, will be used to resolve topological differences across genomic loci for *Colletotrichum* identification. A key outcome of this project is the creation of "COLLETOTRICHUM-GDB", a publicly accessible, user-friendly platform for *Colletotrichum* species identification, supporting future research and management strategies.

WS6.06 - Phylogenomics and adaptive evolution of the *Colletotrichum gloeosporioides* species complex

F. Liu[1,2], *Z. Ma*[1,2], *L. Cai*[1,2]
[1]State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China, [2]University of Chinese Academy of Sciences, Beijing, China

The *Colletotrichum gloeosporioides* species complex (CGSC) is one of the most devastating fungal phytopathogens, which is composed of three main clades: Kahawae, Musae, and Theobromicola. Despite the diversity of CGSC, there is limited understanding on their evolutionary mechanisms. By analysing 49 genomes, we found that the expansion of transposable elements, especially long terminal repeat retrotransposons, facilitates the expansion of genome size and genetic variation. Further analyses suggested that an intra-chromosomal inversion may have been the driving force behind the divergence of Kahawae clade from its ancestor. Within the

Kahawae clade, the narrow-hosted quarantine species *C. kahawae* has undergone extensive chromosomal rearrangements mediated by repetitive sequences, generating highly dynamic lineage-specific genomic regions compared to the closely related broad-hosted species *C. cigarro*. The results of this study highlight the role of chromosomal rearrangements in promoting genetic diversification and host adaptation, and provide new perspectives for understanding the evolution of phytopathogenic fungi.

WS6.07 - Aetiology, epidemiology and environmental influences on almond anthracnose in Portuguese orchards

M. Ramos[1], *P. Arsénio*[1], *R. Baroncelli*[2], *P. Talhinhas*[1]
[1]Instituto Superior de Agronomia, LEAF- Linking Landscape, Environment, Agriculture and Food Research Centre and Associate Laboratory TERRA, Lisboa, Portugal, [2]Università di Bologna, Dipartimento di Scienze e Tecnologie Agro-Alimentari, Bologna, Italy

Almond anthracnose, caused by *Colletotrichum* spp., is a disease that affects the fruit, resulting in sunken, circular, orange-coloured lesions, producing white mycelium and spore masses, ultimately leading to kernel mummification. This disease is becoming increasingly significant in Alentejo, Portugal, due to the recent intensification and large-scale establishment of almond plantations. To assess the impact of the disease, including severity, incidence, and species identification of *Colletotrichum* in almond orchards, in relation to the surrounding landscape and production systems, several almond orchards in Alentejo were sampled between 2022 and 2024. Plant material was collected for the epidemiological, etiological, and genetic characterisation of the disease. The results showed that anthracnose prevalence and incidence were higher in hedged almond orchards compared to those under the intensive system. The cultivars with the highest incidence were ‘Soleta,’ ‘Guara’ and ‘Belona’ although the severity of fruit infections did not significantly differ between cultivars. It was found that almond anthracnose in Alentejo is mostly caused by *C. godetiae* and to a lower extent by C. *acutatum* and by a second lineage of *C. godetiae* (members of the acutatum species complex). These species are not host-specific, indicating potential pathogen movement between wild species and agricultural crops, such as apple, strawberry, blueberry, peach, loquat, and olive. Further research on almond anthracnose will enhance understanding of how inoculum spread, and disease incidence are influenced by environmental, meteorological, ecological, and agronomic factors. This knowledge will contribute to the development of more informed protection strategies, supporting the sustainable intensification of almond cultivation.

WS6.08 - Acidification by germlings initiates inter- and intraspecies conidial anastomosis tube formation

A. Rudolph[1], *N. Rotermund*[2], *A. Fleissner*[2], *D. Nordzieke*[1]
[1]Georg-August University Göttingen, Genetics of Eukaryotic Microorganisms, Göttingen, Germany, [2]University Braunschweig, Fungal Genetics, Braunschweig, Germany

Conidial anastomosis tube (CAT) fusion is a conserved feature in many ascomycete species. There are indications that network formation by CATs increases the fitness of the young forming colony. In addition, the induction of pathogenicity programs and CAT fusions are interwoven processes. The fungus *Colletotrichum graminicola* produces two types of asexual spores, falcate and oval shaped conidia, of which only oval conidia can undergo CAT fusion. Although there is increasing evidence that conserved signals mediates fungal germling attraction also in interspecies interactions, the responsible attractants are unknown.
For the identification of the germling attractant signals, we analyzed secretome harvested during *C. graminicola* germling fusion. Comparison of samples generated by fusion-competent oval and fusion-incompetent falcate conidia shows that pH values of oval conidia samples are more acidic. Germling fusion assays indeed showed that efficient network formation is dependent on pH. By the application of a 3D printed device for the analysis of chemotropic growth, we further identified distinct pH values as chemoattractants for *C. graminicola* germlings whose perception is mediated by the G-protein coupled receptor (GPCR) CgSte3. Moreover, we showed that signal transduction requires the cell wall integrity (CWI) pathway scaffold CgSo, which homologs are essential for germling fusion in other fungi. Similar to *C. graminicola*, *B. cinerea* and *N. crassa* intraspecies interactions are pH-dependent. Together our findings suggest that acidification by germlings is a conserved prerequisite for CAT fusion in different fungal species.

WS6.09 - Mycosporines in spore-type specific development and corn anthracnose spreading

D. Rathi[1], *S. Lewandowski*[1], *D. Nordzieke*[1]
[1]Georg-August University Göttingen, Institute of Microbiology and Genetics, Department of Genetics of Eukaryotic Microorganisms, Göttingen, Germany

Colletotrichum graminicola is a hemibiotrophic plant pathogen that induces anthracnose in *Zea mays*. This fungus produces two types of asexual spores: falcate and oval conidia, which exhibit distinct gene expression patterns, generate distinct metabolites, and undergo unique developmental processes. Interestingly, falcate conidia produces mycosporine molecule, whereas, oval conidia release cell fusion signals.
In cyanobacteria, two parallel biosynthetic pathways were identified for the production of mycosporines. Outgoing from these known genes, we have successfully identified homologs in *C. graminicola,* which are organized in a gene cluster. Interestingly, none of the two pathways from cyanobacteria are fully present in *C. graminicola*, but we have identified genes from both, indicating a merge of both pathways during evolution. Our initial tests involving three putative genes (*Cgddgs*, *CgATP-grasp*, *Cgo-met*) have demonstrated significant impact on germination and germling fusion compared to the wild type (CgM2). Although our initial tests for leaf infection (5dpi) did not reveal any difference between mutants and wild type strains, we identified a regulatory role of mycosporines for the generation of appressoria. Additionally, we are now knocking out the remaining gene of the biosynthesis pathway (*Cgdahp*), also generating double mutant strains, to get an insight into the full biosynthetic pathway in *C. graminicola*. Furthermore, we have initiated a comprehensive biochemical analysis of mycosporine production to gain in-dept understanding of the intermediate molecules formed. This approach will provide

us with valuable insights into the pathogenicity and development of various spore types, shedding light on how these molecules influence fungal growth and infection processes.

WS6.10 - Genomic characterization of pathogenicity genes in Colletotrichum species affecting apple orchards in Northern Italy

S. Iacono[1], *M. Calì*[1], *C. Fiorenzani*[1], *A. Menicucci*[1], *E. Cappelletti*[1], *A. Prodi*[1], *C. Ratti*[1], *R. Baroncelli*[1]
[1]University of Bologna, Department of Agricultural and Food Sciences, Bologna, Italy

Apple cultivation is a cornerstone of northern Italy's agricultural economy, but fungal diseases pose a significant threat to orchard productivity, affecting both fruit quality and yield. Among the most detrimental fungal pathogens are species of *Colletotrichum*, responsible for Apple Bitter Rot (ABR) and Glomerella Leaf Spot (GLS). Previous studies from our group have identified pathogenic *Colletotrichum* species in apple orchards, belonging to three distinct species complexes and exhibiting substantial intraspecific diversity. Some of these species can cause both ABR and GLS, while others are opportunistic pathogens. This diversity presents an ideal platform for investigating the genetic elements associated with pathogenicity. This study aims to identify and characterize pathogenicity genes in *Colletotrichum* species associated with apple diseases, to inform the development of targeted disease management strategies. Seven isolates, representing the pathogen's genetic diversity in northern Italy and capable of causing both ABR and GLS, were selected for genomic analysis. DNA was extracted using a modified CTAB protocol, and genomes were sequenced using Illumina technology. Genome assembly was conducted with SPAdes, followed by gene prediction and annotation. These newly sequenced genomes, along with publicly available data, were used in a comparative genomics framework to identify and analyze putative pathogenicity genes. Comparative genomic analyses are currently ongoing, and results will be presented.

WS6.11 - Bioinformatics-driven identification of candidate pathogenicity-related genes in *Colletotrichum lupini* through comparative genomics and effector profiling

A. Menicucci[1], *C. Fiorenzani*[1], *S. Iacono*[1], *R. Baroncelli*[1,2]
[1]University of Bologna, Department of Agricultural and Food Sciences (DISTAL), Bologna, Italy, [2]University of Naples, Center for Studies on Bioinspired Agro-Enviromental Technology, Portici, Italy

Colletotrichum lupini is a host-specific fungal pathogen responsible for anthracnose in lupin, causing significant agricultural losses. Uncovering the genomic basis of its pathogenicity is essential for developing resistant cultivars and proper disease management strategies.
We employed a bioinformatics pipeline to identify species-specific genes potentially involved in virulence by analysing both lineage-specific regions (LSRs) and species-specific orthogroups (SSOGs) across multiple *Colletotrichum* species.
LSRs were identified by aligning raw sequencing data from closely related species to reference

genomes, detecting zero-coverage genomic regions that may be involved in host specialization or horizontal gene transfer (HGT). In parallel, SSOGs were identified using *OrthoFinder*, revealing protein groups unique to each species, thus providing insights into phylogenomic relationships and proteins likely linked to pathogenicity.
Additionally, a combined bioinformatic workflow was utilized to analyse genes for their roles as candidate effectors, proteins likely involved in host interaction and immune evasion.
Furthermore, to gain a better understanding of the specialisation process, functional categories, often involved in fitness-related or virulence mechanism, such as carbohydrate-active enzymes (CAZymes), peptidases, transporters, and transcription factors (TFs) were annotated using the JGI's fungal genome database for three selected *Colletotrichum* genomes.
This comprehensive analysis identified several candidate genes, of which many within LSRs, SSOGs, and effector regions. These candidate genes are likely critical for host tissue degradation, immune suppression, and nutrient acquisition during infection. These findings provide key insights into the molecular mechanisms of *C. lupini* pathogenicity and could be used to guide the design of novel biocontrol strategies to mitigate agricultural losses.

WS6.12 - High-efficiency multiplex gene targeting in *Colletotrichum* fungi using CRISPR-Cas9 co-editing and dual-marker exchange

N. Kumakura[1], *K. Yonehara*[1,2], *K. Shirasu*[1,2]
[1]RIKEN, Center for Sustainable Resource Science, Yokohama, Japan, [2]The University of Tokyo, Bunkyo, Japan

Colletotrichum fungi cause significant crop losses globally, emphasizing the need to elucidate their infection mechanisms for effective control strategies. Although gene targeting via donor DNA and homology directed recombination (HDR) is feasible in these fungi, it remains hindered by low efficiency, and the number of genes that can be disrupted per strain is limited to 2–3, depending on the availability of selectable markers. To address these limitations, we previously developed a CRISPR-Cas9 system to enhance HDR efficiency, coupled with a *URA3*-based marker recycling method for multiplex gene disruption. This approach, however, was labor-intensive, enabling only single-gene disruptions per transformation with a complex selection marker recycling process. In this study, we established an efficient multiplex gene-targeting method in *Colletotrichum orbiculare* and *C. higginsianum* by integrating CRISPR-Cas9-mediated co-editing with a marker exchange technique. With this co-editing strategy, we achieved the simultaneous disruption of up to four genes in a single transformation. Additionally, our marker exchange approach, which alternates between two selectable markers, eliminates the need for marker removal after each use, enabling sequential transformations in a simplified, streamlined manner. This advanced method significantly accelerates reverse-genetic identification of virulence factors and deepens our understanding of the infection mechanisms in *Colletotrichum* fungi.

WS6.13 - The gene *CgEP4* encodes an effector that plays a key role in *Colletotrichum graminicola* virulence in maize

P. García-Rodríguez[1], *L. Rodríguez-Mónaco*[1], *F.B. Cuevas-Fernández*[1], *S. Becerra*[1], *S. Gutiérrez-Sánchez*[1], *A. Jerez-Vanegas*[1], *F. Rogério*[1], *J. Ortíz-Álvarez*[2], *R. Baroncelli*[3], *E.A. Espeso*[4], *M.R. Thon*[1], *S.A. Sukno*[1]
[1]Universidad de Salamanca - Instituto de Investigación en Agrobiotecnología (CIALE), Villamayor, Spain, [2]Programa "Investigadoras e Investigadores por México" Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCyT), Ciudad de México, Mexico, [3]University of Bologna, Department of Agricultural and Food Sciences (DISTAL), Bologna, Italy, [4]Centro de Investigaciones Biológicas (CIB) Margarita Salas - Consejo Superior de Investigaciones Científicas (CSIC), Department of Molecular and Cellular Biology, Madrid, Spain

Colletotrichum ranks among the most important fungal pathogens, affecting nearly every major crop worldwide. The diseases it causes significantly impact agriculture and the economy, reducing crop yields and threatening global food security. *Colletotrichum graminicola* is the causal agent of maize anthracnose. Its genome contains multiple genes encoding effector proteins that modify plant defense mechanisms to facilitate host infection. Previously, we identified *CgEP1*, a putative effector-encoding gene, through phylogenetic and transcriptomic analyses. Deletion of this gene resulted in a significant reduction of virulence in maize leaves. *CgEP4* is a paralog of *CgEP1* and encodes a protein composed of two alpha-helix. Evolutionary analyses reveal that *CgEP4* originated from a duplication event before the *Graminicola* species complex diverged. Gene expression analyses showed that *CgEP4* is upregulated during the late biotrophic stage of infection. Knockout mutants and complemented strains of *CgEP4* were created and then tested in pathogenicity assays. The results show that *CgEP4* is key to *C. graminicola* infection in maize, affecting both leaf blight and stalk rot disease forms. In addition, phenotypic characterization of the *CgEP4* null mutant showed reductions in spore formation, *in vitro* germination, and growth under diverse stress conditions. Pathogenicity assays revealed that the *CgEP4* mutant had reduced penetration efficiency, with a stronger callose response in maize leaves compared to the wild type. CgEP4 has a nuclear localization prediction but experiments to determine it are underway. In conclusion, our findings provide important insights into the role of *CgEP4* in *C. graminicola* pathogenicity, suggesting potential targets for improved disease control.

WS6.14 - Comparative analysis of the transcriptome of the beneficial endophyte *Colletotrichum tofieldiae* in maize and *Arabidopsis thaliana*

E. Torres[1], *S. Díaz-González*[1], *S. González-Bodí*[1], *C. González-Sanz*[1], *S. Sacristán*[1]
[1]Universidad Politécnica de Madrid, Centro de Biotecnología y Genómica de Plantas, Madrid, Spain

Colletrotrichum tofieldiae is an endophytic fungus originally isolated from *Arabidopsis thaliana*. Different studies have shown the ability of this fungus to colonise the roots of both *A. thaliana*

and other agronomically valuable hosts, providing several fitness advantages to the plants.
In the present study, an RNA-Seq experiment was conducted to obtain transcriptomic data of the fungus interacting with maize at two early time points of the interaction. The results were used to perform a differential gene expression analysis. Functional categories within the differentially expressed genes were examined and functional enrichment analyses using Gene Ontology (GO) terms were performed. The same pipeline was applied to previously published raw transcriptomic data of *C. tofieldiae* in the interaction with *A. thaliana*, allowing the comparison between the findings obtained in both hosts.
The comparative analyses of *Ct*0861 transcriptomes in both host plants and at different timepoints has revealed distinct temporal dynamics, suggesting divergent strategies for the colonisation and establishment of trophic interactions with different hosts. Common trends in the interaction with both hosts are the high induction of genes encoding secreted proteins and transporters during host colonisation, while genes involved in secondary metabolite biosynthesis exhibit low expression levels *in planta*.

WS6.15 - Carbon-for-nutrient exchange dynamics and mutualist reciprocity in *Arabidopsis thaliana* and *Colletotrichum tofieldiae* interactions

J. Gaertner[1], *M. Bucher*[1]
[1]University of Cologne, Institute of Plant Science, Köln, Germany

Arbuscular mycorrhizal symbiosis (AMS) is one of the oldest extant plant-microbe associations. Notwithstanding the benefits of nutrient exchange, AMS requires the plant to release carbon assimilates to its fungal symbiont. It is probably this energy cost to the host associated with AM fungi (AMF) that has led to the loss of AMS in some plant species during evolution. *Brassicaceae* are AMF non-hosts and so is the most commonly used model plant, *Arabidopsis thaliana*. Furthermore, many AMF are difficult to cultivate because of their obligate biotrophy. *A. thaliana* being a non-host for AMF, is a significant complication for research into the molecular mechanisms underlying AMS. *A. thaliana*, however, is known to form a mutualistic relationship with *Colletotrichum tofieldiae*, a facultatively symbiotic free-living ascomycete from a well-studied genus that includes many plant pathogens. In addition, *C. tofieldiae* is readily cultivable and transformable and therefore represents a viable system for investigating the molecular and genetic basis of root endophytism in *A. thaliana*.
In my project, the carbon-for-nutrients dynamic underpinning the plant-fungal nutrient exchange is investigated using stable- and radioisotope labelling of WT and mutant lines of both *A.thaliana* and *C.tofieldiae.* The immediate question to be answered is whether the bidirectional exchange of inorganic phosphate from the fungus and fatty acids from the plant occurs in reciprocity. By strengthening the synthetic symbiotic partnership established in this project, it will be possible to identify both plant and fungal traits that will enable us to construct specific mutualistic symbioses that improve host plant growth and fitness.

WS6.16 - Phyllospheric non-phytopathogenic bacteria promotes the virulence of the Brassicaceae anthracnose fungus *Colletotrichum higginsianum*

K. Tanaka[1], Y. Hayashi[1], S. Yamazaki[1], C. Tanaka[1], F. Ishida[1], M. Yamaguchi[1], N. Takeshita[2], S. Tanaka[1]
[1]Setsunan University, Faculty of Agriculture, Hirakata, Japan, [2]University of Tsukuba, Tsukuba, Japan

Phyllospheric microbiomes are primarily dominated by non-phytopathogenic bacteria. However, their influence on the pathogenicity of plant-pathogenic fungi remains poorly understood. In this study, we isolated cultivable bacteria from the leaves of *Arabidopsis thaliana* and investigated their effects on the pathogenicity of the Brassicaceae anthracnose pathogen *Colletotrichum higginsianum* (Ch). When the isolated bacteria were co-inoculated with Ch, the bacterium *Chitinophaga* sp. significantly promoted lesion formation by Ch. Although there were no significant differences in plant gene expression changes between inoculation with Ch alone and co-inoculation with *Chitinophaga* sp., we observed that the presence of *Chitinophaga* sp. led to the formation of secondary appressoria capable of penetrating plant cells, thereby increasing the frequency of successful penetration. The formation of secondary appressoria was also induced by *Chitinophaga pinensis* and *Flavobacterium* sp., both members of the Bacteroidetes, but not by *Escherichia coli*, *Brucella* sp., or *Cupriavidus* sp., which are members of the Proteobacteria. Furthermore, secondary appressoria formation was induced by the culture supernatant of *Chitinophaga* sp., with its activity remaining unaffected by heat treatment, suggesting that non-proteinaceous molecules may be responsible for this activity. Additionally, the biomass of *Chitinophaga* sp. increased on lesions caused by Ch, indicating that it utilized the exudates from plant cells killed by Ch infection for its growth. Our findings suggest that phyllospheric non-phytopathogenic bacteria may exploit fungal infections to enhance their own growth.

WS6.17 - Host cell death triggered by plant immunity during the necrotrophic phase of hemibiotrophic fungal pathogen infection

K. Yonehara[1,2], N. Kumakura[2], B. Cole[3], H. Morinaka[4], K. Sugimoto[4], G. Coaker[5], K. Shirasu[1,2]
[1]The University of Tokyo, Department of Biological Science, Tokyo, Japan, [2]RIKEN, Plant Immunity Research Group, Center for Sustainable Resource Science, Yokohama, Japan, [3]DOE Joint Genome Institute, Lawrence Berkeley National Lab, Berkeley, United States, [4]RIKEN, Cell Function Research Team, Center for Sustainable Resource Science, Yokohama, Japan, [5]University of California, Davis, Department of Plant Pathology, Davis, United States

Hemibiotrophic fungal pathogens infect living host tissues during the early biotrophic phase and spread within dead host tissues in the later necrotrophic phase. While previous studies suggest that toxic molecules produced by fungi are involved in the host cell death during the necrotrophic phase, it remains unclear whether the host immune system also plays a role in this process. Here, using *Colletotrichum higginsianum*, a hemibiotrophic fungal pathogen infecting *Arabidopsis thaliana*, we demonstrated that plant immune system contributes to the host cell death during the

necrotrophic phase. Our cytological analysis revealed that, in wild-type *A. thaliana*, hypersensitive-response-like cell death, marked by trypan blue staining, occurs in mesophyll cells distant from invaded epidermal cells during the necrotrophic phase. However, this trypan-blue stained mesophyll cell death was absent in a mutant plant lacking a key immune component, while allowing a significant increase in fungal growth. These findings suggest that *A. thaliana* recognizes *C. higginsianum* in epidermal cells and activate a signaling pathway that triggers cell death in distant mesophyll cells. To test this hypothesis, we performed single-nucleus RNA-seq on infected leaves from both wild-type and the mutant plants, identifying candidate genes that contribute to mesophyll-specific cell death. Our study provides insights into how the plant immune system mediates host cell death during the necrotrophic phase of hemibiotrophic pathogens and proposes a potential mechanism for this process.

WS6.18 - A fungal transcription factor *CtBOT6* converts a beneficial root endophyte *Colletotrichum tofieldiae* into an anthracnose leaf pathogen

R. Ujimatsu[1], *J. Takino*[2], *S. Aoki*[3], *M. Nakamura*[1], *H. Haba*[1], *A. Minami*[4], *K. Hiruma*[1]
[1]The University of Tokyo, Department of Life Sciences, Multidisciplinary Sciences, Tokyo, Japan, [2]Hokkaido University, Department of Chemistry, Sapporo, Japan, [3]The University of Tokyo, Department of Integrated Biosciences, Chiba, Japan, [4]Institute of Science Tokyo, Department of Chemistry, Tokyo, Japan

Plant endophytic fungi inhabit their host plants asymptomatically for most of their lifecycle; however, under certain host environments, they can trigger disease, implying their ability to switch lifestyles by modulating virulence factors. The root endophytic fungus *Colletotrichum tofieldiae* (Ct) promotes plant growth under low phosphate conditions, whereas a certain strain inhibits plant growth. Such pathogenic lifestyle is partly attributed to the specific activation of a secondary metabolite biosynthesis gene cluster, designated ABA-BOT cluster, during root colonization. However, the mechanisms by which Ct regulates its virulence, both through the ABA-BOT and the others, and how plants respond to this regulation, remain enigmatic. Here, we identify *CtBOT6*, a transcription factor within the ABA-BOT, as a pivotal regulator of fungal virulence and an inducer of extensive host gene reprogramming. Using Ct*Δbot6* knockout and transgenic lines with varying levels of *CtBOT6* expression in pathogenic and/or beneficial Ct, we show that *CtBOT6* regulates gene expression not only the ABA-BOT but also other virulence-associated genes, such as effectors and carbohydrate-active enzymes. Importantly, *CtBOT6* expression levels are strongly correlated with virulence, and its activation is sufficient to convert the beneficial Ct strain into a leaf anthracnose pathogen, facilitating the completion of its asexual lifecycle. In strong correlation with the observed virulence levels, Ct triggers plant responses, including defense and senescence in both roots and shoots —hallmarks of necrotrophic fungal infection—partially dependent on the host's abscisic acid pathway. Our findings suggest that *CtBOT6* regulates Ct lifestyles along the mutualistic-necrotrophic continuum.

WS1.05 - *Aspergillus* species epidemiology is driven by climate, soil and fungicides

T. Easter[1], *M. Bromley*[1], *J. Rhodes*[2], *M. Brockhurst*[3], *N. van Rhijn*[1,3]
[1]University of Manchester, Manchester Fungal Infection Group, Manchester, United Kingdom, [2]Radboud University Medical Centre, Department of Medical Microbiology, Nijmegen, Netherlands, [3]University of Manchester, Microbial Evolution Research Manchester, Manchester, United Kingdom

Aspergillosis is a severe fungal infection caused by members of the *Aspergillus* genus, primarily *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus flavus*. These soil-based saprotrophs are responsible annually for more than 4 million life-threatening infections and an economic burden of over $10 billion. While *A. fumigatus* is the primary cause of aspergillosis in Europe and the Americas, previous data from our lab have shown that *A. flavus* is more common in Asia and Africa. As the climate changes and global temperatures rise, the impact on the epidemiology of *Aspergillus* species is unknown. To further study the effect of changing environments on *Aspergilli*, we aimed to establish an environmentally relevant soil microcosm. Using CRISPR-Cas9 mutagenesis, we generated genetically barcoded pools of *Aspergillus* species to perform Bar-seq. Bar-seq is a next generation sequencing approach to quantify each fungal isolate within a pooled inoculum before and after growth in our soil microcosms. First, we assessed fitness of n=25 barcoded *A. fumigatus* isolates for their fitness in a compost soil microcosm model. We measured antifungal and fungicide concentrations in these composts and correlated that with fitness and drug resistance. Our results showed that fungicides could be detected in all commercial compost, and a fitness signature of drug resistance could be found. This soil microcosm model will allow us to test more soil-based variables over longer periods of time, and gain a better understanding of how antifungal resistance can develop alongside changing environments.

WS1.06 - Understanding secondary metabolite production based on biosynthetic gene cluster-specific transcription factor binding targets

L. Fan[1], *S. Guo*[1], *L. Pardeshi*[1], *R. Chen*[1], *K.H. Wong*[1]
[1]University of Macau, Macau, China

Fungi possess remarkable capabilities for synthesizing diverse secondary metabolites (SMs) with significant applications in medicine, agriculture, and industry. SM biosynthetic genes are often organized in clusters on fungal genomes. Genome sequencing has identified many novel SM biosynthetic gene clusters (BGCs) in fungi, representing a rich resource for drug discovery. However, most SM BGCs remain transcriptionally silent under standard laboratory conditions, presenting challenges for their identification. Many BGCs contain a transcription factor gene that activates the biosynthetic genes within the cluster under specific conditions. Whether cluster-specific transcription factors can bind and regulate genes outside their cluster is not clear. We

hypothesize that BGC transcription factors may regulate non-BGC genes whose functions could affect the biosynthesis of the intended SM. Therefore, it may be possible to deduce the conditions affecting SM production from its cluster-specific transcription factor target information. To demonstrate this, we conducted Chromatin Immuno-Precipitation followed by Sequencing (ChIP-Seq) to map the genome-wide binding sites of AflR – the well-studied transcription factor controlling the biosynthesis of the harmful mycotoxins sterigmatocystin (ST) and aflatoxin. Our results revealed numerous AflR bindings beyond the ST BGC, exerting control over various physiological processes. More importantly, based on AflR target information, we successfully devised a condition that affects ST production. Taken together, this work provides valuable insights into the regulation of ST and aflatoxin biosynthesis and introduces a novel approach to activate cryptic SM BGCs, enabling the discovery of novel secondary metabolites.

WS1.07 - An improved description of the untranslated regions of poly(A)-tailed RNA from Aspergillus fumigatus

L. Schrettenbrunner[1], *C. Maufrais*[2], *G. Janbon*[2], *M. Blango*[1]
[1]Leibniz-HKI, RNA Biology of Fungal Infections, Jena, Germany, [2]Institute Pasteur, Paris, France

The filamentous fungus *Aspergillus fumigatus* is an opportunistic human pathogen and is categorized in the critical group of the WHO fungal pathogen priority list. Despite its intensely studied genome, relatively little is known about its transcriptome, including transcriptional start/end sites and the functional relevance of regulatory elements like untranslated regions (UTRs). With this project we aim to improve the map of *A. fumigatus* UTRs by experimentally validating and precisely defining the 5' and 3' ends of poly(A) transcripts. After enrichment of poly(A)-tailed RNA, we performed specialized 5' and 3' RNA-end sequencing. Initial screening of aligned reads showed that these were clearly enriched at the respective transcript ends. Peaks were called on the extracted 5' and 3' ends of each read of each method, respectively. High confidence sites were denoted as positions that were found in at least 3 replicates. These sites were then assigned to the closest gene on the reference genome. Finally, we performed manual curation to improve the overall annotation of end sites, which ultimately led to 67% of all genes with an associated high confidence 3' end and 27% of all genes with a high confidence 5' end. In addition to the mapped primary transcriptional ends, we also identified alternative end sites, sites with potential early termination, and 5'/3' end sites within the coding sequences of genes. We hope that this data set will ultimately serve the *A. fumigatus* community as a resource to generate additional hypotheses and facilitate future investigations of the *A. fumigatus* transcriptome.

WS1.08 - Aspergillus fumigatus conidial surface-associated proteome reveals factors for fungal evasion and host immunity modulation

C. Pinzan[1], *C. Valero*[2], *P. de castro*[3], *J. Silva*[1], *K. Earle*[2], *H. Liu*[4], *M. Horta*[1], *O. Kniemeyer*[5], *T. Krüger*[5], *A. Pschibul*[5], *D. Cömert*[5], *T. Heinekamp*[5], *A. Brakhage*[5], *J. Steenwyk*[6], *M. Mead*[7], *N. Hermsdorf*[5], *S. Filler*[4,8], *N. Garzon*[1], *E. Delbaje*[1], *M. Bromley*[2], *H. Cabral*[1], *C. Diehl*[1], *C. Angeli*[9], *G. Palmisano*[9], *A. Ibrahim*[4,8], *D. Rinker*[7], *T. Sauters*[7], *K. Steffen*[7], *A. Gumilang*[7], *A. Rokas*[7], *S. Gago*[2], *T. Reis*[1,10], *G. Goldman*[1,10]

[1]University of Sao Paulo, Ribeirao Preto, Brazil, [2]University of Manchester, Manchester, United Kingdom, [3]University of Sao Paulo, Ribeirão Preto, Brazil, [4]Harbor-UCLA Medical Center, Torrance, United States, [5]Friedrich Schiller University, Jena, Germany, [6]University of California, Berkeley, United States, [7]Vanderbilt University, Nashville, United States, [8]David Geffen School of Medicine at UCLA, Los Angeles, United States, [9]University of Sao Paulo, Sao Paulo, Brazil, [10]National Institute of Science and Technology in Human Pathogenic Fungi, Sao Paulo, Brazil

The section *Fumigati* is composed of *Aspergillus* fungal species presenting variable pathogenicity levels. *Aspergillus fumigatus*, an opportunistic pathogen, belongs to this section and is responsible for approximately 70% of cases of invasive pulmonary aspergillosis (IPA). The establishment of IPA depends on the inhalation of asexual spores (conidia) that trigger host infection. Thus, conidia represent the first point of contact between the fungus and human cells and, therefore, are important for the establishment of IPA. Despite its importance in the initial steps of IPA, there is scarce information about conidial surface proteins of *A. fumigatus* involved in fungal evasion and host immunity modulation. We analysed the conidial surface proteome (surfome) of *A. fumigatus*, two closely related non-pathogenic species, *Aspergillus fischeri* and *Aspergillus oerlinghausenensis*, as well as pathogenic *Aspergillus lentulus*, to identify such proteins. From 62 proteins exclusively detected on the *A. fumigatus* surfome, we constructed null mutants for 42 genes encoding these proteins. Deletion of 33 of these genes altered the fungal susceptibility to macrophage, epithelial cells and cytokine production. The gene encoding a putative glycosylasparaginase was characterized in detail and demonstrated its importance in modulating the levels of host proinflammatory cytokines and contributing to virulence in an immunocompetent murine model of IPA. Other genes are also in the process of being characterized. In summary, our results suggest that the conidial surfome of *A. fumigatus* encompasses proteins that are important for evasion and modulation of the immune response at the onset of fungal infection.
Funding: FAPESP, Brazil and NIH-NIAID, USA

WS1.09 - Flotillin-containing lipid raft microdomains are linked to calcium in *Aspergillus nidulans*

M. Kawashima[1,2], *T. Krüger*[1], *A.A. Brakhage*[1,2]
[1]Leibniz Institute for Natural Product Research and Infection Biology, Department of Molecular and Applied Microbiology, Jena, Germany, [2]Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany

Lipid rafts are tight assemblies of proteins and lipids in a biological membrane and are thought to be involved in many physiological processes such as immune signalling and host-pathogen interactions. However, due to their small sizes exceeding the resolution limit of conventional light microscopy, direct measurement and characterisation of lipid rafts in living membranes remains to be a challenge. While most studies on lipid rafts have been carried out on mammalian cells, here we use the genetic model fungus *Aspergillus nidulans* to allow a more versatile characterisation of lipid rafts at both molecular and organismic levels. In particular we investigate the role of flotillin (FloA), a lipid raft marker conserved across many organisms. Using a

nanoluciferase reporter strain, we demonstrate that the *A. nidulans* flotillin FloA is highly expressed when the fungus is confronted with high calcium stress. Transcriptomic analysis further reveals that repression of *floA* under these conditions leads to the upregulation of numerous mitochondrial genes, suggesting a functional connection between FloA and mitochondrial activity. In addition, in vivo protein-proximity labelling is performed to assess the physical interaction partner proteins of FloA. We also explore the role of FloA in microbial communication through co-cultivation experiments with the soil bacterium *Streptomyces iranensis* and show that the bacterium is able to induce the high expression of FloA, most likely by secreting a natural product. These findings contribute to a deeper understanding of lipid raft dynamics and the organisation of eukaryotic membranes.

WS1.10 - Dynamic molecular dialogues in *A. nidulans* development: Interplay between pheromone producing enzymes and transcriptional regulators

E.S. Xylakis[1], *A. Zhornyak*[1], *L. Moorkamp*[1], *E. Bastakis*[1], *B. Popova*[1], *K. Schmitt*[1], *O. Valerius*[1], *G.H. Braus*[1]
[1]Georg August University, Department of Molecular Microbiology & Genetics, Goettingen, Germany

Internal as well as external stimuli determine the ratio of the further development of vegetatively growing *Aspergillus nidulans* hyphae to more asexual or more sexual development. The timing and balance between sexual and asexual development are controlled by the products of the fatty acid oxygenases PpoA (**P**si (precocious sexual inducer)-**p**roducing **o**xygenase **A**) and PpoC (**P**si-**p**roducing **o**xygenase **C**). Specific cellular Psi factor accumulation represents the molecular cues, which channel the ratio between the distinct transcriptional programs for the asexual or sexual developmental pathways. Therefore PpoA and PpoC indirectly regulate the transcriptomic landscape by producing specific gene expression patterns for either sexual or asexual development. Transcriptional changes driven by Psi factors are essential for ensuring the controlled and regulated progress of each developmental pathway in response to environmental signals. We have analyzed the physical interactions of the Psi-producing oxygenases and important players of fungal transcription. The current status of our study in exploring the interaction dynamics between metabolic enzymes and transcriptional regulators and their consequences for fungal development will be presented

WS1.11 - Metabolic engineering of *Aspergillus violaceofuscus*

C. Wieder[1,2], *C. Laubenstein*[1,2], *L. Fischer*[2], *R. Wiechert*[3], *K. Seipp*[3], *A. Schüffler*[1], *T. Opatz*[3], *E. Thines*[1,2]
[1]Institut für Biotechnologie und Wirkstoff-Forschung gGmBH, Mainz, Germany, [2]Johannes Gutenberg-University, Microbiology and Biotechnology, Mainz, Germany, [3]Johannes Gutenberg-University, Department of Chemistry, Mainz, Germany

Fungal secondary metabolites (SMs) are a rich source for bioactive compounds with various applications in agriculture, biotechnology and medicine. However, discovering novel compounds with desirable bioactivities remains challenging as numerous gene clusters involved in their synthesis remain silent under laboratory conditions. *Aspergillus violaceofuscus* is a fungus with significant biosynthetic potential, containing over 90 predicted biosynthetic gene clusters (BGCs), albeit only few metabolites are produced under standard culturing conditions. In this study, we aimed to engineer the secondary metabolism of *A. violaceofuscus* by creating genetic dereplication strains with reduced metabolic background and overexpressing cryptic biosynthetic genes. We were able to establish an efficient Cas9-mediated microhomology-directed repair (MHDR) protocol, achieving integration rates of up to 90 % and successfully deleted the core genes encoding for the biosynthesis of the major metabolites eupenoxide and himeic acid A. Homologous overexpression of a cryptic NRPS-like coding gene led to the production of a new compound we termed violafuranone A. In conclusion, we demonstrate metabolic manipulation of *A. violaceofuscus* and the potential of this species as a source for novel SMs. These findings pave the way for further investigations into the secondary metabolism of *A. violaceofuscus*.

WS1.12 - Role of the red-light and blue-light photoreceptors in the photoadaptation of transcription in *Aspergillus nidulans*

A. Franco-Cano[1], *G. Gutiérrez*[1], *L.M. Corrochano*[1], *D. Cánovas*[1]
[1]University of Seville, Department of Genetics, Seville, Spain

Fungal responses to light include the regulation of the developmental programs and the regulation of secondary metabolism, among others. The responses to light are mediated by induction of gene expression. However, this induction is transient, and gene expression returns to dark levels in a process known as photoadaptation. *In Aspergillus nidulans*, light sensing requires both the red photoreceptor, the phytochrome FphA, the blue photoreceptors, LreA and LreB, and the UV/blue photoreceptor CryA. Although the *Aspergillus* genomes lack a homolog of *vvd*, the gene responsible for photoadaptation in *Neurospora crassa*, photoadaptation is still observed in *A. nidulans*, suggesting the existence of an alternative regulatory mechanism. To study photoadaptation in A. *nidulans*, the wild-type and photoreceptor mutant strains were exposed to light for different times ranging from 15 to 240 min. RNAseq experiments show that light regulates over 20% of the genome. Clustering analysis of light-regulated genes resulted in six regulatory profiles, which were similar in the wild type and the Δ*lreA* Δ*lreB* mutant, but not in the Δ*fphA* strain. The deletion of *fphA* led to a reduction in the number of light-regulated genes (from 2596 to 1680) with a transcriptional pattern very different to that observed in the wild type. Gene onthology revealed a sequential rewiring of gene expression: metabolic changes come first, possibly due to mitochondrial stress, followed by nucleic acid modification and repair. Based on the expression profiles, our results suggest the existence of novel regulators that could be responsible of photoadaptation in *Aspergillus*.

WP1.1 - Adsorption of cutinase CutL1 to the Langmuir membrane of hydrophobin RolA derived from the fungus Aspergillus oryzae

S. Iio[1], N. Takahashi[1], Y. Terauchi[2], T. Tanaka[3], A. Yoshimi[4,5], M. Mitsuishi[6], H. Yabu[7], F. Hasegawa[8], K. Abe[1]
[1]Tohoku University, Graduate School of Agricultural Science, Sendai, Japan, [2]Yamaguchi University, RCTMR, Ube, Japan, [3]Osaka University, Graduate School of Engineering, Suita, Japan, [4]Kyoto University, Graduate School of Global Environmental Studies, Kyoto, Japan, [5]Kyoto University, Graduate School of Agriculture, Kyoto, Japan, [6]Tohoku University, Graduate School of Engineering, Sendai, Japan, [7]Tohoku University, WPI-AIMR, Sendai, Japan, [8]National Institute of Technology and Evaluation, Tokyo, Japan

Filamentous fungi play an important role in degradation of solid polymers such as proteins and polysaccharides in nature. When a filamentous fungus *Aspergillus oryzae* is cultured using the biodegradable plastic polybutylene succinate-*co*-adipate (PBSA) as the sole carbon source, the fungus co-expresses a hydrophobin RolA and polyesterase CutL1. RolA attached to the surface of PBSA recruits CutL1 via ionic interactions, then promoting the degradation of PBSA. When RolA is adsorbed to a hydrophobic substrate, RolA molecules form an amorphous membrane and then self-assemble to form rod-shaped multimeric structures called rodlet. However, it remains unclear whether the recruitment of CutL1 by RolA takes place on the amorphous or rodlet membrane. In this study, we used the Langmuir membrane to clarify which state of RolA, amorphous or rodlet, interacts with CutL1. RolA solution was spread on the buffer surface in a Langmuir trough and compressed from both sides until reaching the target surface pressure. Then, the surface area of the RolA monolayer was fixed and CutL1 was injected into the buffer. The change in surface pressure was further measured over time. As a result, the surface pressure increased significantly only when amorphous RolA was used. In an experiment using RolA HKK46S, a mutant in which triple positively charged residues were substituted to serine, CutL1 addition scarcely increased the surface pressure of RolA HKK46S amorphous membrane. These results suggest that CutL1 interacts with amorphous RolA via electrostatic interactions, not with RolA rodlet. We discuss the CutL1 recruitment by the form of RolA membranes.

WP1.2 - Study of the value of NL1 as an anti-persulfidation and anti-virulence compound in *Aspergillus fumigatus*

Á. González-Camuesco[1], C. Zhao[2], J. Amich[1]
[1]Instituto de Salud Carlos III, Mycology Reference Centre / National Centre for Micorbiology, Majadahonda, Spain, [2]Metropolitan University Manchester, Manchester, United Kingdom

We previously showed that deleting the cystathionine-γ-lyase (CSE) encoding gene in *Aspergillus fumigatus* caused a decrease in the levels of the post-translational modification persulfidation, which altered the functionality of proteins relevant for fungal pathogenicity, and translated into a reduction in its virulence potential [1]. With the aim of targeting persulfidation

during infection, and therefore reduce fungal virulence, we are now investigating the capacity of the compound NL1, designed against bacterial pathogens [2], to inhibit *A. fumigatus* cystathionine-γ-lyase.
We firstly performed an *in-silico* analysis and found that NL1 docks well into the fungal enzyme. Similarly to the bacterial proteins there is an aromatic amino acid (H120) close to the ligand binding pocket (which is absent in the human counterpart) which function seems to be blocked by NL1 [2]. Accordingly, NL1 efficiently inhibited recombinantly expressed *A. fumigatus* CSE, but not human CSE, *in-vitro*. As expected, this translated into the capacity to reduce persulfidation levels in *A. fumigatus*, as tested on five different strains. In agreement with previous results with the null mutant, we found that NL1 exposure increased *A. fumigatus* susceptibility to peroxide. In contrast to the reports in bacteria, NL1 did not potentiate the action of antifungal drugs (azoles or amphotericin-B) against wild-type or resistant (TR34-L98H) isolates. At the time of submission of this abstract we are investigating the NL1 capacity to reduce fungal virulence using *in-vitro* and *in-vivo* models of infection.
[1] Sueiro-Olivares et al. PLoS Biol. 2021. Doi: 10.1371/journal.pbio.3001247
[2] Shatalin *et al*. Science 2021. Doi: 10.1126/science.abd8377

WP1.3 - Iterative CRISPR/Cas9 genome editing to reduce extracellular protease activity for heterologous protein production in *Aspergillus niger*

M. Arentshorst[1], J. van Dam[1], M. Raats[2], T. Verschuren[2], M. Zhou[2], J. Visser[1], R. Lubbers[1], A. Ram[1]
[1]Leiden University, Institute Biology Leiden, Fungal Genetics and Biotechnology, Leiden, Netherlands, [2]Avans University of Applied Sciences Breda, Center of Expertise Materials and Energy Transition, Breda, Netherlands

The high protein secretion capacity of *Aspergillus niger* has been well recognized and exploited for the production of homologous and heterologous enzymes and other proteins. Genome mining revealed many extracellular or secretion pathway related (Golgi/vacuolar localized) proteases that are potentially harmful for heterologous protein yields. Inferred from experimental data and computational predictions, 60 possible secretion related proteases were identified.
To build a protein expression platform in *A. niger* and to identify proteases harmful for the production of heterologous proteins, a strain lineage of *A. niger* was developed to effectively express the gene of interest (GOI) combined with reduced protease activity. We used iterative CRISPR/Cas9-based genome editing to first delete genes encoding the most abundant secreted proteins (glucoamylase, acid amylase and alpha-glucosidase A), and genes involved in acidification (glucose oxidase and oxaloacetate hydrolase). In this non-acidifying background predicted secretion related proteases were deleted. Here we report a strain lineage consisting of 34 strains in which a total of 60 protease encoding genes were deleted. Gene deletions we verified by diagnostic PCR and representative strains from the lineage were genome sequenced to verify the deletions and to asses chromosome stability. Initial gene deletions were made by replacing the gene with a glucoamylase landing site, allowing targeted integration of the GOI. Using this integration system, up to 10 copies of the GOI can be integrated effectively. The strain

lineage is a powerful tool to identify secretion related proteases that are harmful for the production of heterologous proteins prone to proteolytic degradation.

WP1.4 - Fungal Cutinase revisited: Expression and characterization of novel *Fusarium* Cutinases in *Aspergillus niger* and application in bioremediation

M. Arentshorst[1], *J.-A. Verschoor*[1], *S. Seekles*[1], *T. Regensburg*[1], *K. van den Hondel*[1], *A. Ram*[1], *H. de Winde*[1]
[1]Leiden University, Institute Biology Leiden, Fungal Genetics and Biotechnology, Leiden, Netherlands

Background and aims
Cutinases (EC 3.1.1.74) are widely distributed in fungi, bacteria and plants and act on the natural substrate cutin, a waxy aliphatic polyester acting as protection barrier of plants. In addition, cutinases have been reported to hydrolyze artificial polyesters and toxic xenobiotics such as polyethylene terephthalate (PET), polycaprolactone (PCL), polylactic acid (PLA), and polyhydroxybutyl succinate (PBS). Moreover, cutinases can act as promising stereoselective catalysts in (trans)esterification reactions with high selectivity. Hence, cutinases are powerful tools in synthetic biology and bioremediation and deserve further investigation towards a broader range of ester-containing substrates.

Methods and Results
62 putative endophytic fungal strains were isolated from plants. ITS-sequencing clearly placed the majority of these strains in the genus *Fusarium*. Full genome analysis of the most closely related strains revealed the presence of 3 putative cutinase genes. Cutinase activity was confirmed via para-nitrophenyl butyrate (pNPB) assay. Two strains with considerable cutinase activity were chosen for further analysis. Those strains each contained two different cutinases, with one of each being highly identical. The 3 resulting cutinases were expressed in *Aspergillus niger* using an in-house developed CRISPR/Cas9-based multicopy integration system. Enzyme purification was followed by extensive biochemical analysis of the purified cutinases on various natural and non-natural substrates.

Conclusions
We have identified and characterized 3 novel cutinase enzymes from *Fusarium* spp. The enzymes show different activities on different natural and non-natural substrates, thereby strengthening the plea for an important role of cutinases in synthetic biology and bioremediation.

WP1.5 - An improved method to identify *het* genes of *Aspergillus nidulans*

B. Auxier[1], *A.J. Debets*[1]
[1]Wageningen University & Research, Laboratory of Genetics, Wageningen, Netherlands

Hyphal fusion between two fungal individuals leads to rapid programmed cell death. In the model species *Neurospora crassa* and *Podospora anserina*, this process is known to be controlled by ~10 genes, termed *het* for heterokaryon incompatibility. However the identity of these genes, and their evolutionary origin, is unclear outside of these Sordariomycetes. Many *het* genes are related to genes involved in bacterial defence, and the selection maintaining the extreme *het* gene allelic diversity remains an enigma. To access the dense evolutionary sampling of the *Aspergillus* genus, we developed a method that can rapidly identify *het* genes. Using complementing nitrate auxotrophies, which can be easily induced using UV paired with chlorate selection, we can screen for rare outcrossed progeny that can form heterokaryons with their parents. Pools of compatible progeny can be combined and used for bulk segregant analysis. As a proof of principle, we have identified the *het* genes of *A. nidulans.* The eight *het* genes we identify include loci shared with *Aspergillus fumigatus* as well as undescribed loci. The speed of this method, which can identify *het* genes in weeks instead of the years previously necessary, allows for investigations on a scale not previously possible. We believe, this method can be applied to any species with an accessible sexual cycle and uninucleate conidia. Applying this method to targeted *Aspergillus* species, combined with the abundant genomic resources, we can pinpoint the origins of these genes.

WP1.6 - Characterization of Regulation of Ace2 and Morphogenesis (RAM) network components in Aspergillus fumigatus carbon source-mediated morphogenesis

D.N.J. Bale[1], *A. Martin-Vicente*[1], *H. Thorn*[1,2], *J. Xie*[1,2], *A. Nandi*[1], *U. Perez Cuesta*[1], *J. Fortwendel*[1,3]
[1]University of Tennessee Health Science Center, Clinical Pharmacy and Translational Science, Memphis, United States, [2]University of Tennessee Health Science Center, PhD Program in Pharmaceutical Sciences, Memphis, United States, [3]University of Tennessee Health Science Center, Microbiology, Immunology and Biochemistry, Memphis, United States

Aspergillus fumigatus CotA is a conserved protein kinase of the RAM network that we recently identified as essential for morphogenesis and virulence dependent on host niche-relevant carbon sources. In yeast, the RAM network controls cell proliferation, cell wall integrity, and stress responses and is composed of multiple proteins, including the CotA ortholog, Cbk1, and the additional associated proteins, Mob2, Tao3, Hym1, Sog2, and Kic1 which have not been previously characterized in *A. fumigatus*. To test if any of these RAM components are involved in the carbon source- and morphogenesis-related phenotypes uncovered in our CotA mutant, we first identified single copy orthologs for *hym1*, *kic1*, *sog2*, *tao3*, and *mob2* in the *A. fumigatus* genome through BLAST analyses. Then, using CRISPR/Cas9, we successfully deleted *hym1*, *kic1*, and *tao3* orthologs. After multiple unsuccessful attempts to delete *sog2* and *mob2* orthologs, indicating essentiality, we generated doxycycline-inducible conditional mutants. We then analyzed each strain for growth and morphogenesis in media containing glucose or non-preferred carbon sources (acetate, casamino acids, and ethanol) as the sole source of carbon. Although the deletion of *hym1* and repression of either *sog2* or *mob2* ortholog inhibited hyphal morphogenesis under all carbon sources, no significant differences were observed between the mutant and wild type strains on non-preferred carbon sources, suggesting that the role of CotA in carbon source-

dependent morphogenesis is not controlled by any of these regulatory proteins. Further protein-protein interaction studies will determine how these proteins interact and if their function in regulating CotA activity is conserved in *A. fumigatus*.

WP1.7 - FungiDB: a bioinformatics resource for facilitating data exploration, analysis, and integration for fungal and oomycete species

E. Basenko[1], *O. Harb*[2], *D. Roos*[2], *A. Jones*[1]
[1]University of Liverpool, Institute of Systems, Molecular and Integrative Biology, Liverpool, United Kingdom, [2]University of Pennsylvania, Department of Biology, Philadelphia, United States

FungiDB, a component of the VEuPathDB project, provides a one-stop shop for omics data exploration and analysis for over 300 fungal and oomycete species, including organisms on the WHO fungal priority list. FungiDB integrates diverse data types and enables researchers to interrogate the data using various tools such as the search strategy system, a genome browser, and comprehensive gene pages. Integrated data types include genomic, transcriptomic, proteomic, metabolomic, population-level and phenotypic studies and more. Built-in orthology enables cross-species inferences and enhances the reusability of datasets across the integrated organisms.
Key features of FungiDB include:
- Extensive information on genomes, including gene record pages and automated and expert-curated annotations.
- Tools for conducting comparative genomics, analyzing protein structures, exploring gene regulatory networks, and investigating pathogen-host interactions.
- Integration of publicly available datasets, enabling researchers to conduct in silico experiments and explore data enrichment analyses in the context of existing data.
- Ongoing curation and community-driven enhancements to genomes in the genome editor Apollo to ensure the capture of up-to-date, high-quality annotations by leveraging community expertise.
Future advancements are aimed at integrating AI-driven tools to enhance literature curation and metadata annotation and incorporating tools for new data types.

WP1.8 - Tracking hyphal fusion in highly diverse *Aspergillus fumigatus* populations to identify the formation of multi-drug resistance heterokaryon compatibility groups

M. Bottery[1], *E. Tancredi*[1], *A. Sarkar*[2], *S. Gupta*[3], *N. Osherov*[2], *M. Bromley*[1]
[1]University of Manchester, Division of Evolution, Infection and Genomics, Manchester, United Kingdom, [2]Tel Aviv University, Tel Aviv, Israel, [3]Trinity College Dublin, Dublin, Ireland

Aspergillus fumigatus is a significant human pathogen capable of causing severe pulmonary infections. Heterokaryon formation, achieved through hyphal fusion, promotes the horizontal transfer of ecologically important traits among related individuals. This is particularly important within long-term chronic infections where local genetic diversity, generated through mutation,

can be disseminated within mycelial networks. Stressors such as antifungal treatment can induce hyphal fusion, potentially stimulating the accumulation of multiple resistance alleles within single individuals. However, hyphal fusion is tightly regulated to limit non-self-fusion through a multigenic incompatibility system controlled by heterokaryon incompatibility (*het*) loci, which likely evolved to prevent the spread of selfish genetic elements such as viruses and transposable elements. To investigate hyphal fusion dynamics and identify novel *het* loci, we developed a high-throughput method to track hyphal fusion events between *A. fumigatus* isolates with distinct resistance profiles within large, diverse populations. Our results demonstrate that fusion preferentially occurs between isogenic partners but can also take place with genetically distinct but phylogenetically closely related individuals, suggesting the existence of fusion compatibility groups. By screening genetic loci for high divergence between groups and low divergence within groups, we identified putative novel *het* loci. These results highlight that cell fusion is not restricted to isogenic strains and can promote the sharing of genetically distinct nuclei harbouring different resistance alleles between individuals. This mechanism could contribute to the rapid spread of multiple resistances within *A. fumigatus* populations.

WP1.9 - Biosustainable production of indigoidine dye from polystyrene and polyethylene plastic waste

Z. Bowers[1], *Y. Chen*[2], *E. Oakley*[3], *B.R. Oakley*[3], *T.J. Williams*[2], *C.C.C. Wang*[1]
[1]University of Southern California, Department of Pharmacology and Pharmaceutical Sciences, Los Angeles, United States, [2]University of Southern California, Department of Chemistry, Los Angeles, United States, [3]University of Kansas, Department of Molecular Biosciences, Lawrence, United States

Plastic waste is an acute threat to the environment and human health. The complete elimination of plastics, however, is an inconceivable solution due to their revolutionary impact on daily life and immense economic contribution. Consequently, long-term plastic sustainability is an urgent matter that requires new approaches to achieve, such as the utilization of microorganisms. We previously demonstrated the conversion of polystyrene (PS) and polyethylene (PE), two common plastic polymers with poor recycling rates, into metabolically relevant fungal substrates. Further, the sustainable impact of fungi can be broadened by their ability to create complex secondary metabolites that have the potential to replace our current environmentally hazardous synthetic processes like those used in dye manufacturing. Thus, fungal production of the natural blue pigment, indigoidine, from plastic waste was of interest. We hypothesized that *Aspergillus nidulans* can produce notable titers of indigoidine from post-consumer PS and PE substrates. To accomplish this, the non-ribosomal peptide synthetase (NRPs) responsible for indigoidine biosynthesis was expressed in *A. nidulans*. The engineered strain's ability to utilize PS and PE substrates as sole carbon sources for growth and indigoidine production was determined. Metabolism of PS and PE substrates resulted in indigoidine titers of 624 mg L^{-1} ± 118 mg L^{-1} and 980 mg L^{-1} ± 315 mg L^{-1}, respectively. This study demonstrates the robust nature of *A. nidulans* as a heterologous host, expands the chemical diversity of fungal secondary metabolites from post-consumer plastic substrates, and contributes to the promising potential of fungi for global long-term plastic sustainability.

WP1.10 - Characterisation of the antifungal effects of manogepix on the human-pathogenic mould *Aspergillus fumigatus*

S. Brazil[1], *I. Fouquet*[1], *J. Wagener*[1]
[1]Trinity College Dublin, Clinical Microbiology, Dublin, Ireland

Manogepix is a new antifungal and has been reported to have a fungistatic activity against the opportunistic pathogenic mould *Aspergillus fumigatus*. Manogepix targets Gwt1, an enzyme required to form glycosylphosphatidylinositol (GPI)-anchored proteins in the endoplasmic reticulum. GPI-anchored proteins are localized to the cell wall and play roles in maintaining cell wall strength. We show that manogepix triggers activation of the cell wall integrity (CWI) signalling pathway of *A. fumigatus*, which results in upregulation of cell wall chitin biosynthesis. Deletion of genes that encode key components of CWI pathway typically results in an increased susceptibility to antifungal agents that target the cell wall (e.g., echinocandin antifungals, calcofluor white). Surprisingly, some of these CWI deletion mutants grow better in the presence manogepix when compared to wild type. In agreement with this, overexpression of a key player of *A. fumigatus'* CWI pathway results in reduced growth in the presence of manogepix. Our results support a model where manogepix triggers activation of the CWI signaling pathway, which contributes to the fungistatic effect of manogepix. Furthermore, our data reveal an alternate stress signaling pathway triggered by manogepix which alters the susceptibility of *A. fumigatus* to other antifungal agents.

WP1.11 - Nation-wide air sampling reveals land use is linked to voriconazole resistance and TR_{46} prevalence in *Aspergillus fumigatus* in the Netherlands

B. Briggeman[1,2], *H. Kortenbosch*[1], *F. Reyes-Marquez*[1], *B. Zwaan*[1], *E. Snelders*[1]
[1]Wageningen University, Laboratory of Genetics, Wageningen, Netherlands, [2]National Institute for Public Health and the Environment, Centre for Infectious Disease Research, Diagnostics and Laboratory Surveillance, Bilthoven, Netherlands

Cross-resistance between agricultural and medical triazoles burdens treatment of opportunistic fungal pathogen *Aspergillus fumigatus*. Medical triazoles are the first-line treatment for *A. fumigatus* infections; however, mutations in the *cyp51A* gene confer cross-resistance. Environmentally selected triazole resistance is characterized by a tandem repeat (TR) in the promotor region of this gene with non-synonymous point mutations in the coding region.
In the fall of 2023, Citizen Science project Fungal Radar (Schimmelradar) sent out 500 air sampling kits divided over 100 equal-sized regions in The Netherlands. The seals were selectively cultured, and per location a control, voriconazole (VOR) and itraconazole (ITR) growth treatment was applied. VOR and ITR resistance fractions and the TR haplotypes were determined and linked to publicly available land-use data via optimized generalized linear models.
The median resistance fraction for ITR (3.9%, n= 1,346) and VOR (2.2%, n= 914) was calculated (total n = 98,064). From colonies resistant to VOR or ITR, the TR_{34} (46.5%) and TR_{46} (43.0%)

were most prevalent. Yet, VOR resistance has a more pronounced link to flower bulb cultivation, potato cultivation, and greenhouse horticulture than ITR resistance. Also, the TR_{46} haplotype is spatially heterogeneous, and increased TR_{46} haplotype frequency is associated with local land use, most notably flower bulb cultivation and horticulture. The spread of the TR_{34} haplotype had no land-use association and was more spatially homogeneous. This study demonstrates the differences in relative exposure to triazole-resistant *A. fumigatus* (ranging between 0-20% resistance) and notable differences in exposure to the two most common TR haplotypes.

WP1.12 - Valorization of whiskey side-streams for sustainable mycoprotein production and waste reduction

T.-T. Bui[1], *Ö. Bayram*[1], *J.-C. Jacquier*[2], *Ö.S. Bayram*[1]
[1]Maynooth University, Biology, Maynooth, Ireland, [2]University College Dublin, School of Agriculture and Food Science, Institute of Food and Health, Dublin, Ireland

The whiskey industry is vital to Ireland's economy, contributing to jobs, exports, and preserving cultural heritage. However, whiskey production generates significant waste, including liquid byproducts like spent wash and solid residues like spent grains, which can cause environmental pollution if not managed properly. Poor waste management increases pollution risks and adds operational costs, while missing opportunities for resource recovery. Whiskey production waste is nutrient-rich, containing proteins, fibers, vitamins, and minerals, offering potential as animal feed or soil enhancement, though not suitable for direct use. We screened various edible mushroom and mold strains for their ability to grow in whiskey waste and identified several strains that effectively convert whiskey side streams into edible fungal mycoprotein. These strains produced wet biomass (10-15% weight/volume), which, when dried, yielded 3-5%. The fungal biomass contained over 25% protein by dried weight, as measured by the Kjeldahl method. Additionally, fungal growth reduced the chemical oxygen demand (COD) of the waste, lowering its environmental impact. These findings suggest that fungal biomass from whiskey waste could be repurposed as a nutritious food source for humans and livestock, offering sustainable waste management solutions while addressing environmental challenges.

WP1.13 - Combination of RNA and ChIP-seq reveals regulatory genes of fungal morphogenesis and secondary metabolism controlled by the chromatin binding KERS complex in *Aspergillus nidulans*

T.-T. Bui[1], *S. Shabeer*[1], *P. Agarwal*[1], *C.K.H. Wong*[2], *Ö. Bayram*[1]
[1]Maynooth University, Biology, Maynooth, Ireland, [2]University of Macau, Faculty of Health Sciences, Taipa, Macao, SAR of China

The KERS complex is crucial for epigenetic regulation in Aspergillus nidulans, affecting chromatin remodeling, transcription, and secondary metabolite production. It consists of KdmB (a histone demethylase), EcoA (a histone acetyltransferase), RpdA (a histone deacetylase), and SntB (a chromatin-associated protein). To identify genes dependent on the KERS complex, RNA sequencing (RNA-seq) was performed on deletion mutants of kdmB, ecoA, rpdA, and sntB

compared to the wild-type strain at 20 hours (primary metabolism, PM) and 48 hours (secondary metabolism, SM) of incubation. Analysis revealed significant changes, with 500 to over 4,000 genes differentially expressed in the mutants. Notably, 204 common genes were differentially regulated during PM, and 545 during SM. Of these, 25 genes were downregulated across all mutants at 20 hours, and 104 were downregulated at 48 hours, highlighting the shared regulatory roles of KERS components in activating secondary metabolism, including mycotoxin Sterigmatocystin production. The top five downregulated regulatory genes, bound by the KERS complex, are being deleted and studied for their roles in development and metabolism. These findings improve our understanding of how the KERS complex coordinates fungal development with secondary metabolite production in *A. nidulans*. Current state of the project will be presented.

WP1.14 - The oxylipin-responsive transcription factor ZfpA mediates tolerance against fungicidal tip lysis by β-1,3-glucan synthase targeting antifungals in *A. fumigatus*

D. Calise[1], *B. Chadwick*[2,3], *C. Hull*[1,2], *J. Coon*[2,3], *N. Keller*[1,4]
[1]University of Wisconsin - Madison, Medical Microbiology & Immunology, Madison, United States, [2]University of Wisconsin - Madison, Biomolecular Chemistry, Madison, United States, [3]Morgridge Institute for Research, Madison, United States, [4]University of Wisconsin - Madison, Plant Pathology, Madison, United States

The saprophytic fungus *Aspergillus fumigatus* is responsible for nearly all cases of invasive aspergillosis (IA) in addition to allergic and chronic pulmonary diseases. Treatment of IA is limited to only three approved classes of antifungals with echinocandins serving as an important salvage therapy when azoles fail. Echinocandins, such as caspofungin, are semisynthetic derivatives of natural lipopeptides produced by soil-dwelling Leotiomycetes and Eurotiomycetes fungi. These drugs act by inhibiting the enzyme β-1,3-glucan synthase to impair cell wall biogenesis. Although considered fungistatic against *A. fumigatus*, echinocandins exert both fungistatic inhibition of hyphal growth and fungicidal lysis of apical tip compartments. We found the transcription factor ZfpA mediates protection by the fungal oxylipin 5,8-diHODE against the fungicidal activity of echinocandins. ZfpA also contributes to tolerance of enfumafungin—a novel and structurally dissimilar β-1,3-glucan synthase inhibitor with similar antifungal activity. Interestingly, ZfpA does not protect against the cell wall stressor Congo Red indicating a specific role in β-1,3-glucan targeting antifungal tolerance. To identify ZfpA regulated proteins that prevent apical tip lysis, we performed proteomic analysis of WT, Δ*zfpA*, and OE::*zfpA* strains in response to caspofungin treatment. Candidate proteins were subsequently deleted and mutants assessed for increased susceptibly to caspofungin induced tip lysis.

WP1.15 - Mathematical modeling of fungal growth provides insights into antifungal drug resistance

D. Cánovas[1]
[1]University of Sevilla, Dept of Genetics, Sevilla, Spain

Microbial growth analysis is a crucial tool for microbiologists, providing insights into various strains under different conditions. However, these analyses typically focus on qualitative assessments of quantitative data. To enhance the analysis of fungal growth, I tested five commonly used mathematical growth models using real experimental datasets. One model stood out as the most cost-effective for fitting the data.
To validate the model, I used growth datasets of *Aspergillus nidulans* grown under varying nitrogen concentrations and inoculum sizes. The model and a new parameterization identified three and four parameters, respectively, defining the growth curve. Changes in nitrogen concentration affected both maximum growth and growth rate, while inoculum size inversely correlated with the inflection time point. The model was also useful for studying growth in other ascomycete fungal species, such as *Fusarium* and *Neurospora*. After validation, the model was applied to study the growth of *A. fumigatus* in response to antifungal drugs. Voriconazole at subinhibitory concentrations mainly reduced the growth rate without affecting other parameters. Accordingly, the Δ*nctA* and Δ*nctB* mutants, which display increased azole resistance, showed higher growth rates but no changes in other growth parameters in the presence of voriconazole. Environmental isolates of *A. fumigatus* resistant to azoles due to mutations in the *cyp51A* gene were also tested, and their growth parameters revealed different fitness costs under various growth conditions. This method enables automated characterization of numerous samples simultaneously, offering significant potential for high-throughput antibiotic drug screenings.

WP1.16 - Unveiling the genetic background of azole-resistant and susceptible *Aspergillus fumigatus* environmental isolates of the Basque Country

S. Cendon-Sanchez[1], *L. Fabre*[2], *E. Pelegri-Martinez*[1], *U. Perez-Cuesta*[3], *X. Guruceaga*[4], *A.E. Barber*[2], *A. Ramirez-Garcia*[1], *A. Abad-Diaz-de-Cerio*[1], *A. Rementeria*[1]
[1]University of the Basque Country (UPV/EHU), Department of Immunology, Microbiology and Parasitology, Bilbao, Spain, [2]Institute of Microbiology, Friedrich Schiller University Jena (FSU), Jena, Germany, [3]University of Tennessee Health Science Center (UTHSC), Department of Clinical Pharmacy and Translational Science, Memphis, United States, [4]Institute for Multidisciplinary Research in Applied Biology (IMAB), Public University of Navarre (UPNA), Pamplona, Spain

Aspergillus fumigatus is an opportunistic pathogen, whose infections are treated mainly with azoles. Nevertheless, in the recent years, the fungus has developed resistance mechanisms to the available drugs. This is one of the reasons why WHO included *A. fumigatus* in the group of critical priority fungal pathogens in 2022. The origin of these resistances is both clinical, due to prolonged treatments of chronic patients with medical azoles, and environmental, due to extensive use of azole fungicides in agriculture. In this work, six environmental *A. fumigatus* strains were sequenced (n=2 susceptible; n=4 resistant). Regardless of their origin and susceptibility, phylogeny results showed that the isolates from the Basque Country shared numerous variants compared to the reference genome Af293. The four resistant isolates carried the previously described mutation TR_{34}/L98H in *cyp51A*, the azoles target. Variant calling analysis allowed the identification of 13 genes with possible involvement in triazole resistance based on their function: four play a role in the ergosterol biosynthesis, the pathway targeted by

the azoles, and nine encode for multidrug efflux pumps. To assess the impact of those variants, the expression of some genes was analyzed by RT-qPCR in absence and presence of voriconazole for the six abovementioned strains as well as for the Af293 strain.
This study was funded by project IT1657-22 of the Basque Government. SCS and EPM have received predoctoral grants from the Basque Government. SCS also received the FEMS RTG.

WP1.17 - Extraction and derivatization of chaetoglobosin A from *Chaetomium globosum* for anti-cancer therapy

P.F. Chiu[1], *S. Long*[1], *C.C. Wang*[1,2]
[1]University of Southern California, Pharmacology & Pharmaceutical Sciences, Los Angeles, United States, [2]University of Southern California, Chemistry, Los Angeles, United States

Chaetoglobosin A (Ch A), which consists of (indol-3-yl)methyl, perhydroisoindolone, and macrocyclic ring moieties, is a secondary metabolite derived from *Chaetomium globosum* (*C. globosum*). Ch A is known for inhibiting actin polymerization and disrupting cell division. It has been shown to inhibit the growth of blood cancer cells, PC-3, HCT116, and MDA-MB-231 cell lines, highlighting its potential for clinical application. However, Ch A lacks selectivity, leading to acute toxicity in the spleen and thymus, as well as liver and kidney damage. To address the issue of poor selectivity, conjugating Ch A with a targeting moiety offers a promising solution. In this study, we optimized the production of Ch A by culturing wild-type *C. globosum* in various media. The yield of Ch A increased from 2 mg/L in potato dextrose agar plates to 54 mg/L in oatmeal agar plates when cultured at 25°C for 12 days in the dark. Moving forward, we plan to conjugate Ch A with an antibody using biodegradable or stable linkers to form an antibody-drug conjugate, with the aim of improving Ch A's safety profile and enhancing its clinical potential.

WP1.18 - Co-evolution of biological processes as revealed by whole genus genome sequencing of *Aspergillus*

R.P. de Vries[1], *I.V. Grigoriev*[2], *M.R. Andersen*[3], *S.E. Baker*[4]
[1]Westerdijk Fungal Biodiversity Institute, Fungal Physiology, Utrecht, Netherlands, [2]DOE Joint Genome Institute, Berkeley, United States, [3]Technical University of Denmark, Technical University of Denmark, Lyngby, Denmark, [4]Pacific Northwest National Laboratory, Environmental Molecular Sciences Division, Richland, United States

The rapid expansion of the set of fungal genomes has provided many new insights in the biology of these organisms and their diversity. *Aspergillus* is one of the best studied fungal genera, due to its relevance as an opportunistic pathogen of humans and animals, a spoilage organism of many foods and its wide application in biotechnological processes. In recent years we have generated genome sequences for nearly 300 *Aspergillus* species, providing an unprecedented dataset of a fungal genus.
This dataset has now been used to generate an evolutionary roadmap of biological processes to identify at which level each of these processes has gone through major changes, focusing on the genus, subgenus, section and species level. Rather than studying the conservation of individual

genes or gene groups, we analyzed the evolutionary pattern at the level of process covering the following topics: carbon utilization (CAZy-genes, primary carbon metabolism, sugar transporters), nitrogen utilization (nitrogen and amino acid metabolism), secondary metabolism, stress response, development and propagation (mating, sexual/asexual development, conidiation). Subsequently, the evolutionary patterns of these processes have been compared to reveal which processes co-evolve and which occur at different taxonomic levels. This has provided new insights into fungal evolution, which will also be a template for other whole genus genome projects, such as those of *Penicillium* and *Trichoderma*.

WP1.19 - Organic acid utilization by *Aspergillus niger*

R. de Vries[1], *A. Manassero*[1], *M. Peng*[1]
[1]Westerdijk Fungal Biodiversity Institute, Fungal Physiology, Utrecht, Netherlands

Fungi produce substantial quantities of organic acids under specific stress conditions, leading to their accumulation in the soil. While it is widely presumed that fungi utilize many of these organic acids as a carbon source, particularly by entering the tricarboxylic acid (TCA) pathways, there is a scarcity of experimental evidence supporting this assumption in literature. Employing growth profiling, we conducted an analysis to assess the ability of the *Aspergillus niger* to grow on nine different organic acids as the sole carbon source. The fungus was inoculated on solid minimal medium (MM) plates with the addition of organic acids and it was derived from both mycelium and spores, under two distinct conditions: directly on the organic acid and in the presence of glucose starvation. In each instance, the result was compared to a positive control (glucose) and a negative control (no carbon source). The findings revealed challenges in the utilization of most organic acids as a sole carbon source, both from mycelium and spores, with and without glucose starvation. The phenotypic data was complemented with transcriptomic analysis of transfer cultures to these organic acids to reveal changes in gene expression profiles when the organic acids are the only carbon source present.

WP1.20 - Novel insights into endogenous biotinylation in fungi

Z. Dong[1], *H.P. Leng*[1], *Q. Lan*[1], *K.H. Wong*[1]
[1]University of Macau, Macau, China

Biotin, an essential vitamin, acts as a coenzyme for holocarboxylases that catalyze key steps in gluconeogenesis, amino acid catabolism, and fatty acid synthesis. Biotin is covalently attached to holocarboxylases at the lysine residue in the conserved Met-Lys-Met sequence motif by holocarboxylase synthetase (HCS) through a process called biotinylation. Carboxylase family proteins are generally believed to be the only targets of HCS. However, we and others have observed from Western blot analysis many more biotinylated proteins than expected for carboxylases in different fungal species. Mass spectrometry analysis of streptavidin pull-down showed that many of the endogenous biotinylated proteins in *Aspergillus nidulans* are unrelated to carboxylases and are highly abundant in the cell; e.g. histones and tubulin. Interestingly, unlike carboxylases, these other endogenous biotinylated proteins lack the canonical Met-Lys-Met

biotinylation motif. This raises the question of how biotin is introduced to these proteins and suggests the existence of a non-canonical biotinylation pathway. We are currently testing different potential mechanisms of non-canonical biotinylation and studying the role of these biotinylation events. Endogenous biotinylation is commonly observed in different organisms, including bacteria, various fungal species, and mammalian cells, indicating an evolutionarily conserved mechanism for the non-canonical biotinylation pathway.

WP1.21 - Activation of a sorbicillinoid pathway through deletion of mcrA in Penicillium rubens YAP001

S. Dziedzic[1,2], *J. Shyong*[1], *T.Q.D. Huynh*[3], *C.-K. Lee*[3,4], *C. Kenkel*[5], *C. Wang*[1,2]
[1]University of Southern California, Department of Pharmacology and Pharmaceutical Sciences, Los Angeles, United States, [2]University of Southern California, Department of Chemistry, Los Angeles, United States, [3]Taipei Medical University, School of Pharmacy, Taipei, Taiwan, China , [4]Taipei Medical University, Ph. D Program in Clinical Drug Development of Herbal Medicine, Taipei, Taiwan, China , [5]University of Southern California, Department of Biological Sciences, Los Angeles, United States

Fungal secondary metabolites (SMs) are complex organic compounds comprising a variety of biological activities essential in medicine. Due to the immense potential of SMs to be solutions to currently incurable diseases, it is essential to explore new avenues for upregulation of SMs known to provide useful bioactive properties. In particular, the discovery of sorbicillinoids has led to the detection of compounds containing various bioactivities against human pathogens as well as identification of cytotoxic effects providing potential as an anticancer agent. However, due to the low yield of the production of fungal SMs, we are in pursuit of novel approaches to upregulate the pathways of these valuable metabolites.
Analysis of the fungal genome has established that genes responsible for producing SMs are clustered together, forming biosynthetic gene clusters (BGCs). One strategy for activating BGCs with the objective of upregulation in SM production is through the deletion of the negative global regulator of secondary metabolism, *mcrA*. Previous literature has shown that deletion of *mcrA* in various fungal strains has resulted in stunningly different metabolic profiles with compounds upregulated in the mutated strain. Upon generating a *mcrAΔ* strain in *Penicillium rubens* (YAP001), we found that sorbicillin was not only upregulated, but the mutant strain also produced the dimeric product, trichodimerol, which often exhibits stronger biological activities compared to sorbicillin. This suggests that genetic manipulation of global regulators in filamentous fungi is an effective technique in activating pathways of interest for the purpose of drug discovery.

WP1.22 - *Aspergillus* species epidemiology is driven by climate, soil and fungicides

T. Easter[1], *M. Bromley*[1], *J. Rhodes*[2], *M. Brockhurst*[3], *N. van Rhijn*[1,3]
[1]University of Manchester, Manchester Fungal Infection Group, Manchester, United Kingdom, [2]Radboud University Medical Centre, Department of Medical Microbiology, Nijmegen, Netherlands, [3]University of Manchester, Microbial Evolution Research Manchester, Manchester, United Kingdom

Aspergillosis is a severe fungal infection caused by members of the *Aspergillus* genus, primarily *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus flavus*. These soil-based saprotrophs are responsible annually for more than 4 million life-threatening infections and an economic burden of over $10 billion. While *A. fumigatus* is the primary cause of aspergillosis in Europe and the Americas, previous data from our lab have shown that *A. flavus* is more common in Asia and Africa. As the climate changes and global temperatures rise, the impact on the epidemiology of *Aspergillus* species is unknown. To further study the effect of changing environments on *Aspergilli*, we aimed to establish an environmentally relevant soil microcosm. Using CRISPR-Cas9 mutagenesis, we generated genetically barcoded pools of *Aspergillus* species to perform Bar-seq. Bar-seq is a next generation sequencing approach to quantify each fungal isolate within a pooled inoculum before and after growth in our soil microcosms. First, we assessed fitness of n=25 barcoded *A. fumigatus* isolates for their fitness in a compost soil microcosm model. We measured antifungal and fungicide concentrations in these composts and correlated that with fitness and drug resistance. Our results showed that fungicides could be detected in all commercial compost, and a fitness signature of drug resistance could be found. This soil microcosm model will allow us to test more soil-based variables over longer periods of time, and gain a better understanding of how antifungal resistance can develop alongside changing environments.

WP1.23 - Precise control of *Aspergillus niger* pellet size, heterogeneity and core architecture during shake flask cultivation

K. Engelbert[1], *T.C. Cairns*[1], *T. Kheirkhah*[2], *C. Deffur*[3], *F. Zhang*[3], *H. Winter*[3], *S. Junne*[4], *H. Briesen*[3], *P. Neubauer*[2], *V. Meyer*[1]
[1]Technische Universität Berlin, Applied and Molecular Microbiology, Berlin, Germany, [2]Technische Universität Berlin, Bioprocess Engineering, Berlin, Germany, [3]Technical University of Munich, Process Systems Engineering, Munich, Germany, [4]Aalborg University, Chemistry and Bioscience, Aalborg, Denmark

In submerged culture, filamentous fungi grow in three macromorphological forms: dispersed mycelium, clumps and pellets. The underlying processes that lead to their formation are not yet completely understood and require systematic investigation, as heterogeneous cultures limit the optimization of biotechnological processes.
Therefore, the cell factory *Aspergillus niger* was cultivated under 48 conditions in technical triplicate. The studied process parameters included spore densities, concentration of talc

microparticles, stirrer speeds, and flask shape (+/- baffles). A high-throughput image analysis pipeline was used to analyse thousands of pellets from the resulting populations with pellet diameter, culture heterogeneity, and inter-replicate variation quantified. Newly developed µ-CT technology was used to investigate the pellet's inner architecture in remarkable detail. We used regression modelling to identify multiple parameters that can be used for precise, highly reproducible control of filamentous fungal shake flask growth. Additionally, we reveal three distinct pellet types and propose a new pellet classification system: (i) pellets formed by a single spore core, (ii) pellets formed by multiple spore cores and (iii) pellets formed by multiple mature pellets in later growth phases.
The study analyses the influence of process parameters on pellet populations in a systematic way. The comprehensive data set allowed the identification of simple influencing process parameters. The strength additionally lies in analysing internal pellet architecture, which expands the understanding of pellet formation into three distinct classes. Together, these findings will drastically improve the control and reproducibility of shake flask assays, thus making them accurate models of larger vessel fungal fermentation.

WP1.25 - Control of conidiation in the genus *Aspergillus*: on the centrality and specificity of the master regulator BrlA

F. Wang[1], A. Bereziartu[2], P. Wu[1], R. Chen[1], K. Tan[1], E.A. Espeso[3], D. Cánovas[4], C.K.H. Wong[1], O. Etxebeste[2]
[1]Faculty of Health Sciences, University of Macau, Institute of Translational Medicine, Macao, Macao, SAR of China, [2]Laboratory of Microbiology, Faculty of Chemistry, University of the Basque Country, Applied Chemistry, San Sebastian, Spain, [3]Centro de Investigaciones Biológicas Margarita Salas (CSIC), Dept. of Cellular and Molecular Biology, Madrid, Spain, [4]Faculty of Biology, University of Seville, Department of Genetics, Seville, Spain

Asexual spores are the main vehicle for dissemination of fungi. In *aspergilli*, the transcription factor BrlA plays a central role in conidiophore development and conidia production. Several transcriptional activators bind target sequences in the promoter of *brlA* (*brlA^{p}*) and determine its expression patterns before/during development. The 5'-UTR region of *brlA* is also bound by repressors that inhibit its expression at late stages of conidiophore development and activate sexual development. Here, a mutagenesis procedure was followed to generate: 1) *brlA::HA$_{3x}$* strains that included serial deletions of *brlA^{p}* (spanning ~3 Kb upstream of the *brlAβ* start codon); 2) strains in which the hypothetical binding sites of transcriptional regulators binding *brlA^{p}* were deleted; and 3) a strain bearing a mutation corresponding to a Met1Ile substitution in BrlAβ (BrlAβ and BrlAα differ only in the first 23 amino acids). None of these strains showed the *fluffy* aconidial phenotype characteristic of the Δ*brlA* mutant. Only deletion of the *brlA^{p}* region that includes a uORF caused an inhibition of conidiation, although conidiophores with an aberrant morphology of vesicles and metulae were developed. Sexual development was induced prematurely in this strain, suggesting that BrlA activity goes beyond specifically controlling conidiation. ChIP-Seq results of *BrlA::HA$_{3x}$* and *AbaA::HA$_{3x}$* (after 24h of conidial development) support this hypothesis, showing that BrlA/AbaA bind to promoters of genes encoding activities required for polar-growth, development, cell-wall organization and signaling. We also analyzed

what could be the cause of the phenotypic difference between Δ*brlA* and *brlA*^{p}-ΔuORF mutants, and identified additional genes necessary for conidiation.

WP1.26 - Understanding secondary metabolite production based on biosynthetic gene cluster-specific transcription factor binding targets

L. Fan[1], *S. Guo*[1], *L. Pardeshi*[1], *R. Chen*[1], *K.H. Wong*[1]
[1]University of Macau, Macau, China

Fungi possess remarkable capabilities for synthesizing diverse secondary metabolites (SMs) with significant applications in medicine, agriculture, and industry. SM biosynthetic genes are often organized in clusters on fungal genomes. Genome sequencing has identified many novel SM biosynthetic gene clusters (BGCs) in fungi, representing a rich resource for drug discovery. However, most SM BGCs remain transcriptionally silent under standard laboratory conditions, presenting challenges for their identification. Many BGCs contain a transcription factor gene that activates the biosynthetic genes within the cluster under specific conditions. Whether cluster-specific transcription factors can bind and regulate genes outside their cluster is not clear. We hypothesize that BGC transcription factors may regulate non-BGC genes whose functions could affect the biosynthesis of the intended SM. Therefore, it may be possible to deduce the conditions affecting SM production from its cluster-specific transcription factor target information. To demonstrate this, we conducted Chromatin Immuno-Precipitation followed by Sequencing (ChIP-Seq) to map the genome-wide binding sites of AflR – the well-studied transcription factor controlling the biosynthesis of the harmful mycotoxins sterigmatocystin (ST) and aflatoxin. Our results revealed numerous AflR bindings beyond the ST BGC, exerting control over various physiological processes. More importantly, based on AflR target information, we successfully devised a condition that affects ST production. Taken together, this work provides valuable insights into the regulation of ST and aflatoxin biosynthesis and introduces a novel approach to activate cryptic SM BGCs, enabling the discovery of novel secondary metabolites.

WP1.27 - Role of the red-light and blue-light photoreceptors in the photoadaptation of transcription in *Aspergillus nidulans*

A. Franco-Cano[1], *G. Gutiérrez*[1], *L.M. Corrochano*[1], *D. Cánovas*[1]
[1]University of Seville, Department of Genetics, Seville, Spain

Fungal responses to light include the regulation of the developmental programs and the regulation of secondary metabolism, among others. The responses to light are mediated by induction of gene expression. However, this induction is transient, and gene expression returns to dark levels in a process known as photoadaptation. *In Aspergillus nidulans*, light sensing requires both the red photoreceptor, the phytochrome FphA, the blue photoreceptors, LreA and LreB, and the UV/blue photoreceptor CryA. Although the *Aspergillus* genomes lack a homolog of *vvd*, the gene responsible for photoadaptation in *Neurospora crassa*, photoadaptation is still observed in *A. nidulans*, suggesting the existence of an alternative regulatory mechanism. To study photoadaptation in A. *nidulans*, the wild-type and photoreceptor mutant strains were exposed to

light for different times ranging from 15 to 240 min. RNAseq experiments show that light regulates over 20% of the genome. Clustering analysis of light-regulated genes resulted in six regulatory profiles, which were similar in the wild type and the Δ*lreA* Δ*lreB* mutant, but not in the Δ*fphA* strain. The deletion of *fphA* led to a reduction in the number of light-regulated genes (from 2596 to 1680) with a transcriptional pattern very different to that observed in the wild type. Gene onthology revealed a sequential rewiring of gene expression: metabolic changes come first, possibly due to mitochondrial stress, followed by nucleic acid modification and repair. Based on the expression profiles, our results suggest the existence of novel regulators that could be responsible of photoadaptation in *Aspergillus*.

WP1.28 - Functional analysis of maltose/isomaltose transporters using the nuclear translocation assay of the transcription factor AmyR in *Aspergillus oryzae*

S. Fujita[1], *D.M. Jeong*[1], *K. Gomi*[1]
[1]Tohoku University, Grad. Sch. Agric Sci., Sendai, Japan

Maltose uptake is crucial for amylolytic enzyme production in the industrially-important filamentous fungus *Aspergillus oryzae*. Previously, we reported that MalP acts as a major maltose transporter in *A. oryzae*, and a *malP* disruptant exhibited a significant decrease in maltose consumption and α-amylase activity. Intracellular maltose is converted to isomaltose, which induces the nuclear translocation of the transcription factor AmyR, regulating amylolytic enzyme production. Interestingly, extracellular isomaltose also rapidly induces nuclear translocation of AmyR, although isomaltose is barely incorporated in the cell, suggesting that *A. oryzae* has an isomaltose sensor protein or significantly low-affinity transporter contributing to AmyR activation. In this study, we used the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) to examine whether an isomaltose sensor or transporter exists. Firstly, the nuclear translocation of AmyR was not observed upon the addition of maltose in the presence of CCCP, suggesting that MalP is a proton symporter. Also, the isomaltose-induced nuclear translocation of AmyR was suppressed even at low concentrations of CCCP. This result suggests that isomaltose is not recognized by a sensor but is taken up in quite small amounts by an unidentified sugar transporter(s). We also present the results of similar experiments conducted with the model fungus *Aspergillus nidulans* in which an isomaltose sensor/transporter has been identified[1)].
1) Jeong Da Min et al., The 32nd Fungal Genetics Conference, 293A (2024).

WP1.29 - Genetic dissection of the role of proteins associated with ER-PM contacts in *Aspergillus nidulans*: VapA is essential for growth and polarized recycling of lipid flippases

X. Georgiou[1], *S. Politi*[1], *G. Diallinas*[1,2]
[1]University of Athens, Department of Biology, Athens, Greece, [2]Foundation for Research and Technology, Institute of Molecular Biology and Biotechnology, Heraklion, Greece

Endoplasmic reticulum (ER)-plasma membrane (PM) tethering proteins play a crucial role in eukaryotic cells by anchoring the ER to the PM and other cellular membranes, establishing contact sites that serve as platforms for non-vesicular lipid transfer and lipid homeostasis. This study explores the cellular role of ER-PM tethering proteins in *Aspergillus nidulans* through reverse genetics and quantitative live-cell imaging. By systematically deleting genes involved in ER-PM contact formation, we identify VapA (single homologue of Scs2/22 in *Saccharomyces cerevisiae*) as essential for proper growth and apical distribution of specific polarized membrane cargoes, while it does not affect the steady state localization on non-polarized cargoes, such the UapA purine transporters or the major proton pump ATPase PmaA. More specifically, VapA is required for the apical localization of lipid flippases DnfA and DnfB, as well as the R-SNARE protein SynA. Interestingly, VapA is redundant for the apical localization of chitin synthase ChsB, which is also an apically localized membrane protein. Preliminary findings further suggest that VapA is involved in the local recycling of apical markers at the subapical region necessary for polarity maintenance during hyphal growth. This work paves the way to uncover mechanistic insights into the VapA role in membrane cargo trafficking, thus broadening our understanding of ER-PM contact functions.

WP1.30 - Distinct trafficking routes of polarized and non-polarized membrane cargoes in *Aspergillus nidulans*

G.M. Sagia[1], X. Georgiou[1], G. Chamilos[2,3], S. Dimou[1], G. Diallinas[1,3]
[1]University of Athens, Department of Biology, Athens, Greece, [2]University of Crete, School of Medicine, Heraklion, Greece, [3]Foundation for Research and Technology, Institute of Molecular Biology and Biotechnology, Heraklion, Greece

Membrane proteins are sorted to the plasma membrane (PM) via Golgi-dependent trafficking. However, our recent studies challenged the essentiality of Golgi in the biogenesis of specific transporters. Here, we investigate the trafficking mechanisms of membrane proteins by following the localization of the polarized R-SNARE SynA versus the non-polarized transporter UapA, synchronously co-expressed in wild-type or isogenic genetic backgrounds repressible for conventional cargo secretion. In wild-type, the two cargoes dynamically label distinct secretory compartments, highlighted by the finding that, unlike SynA, UapA does not colocalize with the late-Golgi. In line with early partitioning into distinct secretory carriers, the two cargoes collapse in distinct ERES in a sec31ts background. Trafficking via distinct cargo-specific carriers is further supported by showing that repression of proteins essential for conventional cargo secretion does not affect UapA trafficking, while blocking SynA secretion. Overall, this work establishes the existence of distinct, cargo-dependent, trafficking mechanisms, initiating at ERES and being differently dependent on Golgi and SNARE interactions.

WP1.31 - Identification of potential regulators of the transcription factor AmyR using the screening method based on growth defects caused by *brlA* overexpression in *Aspergillus nidulans*

K. Gomi[1], T. Shintani[1], D.M. Jeong[1], J. Tokashiki[1]
[1]Tohoku University, Graduate School of Agricultural Science, Sendai, Japan

Amylolytic gene expression is regulated by the transcription factor AmyR in *Aspergillus* species, but the factors involved in the functional regulation of AmyR have not been elucidated. Therefore, to identify unknown potential regulators of AmyR, we constructed an *Aspergillus nidulans* strain that overexpressed *brlA*, which is involved in conidiation, under the control of the α-amylase gene promoter. The resulting strain showed a significantly restricted growth in the presence of isomaltose, an inducer of amylase production. Using this strain as a parent, spontaneous mutant strains that recovered growth were isolated on isomaltose-containing agar medium. Consequently, we successfully identified a novel sugar transporter involved in isomaltose transport/sensing through whole genome sequencing of the spontaneous mutants. On the other hand, the screening method we developed can be applied to find unknown factors other than the transporter/sensor, such as cofactors and modifying enzymes, involved in the functional regulation of AmyR. For this purpose, using *A. nidulans* ABPU1/ΔligD (*wA3, argB2, biA1, pyroA4, pyrG89, ΔligD::ptrA*) as a host, we constructed a strain in which two copies of *brlA* overexpression cassette were integrated into the *argB* and *biA* loci, and the *amyR* and isomaltose transporter/sensor genes were integrated into the *pyrG* and *pyroA* loci, respectively. As in the previous study, this strain showed a significantly poor growth in the presence of isomaltose. We could isolate several spontaneous mutant strains that restored growth on isomaltose agar medium using this strain as a parent, and attempted to identify mutated genes by whole genome sequencing.

WP1.32 - Boosting *Aspergillus* antifungal research combining multiplex CRISPR/Cas9 with counter-selection

L.E. Sastré-Velásquez[1], *N. Schiefermeier-Mach*[2], *A. Kühbacher*[1], *B. Mertens*[1], *C. Baldin*[3], *L. Lechner*[2], *G. Diallinas*[4], *F. Gsaller*[1]
[1]Medical University of Innsbruck, Institute of Molecular Biology, Innsbruck, Austria, [2]Health University of Applied Sciences Tyrol/FH Gesundheit Tirol, Research and Innovation Unit, Innsbruck, Austria, [3]Leopold-Franzens University of Innsbruck, Institute of Microbiology, Innsbruck, Austria, [4]National and Kapodistrian University of Athens, Department of Biology, Athens, Greece

Aspergillus fumigatus, the main causative agents of invasive aspergillosis, represents one of the deadliest fungal species worldwide and accounts for hundreds of thousands of deaths each year. In addition to a shortage in the antifungal armory comprising only three drug classes, resistance to the major class employed in the clinical setting, the azoles, is steadily increasing. A comprehensive understanding of the molecular mechanisms driving azole resistance is essential for developing new strategies to combat this problem.
The recent discovery of multiple, endogenous counter-selectable markers enabled site-directed insertion of numerous additional expression cassettes into the genome of *A. fumigatus*, which facilitated research applications that required multigene integration. The shortcoming, gene cassettes had to be transformed sequentially.
In this study, we overcame this obstacle by combining the use of CRISPR/Cas9 with the mentioned markers, successfully integrating multiple expression cassettes in a single

transformation event. Exploiting this new technique, we generated mutants carrying different resistance alleles and strain-specific fluorescent protein tags to simultaneously analyze four strains during azole treatment employing multicolor fluorescence microscopy.
We anticipate that the presented method will bolster a wide range of research applications that require facile and rapid equipment of strains with multiple expression cassettes and will therefore open new avenues in antifungal research.

WP1.33 - Three [2Fe-2S] cluster-binding regions regulate the functional transitions of the *Aspergillus fumigatus* iron regulator HapX for adaptation to iron starvation, sufficiency and excess

S. Oberegger[1], *M. Misslinger*[1], *H. Haas*[1]
[1]Institute of Molecular Biology, Medical University Innsbruck, Biocenter, Innsbruck, Austria

Accurate sensing of cellular iron levels is vital, as this metal is essential but toxic in excess. The iron-sensing transcription factor HapX is crucial for virulence of *Aspergillus fumigatus*, the predominant human mold pathogen. Its absence impairs growth under iron limitation and excess, but not under moderate iron availability, suggesting that HapX switches between three states to adapt to varying iron availability. Previous studies have shown that HapX senses iron excess by [2Fe-2S] cluster binding to three phylogenetically conserved cysteine-rich regions (CRRs), termed CRR-A, -B, and -C.
This study suggests that the HapX state transitions are regulated by the different propensities of the CRRs to coordinate [2Fe-2S] clusters resulting in cumulative occupancies that depend on iron availability. In the iron starvation state, CRR-B and -C lack [2Fe-2S] clusters, the iron sufficiency/neutral state features clusters in CRR-B and/or -C and the iron excess state has clusters in all CRRs. Combinatorial mutation of CRR-B and -C caused synthetic lethality by locking HapX in the iron starvation state, leading to uncontrolled iron uptake, iron accumulation, repression of iron-consuming pathways and impaired iron detoxification. Loss of the C-terminal 27-amino acid region of HapX, which is crucial for the iron starvation state and was found to contain an F-box protein Fbx22-binding degron, rescued the synthetic lethality.

WP1.34 - Beware the air?: Exploring indoor airborne fungal communities and urban chemical pollutants

S. Hemmings[1], *S. Fitzgerald*[1], *L. Barron*[1], *F. Kelly*[1], *M. Fisher*[1]
[1]Imperial College London, London, United Kingdom

In modern western societies, individuals spend up to 90% of their time indoors, yet while outdoor air quality has been extensively studied, the quality of indoor air—particularly the pollutants we inhale at home—has received less attention. Monitoring indoor air quality is essential, especially regarding exposure to mould, volatile organic compounds (VOCs), and particulate matter, all of which are linked to respiratory issues and infectious diseases. As part of the WellHomes project, this study analysed airborne fungal communities in 113 homes in North-West London through passive air sampling conducted between 2022 and 2024, with comparisons to outdoor

environments. Amplicon sequencing revealed a significant prevalence of fungal genera such as *Penicillium* and *Aspergillus* for indoor environments, alongside seasonal variations in fungal community profiles. Quantification of fungal burden using qPCR identified homes with elevated levels of specific fungal pathogens, which were linked to case studies of respiratory issues in occupants. Furthermore, significant correlations were observed between fungal community compositions and VOCs, suggesting potential interactions between biological and chemical pollutants. These findings highlight the need for comprehensive monitoring of indoor environments to better understand the combined effects of biological and chemical pollutants on air quality and public health.

WP1.35 - Establishing *Aspergillus niger* as a production system for azaphilone colourants from fungi

F. Hoberg[1], *L. Lietz*[1], *C. Freidank-Pohl*[1], *V. Meyer*[1]
[1]Technische Universität Berlin, Applied and Molecular Microbiology, Berlin, Germany

Colour is an immanent attribute of almost all items of our daily live. However, most colourants used are of synthetic origin and based on non-renewable petroleum products. Many of these possess irritant, toxic or carcinogenic properties. Thus, the production, dyeing process and use pose health risks to humans when exposed in large quantities. In addition, nature and existing ecosystems can be massively damaged. Due to political bans and social rethinking, demand for natural alternatives has been growing.
Fungi produce a wide range of secondary metabolites (SMs), potentially serving as natural colourants in various industries. In contrast, parallel production of mycotoxin, a relatively slow growth rate or SM production just under specific, potentially unfavourable, conditions, make them irrelevant for an industrial production. A solution is the shift of production into an industrially established heterologous host, i.e. *Aspergillus niger*. *A. niger* has proven to be an efficient heterologous producer for non-ribosomal peptide based SMs with high titers. Further, *A. niger* is capable to provide high quantities of polyketide precursors, rendering it an optimal choice for heterologous gene cluster integration.
Given the capacity of *A. niger* to produce polyketide pigments in high quantities, we aim to establish *A. niger* as a heterologous producer of azaphilones, a promising class of natural colourants within the yellow to red colour spectrum, from the genus *Monascus*. Different strategies of gene expression are investigated and benchmarked against optimal cultivation conditions for *Monascus* species. Results will be presented accordingly.

WP1.36 - Identification and functional analysis of the novel isomaltose sensor/transporter involved in the activation of the transcription factor AmyR in *Aspergillus nidulans*

D.M. Jeong[1], *S. Fujita*[1], *J. Tokashiki*[1], *T. Shintani*[1], *K. Gomi*[1]
[1]Tohoku university, Sendai, Japan

Aspergillus oryzae, or koji mold, is known for its ability to produce high levels of hydrolytic enzymes including amylolytic and proteolytic enzymes, which are used in various industrial applications such as Japanese fermented foods and beverages. In *Aspergillus*, the regulation of amylolytic enzyme gene expression is controlled by the fungal-specific transcription factor AmyR. The induction of AmyR activity is triggered by isomaltose most effectively compared to glucose or maltose. However, the mechanism of AmyR induction in the presence of isomaltose is scarcely studied. Previously, we identified the most putative isomaltose sensor/transporter, AN5050 in *Aspergillus nidulans* through SNP analysis, and showed that deletion of *AN5050* resulted in a significant reduction of amylolytic gene expression in the presence of isomaltose[1)]. In this study, AmyR and AN5050 were tagged with fluorescent proteins to explore their subcellular localization. The AN5050-complemented strain with mCherry showed growth recovery on starch media. Fluorescence analysis revealed that AmyR tagged with eGFP exhibited a significantly delayed nuclear localization in the absence of AN5050. Furthermore, this nuclear localization was observed only when isomaltose was used as an inducer of AmyR, rather than glucose, demonstrating that AN5050 functions as an isomaltose sensor/transporter, although the presence of another minor isomaltose sensor/transporter. Additionally, AN5050 was found to be localized in the transmembrane region. Interestingly, the plasma membrane localization of AN5050 was not affected by the presence of glucose, suggesting that AN5050 does not undergo endocytic degradation.

1) Jeong et al., The 32nd Fungal Genetics Conference, 293A (2024).

WP1.37 - Rapamycin sensitivity is induced by lithium in *Aspergillus fumigatus*: structural, binding, and phenotypic effects of lithium induced rapamycin binding to FKBP12

B.G Bobay[1], D.C. Cole[2], W.J Steinbach[3], P.R Juvvadi[3]
[1]Duke University Medical Center, Department of Radiology, Durham, United States, [2]North Carolina State University, Department of Biological Sciences, Raleigh, United States, [3]University of Arkansas for Medical Sciences, Department of Pediatrics, Little Rock, United States

Antifungal resistance in fungal pathogens is a multifaceted clinical challenge making combination therapy a valuable alternative to monotherapy. The target of rapamycin (TOR), also known as FKBP-rapamycin associated protein (FRAP), is a conserved serine/threonine kinase from yeast to humans. It is a multi-functional kinase regulating key aspects of fungal growth and pathogenicity making it an attractive target for antifungal development and combinatorial regimens with current clinical antifungals. The inhibition of TOR kinase occurs via rapamycin binding to the immunophilin FKBP12, and the FKBP12-rapamycin complex binding to TOR. Despite the essential nature of TOR kinase in *Aspergillus fumigatus*, previous studies have shown that rapamycin (sirolimus; everolimus) or rapamycin analogs (INK128; AZD8055) exhibit poor antifungal activity alone but are synergistic with azoles. To distinguish between the fungal versus mammalian FKBP12 counterparts mediating rapamycin inhibition, we compared the *A. fumigatus* strain expressing native FKBP12 (Af-FKBP12) and human FKBP12 (H-FKBP12). We show that LiCl enhanced rapamycin antifungal activity and was synergistic with the calcineurin inhibitor FK506 in the Af-FKBP12 expression strain. However, the H-FKBP12 expression strain showed sensitivity to rapamycin in the absence of LiCl. Molecular dynamic (MD) simulations were

performed to more accurately characterize FKBP12's solution structure bound to rapamycin and/or (m)Tor (FRAP) in the presence and absence of Li^{1+}. Our MD simulations provided plausible reasons for the observed phenotype of Li^{1+} increasing rapamycin sensitivity in Af-FKPB12 with no observable effect in the h-FKPB12 system. FKBP12-rapamycin interactions distinguishing the fungal versus the human system might be useful in designing fungal-specific rapamycin analogs.

WP1.38 - Regulation of the citrate exporter-encoding *cexA* during *Aspergillus niger* citric acid fermentation

L. Karaffa[1], *A. Márton*[1], *V. Bíró*[1], *A. Tsang*[2], *C.P. Kubicek*[3], *E. Fekete*[1]
[1]University of Debrecen, Debrecen, Hungary, [2]Concordia University, Montreal, Canada, [3]TU Wien, Vienna, Austria

Two critical parameters of the *Aspergillus niger* citric acid fermentation are the high (14%) glucose levels and the suboptimal (<5 ppb) concentration of manganese(II) ions in the culture broth. To investigate the requirement for manganese deficiency, we compared the transcriptome of a hyper-producer *A. niger* strain at Mn(II) ion deficient (5 ppb) and Mn(II) ion sufficient (100 ppb) conditions. Mn(II) deficiency triggered a 110-times upregulation of the citrate exporter-encoding gene *cexA*. To test whether *cexA* upregulation is a derepression, we grew *A. niger* at both manganese deficiency and sufficiency, but with only 1% glucose. No citric acid accumulated and no *cexA* transcript was detected independently of the concentration of manganese(II) ions, suggesting that the metabolism under manganese deficiency may create a metabolite which induces *cexA*. We surmised that this could be citrate itself. To test this, we grew *A. niger* on 1% glucose, and pulsed the culture with citric acid, which led to expression of *cexA* independently of manganese(II) ion deficiency or sufficiency. We conclude that manganese(II) ions are not repressors of *cexA* transcription, but its upregulation is triggered by the accumulation of citric acid or a metabolite related to its metabolism.

WP1.39 - Regulation of itaconic acid accumulation by the extracellular phosphate ion concentration in *Aspergillus terreus*

L. Karaffa[1], *Á.P. Molnár*[1], *I. Bakondi-Kovács*[1], *A. Márton*[1], *V. Bíró*[1], *E. Fekete*[1]
[1]University of Debrecen, Debrecen, Hungary

Itaconic acid is a five-carbon, unsaturated, weak diprotic acid. Its unique chemical properties are roundly exploited by the polymer industry. On industrial scale, itaconic acid is produced from carbohydrates by large-scale submerged fermentation of the filamentous Ascomycete fungus *Aspergillus terreus*. Itaconic acid overflow requires low pH, high dissolved oxygen levels, high initial carbon source concentration, as well as growth-limiting concentrations of nitrogen and manganese(II) ions. The inhibitory effect of manganese(II) ions is particularly critical, as concentrations as low as >5 ppb reduce itaconic acid accumulation by 20%. In this study, fully optimized itaconic acid fermentations performed in 6-L scale bioreactors – where all cultivation conditions including initial phosphate concentration were optimal for maximal volumetric yield –

were compared with fermentations where initial phosphate concentrations were set higher or lower than the optimal value. Fermentations were performed on D-xylose or D-glucose as sole carbon sources. We demonstrate that phosphate ion limitation facilitates itaconic acid accumulation on at least three different grounds, i.e., (1) shifting the carbon flux between biomass and product formation in favour of the latter, (2) attenuating the inhibitory effect of manganese(II) ions, and (3) increasing the expression and activity of the cyanide-resistant alternative oxidase.

WP1.40 - Flotillin-containing lipid raft microdomains are linked to calcium in *Aspergillus nidulans*

M. Kawashima[1,2], *T. Krüger*[1], *A.A. Brakhage*[1,2]
[1]Leibniz Institute for Natural Product Research and Infection Biology, Department of Molecular and Applied Microbiology, Jena, Germany, [2]Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany

Lipid rafts are tight assemblies of proteins and lipids in a biological membrane and are thought to be involved in many physiological processes such as immune signalling and host-pathogen interactions. However, due to their small sizes exceeding the resolution limit of conventional light microscopy, direct measurement and characterisation of lipid rafts in living membranes remains to be a challenge. While most studies on lipid rafts have been carried out on mammalian cells, here we use the genetic model fungus *Aspergillus nidulans* to allow a more versatile characterisation of lipid rafts at both molecular and organismic levels. In particular we investigate the role of flotillin (FloA), a lipid raft marker conserved across many organisms. Using a nanoluciferase reporter strain, we demonstrate that the *A. nidulans* flotillin FloA is highly expressed when the fungus is confronted with high calcium stress. Transcriptomic analysis further reveals that repression of *floA* under these conditions leads to the upregulation of numerous mitochondrial genes, suggesting a functional connection between FloA and mitochondrial activity. In addition, in vivo protein-proximity labelling is performed to assess the physical interaction partner proteins of FloA. We also explore the role of FloA in microbial communication through co-cultivation experiments with the soil bacterium *Streptomyces iranensis* and show that the bacterium is able to induce the high expression of FloA, most likely by secreting a natural product. These findings contribute to a deeper understanding of lipid raft dynamics and the organisation of eukaryotic membranes.

WP1.42 - Post-translational modifications in response to hypoxia in the filamentous fungus *Aspergillus fumigatus*

L. Ivanova[1], *T. Krüger*[1], *A. Bigalke*[1], *T. Heinekamp*[1], *M. Cyrulies*[2], *M. Agler-Rosenbaum*[2,3], *A.A. Brakhage*[1,3], *O. Kniemeyer*[1]
[1]Leibniz Institute for Natural Product Research and Infection Biology, Molecular and Applied Microbiology, Jena, Germany, [2]Leibniz Institute for Natural Product Research and Infection Biology, Bio Pilot Plant, Jena, Germany, [3]Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany

The ability of the filamentous fungus *Aspergillus fumigatus* to adapt to hypoxia is an important virulence trait. The transcription factor SrbA is the central regulator of the fungal hypoxia response. However, little is known about the adaptation to hypoxia on the protein and posttranslational level. In order to get more insights, we performed quantitative proteomics, phospho- and thio redox-proteomics analyses by comparing fungal mycelium grown under either normoxic or hypoxic conditions. Protein were extracted from mycelium and analyzed after tryptic digest by LC-MS/MS. Phosphopeptides were enriched using a TiO_2/ZrO_2 solid phase extraction protocol, while the identification of oxidative thiol modification was determined by the OxICAT technology. We identified in total 5136 proteins, of which 318 proteins and 1674 phosphopeptides showed significantly different abundance upon hypoxia (fold change >4). In particular proteins involved in mitochondrion organization, amino acid metabolism, and lipid metabolic processes increased in abundance under hypoxia. The phosphoproteomic data indicated further that the mitotic cell cycle and autophagy processes were differentially regulated under hypoxic growth. Indeed, phosphopeptides derived from proteins of the Atg1 signaling complex, which is known to initiate autophagosome formation, showed drastic changes in phosphorylation under hypoxia. Redox proteomics revealed 44 cysteine-containing peptides with a decreased and 36 peptides with an increased level of thiol oxidation under hypoxia. A cysteine in the osmotic stress regulating mitogen-activated protein kinase SakA showed a most drastic level of thiol oxidation under hypoxia. Based on these findings, the observed regulatory mechanisms will be investigated further on the genetic level.

WP1.43 - Novel pore-forming aegerolysins and their MACPF partners from fungi

N. Kraševec[1], *L.L. Popovšek*[2], *Č. Pantner*[3], *M. Skočaj*[2], *K. Sepčić*[2]
[1]National Institute of Chemistry, Department of Molecular Biology and Nanobiotechnology, Ljubljana, Slovenia, [2]University of Ljubljana, Biotechnical Faculty, Department of Biology, Ljubljana, Slovenia, [3]University of Ljubljana, Biotechnical Faculty, Department of Microbiology, Ljubljana, Slovenia

The aegerolysin family has received increasing attention in recent years due to its potential applications in biomedicine and biotechnology, despite the limited knowledge of its function. For example, some fungal aegerolysins can serve as probes for the detection, labelling, and imaging of specific membrane lipids, lipid rafts, cancer cells, invertebrates, or parasites. Their genes and their expression, or antibodies produced against aegerolysins, can serve as biomarkers or immunodiagnostic tools for the progression of fruiting body differentiation, fungal pathogens exposure, or infectious disease progression. In combination with larger protein partners, some of them can form pore-forming complexes that can be used to selectively eliminate insect pests or treat certain types of cancer cells.
Although aegerolysins are most abundant in fungi, some of them are also found in other kingdoms of the tree of life. The highly conserved beta-sandwich structure of these low molecular weight proteins is based on low identity primary sequences. Their sequences and copy numbers appear to be species and strain-specific. We have compared their phylogenetic tree with taxonomic distribution of the species. Different combinations of lipids are involved in the interactions of aegerolysins with different target organisms and may involve further interactions

with various larger non-aegerolysin partners that can lead to pore formation. We biochemically characterized four novel aegerolysins and their MACPF protein partners from mushrooms *Heterobasidion irregulare*, *Trametes versicolor*, *Mucidula mucida* and *Lepista nuda* and compared them with the best-studied aegerolysins from the fungal genus *Pleurotus* and the previously studied aegeroysins from *Beauveria bassiana* and *Aspergillus niger*.

WP1.44 - Discovery and biosynthesis of antifungal polyhydroxy-polyketide acrophialocinol

C. Wieder[1,2], *M. Künzer*[1,2], *R. Wiechert*[3], *K. Seipp*[3], *K. Andresen*[2], *A. Schüffler*[1], *T. Opatz*[3], *E. Thines*[1,2]
[1]Institut für Biotechnologie und Wirkstoff-Forschung gGmBH, Mainz, Germany, [2]Johannes Gutenberg-University, Microbiology and Biotechnology, Mainz, Germany, [3]Johannes Gutenberg-University, Department of Chemistry, Mainz, Germany

During ongoing screening efforts for antifungal compounds, *Acrophialophora levis* IBWF 127-08 garnered attention due to the potent antifungal activity of its extracts against a variety of phytopathogenic fungi. Bioactivity-guided isolation led to identification of the main active components as two novel PMA-clade polyhydroxy-polyketides we termed acrophialocinol and its dehydroxylated precursor acrophialocin. Mining the *de novo* sequenced genome of *A. levis* IBWF 127-08 identified a candidate biosynthetic gene cluster *acr*, whose genes were then (co-)expressed in *Aspergillus oryzae* OP12 to heterologously reconstitute the biosynthetic pathway. Metabolite analyses of the transformants revealed that biosynthesis of acrophialocinol may occur via either of the direct precursors acrophialocin or malaysic acid, and involves the hrPKS AcrA, which in turn relies on the *trans*-ER AcrB for alkenyl reduction as well as the truncated NRPS AcrC for hydrolytic product release. While the cytochrome p450 monooxygenase AcrE is required for hydroxylation of a terminal methyl group, α-hydroxylation of the precursors pre-acrophialocin and malaysic acid is catalyzed by the α-ketoglutarate-dependent dioxygenase AcrF, representing an unprecedented reaction in polyhydroxy-polyketide biosynthesis. Furthermore, heterologous expression of the RTA1-like protein coding gene *acrD*, which is conserved across PMA-clade biosynthetic gene clusters, in *A. oryzae* RIB40 conferred resistance to acrophialocin, therefore likely contributing to polyhydroxy-polyketide autoresistance in the native producer.

WP1.45 - Mutation of nitrogen source assimilation in industrial strains of *Aspergillus oryzae*

S. Miki[1,2], *K. Sakai*[1], *T. Nakagawa*[2], *T. Tanaka*[1], *L. Liu*[1], *H. Yamashita*[2], *K.-I. Kusumoto*[1]
[1]Osaka University, Department of Biotechnology, Graduate School of Engineering, Donated Lab of Breeding Engineering for Koji Mold, Suita, Japan, [2]Higuchi Matsunosuke Shoten Co., Ltd., Osaka, Japan

Aspergillus oryzae mutants defective in nitrogen source assimilation are known to grow poorly on Czapek-Dox (CD) medium. In this study, we found an industrial strain of *A. oryzae* that grew very poorly on a CD medium containing sodium nitrate as a nitrogen source. We used media with

various nitrogen components to examine the steps affecting the nitrogen source assimilation pathway of this strain. The strain grew well on the CD medium supplied with nitrite salt or ammonium salt, suggesting that the strain was defective in nitrate assimilation step. To ascertain the gene causing the defect of nitrate assimilation, a gene expression vector harboring either *niaD* or *crnA* of *A. oryzae* RIB40 was introduced into the industrial strain. The industrial strain containing the *crnA* vector recovered its growth. This is the first report that a mutation of *crnA* causes poor growth on CD medium in an industrial strain of *A. oryzae*, and *crnA* can be used as a transformation marker for *crnA* deficient strains.
This work was published in Journal of Bioscience and Bioengineering, 137(4), Pages 231-238 (2024)
[#]The first two authors contributed equally to this work.

WP1.46 - Guardian of the cell: How cryptochrome balances the stress response in Aspergillus nidulans

A. Landmark[1], *R. Fischer*[1]
[1]Karlsruhe Institute of Technology, Karlsruhe, Germany

The perception of light by fungi plays a significant role in adapting to environmental changes like the time of day, rising temperature, or changes in the oxygen level. It relies primarily on the red- and blue-light spectrum and the corresponding light receptors, the red/far-red light-absorbing phytochrome, and the blue-light sensing *White Collar Complex*. These light receptors act independently or together to modulate gene transcription and/or protein activity and impact the fungal development, growth, and metabolite production in response to light stimuli. Members of another blue-light receptor group, the cryptochrome/photolyase family (CPF), play a key role in the processing and transduction of light signals in plants and animals. However, they seem to have only a minor impact on the blue light response in fungi.
Here, we show that the photolyase CryA from *Aspergillus nidulans* modifies the phytochrome activity. CryA, previously described as a cryptochrome-like photolyase, is involved in sexual and asexual development of *A. nidulans*. We show that it uses FAD and MTHF as chromophores and that CryA alters the transcription of blue- and red-light-dependent genes by physically interacting with the phytochrome FphA of *A. nidulans.* It thereby negatively regulates the red-light response. These results provide a deeper insight into the effect of light on fungal development, suggesting a more complex role for fungal CPF members and their coordination with other light receptors.

WP1.48 - Metabolic engineering of *Aspergillus violaceofuscus*

C. Wieder[1,2], *C. Laubenstein*[1,2], *L. Fischer*[2], *R. Wiechert*[3], *K. Seipp*[3], *A. Schüffler*[1], *T. Opatz*[3], *E. Thines*[1,2]
[1]Institut für Biotechnologie und Wirkstoff-Forschung gGmBH, Mainz, Germany, [2]Johannes Gutenberg-University, Microbiology and Biotechnology, Mainz, Germany, [3]Johannes Gutenberg-University, Department of Chemistry, Mainz, Germany

Fungal secondary metabolites (SMs) are a rich source for bioactive compounds with various applications in agriculture, biotechnology and medicine. However, discovering novel compounds with desirable bioactivities remains challenging as numerous gene clusters involved in their synthesis remain silent under laboratory conditions. *Aspergillus violaceofuscus* is a fungus with significant biosynthetic potential, containing over 90 predicted biosynthetic gene clusters (BGCs), albeit only few metabolites are produced under standard culturing conditions. In this study, we aimed to engineer the secondary metabolism of *A. violaceofuscus* by creating genetic dereplication strains with reduced metabolic background and overexpressing cryptic biosynthetic genes. We were able to establish an efficient Cas9-mediated microhomology-directed repair (MHDR) protocol, achieving integration rates of up to 90 % and successfully deleted the core genes encoding for the biosynthesis of the major metabolites eupenoxide and himeic acid A. Homologous overexpression of a cryptic NRPS-like coding gene led to the production of a new compound we termed violafuranone A. In conclusion, we demonstrate metabolic manipulation of *A. violaceofuscus* and the potential of this species as a source for novel SMs. These findings pave the way for further investigations into the secondary metabolism of *A. violaceofuscus*.

WP1.49 - The evolutionary landscape of primary carbon metabolism across the *Aspergillus* genus

J. Li[1], *F. Dekker*[1], *M. Peng*[1], *R.P. de Vries*[1]
[1]Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands

Sugar conversion through primary carbon catabolism in fungi is a complex progress that involves many pathways. As such, detailed insight into sugar metabolism of different species is highly relevant for our understanding of the role of fungi in their natural environment as well as for engineering the sugar metabolic pathways for the production of interesting biochemicals. In a previous FICUS project, we evaluated sugar metabolic models of six taxonomical distant species and revealed that transfer of the sugar metabolic model to other Eurotiomycetes is highly reliable. However, we also noticed significant differences in the transcriptomic response of metabolic genes in Aspergillus niger and Aspergillus nidulans.
As part of the whole genus genome project of Aspergillus, genome sequences for nearly 300 Aspergillus species have been generated. In this project we generated models for primary carbon metabolism for all these species and used them to generate a detailed evolutionary map of this important biological process across the genus, as well as compared to a set of non-Aspergillus reference fungi. This demonstrated the high diversity of primary carbon metabolism among fungi, with frequent gene duplications and losses of genes encoding enzymes catalyzing individual metabolic steps.

WP1.50 - Characterization of GH67 and GH115 α-1,2-glucuronidases (AGUs) for improvement of xylooligosaccharide production

L. Li[1], *P. Sun*[2], *M.A. Kabel*[2], *R.P. Vries*[1]
[1]Utrecht University & Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands,
[2]Wageningen University & Research, Laboratory of Food Chemistry, Wageningen, Netherlands

Xylan, one of the most abundant components of renewable biomass, is a complex heterogeneous polysaccharide composed mainly of a xylosyl backbone with substitutions. Complete saccharification of xylan requires the coordinated action of several hemicellulases. However, our knowledge of these enzyme systems is incomplete and many putative enzymes identified in fungal genomes have not been characterized. In this study, we characterized a set of α-1,2-glucuronidases (AGUs) that remove (4-O-methyl)-glucuronic acid from xylosyl units.
AGUs are so far classified in Glycoside Hydrolase (GH) family 67 and 115. We identified putative AGU-encoding genes in several fungal species, such as *Aspergillus oryzae* and *Penicillium subrubescens* and expressed these in *Pichia pastoris*. The produced enzymes were purified and characterized for their biochemical properties, substrate specificities, product profiles and synergy with xylanases. This project deepens our understanding of the diversity of AGUs and explores their potential to improve xylooligosaccharide production.

WP1.52 - Identification and characterization of enzymes involved in the tannic acid and gallic acid metabolism of *Aspergillus niger*

R. Lubbers[1], *S. Prins*[1], *N. Dimitrov*[1], *J. van Dam*[1], *F. Calcan*[1], *M. Arentshorst*[1], *A. Tsang*[2], *A. Ram*[1]
[1]Leiden University, Institute Biology Leiden, Microbial Sciences, Fungal Genetics and Biotechnology, Leiden, Netherlands, [2]Concordia University, Department of Biology, Montreal, Canada

Tannic acid is a plant polymeric aromatic compound consisting of multiple gallic acid (3,4,5-trihydroxybenzoate) molecules bound to glucose. Certain fungi, such as *Aspergillus niger*, are able to degrade plant biomass by secreting enzymes. For tannic acid, specific tannases are released by *A. niger* that can hydrolyze gallotannins resulting in the release of gallic acid and glucose. Gallic acid is a valuable aromatic compound with many pharmacological and industrial applications but is also a source of pollution. Therefore, understanding the gallic acid metabolic pathway can be of great interest to utilize tannic acid to create gallic acid or to bioremediate gallic acid pollutants. In *A. niger*, gallic acid is further metabolized intracellular as carbon source. Recently, we identified a repressor/regulator (TanX/TanR) complex involved in the regulation of tannases and the gallic acid metabolism.
In this presented work, we used whole genome transcriptomics on the *tanX* deletion strain, in combination with RNA extracted from a tannic acid grown *A. niger* culture to identify genes involved in the metabolism of gallic acid. Four highly induced candidate genes were selected, and deletion strains were made using CRISPR/CAS9. Phenotypic analysis of the strains on multiple aromatic compounds showed growth defects on tannic acid and gallic acid, indicating that these genes encode enzymes required for gallic acid utilization. To biochemically characterize these four enzymes, the genes encoding the enzymes were overexpressed as His-tagged-proteins and complementation studies confirmed that all enzymes were functional. The enzymes were successfully purified and currently analyzed for their activity on gallic acid.

WP1.53 - Functional and physiological characterization of polyol transporters of *Aspergillus niger*

C. Lyra[1,2], *L. Nummela*[1,2], *R. Mewalal*[3], *I. Blaby*[3], *H.N. Maina*[2], *J.T. Pronk*[4], *R. Mans*[4], *I.V. Grigoriev*[3], *M.R. Mäkelä*[1,2]
[1]Aalto University, Espoo, Finland, [2]University of Helsinki, Helsinki, Finland, [3]Lawrence Berkeley National Laboratory, Berkeley, CA, United States, [4]Delft University of Technology, Delft, Netherlands

Aspergillus niger is a crucial contributor to sustainable solutions within the bio-based economy. It converts plant biomass monosaccharides, such as D-galactose and D-xylose, to polyol intermediates through the oxido-reductive and pentose catabolic pathways[a]. Despite extensive *in silico* identification of polyol transporters in *A. niger* and other fungi, most of these proteins remain uncharacterized. To date, only L-arabitol-transporting LatA has been characterized in *A. niger*[b].
We selected ~30 Major Facilitator Superfamily[c] proteins from three phylogenetic groups[d] for characterization in *A. niger* and a *Saccharomyces cerevisiae* platform strain[e]. Since *S. cerevisiae* lacks efficient pathways to metabolize xylitol, sorbitol and mannitol, we engineered the platform strain with the genes required to metabolize these polyols using CRISPR/Cas9-technology. We used the same technology to generate *A. niger* polyol transporter deletion mutants.
Functional characterization showed that part of the recombinant strains expressing *A. niger* polyol transporter genes grow on media containing 0.05% and 0.5% xylitol and sorbitol, and 0.05% mannitol as carbon source. The improvement of *S. cerevisiae* strains for enhanced metabolism and the generation of some of the desired *A. niger* polyol transporter deletion mutants for physiological characterization are still in process.
Classification of sugar transport connecting the exogenous and endogenous processes in *A. niger* could highly enhance our understanding of plant biomass conversion.
[a]Chroumpi *et al. Microb. Biotechnol.* 159:1–10 (2022)
[b]Meng *et al. Biomolecules* 13:1–11 (2023)
[c]Saier *et al. Nucleic Acids Res.* 49(D1):D461-7 (2021)
[d]Xu *et al. Biores Technol.* 391:1–7 (2024)
[e]de Valk *et al. Biotechnol. Biofuels* 15:1–16 (2022)

WP1.54 - Deciphering the role of the key HR1 regulatory domain of protein kinase C (PkcA) in the fungal pathogen *Aspergillus fumigatus*

J.E.M. Campanella[1], *M.C. Rocha*[1,2], *G.A. Sato*[1], *G. Yanes Filho*[1], *A.F. Cunha*[1], *I. Malavazi*[1,3]
[1]Universidade Federal de São Carlos (UFSCar), Genética e Evolução, São Carlos, Brazil, [2]The Hebrew University of Jerusalem - School of Veterinary Medicine, The Robert H. Smith Faculty of Agriculture, Food and Environment, Rehovot, Israel, [3]National Institute of Science and Technology in Human Pathogenic Fungi, Sao Carlos, Brazil

Aspergillus fumigatus is a human pathogen responsible for severe infections, including invasive pulmonaryaspergillosis in immunocompromised individuals. In fungi, the Cell Wall Integrity

(CWI) pathway regulates cell wall homeostasis and is activated by the apical kinase Protein Kinase C (PkcA). Unlike mammalian PKCs, fungal PKCs, such as PkcA, possess unique N-terminal extensions containing tandem HR1 (Homology Repeat) domains namely HR1A and HR1B subdomains. These domains are hypothesized to mediate interactions with Rho-GTPases and are essential for CWI pathway activity. Here, we investigate the role of the PkcA HR1A/HR1B domains in *A. fumigatus*. Mutants of PkcA lacking either HR1A or HR1B domains were generated and complemented using CRISPR-Cas9 technology. Deletion of the HR1A/B domains resulted in severe growth defects, reduced conidiation, and increased susceptibility to cell wall stressors and heat shock. Additionally, the double $pkcA^{\Delta HR1A/B}$ mutant was non-viable. These mutants exhibited altered subcellular localization patterns compared to the full-length PkcA::GFP, with $pkcA^{\Delta HR1A}$ losing its apical localization. HR1A and HR1B were essential for downstream activation of the CWI pathway through the transcription factor RlmA, with HR1B being dominant over HR1A. Recombinant expression of the HR1 domains and full-length PkcA enabled pull-down assays, which revealed that HR1A and HR1B act as effectors for Rho1 and Rho2 GTPases, but not for Rho4. Notably, only GTP-bound Rho1 binds to HR1A, while both GDP- and GTP-bound Rho1 can bind to HR1B. These findings demonstrate that the HR1 domains are pivotal for PkcA function and are critical for fungal development and, stress adaptation, and virulence.

WP1.57 - Genetic manipulation of global regulator mcrA in Penicillium rubens to generate a natural product library for high throughput bioactivity screening

T. Millam[1], *C. Wang*[1], *J. Shyong*[1]
[1]University of Southern California, Pharmacology, Los Angeles, United States

Fungal secondary metabolites (SMs) are diverse organic compounds often associated with medicinal properties that have changed the landscape of medicine. These SMs include the antibiotic, penicillin, and the cholesterol-lowering agent, lovastatin. Through genome sequencing and analysis, it has been shown that natural products are encoded by biosynthetic gene clusters (BGCs) in the fungal genome. Because most BGCs are silent under normal laboratory conditions, it is crucial to find new ways to activate them. Doing so will allow for the discovery of compounds with bioactivity with the potential to have anticancer, antibacterial, and antifungal effects.
Our strain of focus, Penicillium rubens (IMV00188), is part of the Institute of Microbiology Virology collection collected from the Chernobyl nuclear power plant and the surrounding areas. Using the CRISPR-Cas9 genome editing system, we knocked out the negative global regulator, mcrA, which typically suppresses multiple biosynthetic pathways. We confirmed the deletion of mcrA by designing sequencing primers to perform diagnostic PCR and comparing the growth of wild-type and mcrA knockout strains under various culture conditions. Upon analysis of SM production with liquid chromatography-mass spectroscopy (LC-MS) procedures, we found that our mutant strain activates various pathways otherwise not seen in the wild-type strain. Ultimately, we aim to generate a natural product library and use high throughput screening to investigate the bioactive properties of these upregulated and newly produced compounds.

WP1.58 - The multipurpose cell factory *Aspergillus niger* can be engineered to produce hydroxylated collagen

T. Morris[1], *T.C. Cairns*[1], *V. Meyer*[1]
[1]Technische Universität Berlin, Berlin, Germany

Advances in tissue printing and wound healing necessitate a continuous global supply of collagen. Microbial systems are highly desirable to meet these demands as recombinant collagenous proteins can be guaranteed as free from animal viruses. The filamentous cell factory *Aspergillus niger* has been instrumental for decades in the production of organic acids, enzymes and proteins, yet this fungus has not been explored for recombinant collagen production. In this study, we conducted extensive genetic engineering and fermentation optimization to provide proof of principle that *A. niger* can produce hydroxylated collagen.
We used a modular cloning system to generate a suite of cassettes encoding numerous n-terminal secretion signals, biodesigned/native collagen genes and, additionally, various prolyl-4-hydroxylases (P4H) for protein hydroxylation. These were expressed in a previously constructed *A. niger* isolate which is capable of producing the crucial P4H cofactor ascorbic acid. We conducted a wide range of media optimization studies to increase collagen production and hydroxylation levels. Additionally, we deleted an endopeptidase encoding gene, which was likely responsible for degrading secreted collagen. These studies generated an isolate capable of secreting partially hydroxylated collagen to titres of approximately 5mgL^{-1}. Comparative transcriptomic analyses are currently ongoing to identify further candidate genes for genetic and metabolic engineering approaches.

WP1.59 - Development of a novel siderophore-based antifungal for treatment of *A. fumigatus*

C. Murphy[1], *S. Doyle*[1], *K. Walshe*[1]
[1]Maynooth University, Biology, Maynooth, Ireland

As prevalence of antifungal resistance worldwide increases and the effectiveness of current antifungal regimens decrease, there is a greater need for novel antifungals that do not share the same targets as approved antifungal drugs. As such, one avenue for antifungal development is the repurposing of already approved chemotherapy drugs and one such candidate is methotrexate (MTX) which inhibits the folate pathway which is present in all domains of life. However, MTX is used as a chemotherapy treatment for rheumatoid arthritis and suppresses the immune system, thereby mediating infection in patients and therefore, to reduce the risk of infection, MTX must be conjugated to a suitable carrier. Herein, MTX is conjugated to diacetylfusarinine C (DAFC), an analogue of the fungal siderophore triacetyl fusarinine C (TAFC) which is crucial to the virulence of the human pulmonary pathogen *Aspergillus fumigatus*. The DAFC-MTX conjugate is then labelled with iron and gallium to aid its uptake into *A. fumigatus* and *In vitro* assays demonstrate that the both the FeDAFC-MTX and the GaDAFC-MTX conjugate inhibit the growth of *A. fumigatus* in iron-deplete conditions and GaDAFC-MTX exhibits similar antifungal activity against *A. fumigatus* to MTX by itself. Analysis of liquid cultures shows that both MTX

and GaDAFC-MTX inhibit the production of the siderophore TAFC, but the GaDAFC-MTX conjugate by itself inhibits overall siderophore production in *A. fumigatus* demonstrating that not only can *A. fumigatus* growth can be inhibited, but also the virulence of *A. fumigatus* be attenuated which may improve patient outcome.

WP1.60 - Dysbalance of the murine lung microbiome by the human pathogenic fungus *Aspergillus fumigatus*

L. Nikitashina[1,2], X. Chen[3,2], L. Radosa[1], K. Li[3,2], M. Straßburger[4], B. Seelbinder[3], W. Böhnke[5,2], S. Vielreicher[5,2], S. Nietzsche[6], T. Heinekamp[1], I.D. Jacobsen[5,2], G. Panagiotou[3,2], A.A. Brakhage[1,2]
[1]Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Department of Molecular and Applied Microbiology, Jena, Germany, [2]Friedrich Schiller University, Institute of Microbiology, Jena, Germany, [3]Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Department of Microbiome Dynamics, Jena, Germany, [4]Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Transfer Group Anti-infectives, Jena, Germany, [5]Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Research Group Microbial Immunology, Jena, Germany, [6]University Hospital Jena, Electron Microscopy Center, Jena, Germany

The role of the lung microbiome in health and disease remains debated. Here, we demonstrate significant shifts in the lung microbiome and metabolome of mice under immune suppression, following infection with virulent and avirulent strains of *Aspergillus fumigatus*, a major air-borne fungal human pathogen, and after treatment with the antifungal drug voriconazole. Our analysis of the gut-lung axis indicates the crucial role of the gut microbiome for the lung homeostasis mediated through the plasma metabolome. Notably, we observed that invasive aspergillosis induced gut dysbiosis in the mouse model.
In the lung, *A. fumigatus* infection significantly increased abundance of *Ligilactobacillus murinus*, the dominant bacterium in murine lungs, confirmed by isolating live bacteria from the lower respiratory tract. *In vitro*, *L. murinus* is tolerated and even internalized by alveolar epithelial cells. Co-cultivation with *A. fumigatus* enhanced *L. murinus* growth while reducing oxygen levels. This implies that the fungus creates a microaerophilic niche along its hyphae, fostering anaerobic bacterial growth. The fungal-induced promotion of *L. murinus* both *in vivo* and *in vitro* suggests a possible direct impact of *A. fumigatus* on the resident lung bacteria. Further investigation of the lung microbiome during invasive aspergillosis could provide valuable insights into the interdependence of lung microbiota, infection, and the host immune response.

WP1.61 - Characterization of a new glycerol transporter GlpA from *Aspergillus niger*

L. Nummela[1,2], C. Lyra[1,2], H.N. Maina[2], J.T. Pronk[3], R. Mans[3], M.R. Mäkelä[1,2]
[1]Aalto University, Espoo, Finland, [2]University of Helsinki, Helsinki, Finland, [3]Delft University of Technology, Delft, Netherlands

A recent extensive *in silico* study revealed numerous putative polyol transport protein coding gene candidates in fungi, including glycerol transporters[a]. However, the physiological and functional roles of glycerol transporters in filamentous fungi, such as *Aspergillus niger*, remain poorly understood. Here, we characterized the first known glycerol transporter, GlpA, from *A. niger*.
In *A. niger*, a Δ*glpA* strain exhibited reduced growth and glycerol consumption on solid and in liquid media, respectively, compared to the reference strain. In addition, the deletion mutant showed slower uptake of other polyols, including xylitol, mannitol and galactitol. This suggests a central role for GlpA in glycerol uptake in *A. niger*.
To also study the function of GlpA with minimal interference of other transport proteins, we heterologously expressed it in a *Saccharomyces cerevisiae* strain lacking all hexose and disaccharide transporters, and disaccharide hydrolases[b]. A *glpA*-GFP fusion was constructed to confirm the correct localization of GlpA within the yeast plasma membrane by fluorescent microscopy. The growth of the recombinant *S. cerevisiae* strain was tested on different hexoses and polyols and showed a slightly increased growth rate on polyols compared to the negative control.
This study highlights the physiological importance of GlpA in both glycerol and polyol uptake in *A. niger* and advances our understanding of polyol transporters in filamentous fungi.
[a] Xu *et al. Biores Technol.* 391:1–7 (2024)
[b] de Valk SC *et al. Biotechnol. Biofuels* 15:47 (2022)

WP1.62 - Uncover the potential of secreted Luciferases expressed in Aspergillus niger: fusion-proteins and high-throughput screening

K.J. Ost[1], *M. Arentshorst*[2], *A.F.J. Ram*[2], *M.E. Dirks-Hofmeister*[1]
[1]Osnabrück University of Applied Sciences, Food Biotechnology, Osnabrück, Germany, [2]Leiden University, Molecular Microbiology and Biotechnology, Leiden, Netherlands

Aspergillus niger is a filamentous fungus extensively utilized in industrial biotechnology for the production of enzymes, organic acids, and other metabolites. The increasing demand for enhanced protein production and efficient secretion pathways necessitates the development of novel screening methods. Although luciferase technology has been employed in mammalian cells and bacteria for an extended period, only recent advancements have expanded its applicability in fungal biology to investigate gene expression, signal transduction, and metabolic processes. The research landscape is shaped not only by optimization efforts and persistent challenges, but high-throughput methods are currently a primary focus, particularly for the screening of traits pertinent to industrial enzyme production.
In this study, we report successful heterologous expression and secretion of extracellular luciferases in *A. niger*. Using a luciferase-based high-throughput screening assay in 96-well plates, a sensitive method for evaluating the differences in secretion or production efficiency was introduced. This will be highly valuable for screening genetic modifications, for example, across mutant libraries of secretion signals, in future applications. Moreover, fusion of luciferases with homologous or heterologous proteins offers a straightforward approach for determining the secretion and production efficiencies of proteins, both without any enzymatic activity and with activity that is challenging to measure.

The findings of this study indicate that this novel assay addresses the limitations of conventional screening methodologies and may significantly enhance the application of luciferase technology in filamentous fungi. Moreover, the results demonstrate the potential for subsequent research to expand this approach, facilitating improved production systems for *A. niger*.

WP1.63 - Tracking antifungal resistance in A. fumigatus from compost samples in Norway

M.M. Owczarek-Kościelniak[1], *M. Mølmann Kåråsen*[1], *M.T. Noer*[1,2], *I. Skaar*[1]
[1]Norwegian Veterinary Institute, Chemistry and Toxinology Research Group, Ås, Norway, [2]Oslo University Hospital, Department of Microbiology, Oslo, Norway

Fungi from the genera *Aspergillus* are ubiquitous and easily spreading organisms. Particularly *A. fumigatus* poses significant risks to immunocompromised individuals and other at-risk groups. Recent studies and reports point out *A. fumigatus* as an emerging pathogen with an increasing occurrence of azole-resistant strains. Our study employed citizen science and additionally involved several composting facilities to gather compost samples from across Norway in order to enhance our understanding of environmental *A. fumigatus* populations. Over 100 citizen scientists participated, reflecting strong community engagement in this public health initiative. Using selective cultivation, we screened the samples for potentially resistant strains. Through Minimum Inhibitory Concentration (MIC) and broth microdilution method analyses, the susceptibility of the strains was evaluated, allowing for a comprehensive understanding of antifungal resistance patterns. Based on morphological characteristics and calmodulin/β-tubulin sequencing, the taxonomical identity of the strains was verified, ensuring accurate species identification.
Sequencing of the *cyp51A* gene and its promoter region revealed distinct mutation profiles across the samples. Our findings have shown that resistant isolates were present in approx. 24% of the collected samples. Sequencing of *cyp51A* uncovered the TR34/L98H, TR46/Y121F/T289A, and TR46/Y121F/T289A/S363P/I364V/G448S mutations, occurring individually or in combinations, with highly variable MIC values among isolates, ranging to > 32 mg/L. Obtained results underscore the importance of monitoring environmental *A. fumigatus* for antifungal resistance and its implications for public health, highlighting the need for proactive surveillance strategies.

WP1.64 - Microfluidic control reveals chemotropism of fungal hyphae to nutrients and pH

N. Takeshita[1]
[1]University of Tsukuba, Tsukuba, Japan

The importance of fungi in ecological systems and pathogenicity hinges on their ability to search for nutrients, substrates, and hosts. Despite this, the question of whether fungal hyphae exhibit chemotropism toward them remains largely unresolved and requires close examination at the cellular level. Here, we designed a microfluidic device to assess hyphal chemotropism of *Aspergillus nidulans* in response to carbon and nitrogen sources, as well as pH. Within this

device, hyphae could determine their growth direction in a two-layer flow with distinct compositions that were adjacent but non-mixing. Under conditions with and without a carbon source, hyphae changed growth direction to remain in the presence of a carbon source, but it was still difficult to distinguish between differences in growth and chemotropism. Although nitrogen sources such as ammonia and nitrate are important for growth, the hyphae indicated negative chemotropism to avoid them depending on the specific transporters. This fungus grows equally well at the colony level in the pH range of 4 to 9, but the hyphae exhibited chemotropism to acidic pH. The proton pump PmaA is vital for the chemotropism to acid pH, while the master regulatory for pH adaptation PacC is not involved, suggesting that chemotropism and adaptive growth via gene expression regulation are distinct regulatory mechanisms. Despite various plasma membrane transporters are distributed across membranes except at the hyphal tip, the control of growth direction occurs at the tip. Finally, we explored the mechanisms linking these two phenomena, tip growth and chemotropism.

WP1.65 - Stress-responsive Afu4g10610 gene plays a role in cell wall maintenance and osmotic regulation in *Aspergillus fumigatus*

E. Pelegri-Martinez[1], *U. Perez-Cuesta*[2], *S. Cendon-Sanchez*[1], *A. Ramirez-Garcia*[1], *X. Guruceaga*[3], *A. Rementeria*[1]
[1]University of the Basque Country (UPV/EHU), Department of Immunology, Microbiology and Parasitology, Leioa, Spain, [2]University of Tennessee Health Science Center (UTHSC), Department of Clinical Pharmacy and Translational Science, Memphis, TN, United States, [3]Institute for Multidisciplinary Research in Applied Biology (IMAB), Pamplona, Spain

Aspergillus fumigatus is the most pathogenic species among the fungi of the genus *Aspergillus* and has a high incidence and mortality in immunocompromised patients. Therefore, two distinct *in vitro* infection models of *A. fumigatus*, using murine macrophages (RAW 264.7) and human lung epithelial cells (A549), were employed to identify important genes for fungal adaptation during infection. Transcriptomic analyses of co-incubated *A. fumigatus* uncovered 140 fungal genes up-regulated in common between both models. Furthermore, these results were compared with a transcriptomic study of *in vivo* murine infection model previously published by our group, 13 consistently up-regulated genes were identified in all three infection models. Among them, we investigated the Afu4g10610 gene through the deletion mutant strain (*Δ10610*) generated by CRISPR-Cas9 gene-editing technique. This gene encodes a dimeric A/B barrel domain that is potentially involved in stress response. Phenotypic analysis showed increased sensitivity to cell wall stressors and enhanced resistance to osmotic compounds compared to the wild-type strain Af293. Furthermore, RT-qPCR expression analysis showed a significant imbalance in key mediators of the Cell Wall Integrity (CWI) and High Osmolarity Glycerol (HOG) pathways. Although the precise role and interactions of this gene remain to be elucidated, this dimeric protein seems to be involved in these pathways.

WP1.66 - Discovery of novel fungal carbon metabolic enzymes through transfer learning

M. Peng[1], *W. Wennekers*[1], *R.P. De Vries*[1]
[1]Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands

Developing fungal cell factories to convert plant biomass into value-added compounds is crucial for boosting the circular bioeconomy. This often requires engineering of their primary carbon metabolism (PCM) which frequently fails due to our incomplete understanding of the enzymes involved in these metabolic pathways. In this study, we aim to develop a transfer learning based approach to identify the missing metabolic genes by taking advantages of careful curation of high-quality enzymatic knowledge and maximal extraction of relevant features on both the enzymes and their corresponding substrates using pretrained models. The preliminary results revealed that our new approach successfully predicted several promising PCM candidates of *Aspergillus niger* that that can't be identified by commonly used sequence similarity searches. However, the current prediction model requires further optimization to improve its accuracy and generalizability, and additional biochemical experiments are required to further validate the prediction results. We expect this novel computational method will facilitate the prioritizing novel enzymes involved in fungal PCM to facilitate more effective metabolic engineering of fungal cell factories for a broad range of biotechnology applications.

WP1.67 - Discovery and characterization of the first fungal granulin in *Aspergillus fumigatus*

U. Perez-Cuesta[1], *X. Guruceaga*[2], *S. Cendon-Sanchez*[3], *E. Pelegri-Martinez*[3], *A. Martin-Vicente*[1], *H. Thorn*[1,4], *J. Xie*[1,4], *A. Nandi*[1], *D. Bale*[1,4], *A. Ramirez-Garcia*[3], *J. Fortwendel*[1,5], *A. Abad-Diaz-de-Cerio*[3], *A. Rementeria*[3]
[1]University of Tennessee Health Science Center, Clinical Pharmacy and Translational Science, Memphis, United States, [2]Institute for Multidisciplinary Research in Applied Biology, Public University of Navarre, Pamplona, Spain, [3]University of the Basque Country, Department of Immunology, Microbiology and Parasitology, Leioa, Spain, [4]University of Tennessee Health Science Center, PhD Program in Pharmaceutical Sciences, Memphis, United States, [5]University of Tennessee Health Science Center, Deparment of Microbiology, Immunology and Biochemistry, Memphis, United States

Granulin is a secreted growth factor conserved among eukaryotic organisms. In humans, it is related to neuronal, autoimmune, and cancer diseases because it has a role in survival, growth modulation, migration, inflammation, and wound repair. It was thought that fungi had lost this type of domain since there is no sequence homolog, but in this work, we described a conserved protein in filamentous fungi with the same 3D structure. Different approaches using *Aspergillus fumigatus* mutant strains were employed to demonstrate that human and fungal proteins have similar functions and localization. Phenotypic characterization of the deletion strain revealed that the fungal protein is implicated in cell proliferation, polarization, conidiation, morphology, septation, stress resistance, and cell wall integrity. The absence of the gene produced a

significantly lower expression of the cell wall integrity pathway and microtubule and cell end markers-related genes. The protein was found in the secretome being one of the first described extracellular polarization determinants of *A. fumigatus*. Therefore, the protein was localized in the cell membrane during germination and in the external hyphae of solid colonies. Finally, genetic replacement of the fungal protein with human Granulin A confirmed the homology between both proteins since this mutant strain almost phenocopied wild-type strain rescuing the defects observed in the deletion strain.

WP1.68 - Reference pangenomes improve 'omics analysis of fungi by capturing their genetic diversity: a demonstration from *Aspergillus fumigatus*

M. Perrier[1,2], *C. Puerner*[3], *A.A. Golicz*[4], *J.E. Stajich*[5], *R.A. Cramer*[3], *A.E. Barber*[1,2]
[1]Friedrich Schiller University, Institute of Microbiology, Jena, Germany, [2]Friedrich Schiller University, Cluster Balance of the Microverse, Jena, Germany, [3]Dartmouth College, Geisel School of Medicine, Department of Microbiology and Immunology, Hanover, United States, [4]Justus Liebig University, Department of Agrobioinformatics, IFZ Research Center for Biosystems, Land Use and Nutrition, Giessen, Germany, [5]University of California-Riverside, Department of Microbiology and Plant Pathology, Riverside, United States

Fungi harbour a tremendous amount of genomic diversity, including marked differences in gene content even within the same species. A prominent example is *Aspergillus fumigatus*, a ubiquitous environmental mould responsible for an estimated 1.5 million deaths annually. Only 69% of the total genes of the species are conserved in all isolates, with a large number showing presence-absence variation. Due to their absence in the reference strains, the role of these accessory genes in stress resistance, metabolism, and virulence remains unknown. To create a tool that captures species' diversity with the ultimate goal of understanding the function of these accessory genes, we used 26 near-chromosomal level genome assemblies to create a pangenome reference for *A. fumigatus*. This reference has a length of 38 Mbp, 30% longer than the current Af293 reference, and encodes 2,260 ORFs absent in Af293. This novel tool can be used for the unbiased but computationally straightforward analysis of genomic and transcriptomic data from diverse strains. As a demonstration that the graph pangenome better captures *A. fumigatus*' diversity, alignment of genomic and transcriptomic data resulted in notably more reads aligned than the linear reference. Ongoing work uses this new reference for the high-resolution quantification of the genomic adaptations that occur during chronic infection and to understand the role of the accessory genome in the virulence of *A. fumigatus* using a large transcriptomic dataset. This work highlights the value of reference pangenomes for improving our understanding of strain heterogeneity and how it contributes to diverse biological processes.

WP1.69 - Streptomyces small laccase expressed in Aspergillus niger as a new addition for the lignocellulose bioconversion toolbox

P. Punt[1], *A. Sidar*[1], *G. Voshol*[2], *E. Vijgenboom*[1], *A. El-Masoudi*[1]
[1]Leiden University, Microbial Biotechnology, Leiden, Netherlands, [2]GenomeScan, Leiden, Netherlands

Laccases are multi-copper oxidases that are usually composed of three Cu-oxidase domains. Domain one and three house the copper binding sites, and the second domain is involved in forming a substrate-binding cleft. However, Streptomyces species are found to have small laccases (SLAC) that lack one of the three Cu-oxidase domains. This type of SLAC with interesting bioconversion activities have not been reported in Aspergillus niger. In our research, we explored the expression and engineering of the SLAC from Streptomyces leeuwenhoekii C34 in A. niger. Genes encoding two versions of the SLAC were expressed. One encoding the SLAC in its native form and a second encoding the SLAC fused to two N-terminal CBM1 domains. The latter is a configuration also known for specific yeast laccases. Both SLAC variants were functionally expressed in A. niger as shown by in vitro activity assays and proteome analysis. Laccase activity was also analyzed toward bioconversion of lignocellulosic rice straw. From this analysis it was clear that the SLAC activity improved the efficiency of saccharification of lignocellulosic biomass by cellulase enzyme cocktails.

WP1.70 - Re-evaluating the fungal D-glucuronic acid pathway

N. Rahnama[1], *A. Manassero*[1], *P. Chen*[1], *A. Mueller*[1], *R.P. de Vries*[1]
[1]Westerdijk Fungal Biodiversity Institute & Fungal Molecular Physiology, Utrecht, Netherlands

D-glucuronic acid is a common compound in nature that is found as a side-group on the plant cell wall polysaccharide xylan, as well as a component of many gums, such as Arabic and xanthan gum. Its pathway has been well studied in bacteria, but to a much lesser extent in fungi. In recent years, the D-glucuronic acid pathway has been studied in *Aspergillus niger* and several genes encoding the enzymes catalyzing the metabolic steps have been identified. However, deletion mutant strains for these genes showed mostly reduced rather than no growth phenotypes, suggesting that additional enzymes could be involved in this pathway. A similar situation has recently been described for the *A. niger* pentose catabolic pathway, showing that some metabolic steps are catalyzed by at least three different enzymes.
In this study, we aimed to revisit the D-glucuronic acid pathway and identify the full range of genes involved in the pathway in *A. niger*. RNA-seq analysis of *A. niger* wild type strain cultivated on D-glucuronic acid revealed several additional candidate genes for most steps of the pathway. Construction of strains with single and multiple gene deletions were performed to demonstrate their in vivo functionality in the pathway. The resulting strains were compared with respect to phenotypes on D-glucuronic acid and other carbon sources to determine the full scope of genes involved in the pathway, as well as the possible role of these genes in other pathways.

WP1.71 - Aspergillus fumigatus conidial surface-associated proteome reveals factors for fungal evasion and host immunity modulation

C. Pinzan[1], *C. Valero*[2], *P. de castro*[3], *J. Silva*[1], *K. Earle*[2], *H. Liu*[4], *M. Horta*[1], *O. Kniemeyer*[5], *T. Krüger*[5], *A. Pschibul*[5], *D. Cömert*[5], *T. Heinekamp*[5], *A. Brakhage*[5], *J. Steenwyk*[6], *M. Mead*[7], *N. Hermsdorf*[5], *S. Filler*[4,8], *N. Garzon*[1], *E. Delbaje*[1], *M. Bromley*[2], *H. Cabral*[1], *C. Diehl*[1], *C. Angeli*[9],

G. Palmisano[9], A. Ibrahim[4,8], D. Rinker[7], T. Sauters[7], K. Steffen[7], A. Gumilang[7], A. Rokas[7], S. Gago[2], T. Reis[1,10], G. Goldman[1,10]
[1]University of Sao Paulo, Ribeirao Preto, Brazil, [2]University of Manchester, Manchester, United Kingdom, [3]University of Sao Paulo, Ribeirão Preto, Brazil, [4]Harbor-UCLA Medical Center, Torrance, United States, [5]Friedrich Schiller University, Jena, Germany, [6]University of California, Berkeley, United States, [7]Vanderbilt University, Nashville, United States, [8]David Geffen School of Medicine at UCLA, Los Angeles, United States, [9]University of Sao Paulo, Sao Paulo, Brazil, [10]National Institute of Science and Technology in Human Pathogenic Fungi, Sao Paulo, Brazil

The section *Fumigati* is composed of *Aspergillus* fungal species presenting variable pathogenicity levels. *Aspergillus fumigatus*, an opportunistic pathogen, belongs to this section and is responsible for approximately 70% of cases of invasive pulmonary aspergillosis (IPA). The establishment of IPA depends on the inhalation of asexual spores (conidia) that trigger host infection. Thus, conidia represent the first point of contact between the fungus and human cells and, therefore, are important for the establishment of IPA. Despite its importance in the initial steps of IPA, there is scarce information about conidial surface proteins of *A. fumigatus* involved in fungal evasion and host immunity modulation. We analysed the conidial surface proteome (surfome) of *A. fumigatus*, two closely related non-pathogenic species, *Aspergillus fischeri* and *Aspergillus oerlinghausenensis*, as well as pathogenic *Aspergillus lentulus*, to identify such proteins. From 62 proteins exclusively detected on the *A. fumigatus* surfome, we constructed null mutants for 42 genes encoding these proteins. Deletion of 33 of these genes altered the fungal susceptibility to macrophage, epithelial cells and cytokine production. The gene encoding a putative glycosylasparaginase was characterized in detail and demonstrated its importance in modulating the levels of host proinflammatory cytokines and contributing to virulence in an immunocompetent murine model of IPA. Other genes are also in the process of being characterized. In summary, our results suggest that the conidial surfome of *A. fumigatus* encompasses proteins that are important for evasion and modulation of the immune response at the onset of fungal infection.
Funding: FAPESP, Brazil and NIH-NIAID, USA

WP1.72 - Arginoketides mediating cross-kingdom microbial interactions are modified by a fungal oxidase

M. Rosin[1,2], L. Bulmann[1], M. Stroe[1,3], P. Hortschansky[1], T. Krüger[1], O. Kniemeyer[1], V. Schroeckh[1], A. Brakhage[1,2]
[1]Leibniz Institut for Natural Product Research and Infection Biology - HKI, Molecular and Applied Microbiology, Jena, Germany, [2]Friedrich Schiller University Jena, Jena, Germany, [3]BASF SE Ludwigshafen, Present Adress, Ludwigshafen, Germany

Soil provides a habitat for various microorganisms living in close proximity to each other. The microbial interactions within such a soil community lead to the inclusion or exclusion of organisms in a microbiome. These interactions often occur *via* natural products (Brakhage, 2013; Krespach et al., 2023). An example is given by the arginoketide azalomycin F (AzF), produced by the soil-dwelling actinobacterium *Streptomyces iranensis*. AzF impacts the surrounding

microorganisms, *e.g.*, it triggers the induction of otherwise silent biosynthesis gene clusters (BGC) in the fungi *Aspergillus nidulans* and *Aspergillus fumigatus* or acts as toxin towards the green alga *Chlamydomonas reinhardtii* (Krespach et al., 2023, 2020).
Here, we provide evidence that *A. fumigatus* modifies the arginoketide signal by secretion of an oxidase. The modified AzF is not able to induce the *ors* BGC in *A. nidulans* and showed much reduced toxicity against fungi and the green algae *C. reinhardtii*. Expression of the oxidase gene which we named *arkO* (arginoketide oxidase) is highly induced by presence of AzF and the ArkO protein is released by the fungus into the extracellular milieu only after exposure to azalomycin F. Purification of the enzyme and analysis of its activity indicated that the protein acts specific towards AzF and similar arginoketides as monazomycin, desertomycin A and linearmycin A.
Brakhage AA. Nat Rev Microbiol. 2013 Jan;11(1):21-32.
Krespach, *et al. ISME J* **14**, 2794–2805 (2020).
Krespach*, Stroe*, Netzker, Rosin, *et al. Nat Microbiol* **8**, 1348–1361 (2023).

WP1.73 - Growth response of *Aspergillus oryzae* to soil component humic acid and elucidation of its mechanism

K. Sakai[1], *L. Liu*[1], *K.-I. Kusumoto*[1]
[1]Osaka University, Engineering, Suita, Japan

Aspergillus oryzae is a filamentous fungi used in the production of traditional fermented food products such as sake, soy sauce, and miso for hundreds of years. *A. oryzae* was thought to be domesticated from saprophytic ancestor strain by humans to adapt for fermentation and brewing after isolated from environment. In recent years, several reports of *A. oryzae* isolation from environmental samples have suggested that *A. oryzae* may possesses mechanisms to respond to and avoid various environmental stresses. We used humic acid (HA) as one of the environmental components and found that HA affected the growth of *A. oryzae* differently depending on the strain (growth promotion, growth inhibition, or no change) [Liu *et al*., JGAM (2023)]. Since growth responses and genetic classification were somewhat correlated, genetic changes during domestication may contributed to HA responses. To elucidate the mechanisms of HA response, genome sequences comparison was performed between strains that showed different HA responses and RNA-seq analysis under the cultivation condition with or without HA. Several candidate genes thought to be involved in HA response were selected, especially growth promotion, and constructed disruption strains. Among the disruptants, some with weakened HA responses were obtained, and we are investigating these strains in detail.

WP1.74 - An improved description of the untranslated regions of poly(A)-tailed RNA from Aspergillus fumigatus

L. Schrettenbrunner[1], *C. Maufrais*[2], *G. Janbon*[2], *M. Blango*[1]
[1]Leibniz-HKI, RNA Biology of Fungal Infections, Jena, Germany, [2]Institute Pasteur, Paris, France

The filamentous fungus *Aspergillus fumigatus* is an opportunistic human pathogen and is categorized in the critical group of the WHO fungal pathogen priority list. Despite its intensely studied genome, relatively little is known about its transcriptome, including transcriptional start/end sites and the functional relevance of regulatory elements like untranslated regions (UTRs). With this project we aim to improve the map of *A. fumigatus* UTRs by experimentally validating and precisely defining the 5' and 3' ends of poly(A) transcripts. After enrichment of poly(A)-tailed RNA, we performed specialized 5' and 3' RNA-end sequencing. Initial screening of aligned reads showed that these were clearly enriched at the respective transcript ends. Peaks were called on the extracted 5' and 3' ends of each read of each method, respectively. High confidence sites were denoted as positions that were found in at least 3 replicates. These sites were then assigned to the closest gene on the reference genome. Finally, we performed manual curation to improve the overall annotation of end sites, which ultimately led to 67% of all genes with an associated high confidence 3' end and 27% of all genes with a high confidence 5' end. In addition to the mapped primary transcriptional ends, we also identified alternative end sites, sites with potential early termination, and 5'/3' end sites within the coding sequences of genes. We hope that this data set will ultimately serve the *A. fumigatus* community as a resource to generate additional hypotheses and facilitate future investigations of the *A. fumigatus* transcriptome.

WP1.75 - Enhancing secondary metabolite production in Penicillium camemberi through genetic manipulation of global regulator mcrA for high-throughput screening

A. Shih[1], *J. Shyong*[1], *S. Dziedzic*[1], *C. Wang*[2,1]
[1]Alfred E. Mann School of Pharmacy and Pharmaceutical Sciences, University of Southern California, Pharmacology and Pharmaceutical Sciences, Los Angeles, United States, [2]Dornsife College of Letters, Arts, and Sciences, University of Southern California, Chemistry, Los Angeles, United States

Fungal secondary metabolites (SMs) are complex organic compounds of ranging biological activity with potential applications in medicine, industry, and agriculture[1]. Prime examples of these compounds include penicillin, aflatoxin, and lovastatin. These secondary metabolites are encoded by biosynthetic gene clusters (BGCs) most of which are silent under normal laboratory conditions. We are constantly exploring new biological methods to activate silent BGCs because SMs have great potential for application in drug development due to their various bioactive characteristics. For instance, Penicillium has been known to produce a wide array of SM compounds including mycotoxins, immunosuppressants, and cholesterol reducing agents[2]. The Wang lab has previously shown that deletion of mcrA, a negative global regulator of secondary metabolism, can activate normally silenced BGCs [3]. The focus of this project was to knockout mcrA in Penicillium camemberti (IMV00769) and generate a new metabolic profile for discovery of new compounds. We used the CRISPR-Cas9 genome editing system to knockout mcrA and confirmed the knockout with diagnostic PCR and growth on selective plates. Then, we grew the wild-type (WT) and mcrA knockout (mcrAΔ) strains in various conditions and extracted them using ethyl acetate. These extracts were analyzed with Liquid Chromatography– Mass Spectrometry (LCMS) to generate metabolic profiles for the WT and mcrAΔ strains. The mcrAΔ strain produced vastly different metabolic profiles compared to the WT in multiple tested

conditions. Ultimately, the different metabolites produced by the mcrAΔ strain will be further isolated and tested for potential pharmacological and industrial application.

WP1.76 - Discovery of penicillic acid as a chemical probe against tau aggregation in Alzheimer's Disease

J. Shyong[1], J. Wang[1], T.Q.D. Huynh[2], M. Fayzullina[1], B. Yuan[1], C.-K. Lee[2,3], T. Minehan[4], P. Seidler[1], C. Wang[1,5]
[1]University of Southern California, Pharmacology and Pharmaceutical Sciences, Los Angeles, United States, [2]Taipei Medical University, College of Pharmacy, Taipei, Taiwan, China , [3]Taipei Medical University, Ph.D. Program in Clinical Drug Development of Herbal Medicine, Taipei, Taiwan, China , [4]California State University, Northridge, Chemistry and Biochemistry, Northridge, United States, [5]University of Southern California, Chemistry, Los Angeles, United States

Alzheimer's Disease (AD) is a neurodegenerative disorder proven to be caused by the aggregation of protein tau into fibrils, resulting in neuronal death. The irreparable neuronal damage leads to irreversible symptoms with no cure; therefore, disaggregation of these tau fibrils could be targeted as a therapeutic approach to AD. Here we have developed a fungal natural product library through the genetic modification of global regulator *mcrA* to screen for secondary metabolites that have bioactive potential towards AD tau. Our initial screenings indicate that penicillic acid demonstrates anti-aggregation activity towards tau, while further *in vitro* experiments reveal that penicillic acid directly inhibits tau by disaggregating fibrils. Although penicillic acid possesses blood-brain barrier penetrability properties that are computationally predicted to be favorable, it is presumed to contain some mutagenic effects as well. To address this, we used the backbone of penicillic acid as a chemical probe to discover similar compounds that can inhibit AD tau aggregation with limited mutagenicity. This work suggests the potential of discovering chemical probes through natural product screening for small-molecule drug discovery of tauopathies.

WP1.77 - Activation of cryptic biosynthetic gene clusters through genetic manipulation of global regulator mcrA in Penicillium expansum

A. LaCorte[1], J. Shyong[1], S. Dziedzic[1], C. Wang[1,2]
[1]University of Southern California, Department of Pharmacology and Pharmaceutical Sciences, Los Angeles, United States, [2]University of Southern California, Department of Chemistry, Los Angeles, United States

Fungal secondary metabolites (SMs) are organic compounds produced by fungi that are not essential for survival, but provide ecological advantages against other microbes. Due to the potency of these SMs used for defense and the complexity of the compound structures, many metabolites often have biological activities beneficial to humans. Previous studies have demonstrated that SMs are produced by groups of genes working together, forming biosynthetic

gene clusters (BGCs); however, because many BGCs are silent under normal laboratory conditions, additional SMs have yet to be discovered and tested for potential bioactivity. Targeting a negative global regulator of fungal secondary metabolism, multicluster regulator A (mcrA), will allow for the upregulation of SMs and the possibility for discovery of new natural products.
Using a combinational approach of one-strain-many-compounds (OSMAC) and genetic manipulation via the CRISPR Cas9 genome editing system, we aimed to knock out mcrA in Penicillium expansum (IMV00074). Successful transformation of the strain resulted in the activation of multiple silent BGCs in various culture conditions as shown in our liquid chromatography-mass spectroscopy (LC-MS) analysis. This demonstrated the emergence of new compounds and the upregulation of others in the mcrAΔ strain, creating a natural product library, on which we will perform high throughput screening for novel bioactive properties.

WP1.78 - Multi-omics analysis of a fungal cell factory producing recombinant enzyme controlled by a constitutive or inducible promoter

J.V. Bernardi[1,2], *E.P. Antoniel*[1], *M.F. Cavalheiro*[2], *L.O.S. Silva*[1], *M.M. Brandão*[2], *A. Damasio*[1]
[1]UNICAMP, Biochemistry and Tissue Biology, Campinas, Brazil, [2]UNICAMP, Center for Molecular Biology and Genetic Engineering, Campinas, Brazil

Aspergilli are filamentous fungi known for their high secretion capacity, making them effective cell factories for industrial enzyme production. However, some challenges can limit enzyme titers, so optimizing fungal strains is crucial to enhance enzyme production. This study compares two *Aspergillus nidulans* strains expressing α-L-arabinofuranosidase (AbfA) from *Aspergillus fumigatus*, controlled by either a constitutive (glyceraldehyde-3-phosphate dehydrogenase promoter, pgpdA, from *A. nidulans*) or an inducible (glucoamylase promoter, pglaA, from *Aspergillus niger*) promoter. The strain with the constitutive promoter showed 0.71-fold higher AbfA secretion and 4.11-fold increased enzyme activity. However, higher *abfA* mRNA expression (5.49-fold) was detected by qPCR in the strain with the inducible promoter. To further understand why the strain with higher mRNA levels secretes the lowest amount of enzyme, we performed RNA-seq and proteomic. Transcriptomic confirmed higher *abfA* levels in the pglaA::abfA strain (2.65-fold). Gene ontology enrichment revealed that oxidative stress was overrepresented under the control of the pglaA, while amino acid biosynthesis was highlighted for the pgpdA. Transcript levels of unfolded protein response genes were similar in both strains, suggesting that misfolded proteins may not limit AbfA secretion in the pglaA::abfA strain. Co-expression network identified genes whose expression regulation was directly associated with AbfA production, and their roles in fungal cell factories are under investigation. Proteomic data showed differential expression of proteins involved in signaling, DNA packaging, and protein synthesis. AbfA was not found in the intracellular proteome, indicating effective secretion. The transcriptomic-proteomic correlations will contribute to unraveling potential bottlenecks that limit AbfA control by the inducible promoter.

WP1.79 - Elucidating the antifungal modes of action of G-quadruplex-stabilising ligands in A. fumigatus

I. Storer[1], *G. Middleton*[1], *E. Barclay*[2], *J. McColl*[3], *B. Lezé*[1], *Z. Waller*[4], *S. Bidula*[1]
[1]University of East Anglia, School of Chemistry, Pharmacy and Pharmacology, Norwich, United Kingdom, [2]John Innes Centre, Bioimaging Facility, Norwich, United Kingdom, [3]University of East Anglia, Henry Wellcome Laboratory for Cell Imaging, Norwich, United Kingdom, [4]University College London, School of Pharmacy, London, United Kingdom

Invasive aspergillosis, caused by fungal pathogens in the genus *Aspergillus*, causes almost 2 million deaths each year. Due to the emergence of antifungal resistance, the drugs we use to treat fungal infections are becoming increasingly ineffective. Therefore, new drugs with novel mechanisms of action are urgently needed. We have shown that ligands that stabilise G-quadruplexes (G4s), four-stranded secondary structures found in DNA and RNA, prevent the growth of *A. fumigatus, Candida* spp., and dermatophytes. Here, we show that the G4-stabilising ligand, PhenDC3, increased the number of stable G4s in A. fumigatus RNA. Notably, spores treated with PhenDC3 became swollen, but did not germinate. This increase in spore size was associated with a significant increase in the thickness of the cell wall. Similarly, another G4-stabiliser, pyridostatin, prevented germination and spore swelling. TEM imaging indicated that PhenDC3 could significantly impact organelle organisation. These impacts were explored further by imaging the nuclei, mitochondria, cell membrane, peroxisomes, and vacuoles. Finally, we investigated transcription using RNAseq and uncovered differential expression of genes associated with primary metabolism upon PhenDC3 treatment. These genes are predicted to contain G4s by prediction software, suggesting G4 sequences in these genes were stabilised by PhenDC3, preventing transcription. This work describes the first steps in identifying the target or targets of G4-stabilising ligands PhenDC3 and pyridostatin to guide the design of fungal-specific DNA/RNA-binding antifungal agents.

WP1.80 - Improved hyphal dispersion strain of *Aspergillus oryzae* with decreased wall-growth in the liquid fermentation

S. Susukida[1], *K. Muto*[1], *K. Miyazawa*[1], *A. Yoshimi*[2,3], *F. Hasegawa*[4], *K. Abe*[1]
[1]Tohoku University, Graduate School of Agricultural Science, Sendai, Japan, [2]Kyoto University, Graduate School of Agriculture, Kyoto, Japan, [3]Kyoto University, Graduate School of Global Environmental Studies, Kyoto, Japan, [4]National Institute of Technology and Evaluation, Tokyo, Japan

Aspergillus oryzae has been widely used in the industrial production of enzymes. In liquid fermentation, the mycelial morphology increases the viscosity of culture liquid, causing poor mixing. In addition, mycelial growth on the bioreactor wall (wall-growth) leads to unstable operation and reduces liquid volume, which can limit yields. Previously, we have shown that the hyphal dispersed mutant of *A. oryzae* lacking both α-1,3-glucan and galactosaminogalactan (AGΔ-GAGΔ) exhibited reduced culture viscosity and increased recombinant enzyme production. However, wall-growth remained a challenge. Hydrophobin RolA, an amphipathic

protein on the cell surface of *A. oryzae*, makes surfaces of hyphae and conidia hydrophobic and contributes to their attachment on solid surfaces. In this study, we generated a strain deficient in the hydrophobin RolA (AGΔ-GAGΔ-RolAΔ) using the AGΔ-GAGΔ strain as the parental strain, aiming to reduce wall-growth and improve productivity.
When cultured in Sakaguchi flasks with 100 mL minimal medium, the AGΔ-GAGΔ-RolAΔ strain reduced wall-growth by 32% and increased recombinant enzyme activity by 13% compared to the AGΔ-GAGΔ strain after 72 hours. The liquid volume decreased less in the AGΔ-GAGΔ-RolAΔ strain, with the final volume being 6% greater, leading to a 16% increase in total enzyme activity. In a 5 L bioreactor, wall-growth was reduced by 20% in the AGΔ-GAGΔ-RolAΔ strain compared to the AGΔ-GAGΔ strain at 72 hours of fermentation. These findings suggest that the loss of RolA reduced wall-growth and increased culture volume at the end of the fermentation, resulting in the enhancement of total enzyme production.

WP1.81 - Building a genetic map of aflatoxin biosynthesis to accelerate novel strategies for transgenic and biological Control

B. Bluhm[1], *T. Marshall*[1], *K. Swift*[1]
[1]University of Arkansas Division of Agriculture, Department of Entomology and Plant Pathology, Fayetteville, United States

Conventional breeding has revolutionized modern corn production but has not yet provided reliable resistance to aflatoxin. Transgenic approaches can accelerate the deployment of aflatoxin resistance, but a key bottleneck is an incomplete understanding about which fungal genes should be targeted. Relatedly, one of the most effective tools currently available to combat aflatoxin is biological control via the application of atoxigenic strains of *Aspergillus flavus*. Molecular dissection of chemical communication among strains could enable development of novel inhibitors of aflatoxin biosynthesis; however, fungal genes underlying biological control have not been identified. The overarching goal of this project is to globally map the genetic regulation of aflatoxin biosynthesis and atoxigenic suppression in *A. flavus*. To this end, we are taking a two-pronged approach. First, we are harnessing naturally occurring genetic and phenotypic diversity to dissect the regulation and suppression of aflatoxin biosynthesis via population genetics. In a complementary approach, we are utilizing a blend of forward and reverse genetics to identify genes involved in the regulation of aflatoxin biosynthesis. Results from these parallel activities will be integrated to provide a comprehensive genetic map of aflatoxin biosynthesis in *A. flavus*.

WP1.82 - High-throughput identification of genetic factors driving tissue invasion in *Aspergillus fumigatus* through an integrated *in vitro* and *ex vivo* screening platform

S.Y. Tang[1], *C. Valero*[2], *N. Van Rhijin*[2], *M. Bromley*[2], *C. Zhao*[1,2]
[1]Manchester Metropolitan University, Life Sciences, Manchester, United Kingdom, [2]University of Manchester, Manchester Fungal Infection Group, Manchester, United Kingdom

Over 10 million people globally suffer from lung diseases caused by *Aspergillus fumigatus*, with limited treatment options and increasing antifungal resistance. Understanding the genetic factors that enable *A. fumigatus* to penetrate host tissues is crucial for developing new therapies, yet these genomic determinants remain largely unexplored.
As part of the *A. fumigatus* genome-wide knockout program (COFUN), we have completed the generation of a library that consists of over 6000 genetically dual-barcoded null mutants. Recently, we developed a novel high-throughput screening platform, using competitive fitness profiling in an *in vitro* barrier model to identify key genetic factors involved in invasive capacity. To expand this screening approach, we also incorporated a second-stage, high-throughput *ex vivo* screening employing a porcine corneal infection model of fungal keratitis (FK), which mimics tissue invasion more closely. This is designed to screen mutants identified from the *in vitro* stage, providing a more clinically relevant assessment of tissue invasion.
As a proof of concept, a subset of the COFUN collection, containing knockout mutants of protein kinases, was screened through this integrated pipeline. Key kinases critical to barrier penetration and tissue invasion were identified, including YakA, a known stress-activated kinase that plays a crucial role in septal plugging and contributes to the pathogenicity of *A. fumigatus*.
This study showed that the our methodology can provide a powerful tool for uncovering fungal invasion mechanisms and identifying potential therapeutic targets for FK and other invasive fungal infections.

WP1.83 - Synthetic strategies to optimize octatrienoic acid production in Aspergillus nidulans

M. Tangalos[1,2], *C. Jenkinson*[3], *B. Miller*[1,2], *P.F. Chiu*[1], *C. Wang*[1], *B. Oakley*[3]
[1]University of Southern California, Pharmacy and Pharmaceutical Sciences, Los Angeles, United States, [2]University of Southern California, Chemistry, Los Angeles, United States, [3]Kansas University, Molecular Biosciences, Lawrence, United States

Aspergilli produce many commercially valuable and bioactive secondary metabolites (SMs). Because SM biosynthesis typically occurs at low levels, promoters from native housekeeping or primary metabolism genes have been used to upregulate target SMs. Optimizing current platforms and exploring new promoters are powerful opportunities to enhance SM production for the industrial scale.
We compared the use of simple promoter systems against positive feedback systems to upregulate (2Z, 4Z, 6E)-octa-2,4,6-trienoic acid (ZZE-OTA), an intermediate of the (+)-asperlin pathway. In the simple promoter systems, the inducible *alcA*(p) and the constitutive *gpdA*(p), from the alcohol dehydrogenase I and G3P dehydrogenase clusters, were used to directly drive ZZE-OTA biosynthesis. In the positive feedback system, *alcA*(p) and *gpdA*(p) are used to promote *afoA*, which encodes a transcription factor from the asperfuranone cluster. Promoters from the asperfuranone cluster were then engineered to drive ZZE-OTA biosynthesis, as well as more *afoA*, activated by the AfoA transcription factor.
RNA-seq analysis revealed additional strong promoter candidates to drive the AfoA system: a gene involved in thiamine biosynthesis (*nmtA*), the transcription elongation factor 1 gene (*tefA*), and a highly expressed gene that encodes metallothionein (*mtnA*). The constitutive AfoA feedback systems overwhelmingly outperform the simple promoter systems in ZZE-OTA output.

Amongst the constitutive AfoA feedback systems, the novel *mtnA*(p) outperformed the others, revealing an optimized strategy for ZZE-OTA production.
Ongoing efforts include employing a palladium-acetate catalyst to isomerize ZZE-OTA to (2E, 4E, 6E)-octa-2,4,6-trienoic acid (EEE OTA), a known promoter of melanogenesis. We hope to next compare melanogenesis bioactivity between ZZE-OTA and EEE-OTA.

WP1.84 - Activity-based profiling of *β*-mannanases in *Aspergillus niger*

M. Tedeschi[1,2], *V.A.J. Lit*[2], *P. Kooloth Valappil*[1], *M. Arentshorst*[1], *J. C. R. Brzoskowski*[2], *T. Gote*[2], *B.I. Florea*[2], *Z. Armstrong*[2], *H.S. Overkleeft*[2], *A.F.J. Ram*[1]
[1]Leiden University, Institute of Biology Leiden, Microbial Sciences, Fungal Genetics and Biotechnology, Leiden, Netherlands, [2]Leiden University, Leiden Institute of Chemistry, Bio-organic Synthesis, Leiden, Netherlands

β-Mannanases are endo-acting glycosyl-hydrolases (GHs) capable of hydrolyzing *β*-1,4 linkages of mannan-containing polysaccharides. *Aspergillus* species are amongst the main producers of these enzymes, which have applications in many industrial sectors. A specific *β*-mannanase of *Aspergillus niger* (*An*ManA) was successfully identified and characterized by activity-based protein profiling (ABPP). ABPP is an effective tool to study GHs in complex mixtures. ABPP relies on a simple yet powerful concept in which activity-based probes (ABPs) irreversibly inhibit an enzyme by covalently binding to its active site. ABPs are endowed with a reporter entity (either a fluorophore like Cy5 or a capture agent like biotin) which allows detection and isolation of the enzyme bound to the ABP. In this study ABPs for *β*-mannanases were developed. Mannobiose and mannotriose were chosen as recognition elements, and an epoxide was employed as electrophilic warhead. The synthesized ABPs were evaluated on *A. niger* secretomes obtained from cultivations on mannan-containing substrates. *An*ManA was pulled-down from the secretome with a biotinylated ABP and identified by LC-MS. The ABPs were also employed to test the temperature and pH stability of the labelled enzymes directly in the secretome. Furthermore, *An*ManA was overexpressed in a genetically modified strain of *A. niger*. The recombinant mannanase was purified from the secretome of the overexpressing strain. Finally, the active site nucleophile of ManA was experimentally identified by ABPP using LC-MS/MS. This research highlights the utility of ABPP in the identification and characterization of fungal mannan-degrading GHs and its applicability in the field of industrial biotechnology.

WP1.85 - Identification of novel Aspergillus fumigatus SIN kinase interactors through near-neighbor analysis

H. Thorn[1,2], *A. Martin-Vicente*[1], *J. Xie*[1,2], *A. Nandi*[1], *D. Bale*[1,2], *U. Perez-Cuesta*[1], *J. Fortwendel*[1,3]
[1]University of Tennessee Health Science Center, Clinical Pharmacy and Translational Medicine, Memphis, United States, [2]University of Tennessee Health Science Center, PhD Program in Pharmaceutical Sciences, Memphis, United States, [3]University of Tennessee Health Science Center, Microbiology, Immunology and Biochemistry, Memphis, United States

Aspergillus fumigatus is a leading cause of invasive fungal infections, and novel therapeutic strategies are urgently needed. Previous work in our laboratory suggests that septation inhibition may be one such strategy in combination with echinocandin treatment, as loss of septation renders *A. fumigatus* avirulent and unable to tolerate echinocandin stress at concentrations where the drugs would normally exert fungistatic activity. We have shown that the terminal member of the Septation Initiation Network (SIN) signaling complex, composed of the kinase SidB and its activator MobA, is essential for proper septation, virulence, and echinocandin response. However, the downstream effectors of this complex remain largely unknown. Proteins that interact with the SIN are likely important effectors for proper septation, so we have employed proximity-based labeling of the SidB/MobA kinase module using TurboID for the first time in A. fumigatus. LC-MS/MS of lysates from TurboID-tagged culture indicates that SidB and MobA have very similar interaction profiles, as expected based on our previous genetic analysis revealing that MobA is essential for SidB's role in septation. We found that samples from tagged strains shared 507 proteins overrepresented compared to the control. Of these, we have selected 17 candidate proteins for further characterization based on predicted roles in cell wall synthesis or cytoskeletal dynamics. Genes encoding candidate proteins were deleted, and several deletion mutants exhibited growth defects. This work demonstrates successful adaptation of the TurboID technology to *A. fumigatus*, which may be used in the future to delineate other molecular pathways involved in pathogenesis.

WP1.86 - Combination therapy suppresses the emergence of resistance in Aspergillus fumigatus

J. Tindale[1], *A. Anna*[1], *C. Valero*[1], *M. Bromley*[1], *M. Bottery*[1]
[1]University of Manchester, Manchester Fungal Infection Group, Division of Evolution, Infection and Genomics, Manchester, United Kingdom

The fungal pathogen *Aspergillus fumigatus* is responsible for invasive and chronic diseases that causes an estimated 300,000 deaths each year. Therapeutic options for treating aspergillosis are limited to only a few classes of antifungal drugs. Treatment of chronic disease is characterised by prolonged azole therapy, often resulting the evolution of azole-resistant strains. As azoles are the first-line antifungal for aspergillosis and the only orally available class, new treatment strategies are crucial to prevent resistance. Combination therapy is one promising solution that has already demonstrated clinical effectiveness through reducing fungal burden and improving patient outcomes. The application of this strategy in the context of suppressing the emergence of resistance is yet to be explored. Using a fluctuation assay, we demonstrated that the rate of spontaneous resistance was significantly lower in response to combination treatment of paired antifungals compared to the respective drugs individually. We show using combinations of two antifungals either from the same (voriconazole and itraconazole) or different (itraconazole plus the novel orally bioavailable antifungal olorofim) classes, there is a lower probability of acquiring mutations that confer resistance to both drugs simultaneously. We conducted whole genome sequencing on resistant isolates to uncover mechanisms of cross-resistance to compounds from both the same and different antifungal classes. Combination therapy through the use of multiple antifungal drugs may provide a promising strategy to reduce the risk of antifungal resistance emerging during the treatment of aspergillosis.

WP1.87 - Efficient protoplast preparation method of *Aspergillus* section *Nigri* using α-1,3-glucanase and α-1,3-glucan synthesis gene disruption strain

J. Tokashiki[1], *O. Mizutani*[2], *K. Gomi*[1]
[1]Tohoku, Agricultural, Sendai, Japan, [2]University of the Ryukyus, Okinawa, Japan

Transformation of Aspergillus filamentous fungi generally require the preparation of protoplasts. For most filamentous fungal strains including Aspergillus oryzae, Aspergillus nidulans and so on, protoplasts can be easily prepared using a mixture of enzymes such as cellulase, β-glucanase, and chitinase, as expected from the polysaccharides that constitute of the cell walls. However, Aspergillus section Nigri is considered to be difficult to readily form protoplasts compared to A. oryzae and A. nidulans. Here, we compare the protoplast generation methods of several strains from section Nigri between a conventional method and one with α-1,3-glucanase preparation. Consequently, the method using α-1,3-glucanase was applied to prepare protoplasts from the Aspergillus luchuensis RIB2604 strain, which is particularly difficult to form protoplasts. Based on the result, the α-1,3-glucan synthesis gene disruption was suggested to be effective in efficient protoplast preparation. Therefore, α-1,3-glucan synthesis gene was disrupted in A. luchuensis, resulting in easier and efficient protoplast preparation even in the absence of α-1,3-glucanase.

WP1.88 - Development of new and highly efficient *Agrobacterium*-mediated platforms for genetic manipulation in some important filamentous fungi

V.-T. Tran[1,2], *H.-D. Thai*[1], *M.T. Trinh*[1], *L.T.D. Mai*[2], *T.X. Vu*[1,3]
[1]University of Science, Vietnam National University, Hanoi, National Key Laboratory of Enzyme and Protein Technology, Ha Noi, Vietnam, [2]University of Science, Vietnam National University, Hanoi, Faculty of Biology, Hanoi, Vietnam, [3]National Center for Technological Progress, Ministry of Science and Technology of Vietnam, Center for Experimental Biology, Hanoi, Vietnam

Fungi play important roles in nature and human life. Many beneficial species such as *Aspergillus oryzae*, *Aspergillus niger*, *Penicillium rubens* (*P. chrysogenum*), *Cordyceps militaris*, etc. are industrially exploited for the production of antibiotics, enzymes, organic acids, and other bioactive substances. Meanwhile, fungal plant pathogens including the citrus postharvest pathogen *Penicillium digitatum* represent as main causes of serious losses in agricultural production. Therefore, new approaches for molecular inspections of the fungi can help improve the production of the desired products or identify specific targets for controlling the fungal pathogen on citrus. We have developed highly efficient systems for genetic manipulation in some important filamentous fungi using *Agrobacterium tumefaciens*-mediated transformation (ATMT). We report for the first time new ATMT systems based on the uridine/uracil auxotrophy for *A. oryzae*, *A. niger*, *P. rubens*, and *C. militaris*. Our ATMT systems achieve high transformation yields of over 1000 transformants per 10^6 spores. We have also succeeded in developing the dual auxotrophic marker ATMT systems based on the uridine/uracil and histidine auxotrophy in *A.*

oryzae, *A. niger*, and *C. militaris*. These systems are effective for gene targeting with a gene deletion efficiency of over 90%, especially in *A. oryzae* and *A. niger*. Additionally, we have also improved the ATMT systems based on antibiotic resistance markers for *A. niger*, *P. rubens*, and *P. digitatum* to serve gene function studies in wild-type fungi. Our developed ATMT systems provide new genetic platforms to construct fungal mutants for producing beneficial metabolites and gene function characterization in the relevant filamentous fungi.

WP1.89 - Investigation of a novel azole-resistance mechanism in *Aspergillus fumigatus*

M. Uchida[1], *S. Shibata*[1], *T. Arazoe*[2], *T. Kamakura*[2], *H. Takahashi*[1,3]
[1]Chiba University, Medical Mycology Research Center, Chiba, Japan, [2]Tokyo University of Science, Department of Applied Biological Science, Noda, Japan, [3]Chiba University, Molecular Chirality Research Center, Chiba, Japan

Aspergillus fumigatus is a pathogenic filamentous fungus that causes aspergillosis, with an estimated 600,000 deaths annually. Azoles are the most commonly used antifungals for treating this fungus, but azole resistance has been recognised in many clinical isolates, making treatment more difficult. A typical resistance mechanism is mutations on the coding sequence or promoter region of *Cyp51*, which codes the targeted enzyme involved in ergosterol biosynthesis. In addition, it has also been reported that mutations in the HMG-CoA reductase *Hmg1* could lead to azole resistance. On the other hand, many azole-resistant isolates have been isolated in which there is no mutation in these genes, or the resistance is so high that it cannot be explained by these genes alone, suggesting other unknown mechanisms.
This study aims to identify a novel mechanism of azole resistance using genomic recombination by mating. Crossings between azole-susceptible and -resistant isolates (without known mutation) with all combinations resulted in a successful acquisition of progenies between one susceptible (S) and two resistant (R-1 and R-2) isolates, with over 160 F_1 progeny each. The comparative genomic analysis of five susceptible and five resistant progenies from the S×R-1 identified a single genomic region of 24 kb, predicted to be responsible for the resistance. This region contained 17 genes, of which 6 had single amino acid substitutions, and 2 had frameshift mutations. Disruptants and point mutants of these genes are being generated to verify their association with azole resistance.

WP1.90 - High recombination rates in *Aspergillus fumigatus* allows for bulk quantitative trait locus (QTL) mapping of known and novel azole resistance and fitness traits

C. Valero[1], *M. Bottery*[2], *B. Auxier*[3], *F. Stanford*[4], *P.S Dyer*[4], *M. Bromley*[1]
[1]The University of Manchester, Manchester Fungal Infection Group, Manchester, United Kingdom, [2]The University of Manchester, Manchester Fungal Ingection Group, Manchester, United Kingdom, [3]Wageningen University, Wageningen, Netherlands, [4]University of Nottingham, Nottingham, United Kingdom

Aspergillus fumigatus is an environmental fungus that can cause life-threatening or debilitating lung diseases. The number of *A. fumigatus* human infections resistant to the first-line treatment azole drugs have increased over last years which has been linked to the widespread use of azoles fungicides in agriculture. Azole resistance is primarily caused by variants in the gene coding the azole target Cyp51A, however alternative and complementary non-target variants are increasingly recognized as the cause for antifungal resistance which are potentially coupled with variants associated with increased fitness. Recently, it has been demonstrated that *A. fumigatus* harbours the highest known rate of meiotic crossovers during sexual reproduction generating a highly recombinant progeny which allows for fine mapping of traits of interest. Here, we have developed a high-throughput bulk QTL mapping approach to identify variants causing azole resistance in *A. fumigatus*. An azole sensitive strain was crossed with an environmental strain with known mechanism of azole resistance (*cyp51A*$^{TR34/L98H}$) and pooled F1 progeny was exposed to voriconazole (0.5µg/ml). Using a custom QTL bioinformatic pipeline we were able to identify not only the variant conferring azole resistance (*cyp51A*$^{TR34/L98H}$) but also complementary variants contributing to general fitness. This technique offers a great potential for identifying the underlying mechanism of complex polygenic traits such as antifungal resistance and fitness.

WP1.91 - A CRISPR/Cas9-based multicopy integration system for increased glucoamylase production in *Aspergillus niger*

J.L. van Dam[1], *S. Forrer*[1], *M. Arentshorst*[1], *P.K. Valappil*[1], *J. Visser*[1], *R.J.M. Lubbers*[1], *A.F.J. Ram*[1]
[1]Leiden University, Microbial Sciences, Leiden, Netherlands

The filamentous fungus *Aspergillus niger* is well known for its high protein secretion capacity and is therefore a preferred host for protein production. Glucoamylase is one of the highest expressed genes in *A. niger* and the promoter and terminator regions of glucoamylase are often used to drive the expression of (heterologous) genes of interest. Moreover, the introduction of multiple copies of such gene constructs is known to further boost product yields.
To increase glucoamylase production in *A. niger*, we have designed a CRISPR/Cas9-based gene targeting method to integrate up to six copies of the *glaA* gene to predetermined sites in the genome. Genes encoding extracellular enzymes such as alpha-amylase and alpha-glucosidases or proteases (PepA and PepB), were deleted and replaced by a Glucoamylase Landing Site (Gla_LS). Each Gla_LS consists of the *glaA* promoter and the *glaA* terminator region. In between the *glaA* promoter and *glaA* terminator regions a unique DNA sequence was introduced for which a unique Cas9 compatible guide RNA was designed. A strain lineage in a non-homologous end joining mutant background was made in which up to six Gla_LS were constructed. As a proof of principle, an *A. niger* strain in which six copies of the glucoamylase gene were introduced was subsequently analyzed for glucoamylase production.
We successfully used the expression platform to generate glucoamylase hyperproducing strains of *A. niger*. The expression platform is currently exploited for the expression of heterologous proteins.

WP1.92 - Mutations in the AmyR transcription factor leading to constitutive expression of starch degrading enzymes in *Aspergillus niger*

J.L. van Dam[1], *P.K. Valappil*[1], *M. Arentshorst*[1], *R. Rijnders*[1], *L. Kruithof*[1], *A. Faber*[1], *R.J.M. Lubbers*[1], *J. Visser*[1], *P.J. Punt*[1,2], *A.F.J. Ram*[1]
[1]Leiden University, Microbial Sciences, Leiden, Netherlands, [2]Ginkgo Bioworks NL, Zeist, Netherlands

The expression of starch degrading enzymes (SDEs) by filamentous fungi is tightly controlled. In *Aspergillus niger* the expression of SDEs is induced by maltose and glucose and dependent on the AmyR transcription factor. Detailed knowledge on the mechanism(s) that keep AmyR inactive during non-inducing conditions and result in its activation in the presence of inducer, are lacking.
To fill this gap, we have developed dual and single reporter strains to study the regulation of SDEs. Promoter sequences of the glucoamylase (*glaA*), acid amylase (*aamA*) and alpha-glucosidase (*agdA*) genes were used to make reporter strains using both the acetamidase gene (*amdS*) and the luciferase gene (lux_{613}). The dual reporter strains containing the *PaamA-amdS* and *PaamA-lux*) reporters were selected to isolate constitutive mutants by screening for mutants that grow well on acrylamide plates under non-inducing conditions. Trans-acting mutants were identified using the luciferase reporter and/or by performing AZCL-amylase plate assays.
In total six mutants were identified that had point mutations in the *amyR* gene resulting in specific amino acid changes. The mutations and resulting amino acid changes were not confined to a specific region of the AmyR protein but scattered over the AmyR protein sequence. Reintroduction of the strongest AmyR mutation in a strain expressing multiple copies of the *glaA* gene confirmed that this mutation in AmyR leads to a constitutively active AmyR transcription factor. This finding is currently exploited to increase production of other homologous and heterologous proteins in *A. niger*.

WP1.93 - Multi-drug resistant *Aspergillus fumigatus* are more fit at sub-inhibitory concentrations of DHODH inhibitors

N. van Rhijn[1], *J. Rhodes*[2], *C. Valero Fernandez*[1], *M. Bottery*[1], *M. Fisher*[3], *M. Bromley*[1]
[1]University of Manchester, Manchester Fungal Infection Group, Manchester, United Kingdom, [2]Radboud UMC, Nijmegen, Netherlands, [3]Imperial College London, London, United Kingdom

Pesticides, including fungicides, are extensively used in agricultural practice to protect plants from unwanted growth of weeds, plant pathogens and other pests. Dual use of antifungals in the environment and in the clinic, with similar mode of actions, has been shown to drive the development of resistance. Although not a plant pathogen, *A. fumigatus* is ubiquitous in the environment and therefore exposed to agricultural fungicides. Extensive use of triazoles in the environment has led to high rates of resistance found in clinical *A. fumigatus* isolates. These resistant isolates are not only triazole resistant, but we also show that multi-drug resistant *A. fumigatus* to several fungicides are common. It is critical that the use of novel antifungals and

fungicides remains effective. Olorofim is a novel antifungal for clinical use, targeting the essential protein DHODH, for which resistance is rare. Recently, several agricultural DHODH inhibitors, including ipflufenoquin , have gone through the approval process. We show here through Bar-seq experiments, in which we compete 180 genetically barcoded environmental and clinical isolates of *A. fumigatus*, that we can identify azole and multi-drug resistant signatures. Furthermore, we show that multi-drug resistant strains are more fit at sub-inhibitory concentration of ipflufenoquin and olorofim. On solid plate transfer experiments, we show that after one passage, multi-drug resistant strains take up the majority of the spore producing population. Our results highlight the potential dangers of using DHODH inhibitors in agriculture and selecting for multi-drug resistant strains upon sub-inhibitor concentrations of DHODH inhibitors.

WP1.94 - RNA-binding protein SsdA shows dynamic localisation and transport during hyphal growth in *Aspergillus nidulans*

D. Modaffari[1], *E.W. Wallace*[1], *K.E. Sawin*[1]
[1]University of Edinburgh, School of Biological Sciences, Edinburgh, United Kingdom

Filamentous fungi grow through highly polarized hyphal extension, requiring precise spatial coordination of vesicle fusion and cell wall material delivery at the growing tip. While mRNA transport along hyphae is well-documented in fungi, the relationship between RNA regulation and polarized growth remains poorly understood. Using *Aspergillus nidulans* as a model organism, we characterized SsdA, a putative RNA-binding translational repressor homologous to *Saccharomyces cerevisiae* Ssd1.
Bioinformatic analysis revealed that putative SsdA RNA targets are enriched for cell wall-related proteins, suggesting a role in regulating fungal cell wall synthesis. Live-cell fluorescence microscopy demonstrated that SsdA moves along microtubules in association with early endosomes, dependent on the endosomal hitchhiking-mediator PxdA. Notably, we observed that SsdA particles are absent from growing hyphal tips.
Our findings describe the dynamic localization pattern of SsdA during hyphal growth. We hypothesise SsdA might regulate tip-specific translation by repressing target mRNAs during transport and releasing this repression at hyphal tips. Ongoing work is investigating the dynamics of SsdA's RNA targets and the role of its upstream kinase CotA.

WP1.95 - Immunoassay to detect urinary siderophore Triacetylfusarinine-C (TAFC) as a diagnostic biomarker of Invasive Aspergillosis

K. Walshe[1]
[1]Maynooth University, Maynooth, Ireland

The incidence of global fungal infection including Invasive Aspergillosis (IA) is expected to grow. Given the recent recognition that IA affects over 2 million people with COPD, the world's population is rapidly aging, and the high incidence of death caused by Aspergillosis in patients

with COVID-19 in ICU, non-invasive methods that enable rapid identification of infection will ideally avoid the need for disruption of care. The WHO has emphasized that Aspergillus fumigatus detection is key to facilitating faster and more accurate diagnosis of IA . However, even with the currently available rapid tests for detection of Aspergillus antigen, which require specimen pre-treatment, new methods for improved ease of detection with increased sensitivity and specificity are essential. Recent data has demonstrated that detection of biomarker Triacetylfusarinine C (TAFC), a virulence-associated metabolite produced by A. fumigatus, can be detected in the urine of infected patients with very high sensitivity and specificity using mass spectrometry. Until now, monoclonal IgG against A. fumigatus TAFC has proven difficult to produce. However, using a newly-generated recombinant monoclonal TAFC-specific IgG, we have developed a rapid, sensitive immunodiagnostic ELISA which demonstrates potential to detect TAFC at clinically-relevant levels directly from urine samples. The TAFC ELISA has high specificity, is reproducible and demonstrates excellent recovery of spiked TAFC in urine. Unlike other Aspergillus antigen assays, no sample pre-treatment is required. A proof-of-concept TAFC lateral flow device (TAFC LFT) has also been demonstrated.

WP1.96 - Carbon fiber reinforced polymers composite recycling and thermoset matrix up-cycling utilizing an engineered strain of Aspergillus nidulans

C. Wang[1], C. Olivar[2], Z. Yu[3], B. Miller[1], M. Tangalos[1], C. Jenkinson[4], S. Nutt[3], B. Oakley[4], T. Williams[2]
[1]University of Southern California, Pharmacology and Pharmaceutical Sciences, Los Angeles, United States, [2]University of Southern California, Chemistry, Los Angeles, United States, [3]University of Southern California, Chemical Engineering, Los Angeles, United States, [4]University of Kansas, Molecular Biosciences, Lawrence, United States

Carbon fiber reinforced polymers (CFRPs, or composites) are increasingly replacing traditional manufacturing materials used in automobile, aerospace, and energy sectors. With this shift, it is vital to develop end-of-life processes for CFRPs that retain the values of both the carbon fibers and the polymer matrix. Here we demonstrate a strategy to upcycle pre- and postconsumer polystyrene-containing CFRPs, cross-linked with unsaturated polyesters or vinyl esters, to benzoic acid. The thermoset matrix is upgraded via biocatalysis utilizing an engineered strain of the filamentous fungus Aspergillus nidulans, which gives access to valuable secondary metabolites in high yields, exemplified here by (2Z,4Z,6E)-octa-2,4,6-trienoic acid. Reactions are engineered to preserve the carbon fibers with much of their sizing so that the isolated carbon fiber plies are manufactured into new composite coupons that exhibit mechanical properties comparable to those of virgin manufacturing substrates. In sum, this represents the first system to reclaim a high value from both the fiber fabric and polymer matrix of a CFRP.

WP1.97 - Saintopin biosynthesis implies a non-canonical polyketide cyclization mechanism

C. Wieder[1,2], S. Galwas[2], R. Wiechert[3], K. Seipp[3], K. Andresen[2], A. Schüffler[1], T. Opatz[3], E. Thines[1,2]

[1]Institut für Biotechnologie und Wirkstoff-Forschung gGmBH, Mainz, Germany, [2]Johannes Gutenberg-University, Microbiology and Biotechnology, Mainz, Germany, [3]Johannes Gutenberg-University, Department of Chemistry, Mainz, Germany

In ongoing effort to discover new natural products, we isolated the fungal strain IBWF 003-21, which exhibits vibrant purple-pigmented conidia. We isolated the compound responsible for the coloration and identified it as the tetracyclic polyketide saintopin, a topoisomerase I/II inhibitor first reported in 1990, that has not been investigated much since. The chemical structure of saintopin differs from that of other fungal napthacenediones, implying a divergent biosynthetic mechanism. Genome mining identified six non-reducing polyketide synthases (nrPKS), that were introduced into the heterologous host *Aspergillus oryzae* OP12 for product analysis. Expression of *stpA* led to the production of a tetracyclic pyrone similar to saintopin, making it the prime candidate for saintopin biosynthesis. Deletion of *stpA* in the native producer abolished the production of saintopin, confirming its involvement in saintopin biosynthesis. A metallo-β-lactamase-like thioesterase (*stpB*) and a flavin-dependent monooxygenase (*stpC*), which are canonically required for biosynthesis of fungal napthacenediones did not cluster with *stpA* but were found elsewhere in the genome. Coexpression of the accessory genes led to heterologous production of saintopin in *A. oryzae*, elucidating the biosynthesis. While the exact mechanism for the cyclization of saintopin remains elusive, we provide first evidence of a new cyclization mechanism divergent from that of other fungal napthacenediones.

WP1.98 - Evolutionary transcriptomics to understand conidial development and germination of pathogenic and non-pathogenic Aspergillus species

P. Wu[1], *K.H. Wong*[1]
[1]University of Macau, Macao, China

Fungal conidia are the main infectious propagules of many human fungal pathogens. Inhalation of conidia by the host is a major entry route for pathogenic fungi, such as *Aspergillus fumigatus*, that cause lung and systemic infections. In order to cause lung infection, the inhaled conidia have to germinate and grow within the lung of a host. Conidial size and germination are believed to be important attributes of pathogenic species. Therefore, understanding conidia development and germination has important medical implications. In this study, we performed transcription profiling to study conidial development and germination in the human pathogen *Aspergillus fumigatus* and the non-pathogenic research model *Aspergillus nidulans*. Comparative and evolutionary transcriptomic analysis revealed that the transcriptomes of the two species during conidial germination are highly conserved, including numerous ancient genes with a pattern reminiscent of the “Hourglass” evolution model. In contrast, the conidiation process involved many modern genes, conforming to a “Reverse Funnel-like Model” of evolution. These findings suggest that the pathogenic features of *A. fumigatus* may have evolved, at least in part, from conidiation rather than germination. Evolutionary transcriptomic analysis of additional non-pathogenic and pathogenic species will provide further insights into the evolution of fungal

pathogenicity. These findings have important implications for combating fungal infections and developing novel antifungal strategies.

WP1.99 - Dynamic molecular dialogues in *A. nidulans* development: Interplay between pheromone producing enzymes and transcriptional regulators

E.S. Xylakis[1], *A. Zhornyak*[1], *L. Moorkamp*[1], *E. Bastakis*[1], *B. Popova*[1], *K. Schmitt*[1], *O. Valerius*[1], *G.H. Braus*[1]
[1]Georg August University, Department of Molecular Microbiology & Genetics, Goettingen, Germany

Internal as well as external stimuli determine the ratio of the further development of vegetatively growing *Aspergillus nidulans* hyphae to more asexual or more sexual development. The timing and balance between sexual and asexual development are controlled by the products of the fatty acid oxygenases PpoA (**P**si (precocious sexual inducer)-**p**roducing **o**xygenase **A**) and PpoC (**P**si-**p**roducing **o**xygenase **C**). Specific cellular Psi factor accumulation represents the molecular cues, which channel the ratio between the distinct transcriptional programs for the asexual or sexual developmental pathways. Therefore PpoA and PpoC indirectly regulate the transcriptomic landscape by producing specific gene expression patterns for either sexual or asexual development. Transcriptional changes driven by Psi factors are essential for ensuring the controlled and regulated progress of each developmental pathway in response to environmental signals. We have analyzed the physical interactions of the Psi-producing oxygenases and important players of fungal transcription. The current status of our study in exploring the interaction dynamics between metabolic enzymes and transcriptional regulators and their consequences for fungal development will be presented

WP1.100 - Dominant negative effect on UPR and RIDD by expression of RNase-inactive IreA in *Aspergillus oryzae*

M. Yokoyama[1], *K. Gomi*[1], *T. Shintani*[1]
[1]Tohoku University, Graduate School of Agricultural Science, Sendai, Japan

Aspergillus oryzae is a promising host for recombinant protein production due to its high protein secretion capacity, but the productivity of heterologous proteins is significantly low. This may be due to the removal of mRNAs and/or proteins by quality control mechanisms in the host during gene expression and protein secretion. Therefore, we focused on Regulated IRE1-dependent decay (RIDD), mRNA degradation mechanism in the endoplasmic reticulum (ER). Under ER stress, the ER transmembrane sensor protein IreA is activated through oligomerization and phosphorylation, and specifically degrades secretory protein mRNAs targeted to the ER membrane by its RNase activity. IreA is also involved in the splicing of *hacA* mRNA in the unfolded protein response (UPR), and its gene disruption is lethal. In this study, we introduced mutation into the RNase domain of IreA and we analyzed the effects on *hacA* splicing and RIDD. Mutations were introduced into highly conserved amino acids in the RNase domain identified

from the multiple alignments with other organisms. The resulting *ireA* mutants were expressed with its own promoter in a strain that can suppress expression of host-derived *ireA* by addition of thiamine. Mycelial growth was not complemented by expression of the *ireA* mutants in the presence of thiamine, but rather was even more inhibited. Furthermore, expression of mutant *ireA* reduced *hacA* splicing ability and RIDD of amylase mRNA under ER stress condition even when endogenous *ireA* was not suppressed. These results indicate that the expression of RNase-inactive IreA has a dominant-negative effect.

WP1.101 - Atypical cell death phenomenon induced by cell fusion revealed by pairing diverse strains in the industrial fungus *Aspergillus oryzae*

Y. Zou[1], *T. Katayama*[1], *K. Iwashita*[2], *J.-I. Maruyama*[1]
[1]The University of Tokyo, Department of Biotechnology, Tokyo, Japan, [2]National Research Institute of Brewing, Hiroshima, Japan

In filamentous fungi, cell fusion between genetically incompatible strains results in cell death, a phenomenon referred to as heterokaryon incompatibility. In *Aspergillus oryzae*, an industrial filamentous fungus used in Japanese food fermentation, there are numerous diverse strains for different purposes such as sake, soy sauce, and *miso* production. Our previous study revealed that *A. oryzae* strains can be classified into compatible groups, and cell death was typically detected in both fused cells derived from incompatible strains. In this study, we extensively examined diverse *A. oryzae* strains in heterokaryon incompatibility and assessed cell death phenomena. To explore the connection between the phylogeny and overall diversity in *A. oryzae*, multiple strains were selected from each of phylogenetic clades. Compatibility analysis by protoplast fusion revealed that compatible group classification exhibited a consistency with the phylogenetic clades. Additionally, co-culturing was performed to analyze heterokaryotic cell formation through cell fusion; strains from distinct phylogenetic clades did not form heterokaryon cells detected. However, unexpectedly, some of strain pairings within the same phylogenetic clade failed to produce heterokaryotic cells, which conflicted with the compatibility groupings by protoplast fusion. During cell fusion in such strain parings, either of the fused cells underwent cell death. These findings revealed an atypical cell death phenomenon induced by cell fusion under specific pairings of the diverse *A. oryzae* strains.

WP1.102 - Integrated study of fungal secondary metabolite and exoenzyme profiles, elucidating evolution of fungal interaction with competitors, substrate and host

L. Lange[1], *J.C. Frisvad*[2]
[1]Technical University of Denmark, Valby, Denmark, [2]Technical University of Denmark, Denmark/BioEngineering, Kongens Lyngby, Denmark

The secondary metabolite- and exoenzyme-profiles are studied separately, by researchers with different expertise, methods and objectives. In the current study we aim at making integrated

secretome analysis, including both metabolites and exoenzymes. This approach is inspired by evolution, exerting selection pressure on the entirety of the interaction profile; composed of both biologically active metabolites and enzyme-proteins. This study aims at testing the hypothesis that *Aspergillus* species, with strong degrading capacity, also have a strong profile of biologically active metabolites - e.g. giving added benefit as the fungus has a large amount of degraded substrate to defend against microbial foraging. In carrying out such integrated analysis, we take point of departure in *Aspergillus* genome-sequences, annotated to integrated F:F-observations (EC Function:Protein Family). Based on this the total number of CAZyme F:F-observations and the total number of unique observations (= function specificity diversity), can be summed up. Both scorings enable ranking, of enzyme profiles of all analyzed *Aspergillus* species, from highest to lowest. For integrated metabolite- and exoenzyme profiles, we selected four groups of *Aspergillus* species. Scoring highest, in enzyme degrading capacity (i), or in number of unique functions (ii); or scoring lowest in enzyme degrading capacity (iii), or lowest in number of unique functions (iv). Among these four groups of *Aspergillus* species, we selected species with secreted, well-characterized metabolite profiles, as analyzed by chromatography/mass spectrometry, together with exoenzyme profiles. The result from such integrated analysis of secondary metabolites and exoenzymes will provide an attempt to elucidate evolution of the *Aspergillus* interaction secretome.

WP1.103 - Multi-drug and -fungicide resistance in the pathogen *Aspergillus fumigatus*

M. Momany[1], *B.N Celia-Sanchez*[1], *B Mangum*[1,2], *L.F Gómez Londoño*[2], *C Wang*[2], *B Shuman*[1], *M.T Brewer*[2]
[1]University of Georgia, Fungal Biology Group & Plant Biology Dept., Athens, United States,
[2]University of Georgia, Fungal Biology Group & Plant Pathology Dept., Athens, United States

Fungi are important pathogens of plants and people. Fungi destroy 125 million tons of food crops annually, about 20% of the global food crop total. Invasive fungal infections kill 2.5 million people each year, with the pathogen *Aspergillus fumigatus* responsible for 1.8 million of those deaths. Azole compounds account for 25% of fungicides used on pathogens of crop plants and are also the first-line treatment used to combat *A. fumigatus* infections of people.
A. fumigatus resistance to azole antifungal drugs, a recognized problem in Europe and Asia for twenty years, has recently been gaining attention in the U.S. We isolated azole-resistant *A. fumigatus* from soil, plant debris, and compost in agricultural environments in seven U.S. states. We tested isolates for sensitivity to azole fungicides and antifungals along with other fungicides used only on crop plants. We found multi-drug and -fungicide resistant *A. fumigatus* is widespread in U.S. agricultural environments. Analysis including publicly-available sequence data showed that the U.S. isolates belong to three defined world-wide clades, with multi-drug and -fungicide resistant isolates largely in a single clade. Our analysis also showed high levels of recombination among isolates. Strikingly, clinical isolates of pan-azole resistant *A. fumigatus* also contained alleles conferring resistance to fungicides that have only been used on crop plants, showing that these clinical isolates have a clear agricultural history.

Author Index

Presentations where the author is the presenting author are in bold.

C

G

N

O