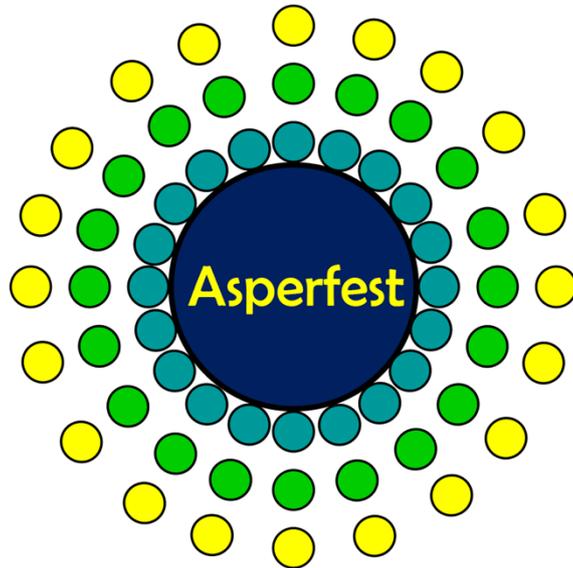


The 22nd International Aspergillus Meeting

Asperfest 22

March 16-17, 2026

Asilomar Conference Center, Pacific Grove CA, USA.



Aspergillus Genomes Research Policy Group (AGRPG)

An Aspergillus Genomics workshop was held at the March 2003 Asilomar Fungal Genetics meeting. From discussions in that workshop it was obvious that our community needed to organize to fully exploit genomics resources. A provisional Aspergillus Genomes Research Policy Committee (AGRPC) was conscripted and charged with creating a structure for community-wide coordination and organizing an annual meeting. The First Aspergillus Meeting was held in Copenhagen, April 21, 2004, as a satellite meeting of the European Congress on Fungal Genetics-7. In addition to scientific presentations, bylaws were approved, community research directions were discussed and the 2004 AGRPC was elected. The name Aspergillus Genomes Research Policy Group was adopted for the community. The objectives of the AGRPG are: (1) Provision of an educational and discussion forum for issues pertaining to Aspergillus genomics, in its widest sense, and for the various Aspergillus research communities; (2) Influencing grant making bodies and other institutions on behalf of the various Aspergillus research communities; (3) Coordinating research activities internationally, as and when required, to further the science base of the Aspergillus genus. For more information on the activities of the AGRPG and other Aspergillus news see our homepage at FGSC (<http://www.fgsc.net/Aspergillus/asperghome.html>).

2025 AGRPC

Amelia Barber, 2023-2025, Friedrich Schiller University, Germany; amelia.barber@uni-jena.de
Fabio Gsaller, 2023-2025, Medical University of Innsbruck, Austria; fabio.gsaller@i-med.ac.at
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Kap-Hoon Han, 2025-2027, Woosuk University, South Korea; khhan@woosuk.ac.kr

THANKS TO OUR MEETING SPONSORS!



MERCK complex and downstream regulatory genes operate development and secondary metabolism in *Aspergillus nidulans*.

Pranay Agarwal, *Maynooth University, Ireland.*

Global patterns of diversity and distribution in *Aspergillus* fungi are driven by human and environmental influences.

Olivia Riedling, *Vanderbilt University, USA.*

Strain-specific redox balancing for *Aspergillus fumigatus* pathoadaptation and antifungal drug susceptibility.

Katherine Quinn, *Geisel School of Medicine, Dartmouth College, USA.*

An *Aspergillus fumigatus* homeobox transcription factor provides resistance to copper stress and alters lipid metabolism through oxylipin signaling.

Harrison Estes, *University of Wisconsin-Madison, USA.*

Mutants escaping from growth inhibition between colonies of the same strain in filamentous fungi.

Yuya Hamanaka, *The University of Tokyo, Japan.*

10:00-10:30 Coffee Break and Posters

10:30 Session II

Jun-ichi Maruyama, The University of Tokyo, Japan

The dynamic idiomorph - a new role in heterokaryon incompatibility and diversification of mating-type regions.

Paul Dyer, *University of Nottingham, United Kingdom.*

AI-driven optimization of growth factor manufacturing from waste by *Aspergillus oryzae*.

Ofer Prinz Setter, *University of California, Berkeley, USA.*

Role of pyrimidines transport in the adaptation to the novel antifungal olorofim in *Aspergillus fumigatus*.

Clara Valero, *The University of Manchester, United Kingdom.*

The *Aspergillus fumigatus* growth determinant Afu6g07200 is the first granulin described in fungi.

Uxue Perez Cuesta, *University of Tennessee Health Science Center, USA.*

11:15 Tools/Resources

Amelia Barber, Friedrich Schiller University, Germany

Aspergillus fumigatus Af293/CEA10 community sequencing initiative.

Grant Nickles, *University of Wisconsin-Madison, USA.*

BRC Analytics.

Scott Cain, *The Pennsylvania State University, USA.*

FungiDB.

Omar Harb *FungiDB*

JGI MycoCosm pan-Aspergillus genomics resources
Sajeet Haridas, DOE Joint Genome Institute

12:10 Community directions: Discussion; Elections.

Discussion; Elections

Mike Bromley
University of Manchester, UK

12:30PM - 1:30PM Lunch

Crocker Dining Hall.

1:30PM Session III: Talks from Abstracts

Kap-Hoon Han
Woosuk University, South Korea

Molecular architecture and dynamic remodeling of fungal cell walls revealed by Advanced Solid-State NMR Spectroscopy.

Isha Gautam, *Michigan State University, USA.*

FluG confers conidial heterogeneity in *Aspergillus fumigatus*.

Ken Miyazawa, *National Institute of Infectious Diseases, Japan.*

Role of the *osaA* transcription factor gene in development, secondary metabolism and virulence in the mycotoxigenic fungus *Aspergillus flavus*.

Farzana Ehetasum Hossain, *Northern Illinois University, USA.*

Tissue-dependent role for cell wall regulatory pathways on *Aspergillus fumigatus* virulence.

Kevin Fuller, *University of Pittsburgh, USA.*

2:15 Pontecorvo Lecture. (Sponsored by CropLife International)

Mike Bromley
University of Manchester, UK

Tête-à-tête with *Aspergillus* .

Gerhard Braus,
Göttingen University, Germany.

2:45PM Election results; other discussion items

Mike Bromley
University of Manchester, UK

Novonesis student poster prize presentation

Clara Valero
University of Manchester, UK
Representative, Novonesis, Denmark

3:00PM Dismiss (Remove posters)

List of Posters

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Pranay Agarwal, Thuc Bui Tri, Iveta Sutkute, Eszter Sas, Saba Shabeer, David Fitzpatrick, Chris Koon Ho Wong, Rebecca Owens, Takeshita Norio, Kebin Kavanagh, Ozlem Sarikaya Bayram
- 2. Assessment of filamentous fungi with dye-degrading potential isolated from Basque estuaries**
Ziortza Agirrezabala Urkia, Ainara Otamendi, Laura Alonso-Sáez, Miren Ostra, Oier Etxebeste
- *3. Transition to an oxygen-limited phase in stirred-tank fermentation causes a transient increase in intracellular ROS and triggers respiratory rebalancing including an alternative oxidase-mediated bypass in *Aspergillus oryzae***
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- 4. Integrative Sequence–Structure Mining of Fungal Polyurethane-Degrading Enzymes Through a One Health Framework**
Saliha B Ardic, **Gunseli Bayram Akcapinar**
- 5. Rapid shift in azole resistance of *Aspergillus fumigatus* during isolate processing**
Balazs Brankovics, Bárbara Casella Amorim, Wellington Santos Fava, Manuel Leeuwerik, Plínio Trabasso, Marcia S. C. Melhem, María Luiza Moretti, Luciana Trilles, Beatriz Bustamante, Claudia M. Parra-Giraldo, Laura Castañón-Olivera, James Venturini, the Latin American Medical Mycology Network, Wieland Meyer
- 6. KERS Complex and Downstream Regulators Control Development and Secondary Metabolism in *Aspergillus nidulans***
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- *7. Exploring the interaction between the septin cytoskeleton and the cell wall integrity pathway MAPKs MpkA and MkkA in *Aspergillus fumigatus***
Rebecca J Busch, José Vargas-Muñiz
- 8. Quantitative single-molecule FISH reveals differential subcellular localization of *flb* family mRNA in the filamentous fungus *Aspergillus niger***
Xiaoyi Chen, Zixu Wang, Hans J.J.P.A.de Cock, Han H.A.B Wosten, Evelina Tutucci
- 9. The impact of geospatial variables and local mycobiota on the landscape of drug-resistant *Aspergillus fumigatus* in the United Kingdom**
Harry Chown, Samuel J Hemmings, Rodrigo Leitaó, Jennifer M. G. Shelton, Isobel C Stanton, Rachel Payne, Ahmed Nawaz, Huw Davis, Nichola J Hawkins, Abdullah Shahid, William D Pearse, Kostya Kanyuka, Toni Gladding, Andrew Singer, Leon P Barron, Matthew C Fisher
- 10. Molecular Basis of a Bacterial ‘Toxin Sponge’ that Protects its Fungal Partner from Antimicrobial Compounds**
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- 11. A long non-coding RNA mediates crosstalk between fungal thermal adaptation and azole drug response in *Aspergillus fumigatus***
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Milton Drott, Yen-Wen Wang, E. Anne Hatmaker, Nayanna M. Mercado-Soto, J. Mitch Elmore, Dianiris Luciano-Rosaro, Jae-Hyuk Yu, Antonis Rokas, John G. Gibbons, Anna Huttenlocher, Kunlong Yang, Nancy P. Keller
- 13. The dynamic idiomorph – a new role in heterokaryon incompatibility and diversification of mating-type regions**
Harry Chown, Felicia A Stamford, Asaf Salamov, Edoardo Tancredi, Scott Baker, Johanna Rhodes, Matthew Fisher, Michael Bottery, Igor Grigoriev, Ronald de Vries, Mike Bromley, **Paul S Dyer**
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Justin L Eagan, Samuel O'Donnell, Mira Syahfrien Amir Rawa, Sung Chul Park, Jin Woo Bok, Nancy P Keller, Emile Gluck-Thaler

- *15. An *Aspergillus fumigatus* homeobox transcription factor provides resistance to copper stress and alters lipid metabolism through oxylipin signaling**
Harrison P. Estes, Sung Chul Park, Benjamin Chadwick, Grant Nickles, Jin Woo Bok, Joshua Coon, Nancy P. Keller
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Becca L Wells, Joshua Cuozzo, Manali M Kamath, Gautham Ramakrishnan, Shi Ying Tang, Can Zhao, Kevin K Fuller
- *19. Molecular architecture and dynamic remodeling of fungal cell walls revealed by Advanced Solid-State NMR Spectroscopy**
Isha Gautam, Fredrick Mentik-Vigier, Jean-Paul Latge, Ramon Alberto Batista
- *20. Mutants escaping from growth inhibition between colonies of the same strain in filamentous fungi**
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- *29. Fitness effects of multi-fungicide resistances in *Aspergillus fumigatus***
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***33. Roles of aneuploidy in *Aspergillus fumigatus* unstable adaptation and antifungal resistance**

Anna E. Lehmann, Enrique Aguilar Ramirez, Nancy P. Keller, Joseph Heitman

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Ling Lu

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Ken Miyazawa, Takashi Umeyama, Shogo Takatsuka, Yasutaka Hoshino, Takanori Horiguchi, Yasunori Muraosa, Yoshitsugu Miyazaki

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Katherine D Mueller, Eun Young Huh, Chang Hwan Choi, Soo Chan Lee

39. *Aspergillus fumigatus* Af293/CEA10 community sequencing initiative

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***45. Strain-specific redox balancing for *Aspergillus fumigatus* pathoadaptation and antifungal drug susceptibility**

Katherine G Quinn, Robert A Cramer

***46. Global patterns of diversity and distribution in *Aspergillus* fungi are driven by human and environmental influences**

Olivia L. Riedling, Kyle T. David, Antonis Rokas

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Thomas J C Sauters, Allyson M Ray, Adiyantara Gumilang, Phoebe Lin, Ann Tate, Antonis Rokas

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David C. Rinker, **Thomas Sauters**, Karin Steffen, Adiyantara Gumilang, Huzefa Raja, Manuel Grimaldo, Thaila Reis, Patricia Alves de Castro, Camila Figueiredo Pinzan, Gustavo Goldman, Nicholas Oberlies, Antonis Rokas

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Jamie Tindale, Dillan Cara, Xijie Wang, Clara Valero Fernandez, Norman van Rhijn, Mike Bottery, Mike Bromley

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Heather D. Forster, Sara M. Hopkins, Cameron C. Hunter, Joel T. Steyer, **Richard B. Todd**

59. Role of pyrimidines transport in the adaptation to the novel antifungal olorofim in *Aspergillus fumigatus*

Clara Valero, Atisak Jiaranaikulwanich, Michael Bottery, Sara Gago, Michael Bromley

60. Epigenetic rewiring of the low-oxygen response by an Inhibitor of Growth (ING) family Protein drives *Aspergillus fumigatus* disease progression

Sandeep Vellanki, Katherine M Bultman, Robert A. Cramer

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Lyric A Wardlaw, Kurt A Dahlstrom

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Haruno Watanabe, Nuo Li, Kyoko Kanamaru, Makoto Kimura, Tetsuo Kobayashi, Tetsuya Kimura, Emi Kunitake

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Masaki Yokoyama, Sohta Sagara, Katsuya Gomi, Takahiro Shintani

65. Pseudouridine modification regulates development, azole resistance and genome stability in *A. fumigatus*

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Silai Zhang, Sohta Sagara, Hayato Takebayashi, Katsuya Gomi, Takahiro Shintani

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(*Student presenters in bold)

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Abstracts

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* denotes a student poster presenter

*1. MERCK complex and downstream regulatory genes operate development and secondary metabolism in *Aspergillus nidulans*

Pranay Agarwal¹, Thuc Bui Tri¹, Iveta Sutkute¹, Eszter Sas¹, Saba Shabeer¹, David Fitzpatrick¹, Chris Koon Ho Wong², Rebecca Owens¹, Takeshita Norio³, Kebin Kavanagh¹, Ozlem Sarikaya Bayram¹

¹Biology, Maynooth University, ²Health sciences, University of Macau, ³Life and Environmental Sciences, University of Tsukuba

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 (20 h of growth) and the SM stage (48 h of growth) in deletion mutants and wild type (WT). Comparison of deletion mutants with the wild type identified 214 genes commonly downregulated at the SM stage across all mutants. The top five most differentially regulated genes with regulatory functions (e.g., transcription factors, chromatin-binding proteins, and kinases) were selected for further functional characterization. The bZIP transcription factor mdrC, which is consistently downregulated in all MERCK mutants and is directly bound by RstB (R from MERCK), is required for normal growth, epigenetic regulation, transcriptional control of regulatory genes, stress response, sporulation and spore germination, sexual fruiting-body formation and maturation, as well as secondary-metabolite production. Protein pull-down experiments using MdrC-GFP and MdrC-3xHA tagged strains revealed that it binds with proteins involved in the synthesis of SMs antibiotic 2,4-dihydroxy-3-methyl-6-(2-oxopropyl)benzaldehyde (DHMBA) and Aspercryptins along with key proteins involved in amino acid metabolism. Collectively, the data indicate that the MERCK complex and its downstream regulatory network integrate developmental pathways with the regulation of secondary-metabolite production in *A. nidulans*.

2. Assessment of filamentous fungi with dye-degrading potential isolated from Basque estuaries

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Contamination of aquatic environments has become a big issue over the last years. Coloured wastewater effluents released by several industries, especially the textile sector, may not only be mutagenic, carcinogenic, or toxic, but also block light penetration into the water, restricting photosynthetic processes, inhibiting the growth of aquatic biota, and interfering with gas solubility in water. Filamentous fungi derived from marine environments are well known as a potential genetic resource for various biotechnological applications, including bioremediation. In this context, this work focused on the isolation of filamentous fungi from sediment samples collected from estuaries of the Basque Country. Their phenotypic characterization led to the identification of strains potentially capable of degrading industrial dyes. Three isolates belonging, based on ITS sequencing, to the genera *Acremonium* (M38), *Monodictys* (M63), and *Cladosporium* (M104) were selected for further study due to their potential to degrade and/or adsorb industrial dyes such as Remazol Brilliant Blue R (Reactive Blue 19), Congo Red (Direct Red 28) or Indigo Carmine (Acid Blue 74). Genomics, transcriptomics and proteomics analyses of those strains will provide key information on the genome features of those species and the mechanisms and activities induced for the degradation/adsorption of these dyes. In summary, this study shows that marine fungi from the Basque coast could be a valuable source for the development of new biotechnological tools for reducing dye pollution and protecting aquatic environments.

*3. Transition to an oxygen-limited phase in stirred-tank fermentation causes a transient increase in intracellular ROS and triggers respiratory rebalancing including an alternative oxidase-mediated bypass in *Aspergillus oryzae*

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During fermentation in stirred-tank bioreactors (STBR), filamentous fungi are frequently exposed to oxygen-limited conditions. However, the response to such conditions, particularly during short-term exposure (≤ 6 h), remains poorly understood. To address this gap, we conducted short-term multi-omics profiling of *Aspergillus oryzae* hyphal dispersion strain grown under the condition in which dissolved oxygen (DO) concentration was transiently controlled towards 0% in the 4 L STBR.

Transcriptome analysis revealed that genes encoding respiratory chain complexes I–III were temporarily down-regulated within 1 h after DO depletion (DO < 1%: hypoxia) followed by enhanced expression at later phases. In contrast, complex IV transcripts were immediately up-regulated under hypoxia. This rapid induction of complex IV genes likely reflects an enhancement in oxygen utilization capacity, thereby increasing the likelihood of capturing scarce oxygen under hypoxic conditions. However, biological interpretations of the observed transient down-regulation of complexes I–III genes were unclear. Based on a known phenomenon that the levels of reactive oxygen species (ROS) increases in respiratory chain under hypoxia, we hypothesized that elevated ROS may suppress the

expression of complexes I–III genes. As a result of a ROS assay, a transient rise in intracellular ROS was observed under hypoxia. Furthermore, metabolome analysis showed a decrease in the reduced/oxidized-glutathione ratio under hypoxia, aligning with the ROS assay results.

To further investigate the relationship between respiratory chain and intracellular ROS, we focused on an alternative oxidase (AOX) of which gene expression showed immediate up-regulation under hypoxia. Generally, AOXs bypass complexes III–IV and can limit the generation of ROS, but their functions in *A. oryzae* remain unclear. Interestingly, a deletion of an AOX gene prolonged the ROS elevation. Consistent with the prolonged ROS elevation, complexes I–III genes remained lower expression levels compared to pre-hypoxia, further supporting our hypothesis. These results suggest a biological function of AOX in *A. oryzae* in mitigating the elevated ROS caused by hypoxia in the STBR fermentation.

Overall, our results provide new insights showing that early hypoxia temporarily increases intracellular ROS levels, and the respiratory rebalancing including a strong AOX induction buffers the oxidative stress in *A. oryzae* in the STBR fermentation.

4. Integrative Sequence–Structure Mining of Fungal Polyurethane-Degrading Enzymes Through a One Health Framework

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Plastic has become deeply embedded in modern life, yet its accumulation in natural ecosystems—particularly polyurethane (PU), a chemically diverse and highly durable polymer—posed escalating environmental and public health risks. Although PU's industrial value stemmed from its resilient urethane, ester, and ether linkages, these same features made it a persistent pollutant for which conventional chemical remediation methods remained inefficient and environmentally burdensome.

In this study, we applied a systematic, bioinformatics-driven approach grounded in the One Health concept to discover and prioritize novel PU-degrading enzymes, with a particular emphasis on fungal lineages. We curated a comprehensive dataset of reported bacterial and fungal enzymes with activity on PU-associated bonds. Their physicochemical properties were characterized using ExPASy ProtParam, and high-confidence structural models were generated with AlphaFold. Comparative analyses using Datamonkey and MEME Suite identified conserved motifs and residues under positive selection that could underpin catalytic efficiency.

Guided by these molecular signatures, we performed large-scale sequence-based mining across NCBI, Swiss-Prot, EMBL-EBI, and especially the JGI MycoCosm portal, which provided extensive genomic coverage of ecologically diverse fungi. In parallel, structure-based searches using FoldMason identified protein architectures with active-site features favorable for urethane-bond cleavage.

By integrating evolutionary, structural, and sequence-level insights, we constructed a focused library of high-priority candidate enzymes enriched in degradation-associated motifs and stable folds.

5. Rapid shift in azole resistance of *Aspergillus fumigatus* during isolate processing

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Within the framework of the LatAsp (Latin American *Aspergillus fumigatus* Azole Resistance Survey in 12 countries, 20 laboratories), a large number of air samples were collected to assess the genetic diversity of *A. fumigatus* in Latin America and the percentage of azole resistance. During processing, some isolates showed discrepancies between their MIC profiles and their *cyp51A* genotypes. Repeating MIC assays yielded inconsistent results, with later assays showing higher MIC values than earlier ones. Several of these isolates were submitted for whole genome sequencing in duplicate, using DNA extracts from subcultures from earlier and later MIC assays. The initial hypothesis was that these isolates represented a population of strains, and different experiments corresponded to different strains from the population.

DNA extracts were sequenced using Illumina NovaSeq X. Sequencing reads were quality-filtered. Followed by read mapping using bwa against the reference genome, Af293. The sequences were also *de novo* assembled using SPAdes assembler. Relatedness of the genomes was estimated using whole genome SNP tree, average nucleotide identity of genome assemblies, and microsatellite genotyping (STRAf in the lab and *in silico* for genomes).

The sequencing replica results can be classified into three sets: i) showing nearly identical genomes including *cyp51A* sequence, ii) showing two completely divergent genomes confirming our initial hypothesis, and iii) showing highly similar genomes that deviated in their *cyp51A* sequences. This final set of replicas, having nearly identical genomes but differing in the *cyp51A* sequences, was further investigated. Closer analysis of the mapping data for the *cyp51A* gene region of these replicas showed 100% support for the absence of the resistance-associated SNP in the wildtype genome and 100% support for the SNP in the non-wildtype one. Some of these isolates had a similar genetic background, indicative of a clonal lineage. However, individual events involve different resistance-

conferring mutations. In addition, this pattern has also been found with other genetic backgrounds besides the clonal lineage.

The results raise the question of whether closely related strains that differ in their susceptibility to azoles are distributed in the air together, or whether azole resistance can emerge so rapidly in the laboratory. Further experiments on these wildtype isolates could shed light on the rate of resistance development.

6. KERS Complex and Downstream Regulators Control Development and Secondary Metabolism in *Aspergillus nidulans*

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The KERS complex is an important epigenetic regulator in *Aspergillus nidulans*, integrating chromatin remodeling with transcriptional control and secondary metabolism. Composed of KdmB (histone demethylase), EcoA (histone acetyltransferase), RpdA (histone deacetylase), and SntB (chromatin-associated protein), the complex influences both primary and secondary metabolic levels. To distinguish KERS-dependent genes, RNA-seq was performed on $\Delta kdmB$, $\Delta ecoA$, $\Delta rpdA$, and $\Delta sntB$ mutants and compared with the wild type at 20 hours (primary metabolism) and 48 hours (secondary metabolism). Differential expression analysis revealed broad transcriptional changes, with 500 to over 4,000 genes affected per mutant, including 204 genes commonly altered during primary metabolism and 545 during secondary metabolism; of these, 25 genes were consistently downregulated across all mutants at 20 hours and 104 at 48 hours. Among the strongest candidate genes is *kdrD*, which is consistently downregulated in all KERS mutants and directly bound by the complex. *kdrD* is identified to regulate fungal development, secondary metabolism, spore formation, and germination, and a loss-of-function analysis is underway to determine how its absence affects these processes and how this may relate to the defects observed in the KERS mutants. Overall, these results highlight how the KERS complex and its downstream regulator genes coordinate fungal development with secondary metabolite production in *A. nidulans*.

7. Exploring the interaction between the septin cytoskeleton and the cell wall integrity pathway MAPKs MpkA and MkkA in *Aspergillus fumigatus

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Aspergillus fumigatus is the primary etiological agent behind invasive aspergillosis (IA), one of the most deadly and costly invasive fungal diseases. IA is notoriously difficult to treat due to increasing resistance to antifungal drugs and limited therapy options. The echinocandins, including caspofungin, target the cell wall, but are only fungistatic against *A. fumigatus*. This fungistatic response requires signaling through the cell wall integrity (CWI) pathway. Similarly, the septin AspB is also required for caspofungin fungistatic effect. Septins are a family of highly conserved eukaryotic GTP-binding proteins. Despite their functions varying across species, they notably act as scaffolds to recruit proteins for specific cellular functions. Previous epistasis analyses in *A. nidulans* also showed a connection between MpkA and the septins. Furthermore, our preliminary proteomics pulldown data showed that the CWI pathway kinases, MkkA and MpkA, co-immunoprecipitated with AspB. Based on this, we hypothesize that the *A. fumigatus* septin cytoskeleton contributes to CWI signaling. To test this hypothesis, we performed epistasis analyses between the *aspB*, *mpkA*, and *mkkA* genes and found that the $\Delta aspB\Delta mpkA$ and $\Delta aspB\Delta mkkA$ double deletion strains had reduced susceptibility at sub-lethal concentrations compared to the $\Delta mpkA$ and $\Delta mkkA$ single deletion strains. Western blot analyses showed that the $\Delta aspB$ strain had less total MpkA, but more phosphorylated MpkA under basal conditions. We will determine if loss of septins during caspofungin exposure prevents CWI signal to the nucleus using a strain that expresses MpkA tagged with GFP in the wild-type and $\Delta aspB$ strains. This work will help uncover the connection between the CWI pathway and septins, as well as provide insight into unlocking the fungicidal potential of caspofungin for use against IA.

8. Quantitative single-molecule FISH reveals differential subcellular localization of *flb* family mRNA in the filamentous fungus *Aspergillus niger*

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Aspergillus niger is a filamentous fungus important in carbon cycling due to its ability to degrade diverse organic materials. During growth, it forms colonies with distinct regions, where peripheral zones show enhanced growth and protein secretion. While colony heterogeneity is well described, it is unclear whether spatial organization arises from localized gene expression at the hyphal level. To investigate this, we analyzed the spatial distribution of the Flb proteins and their mRNAs in *A. niger* using reporter proteins and single-molecule FISH. The *flb* gene family (*flbA*–*flbE*) regulates growth, sporulation, and secretion. FlbA and its mRNA were evenly distributed, but FlbB–E showed distinct spatial patterns. Notably, *flbD* mRNA localized at hyphal tips, while its protein was nuclear throughout the hyphae. This mRNA localization was disrupted in $\Delta flbE$ and $\Delta rrm4$, reducing nuclear FlbD levels. These findings suggest that spatial mRNA localization affects protein distribution, possibly contributing to *A. niger*'s growth and colony organization.

9. The impact of geospatial variables and local mycobiota on the landscape of drug-resistant *Aspergillus fumigatus* in the United Kingdom

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Aspergillus fumigatus is a major aetiological agent of the disease aspergillosis, which is responsible for the annual deaths of an estimated 2 million people, globally. *A. fumigatus* occupies a soil niche environment and is exposed to agricultural fungicides. Agricultural fungicides share similar mechanisms of action as clinical antifungals. Therefore, resistance towards fungicides leads to inefficacy of clinical therapeutics. The widespread application of fungicides has led to a growing incidence of fungal antimicrobial resistance (fAMR). Drug-resistant *A. fumigatus* has been identified in up to 49% of clinical and environmental samples. Environmental reservoirs, termed “hotspots”, of drug-resistant *A. fumigatus* have been identified in sources such as retail plant products, fruit and compost. However, associations between local environmental variables, ecological associations with other fungi and the development of hotspots remain unexplored in the United Kingdom. Here, we performed fAMR surveillance of *A. fumigatus* across 94 sites including farms, industrial composters and private residences in the UK. A combination of terrestrial and aerial samples were collected to identify hotspot locations and airborne exposure sites. We were able to calculate the resistance fraction from each site and associate this with geospatial variables including temperature, fungicide application and rainfall. Using a Bayesian statistical framework, we have been able to identify the impact of land usage on the prediction of fAMR generation. Furthermore, through the incorporation of 163 whole-genome sequenced isolates from aerial sampling into phylogenetic generalised linear mixed models (PGLMM), to uncover whether occupancy in the air is not only driven by environmental gradients and resistance profiles but also evolutionary constraints. Additionally, we have performed mycobiome analysis of terrestrial samples to uncover the ecological interactions that may drive the development of resistance. These results highlight the impact of anthropogenic behaviours, microbial communities and environmental conditions on the emergence of fAMR in *A. fumigatus*. Understanding variables driving the generation of fAMR hotspots will offer novel avenues for targeted intervention through evidence-based-policies to help mitigate the development of fAMR and ultimately improve therapeutic outcomes.

10. Molecular Basis of a Bacterial ‘Toxin Sponge’ that Protects its Fungal Partner from Antimicrobial Compounds

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Microbial community composition is determined by competition among resident microbes where community-produced antimicrobial compounds (AMCs) play a large role. Our lab studies a physically associated, bacterial-fungal pairing in which the novel bacterium *Paraburkholderia edwinii* acts as a toxin sponge to protect its fungal partner, *Aspergillus calidoustus*, from natural AMCs like phenazine-1-carboxylic acid (PCA). We have found that *P. edwinii* acts as a ‘toxin sponge’ by sequestering and reducing the toxic molecule while forming aggregates within the fungal mycelium. Further, *A. calidoustus* lowers the local pH during stress, activating the protection program in *P. edwinii*. We have utilized this fact to create a colorimetric assay whereby the bacterial protection program can be activated in the absence of the partner fungus, enabling mutagenic screens for genes involved in protection. We have identified the regulon of a key transcription factor that promotes both association with the fungal host during stress and detoxification of PCA.

To fully establish this as a model system, our group has also rendered *A. calidoustus* genetically tractable and conducted transcriptomic studies to determine which fungal genes are implicated in the defense against PCA without, and with, the partner bacterium. Finally, we have expanded the work to show *P. edwinii* can protect *A. calidoustus* from synthetic, clinically relevant, antifungal drugs such as voriconazole, and have demonstrated that key bacterial genes implicated in PCA protection are not relevant in voriconazole protection. Taken together, our results raise the possibility that protective bacteria are a major factor in determining which fungi are able to persist in environmental and infectious microbial communities.

11. A long non-coding RNA mediates crosstalk between fungal thermal adaptation and azole drug response in *Aspergillus fumigatus*

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Fungal pathogens are remarkable in their ability to adapt to mammalian (37°C) and avian physiological temperatures (38-42°C) to cause infection. Saprophytic mold *Aspergillus fumigatus* (AF) is unique in its ability to adapt to temperature and can grow above 50°C. Here, we show that AF adaptation to increased temperature results in morphological changes and, more importantly, increased fungal growth in presence of azoles. Importantly, this is a reversible phenotype and is mediated by a long non-coding RNA whose RNA levels negatively correlate with temperature. Our data show that lncRNA levels differ during saprophytic phase (25°C), mammalian infection phase (37°C) and increased temperature exposure (42°C), thus acting as a link between temperature adaptation and fungal azole response. Furthermore, the overexpression of lncRNA in azole-resistant isolates increases fungal azole susceptibility and, importantly, improves the azole treatment outcomes in a murine model of infection, clearly showing the role of lncRNA in infection outcomes for azole drug-resistant isolates. Through our adaptation experiment, we also

show that fungal adaptation to sub-MIC voriconazole concentration is a precursor to the development of a non-reversible novel drug resistance mechanism that is lncRNA dependent. Taken together, these data show that *A. fumigatus* adapts to increased temperature by downregulating lncRNA levels that impacts fungal azole response and the subsequent exposure to azoles results in evolution of non-CYP51-dependent azole resistance.

12. Population-Specific Transcriptomic Rewiring Underlies Secondary Metabolic Diversification in *Aspergillus flavus* and the Domestication of *Aspergillus oryzae*

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The genomic signatures of microbial domestication remain poorly understood within the context of natural population variation. Here, we demonstrate that *Aspergillus oryzae*, the filamentous fungus used in soy sauce production, shares more recent ancestry with a predominantly northern, largely non-aflatoxigenic population of *Aspergillus flavus* (population C). Strikingly, *A. oryzae* isolates also overlap with the recently described, clinically enriched *A. flavus* population D, suggesting the possibility of multiple domestication events. Although *A. oryzae* exhibits reduced virulence compared to *A. flavus*, all isolates tested retained pathogenicity in a zebrafish infection model. At the transcriptomic level, *Aspergillus* populations are significantly differentiated, with distinct responses to population density, indicating that population-specific transcriptomes adapt differently to ecological conditions. These differences extend beyond gene content and are not always explained by phylogenetic relationships, suggesting that phenotypic diversification occurs through the rapid reorganization of transcriptomic architectures. For example, *A. oryzae* displays significantly elevated expression of a module enriched for carbohydrate metabolism. Population-specific variation is also evident among secondary metabolite (SM) gene clusters. While *A. oryzae* shows markedly reduced expression of specific SM genes, particularly those involved in aflatoxin biosynthesis, this trend does not extend across the entire secondary metabolome. Using machine-learning-based gene regulatory network inference, we identified population-specific transcriptomic differences linked to distinct transcription factors, with evidence for both cis- and trans-acting regulatory divergence, but no changes in global regulators such as *laeA*. Together, these findings provide new insights into the domestication of *A. oryzae*, its global significance, and the microevolution of fungal secondary metabolic pathways.

13. The dynamic idiomorph – a new role in heterokaryon incompatibility and diversification of mating-type regions

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The idiomorph region is defined within fungal genomes as a region where at a given locus two highly dissimilar stretches of DNA may be present in different isolates. This contrasts with standard loci where similar, although slightly divergent, alleles may be found. The term was first introduced in the early 1990s and has since been widely used to describe mating-type (MAT) regions in filamentous ascomycete fungi. Here, highly dissimilar stretches of DNA approx. 2-5 kb in size are present at the MAT locus in MAT1-1 or MAT1-2 isolates of heterothallic species, with these idiomorph regions typically containing between one to three ORFs. Thus, idiomorphs are primarily known for their role in sexual compatibility. In a first project involving pangenome analysis of the opportunistic pathogen *Aspergillus fumigatus*, an idiomorph-like locus was detected on chromosome 2 whilst searching for highly divergent allelic regions that might have a role in heterokaryon incompatibility. The idiomorphs consisted of highly dissimilar DNA regions of 2.0 or 3.2 kb in different isolates, bordered by common conserved flanking sequences. The different idiomorphs each contained at least one putative ORF. The functional role of the region was investigated by CRISPR-Cas9 mediated gene transfer using nit mutants. Different idiomorph combinations either allowed hyphal fusion or resulted in hyphal incompatibility i.e. genes present at this region were responsible for heterokaryon incompatibility, with the genes termed *hetF1* and *hetF2*. Therefore, a novel role for idiomorphs in asexual heterokaryon fusion is now described. In a second project involving whole genome analysis of 292 *Aspergillus* species the structural organisation of the MAT locus and associated idiomorph region was investigated. A high diversity of six different MAT locus arrangements was discovered. Heterothallic species contained a typical idiomorph region with either MAT1-1 or MAT1-2 genes present. By contrast, homothallic species showed either incorporation of both MAT1-1 and MAT1-2 genes at the MAT locus, various forms of MAT gene incorporation elsewhere in the genome outside of the idiomorph region, or in one remarkable instance movement of both MAT1-1 and MAT1-2 out of the idiomorph region, leaving a locus with the conserved synteny of a MAT locus but without any MAT genes present. Results overall show that idiomorph regions can be highly dynamic and have roles both in sexual and asexual incompatibility in fungi.

14. Fungal *Starships* as Hotbeds of Sub-functionalization: Divergent Roles of the *LaeA* Paralogs on *Starship Janus*

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Fungal *Starships* are a recently discovered superfamily of large, mobile DNA elements that not only encode a mechanism for their transposition but also carry a variety of other genes as genetic cargo. We have identified an intriguing pattern across several fungal species where paralogs of non-mobile core genes are repeatedly found in *Starships*. Yet we do not know how processes of phenotype evolution via gene duplication play out in the context of *Starships*: are *Starships* hotbeds of neo-functionalization or is sub-functionalization more common when compared with other related sequences in the non-mobile genome? Here, we test the hypothesis that paralogs carried as *Starship* cargo uniquely contribute to the evolution of phenotypic variation compared with other related loci. *Starship Janus* in *A. fumigatus* carries two paralogs of the master regulator *LaeA* whose activities profoundly impact fungal secondary metabolism, virulence and development. These two *Starship*-associated *LaeA*-like proteins (*sLaeA* and *sLaeB*) have canonical methyltransferase domains and are more closely related to *LaeA* than any other methyltransferase, suggesting they may also participate in global regulation of important phenotypes. We developed deletion strains of each *Starship*-associated paralog individually and in combination in both the wild type and *laeA* deletion backgrounds. Preliminary data suggests loss of *sLaeA* reduces sporulation and is additive to the already reduced spore production observed in the *laeA* deletion background. Metabolomic analysis did not suggest further alterations to the chemical profiles beyond the extensive changes of *laeA* loss alone. Current efforts include assessing these strains with transcriptomics and proteomics to identify pathways that may be shared between *LaeA* and the *Starship*-associated paralogs to quantify the extent of neo- vs sub-functionalization. Although still preliminary, our data support the conclusion that *Starship*-associated *LaeA*-like proteins and their counterpart *LaeA* are similarly involved in fungal development but do not share a global regulatory role in secondary metabolism, supporting a model where duplicated *Starship* cargo contributes to fungal evolution primarily through the process of sub-functionalization.

*15. An *Aspergillus fumigatus* homeobox transcription factor provides resistance to copper stress and alters lipid metabolism through oxylipin signaling

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Fungal pathogens like *Aspergillus fumigatus* must maintain copper homeostasis during infection, balancing copper's essential role as an enzyme cofactor against its toxicity when weaponized by host immune cells. While copper transporters have been well characterized, the regulatory mechanisms coordinating copper stress responses with broader metabolic adaptations remain poorly understood. We identified *HbxH*, a previously uncharacterized homeobox transcription factor, which when overexpressed (OE::*hbxB*) increases both germination and biomass in copper starvation and toxicity up to fourfold. To investigate the mechanisms underlying this phenotype, we performed untargeted proteomics under copper starvation, identifying 522 differentially expressed proteins between the deletion (Δ *hbxB*) and OE::*hbxB*. Multiple predicted lipid biosynthetic and regulatory proteins were significantly altered in OE::*hbxB*, prompting lipid visualization using Nile Red fluorescence microscopy. OE::*hbxB* displayed pronounced lipid accumulation and increased lipid droplet abundance across all tested conditions. Additionally, metabolomics revealed the oxylipin 5,8-diHODE was increased by approximately 30-fold in the overexpression. Strikingly, exogenous addition of 5,8-diHODE to wild type and Δ *hbxB* phenocopies the increased growth and lipid accumulation of OE::*hbxB* in copper stress. These findings reveal an unexpected link between copper homeostasis and lipid metabolism mediated by oxylipin signaling. Future investigation aims to determine if lipid accumulation itself confers copper stress resistance and understand these effects using *in vivo* and *in vitro* host-pathogen interaction models.

16. Control of conidiation in *Aspergillus nidulans*: On the centrality and specificity of the master regulator BrIA

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Asexual spores are the main vehicle for dissemination of fungi. *Aspergillus nidulans* is one of the most commonly used reference systems for the study of the genetic and molecular control of asexual development. According to the generally accepted model, in *A. nidulans*, BrIA is a master transcription factor of conidiophore development and conidia production linking upstream regulators of the UDA pathway with downstream regulators of the central developmental pathway. This talk will discuss specific features of this model, and whether it is extrapolable or not to other clades of the fungal tree. It will also present recent results on the study of the role that BrIA and other transcriptional regulators have in conidiation and introduce recently identified genes necessary for asexual

development. Future approaches to the topic will also be suggested, as stimulating elements contributing to the understanding of how BrIA, in coordination with other regulators of growth and development, controls conidiation.

***17. Conservation of the regulatory pathway for conidiophore development across species of the *Aspergillus* genus**

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Ascomycete fungi reproduce asexually by conidiation, producing clonal spores. The morphogenetic processes leading to the production of a conidiophore, the structure bearing the asexual spores or conidia, are well defined in the model fungus *Aspergillus nidulans*. The central regulatory pathway (CRP) consists of three proteins: BrIA, which is required for vesicle formation, AbaA, which is required for sterigmata formation, and WetA, which is required for conidial maturation. This CRP includes self- and cross-regulatory loops and is also regulated by additional proteins to ensure conidiation only happens when proper signals are present in the environment.

In this work, we examined the conservation of the CRP proteins as well as associated conidiation regulators in nearly 300 species of the genus *Aspergillus* by first characterizing the corresponding orthologs across the genus. Then, the promoter regions of these orthologs were inspected, finding a variable degree of conservation in the self- and cross-regulatory loops described in *A. nidulans*. These orthologs were further analyzed for variability in their protein sequences, unraveling several group determinant residues across BrIA and AbaA, some of which mapped to or nearby to their DNA binding regions. A complementary evolutionary model based on selected conidiophore characteristics described in the *Aspergillus* genus suggests an evolution through specialization with similarities to what was described originally by Darwin's finches. Together, these results suggest that different versions of BrIA and AbaA might participate in the determination of some of the conidiophore morphologies – such as sterigmata arising in one or two series –, particularly in species belonging to subgenus Fumigati or Nidulantes, as these groups represent highly specialized proteins, as shown by our results.

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18. tissue-dependent role for cell wall regulatory pathways on *Aspergillus fumigatus* virulence.

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Aspergillus fumigatus is a common agent of invasive lung and corneal infection, both of which are associated with poor antifungal treatment outcomes. We reason that pathways that regulate fungal adaptation or growth within the host environment may serve as novel drug targets, yet the extent to which such pathways vary across host tissues (e.g. the lung vs the cornea) is poorly understood. Here, we demonstrate that deletion of the carbon catabolite repressor gene (*creA*) in *A. fumigatus* results in a marked reduction in cell wall chitin, which is an important mediator of wall rigidity. Interestingly, whereas $\Delta creA$ was able to grow through explanted lung tissue and establish infection in a murine model of invasive pulmonary aspergillosis (IPA), the mutant could not penetrate into explanted cornea and was avirulent in an *in vivo* model of fungal keratitis (FK). We reasoned that salient difference between the two host sites is matrix density, where the fungus requires high wall rigidity (increased chitin) to penetrate the dense collagen matrix of the cornea, and less rigidity to penetrate the relatively spongy lung parenchyma. We further reasoned that this may explain why others have shown that an *mpkA* mutant, which is defective in its ability to upregulate chitin in response to cell wall stress, is fully virulent in the lung. To explore if *MpkA* is important for virulence in the cornea, we generated an *mpkA* deletion in our background and confirmed expected phenotypes, including a marked hypersensitivity to cell wall stressing drugs, including Congo red and caspofungin, as well as severely stunted radial growth on agar. Interestingly, however, we found that growth (biomass accumulation) of $\Delta mpkA$ was indistinguishable from the wild-type and complemented strains in shaking broth culture, suggesting that growth of the mutant varies as function of the environmental growth matrix. Critically, and in contrast to reports from the lung, $\Delta mpkA$ was unable to establish infection in the murine cornea. Ongoing studies seek to determine if this loss of virulence for $\Delta mpkA$ can be directly tied to reduced cell wall chitin and if chitin synthesis in the wild-type strain is different within the cornea and lung microenvironments. Nevertheless, our current results suggest that virulence pathways, and by extension novel antifungal targets, may vary as a function of host niche, with cell wall regulatory pathways being particularly attractive targets in the setting of FK.

***19. Molecular architecture and dynamic remodeling of fungal cell walls revealed by Advanced Solid-State NMR Spectroscopy**

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Invasive fungal infections remain a major cause of morbidity and mortality among immunocompromised patients, yet current antifungal drugs are limited by toxicity, poor efficacy and rapid emergence of resistance. The carbohydrate-rich cell wall is an attractive target as it is absent in humans; however, its structural organization, dynamics, and remodeling pathways remain poorly understood. To address this gap, we employed multidimensional solid-state NMR

(ssNMR), a nondestructive atomic-resolution technique, to characterize the architecture, packing, and adaptive restructuring of fungal cell walls directly in intact cells across pathogenic and extremophilic species. Using $^{13}\text{C}/^{15}\text{N}$ isotopic labeling and Fast magic-angle spinning, and hydration-relaxation NMR methods, we resolved the composition, linkages, and dynamic of major polysaccharides, including β -1,3-glucan, β -1,6-glucan, chitin, chitosan, galactomannan, and galactosaminogalactan in *Aspergillus fumigatus*, *Candida albicans*, and *Rhizopus delemar*. Proton-detected ssNMR on partially deuterated *Aspergillus* cell wall revealed pronounced polymorphism and conformational heterogeneity in glucan and chitin networks, identifying multiple packing states that coexist within the native wall. Second, we characterized the structural responses of *A. fumigatus* to caspofungin, demonstrating a coordinated remodeling program in which β -1,3-glucan depletion is compensated by increased chitin, chitosan, and new α -glucan and branched glucan conformers that stabilize the drug-stressed wall. Third, we examined *Rhizopus* germination and uncovered a temporally ordered remodeling trajectory: early loosening of β -glucans, progressive chitin-to-chitosan conversion, and increasing wall rigidification as spores transition from dormancy through swelling to polarized hyphal growth. Finally, to understand adaptation under extreme abiotic pressure, we investigated the halophilic fungus *Aspergillus atacamensis*, revealing that hypersaline conditions induce the incorporation of extracellular salt microcrusts, reshape hydration profiles, and reorganize glucan assemblies into densely packed, mechanically resilient architectures that support survival in salt-saturated environments. Altogether, this research provides a molecular blueprint of how fungal cell walls reorganize during drug stress, development, and extreme environments, revealing structural signatures that could guide the creation of more effective, targeted, and broadly applicable antifungal therapies.

***20. Mutants escaping from growth inhibition between colonies of the same strain in filamentous fungi** **Yuya Hamanaka¹, Takuya Katayama², Jun-ichi Maruyama³**

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In filamentous fungi, growth inhibition occurs between colonies of different species or strains, known as “antagonistic effect”, which has been studied for a hundred years in terms of a biological control. In contrast, we found that colony-colony growth inhibition occurs between the same strain in *Aspergillus oryzae*. This phenomenon would mean the presence of self/non-self recognition even between colonies of the same strain. Previously, we demonstrated that the inhibition involves FlbA/FadA-mediated G-protein signaling. In this study, to further elucidate mechanisms regulating colony-colony growth inhibition, we performed mutant screening using UV-induced mutagenesis. Under the multi-spot inoculation, colony growth normally stopped without contacting to each other. In the screening, 73 mutants that extended into the growth-inhibited zones were isolated, and thorough a subsequent analysis by a paired-culture 40 mutants escaping from colony-colony growth inhibition were obtained. As some of the mutants exhibited conidiation-deficient phenotype typically seen in $\Delta flbA$, gene sequencing revealed that mutations in the *flbA* were commonly found, which supports the importance of FlbA/FadA-mediated G-protein signaling. Furthermore, whole-genome sequencing identified mutations in several uncharacterized genes. Reintroduction of the wild-type genes restored the inhibition, suggesting their involvement in the regulation of colony-colony growth inhibition. Collectively, mutant screening revealed candidate genes involved in the inhibition, which would help clarify the overall regulatory mechanism underlying colony-colony growth inhibition between the same strain.

21. GATA-type regulator NsdD-mediated species-specific rewiring of gene regulatory networks in *Aspergillus* **Kap-Hoon Han¹, Jae-Hyuk Yu²**

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NsdD is a conserved GATA-type transcription factor that governs development and secondary metabolism in *Aspergillus*, yet its species-specific regulatory mechanisms remain unclear. Using network-based multiomics analyses in *Aspergillus nidulans* and *Aspergillus flavus*, we show that NsdD functions in a cell type- and species-dependent manner. Cross-complementation of *A. flavus* $\Delta nsdD$ with the *A. nidulans* *nsdD* gene partially rescued developmental and transcriptional defects, indicating evolutionary divergence of function. Genome-wide ChIP-seq identified 502 direct NsdD targets in *A. nidulans* and 674 in *A. flavus*, including key developmental and metabolic regulators. A conserved binding motif (5'-GATCT-3') was defined as the NsdD response element. Network reconstruction revealed conserved core modules but extensive regulatory rewiring between species, contributing to differences in asexual/sexual morphology and sterigmatocystin/aflatoxin biosynthesis. These findings provide the first comparative map of NsdD-mediated gene regulatory networks in filamentous fungi and highlight how evolutionary changes enable a conserved TF to acquire species-specific roles.

22. FungiDB: Tools for Genomic-Scale Data Exploration, Integration, and Discovery

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Modern biological research—spanning biomedical, model organism, and biotechnology—is increasingly driven by Big Data: genome sequences, population-level diversity analyses, and an ever-expanding range of multi-omics datasets. Effectively collecting, integrating, and interpreting these data is vital to ensure FAIR (Findable, Accessible, Interoperable, Reusable) access and to accelerate discovery across diverse areas of life science research.

The Eukaryotic Pathogen & Vector Genomics Resource (VEuPathDB.org)—including FungiDB.org—offers a robust, sustainable platform for data mining and discovery, supporting thousands of researchers in the eukaryotic microbe research community.

At the Fungal Genetics Conference, the VEuPathDB team will be available at a help desk during all poster sessions to demonstrate database features, introduce new tools, and engage with the community to shape future priorities.

Highlights include:

1. **Comprehensive multi-omics data access:** Explore genes, genomes, annotations, population diversity, comparative genomics, epigenetics, transcriptomics, proteomics, metabolomics, phenotypes, AlphaFold structures, and more.
2. **Integrated data analysis (in silico experiments)** with tools for pathway exploration, orthology-based functional inference, and subcellular localization.
3. **AI-powered expression summaries:** A new experimental feature that automatically analyzes transcriptomics datasets for each gene and generates concise summaries with biological relevance scores. Experiment results are organized into thematic patterns for rapid interpretation.
4. **AI-assisted gene function discovery** that leverages literature analysis to generate user-submitted comments and expand functional annotations.
5. **Expanded support for oomycete and fungal phytopathogens**, in collaboration with Ensembl and backed by a three-year BBSRC award.
6. **OrthoMCL-7 (OrthoMCL.org)**, featuring updated UniProt proteomes, OrthoFinder-based clustering, customizable phylogenetic trees, and improved visualization of protein superfamilies.
7. **Commitment to sustaining open access:** VEuPathDB remains freely accessible; however, resource development and support requires support. A new subscription model helps ensure long-term sustainability, and most funding agencies allow budget allocations for website access and data dissemination.

FungiDB continues to evolve as a bioinformatics platform leveraging AI, community expertise, and new partnerships to transform large-scale biological data into actionable insights.

*Abstract submitted on behalf of the entire VEuPathDB team.

23. Proteomics of programmed cell death in *Aspergillus fumigatus* conidia to design fluorescent viability reporter

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Airborne conidia of the human-pathogenic fungus *Aspergillus fumigatus* are the main cause of invasive pulmonary aspergillosis particularly in immunocompromised individuals. After inhalation, alveolar macrophages constitute the first line of defense by phagocytosing and intracellularly processing conidia; however, *A. fumigatus* can partially evade this immune response, making improved therapeutic strategies necessary. A major challenge in studying early host–pathogen interactions is the lack of reliable markers to distinguish viable from dead conidia in situ, as both exhibit only minimal morphological differences during the initial hours post-infection. Developing a reporter strain expressing a fluorescently tagged cell-death–associated protein would allow real-time visualization of conidial death by microscopy. To identify suitable candidate proteins for such a reporter system, we characterized programmed cell death (PCD) in *A. fumigatus* conidia. An in vitro cell death assay was established using multiple PCD-inducing compounds, treatment durations, and culture conditions. Among the tested inducers, hydrogen peroxide in nutrient-rich medium exhibited the highest killing efficiency on resting conidia, compared to amphotericin B and voriconazole. These cell-death inducing conditions were subsequently used for comparative proteomic analyses of resting and swollen conidia. The resulting data revealed a set of cell-death–associated proteins with differential abundance during PCD. Based on these findings, fluorescent reporter strains expressing a fluorescent protein fused to selected candidate proteins were generated. This integrated approach provides new insights into the molecular features of PCD in *A. fumigatus* conidia and establishes a foundation for the development of robust live/dead reporter strains. Such tools will facilitate in situ tracking of conidial death during host–pathogen interactions and may support the evaluation of antifungal interventions and the development of novel therapeutic strategies.

24. Retrotransposon-mediated transition from sexual to asexual development in the katsuobushi fungus, *Aspergillus chevalieri

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Katsuobushi, a dried bonito, is a traditional Japanese fermented food. A particular type of katsuobushi, known as karebushi, is produced through repeated cycles of cultivating xerophilic fungi on the surface of bonito fillets. These processes reduce the moisture content and refine the flavor of karebushi. *Aspergillus chevalieri* is one of the predominant fungi on the surface of karebushi. This species includes two types that differ in their life cycles: a teleomorphic type, which produces cleistothecia (sexual fruiting bodies), and an anamorphic type, which produces conidia (asexual spores) under identical environmental conditions, such as on minimal agar medium. This contrasts with the model fungus *Aspergillus nidulans*, which switches between sexual and asexual development depending on environmental factors such as oxygen availability and light. When cultivated on minimal agar medium, the teleomorphic strain of *A. chevalieri* initially produces cleistothecia. However, upon prolonged incubation, it partially begins to form conidia. Conidia isolated from these cultures, when subcultured on minimal agar medium, consistently exhibited an anamorphic phenotype, indicating a loss of sexual fertility. Comparative genomic analysis between the

teleomorphic strain and its anamorphic mutant using PacBio long-read sequencing revealed the insertion of an approximately 5.8-kb retrotransposon upstream or inflame of a gene encoding an HMG-box transcription factor in the anamorphic mutant. Disruption of this transcription factor gene in the teleomorphic strain resulted in the acquisition of an anamorphic phenotype, whereas complementation restored the teleomorphic phenotype. These findings indicate that this transcription factor is essential for sexual reproduction in *A. chevalieri*. Furthermore, expression levels of the mating-type genes *MAT1-1* and *MAT1-2* were reduced in the anamorphic mutant, suggesting that this transcription factor regulates *MAT* genes expression.

25. Role of the *osaA* transcription factor gene in development, secondary metabolism and virulence in the mycotoxigenic fungus *Aspergillus flavus

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Aspergillus flavus is an opportunistic fungal pathogen that affects numerous economically relevant oil-seed crops, including corn, cotton, peanuts, and various tree nuts. While colonizing the plant tissues, *A. flavus* generates several powerful mycotoxins, most notably aflatoxin B₁, considered the most carcinogenic natural compound known. The infection of crops by *A. flavus* and the resulting aflatoxin contamination pose significant global challenges to both public health and the economy. Current control measures are inadequate to mitigate the harmful effects of *A. flavus*. Molecular genetic research may reveal new targets for the development of innovative strategies to combat the negative effects of this pathogen. Here, we characterized a putative WOPR transcription factor gene, *osaA*, in this agriculturally relevant fungus. Our study revealed that *osaA* is a repressor of conidiation in *A. flavus*. In addition, *osaA* is required for normal production of sclerotia. Importantly, removal of the *osaA* gene results in a decrease in aflatoxin B₁ production. Unexpectedly, transcriptome analysis revealed that while several aflatoxin biosynthetic genes were upregulated in the mutant, although the actual toxin levels were reduced, suggesting that *osaA* may influence aflatoxin production through post-transcriptional or cofactor-regulated mechanisms. Other *osaA*-dependent changes in secondary metabolism were observed, including a significant decrease in cyclopiazonic acid production. In addition, deletion of *osaA* resulted in upregulation of genes involved in the production of aspirochlorine, a potent anti-fungal compound. Importantly, *osaA* is indispensable for normal *A. flavus* seed colonization; deletion of *osaA* significantly reduced fungal burden in corn kernels. Furthermore, aflatoxin B₁ production in seeds also decreased in the absence of *osaA*. The *osaA* mutant presented alterations in oxidative stress and temperature sensitivity, as well as a decrease in cell wall chitin content, which could contribute to the decrease in pathogenicity in this strain. Understanding the regulatory scope of *osaA* provides new insight into fungal biology and emphasizes its potential as a molecular target for controlling fungal seed colonization and mycotoxin contamination.

26. Improved recombinant protein production and culture rheology in pure oxygen aeration fermentation using a hyphal dispersion strain of *Aspergillus oryzae

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Aspergillus oryzae is a filamentous fungus widely used as an industrial production host in submerged cultivation. Following inoculation, wild-type strains rapidly form spore aggregates that develop into pelleted mycelia, which can impose substantial diffusion constraints on oxygen and nutrients. In contrast, dispersed mycelia enhance mass transfer but markedly increases culture viscosity and non-Newtonian properties, thereby compromising mixing efficiency. We previously reported that deficiency in the cell-surface polysaccharides α -1,3-glucan (AG) and galactosaminogalactan (GAG) generates a hyphal dispersion strain (AG Δ -GAG Δ) exhibiting reduced culture viscosity and enhanced recombinant protein production. Such rheological control is particularly crucial in large-scale fermentations, where excessive viscosity markedly increases mixing energy requirements and limits bioprocess scalability. Although AG Δ -GAG Δ strain represents a promising host for industrial enzyme manufacturing, the reduction in culture viscosity remains insufficient, indicating a need for further rheological optimization. In this study, we sought to further enhance recombinant enzyme production in stirred-tank fermentation by cultivating either the wild-type or AG Δ -GAG Δ strain under air or pure-oxygen aeration. Comparative analyses were conducted on recombinant enzyme productivity, culture viscosity, and pellet size distribution. Pure oxygen aeration increased enzyme productivity compared with air aeration and induced a morphological shift in the AG Δ -GAG Δ strain toward finer pellets. Quantitative pellet analysis revealed that pelleted mycelia accounted for 0 % of total biomass up to 36 h of cultivation but increased significantly thereafter. Most wild-type strain pellets were larger than 700 μ m in diameter, whereas those of AG Δ -GAG Δ strain ranged from 200 to 700 μ m. Rheological measurements revealed that decreases in viscosity and non-Newtonian behavior of culture correlated with increased abundance of finer pellets. The formation of smaller pellets in AG Δ -GAG Δ strain likely alleviates intrapellet diffusion limitations for oxygen and nutrients. Collectively, these findings demonstrate that pure-oxygen aeration in AG Δ -GAG Δ strain improves both culture rheology and recombinant enzyme productivity, underscoring its potential to enhance mixing efficiency and reduced agitation power in filamentous fungal bioprocesses.

27. A novel beta-propeller protein in *Aspergillus nidulans* required for microtubule stability

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We discovered a fungal-specific protein required for microtubule (MT) stability, *wmcA* (WD-repeat mitotic catastrophe) in *Aspergillus nidulans*. Cells lacking *wmcA* exhibit striking abnormalities: the cells are extremely cold sensitive and undergo mitotic catastrophe characterized by the absence of microtubules and consequent lethal fragmentation and dispersion of chromosomes. Several lines of evidence point to a role in MT homeostasis, including (1) at the non-permissive temperature, absence of GFP-tagged MTs and mislocalization of the MT (+)-end binding protein *ebA::GFP*, and (2) genetic interactions with a null mutation in Tubulin-binding Cofactor A (TBCA) and with a MT-hyperstabilizing mutation in beta-tubulin. Generation of suppressors of the cold-sensitivity of *delta-wmcA* revealed additional genetic interactions involving translation and ubiquitin-mediated processes. Localization studies demonstrate that *wmcA* is both cytoplasmic and nuclear. Functional domain studies show that the *wmcA* N-terminus prior to the first WD repeat is essential, whereas the highly conserved C-terminus following the last WD repeat is fully dispensable. Given that WD-repeat proteins typically serve as scaffolds in multi-protein complexes, and that cold-sensitive mutations, such as occur in the *delta-wmcA* mutant, often reflect the destabilization of protein complexes at low temperature, it is likely that *wmcA* functions to stabilize a multi-protein complex. Colabfold modeling suggested beta-tubulin and/or TBCA as candidate physical interactors with *wmcA::GFP*. Current efforts are aimed at testing these candidates by co-IP to identify *bona fide* binding partners and thereby gain insight into the mechanism by which this previously unstudied protein influences MT stability and dynamics.

28. Transcriptomic Insights into Isomaltose-induced amyolytic enzyme production in *Aspergillus nidulans*

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Amyolytic enzymes, which degrade starch, are of great importance in both industrial applications and fundamental research. In *Aspergillus* species, the expression of amyolytic enzyme genes is regulated by the fungal-specific transcription factor AmyR. Among known inducers, isomaltose is the most effective and direct activator of AmyR compared to glucose or maltose. We previously showed that isomaltose uptake is mediated by proton-coupled symporter¹⁾ and identified a novel isomaltose transporter, *ImtA*, in *Aspergillus nidulans* through spontaneous mutagenesis²⁾. However, isomaltose uptake pattern and further isomaltose-mediated AmyR activation mechanism remain poorly understood. In this study, HPLC analysis demonstrated that isomaltose is slightly taken up inside the cell in *Aspergillus oryzae* and *A. nidulans*, and this uptake is dependent on the novel isomaltose transporter *ImtA*. Altogether, a tiny amount of isomaltose is transported into the cell and subsequently triggers AmyR nuclear localization to activate amyolytic gene expression in *Aspergillus* species. RNA-seq analysis further revealed differentially expressed genes in the isomaltose transporter-deficient or AmyR-deficient strain in the presence of isomaltose to make clear observation of isomaltose-mediated AmyR activation pathway. Interestingly, the expression of amyolytic genes including *amyR* is dependent on *ImtA* in the presence of isomaltose.

1) Shoki Fujita et al., *Biosci Biotechnol Biochem*, 89, 1391–1399 (2025)

2) Jeong et al., The 33rd Conference on Fungal Genetics and Molecular Biology (P-42) (2024).

*29. Fitness effects of multi-fungicide resistances in *Aspergillus fumigatus*

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Aspergillus fumigatus is an opportunistic human pathogenic fungus mainly found in plant-degrading material. Although not a plant pathogenic fungus, within this substrate it is exposed to multiple classes of agricultural fungicides. It has been shown that exposure to agricultural triazoles leads to cross-resistance of clinical triazoles, but the evolutionary impact of exposure to other fungicide classes is unknown. Recent genomic surveys have shown correlations of variants assumed to lead to resistance between DMI, QOI, SDHI, and MBC fungicides, suggesting ongoing adaptation to current agricultural practices. To identify the full diversity of genetic resistances, we used literature to identify target genes and screened for common (>0.3%) non-synonymous variants across 1500 available *A. fumigatus* genomes. We identified 12 resistance variants, spread across seven genes, with five resistant variants being shown for the first time. Natural isolates that contain one resistance likely contain multiple, resulting in multi-fungicide resistance phenotypes. On the other hand, certain resistance variants like *sdhB* H270Y and *sdhC* S105I were never found together, suggesting negative fitness costs of some combinations, particularly alleles of the same gene or in members of the same protein complex.

To test the impact of these resistance variants, we introduced point mutations with CRISPR/Cas9 using co-transformation of a transient hygromycin resistance plasmid. All predicted resistance variants caused resistance, including newly described alleles to the aminopyrimidines class of fungicides. We did not observe a fitness cost for single mutants or naturally co-existing combinations. However, by constructing combinations not observed to occur in nature, we show significant fitness reductions. For instance, multiple variants in the SDH complex are not tolerated (more than 40% growth reduction), and likewise within beta-tubulin, the target of MBCs (more than 70% growth reduction). Our results indicate that natural selection is shaping a combination of resistance variants in different genes and for different fungicide classes and provides additional evidence that the continued use of non-triazole fungicides are indirectly selecting for triazole resistance.

30. Signs of ongoing reproductive incompatibility in *Aspergillus fumigatus

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Wageningen University

Reproductive isolation is the inability of two populations to produce viable, fertile, sexual offspring due to biological barriers. In *Aspergillus fumigatus*, an important human fungal pathogen, studies have shown that antifungal resistant isolates cluster separately from sensitive isolates in phylogenetic analyses irrespective of geographic location, indicating a lack of current gene flow. Reproductive isolation could occur through prezygotic barriers, which prevent fertilization, or postzygotic barriers which act after a hybrid zygote has formed. Crosses within- and between-clusters are equally fertile, indicating a lack of prezygotic barriers.

To identify potential reproductive barriers, we used 102 isolates to perform 286 sexual crosses, 60 of which produced offspring. Of the 60 crosses, 13 showed unfit progeny with reduced mycelial growth, no sporulation, or both. We identified three main phenotypic segregation ratios: (a) 1:1 segregation of sporulating and non-sporulating offspring, (b) 1:1:1:1 segregation involving differences in both sporulation and size, and (c) a small percentage (5–20%) of non-sporulators. These repeated phenotypic ratios do not appear linked to resistance background, equally found within and between cluster crosses. Surprisingly, these phenotypes are not found in either parent (both wild type), pointing towards a non-Mendelian mode of inheritance. We hypothesize that differential adaptation in phylogenetic clusters may lead to low-fitness hybrid progeny, which are outcompeted in nature.

To identify the genetic elements causing this segregation, we used BSA on a cross showing the 1:1 ratio of sporulating and non-sporulating offspring. We identified a 70-kb locus on chromosome 1 linked to the phenotype, containing 19 genes. Notably, in the *brlA* gene, a known regulator of asexual development, all non-sporulating offspring carried an identical deletion of ~45 bp, not shared with the sporulating offspring. This deletion is not found in either parent but only occurs in one parental genome. BSA analysis of the 1:1:1:1 ratio of offspring indicates a mechanism outside of *brlA*. Crosses of closely related species also show the same phenotype, but BSA also indicates a non-*brlA* mechanism. Our data shows for the first time a post-zygotic reproductive mechanism, that is surprisingly widespread in a sexually reproducing species. The importance of this mechanism will depend on the contribution of sexual and asexual reproduction in this species.

***31. Parallel comparative transcriptome analyses of bacterial–fungal interactions (BFI) reveal the determinants of BFI directionality**

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Bacteria and fungi coexist in diverse environments, exhibiting either antagonistic or cooperative relationships over space and nutrients. Bacterial–Fungal Interactions (BFI) play a crucial role in shaping the structure and function of microbial communities. However, the underlying mechanisms remain largely unexplored. The knowledge gap persists as most studies have focused on specific model systems or individual pairwise interactions, lacking comprehensive cross-combinatorial comparisons. In this study, we conducted a systematic screening of 828 bacterial–fungal pairings and selected three each of fungal and bacterial species displaying prominent interaction traits (e.g., antagonism, mutualism, neutrality). By comparing the transcriptomic dynamics induced by a range of microbial pairings, we have identified gene regulations unique to each interaction. The trends of gene expression across all samples revealed orthologous genes contributing to interaction traits. Genes involved in secondary metabolite production, oxidative stress responses, and vitamin B biosynthesis were regulated specific to the interaction type. We propose a dual interaction model in which fungal hyphae in contact with harmful bacteria release defensive metabolites, while concurrently fostering spatial and chemical interactions with beneficial or neutral bacteria. Our findings highlighted the commonly altered responses and the pronounced differences arising from subtle phylogenetic variations, enabling a deeper understanding of the mechanisms underlying the specificity of BFI. This contributes to elucidating the intricate dynamics and functional roles of microbial communities across diverse fields, including environmental science, agriculture, and biomedical applications.

32. Proteomic profiling of antifungal drug responses of *Aspergillus fumigatus* indicates drug-specific signatures and modes of action

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The human fungal pathogen *Aspergillus fumigatus* has become increasingly resistant to clinically used antifungals agents, in particular to azole-based drugs. This has led to high mortality rates in patients with azole-resistant invasive aspergillosis. The discovery of new antifungal agents, especially those with novel modes of action (MoA), is therefore urgently needed. However, due to the closer evolutionary relationship between cells of fungi and humans in comparison to bacteria, the number of fungal-specific targets for drug development is rather limited. This often leads to the rediscovery of drugs with known MoA. To address this challenge and to learn more about how antifungal agents affect fungal cells, we employed expression-based proteomics to construct a comprehensive drug-specific proteomic response library based on the profiling of 14 anti-*Aspergillus fumigatus* compounds. Central to this effort was the development of a robust, standardized workflow from cultivation to LC-MS/MS data evaluation. Our analysis revealed that the strongest proteomic responses were linked to processes involved in 'detoxification' and 'defence' against environmental insults. Our analysis allows to distinguish between synthetic drugs and natural products. Based on our response library, we successfully identified MoA-specific signature proteins, enabling the prediction of similarities

between MoAs of compounds. Our strategy also uncovered drug-specific induction patterns of biosynthetic gene clusters (BGCs). We also validated our approach by using three additional drugs, including azalomycin F, a natural product with a predicted new MoA. Notably, we identified the drug-specific increase of poorly characterised transcription factors, which suggests complex regulatory circuits of antifungal drug resistance.

***33. Roles of aneuploidy in *Aspergillus fumigatus* unstable adaptation and antifungal resistance**

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Aneuploidy causes genome plasticity and enables adaptive responses that confer drug resistance in eukaryotes ranging from fungal pathogens to human cancer cells. *Aspergillus fumigatus* is a soil-resident fungus and the most common etiologic agent of invasive fungal infections globally. Aspergillosis has an alarmingly high mortality rate due in part to treatment failure resulting from widespread antimicrobial resistance and an ability of the fungus to dynamically adapt to changing environmental and host conditions. Our understanding of these treatment failures is hindered by gaps in knowledge about how this organism adapts to resist antimicrobial stress. We find that exposure to FK506, an antifungal and immunosuppressive compound produced by *Streptomyces*, selects for whole-chromosome aneuploidy in this fungus. These chromosome duplications also reduce susceptibility to clinically-used azole antifungals, and whole-chromosome and segmental aneuploidies arise independently directly in response to azole exposure and confer resistance. Transcriptomic analysis revealed that Chr7 disomy leads to marked induction of the normally-silent neosartoricin biosynthetic gene cluster in response to FK506 exposure, and we demonstrate that constitutive genetic induction of this cluster recapitulates in the euploid background the response to FK506 in the aneuploid state. We further show that this response is independent from the production of neosartoricin, suggesting a role for cross-pathway metabolic changes resulting from the activation of this gene cluster or from the aneuploid state. In the aneuploids that arose in response to voriconazole, we find that a single duplication of the left arm of Chr2 is sufficient to confer a high level of resistance, and that the expression of known azole resistance genes is not altered in this strain. In both FK506-resistant and voriconazole-resistant aneuploids, culture in the absence of drug leads to aneuploid chromosome loss and rapid reversion to a euploid, drug-susceptible state. These results uncover a novel mechanism of rapid and reversible antimicrobial stress adaptation in *A. fumigatus* and demonstrate the utility of aneuploid strains to identify novel genetic contributions to antifungal adaptation.

34. Azole-resistant development in *Aspergillus fumigatus* and its related antifungal strategy with novel nano-salt particles used as the aerosolized disinfectant

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The incidence of invasive fungal infections has increased steadily, driven by the growing population of immunocompromised patients. *Aspergillus fumigatus* is one of leading fungal pathogens and allergen of humans worldwide while invasive pulmonary aspergillosis is one of deadliest fungal infections with high mortality rates. The management of invasive fungal infections is further complicated by difficulties in accessing appropriate antifungal agents and the rising prevalence of antifungal resistance in clinical settings. This study focuses on how development and evolution in these *A. fumigatus* isolates embedding antifungal drug resistance/tolerance and persistent and then to enable the development of new therapeutic strategies for life-threatening fungal infections. Currently, it has been recognized that fungi primarily enter the human body through the respiratory tract via airborne transmission resulted in invasive fungal infections. However, related safety aerosolized disinfectants and related technologies are limited. Sodium chloride, a vital inorganic salt, plays a key role in regulating biological fluid balance. At high concentrations, it also exhibits potent disinfection properties. This study focuses on the design and synthesis of novel nano-salt particles. Using anti-solvent precipitation coupled with high-energy nanonization, we produced ultrafine sodium chloride powder at the nanoscale. These nano-salt particles are intended for use as aerosolized disinfectants against predominantly existed almost all test human fungal pathogens including antifungal azole susceptible and resistant *Aspergillus* spp, *Candida albicans* and *Cryptococcus neoformans*. Notably, the fungicidal mechanism of action against fungal conidia of *A. fumigatus* has been investigated. Data demonstrated that these novel nano-salt particles can disrupt the cell wall and membrane's integrity by creating pores and then leads to leakage of essential cellular contents and ultimately resulted in cell lysis and death. Findings in this study assess the disinfectant efficacy of this innovative nano-formulation and explore its potential practical applications.

35. Decoding Phenazine Stress Responses in *Aspergillus calidoustus* During Protection by *Paraburkholderia edwinii

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The composition of microbial communities is modulated by a number of factors, including the antimicrobial-compounds(AMC) secreted by several members of the community. Phenazines, a category of redox-active compounds produced by a wide variety of soil bacteria, are generally harmful to fungi. In spite of this, from a phenazine-rich niche, Dahlstrom & Newman (2022) reported the co-isolation of *Aspergillus calidoustus* with a protective bacterium, *Paraburkholderia edwinii*. Further-work also showed that the novel species *Paraburkholderia edwinii* can protect *Aspergillus calidoustus* from phenazine-1-carboxylic acid (PCA) by absorbing the toxin and sequestering it in a reducing environment. Our research builds on this base by identifying which *A. calidoustus* genes aid in survival when under PCA stress in the presence of the protective bacterium. We identified a group of genes that

are particularly elevated during co-culture with *P. edwinii* during PCA exposure using RNA-seq. These include genes involved in membrane transport, ROS detoxification, stress response pathways, and a number of transcription factors. We have also identified two specific candidate genes to focus on. The first, CDR1_2, has a high degree of similarity to Cdr1B, an ABC efflux transporter in *Aspergillus fumigatus* that is known to mediate azole resistance by expelling itraconazole and other azoles. The second potential candidate encodes a ROS-detoxifying enzyme, which may help *A. calidoustus* reduce oxidative stress brought on by phenazine exposure accompanied the protective partnership. We are now employing CRISPR–Cas9 with homology-directed repair (HDR) to create targeted knockouts in order to experimentally confirm these candidates. Using PEG-mediated transformation, editing constructs are introduced into fungal protoplasts to accomplish accurate gene replacement. The PCA-stress phenotypes of the resulting mutants will be evaluated alone and in co-culture with *P. edwinii*.

This study offers insight into how fungi survive in chemically harsh environments by defining the fungal genetic responses to phenazine stress in the presence of a protective bacterial partner. In a larger sense, these results may shed light on how fungal-bacterial interactions play a role in the development or alteration of antifungal resistance.

***36. Mariner-Based Transposon Mutagenesis Identifies *Paraburkholderia edwinii* Genes Required for Protecting *Aspergillus calidoustus* Under Phenazine Stress**

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The soil bacterium *Paraburkholderia edwinii* possesses the ability to protect the filamentous fungus *Aspergillus calidoustus* from fungistatic, redox-active molecules called phenazines. Phenazines are produced by bacteria within microbial communities and have an effect on shaping the species-level composition of microbial communities found in various environmental and healthcare settings. *P. edwinii* has the ability to form aggregates within the mycelium of *A. calidoustus*, where it sequesters and reduces a common phenazine called phenazine-1-carboxylic acid (PCA) into a less toxic form. This bacterial protection response is triggered by the acidic microenvironment created by *A. calidoustus* during PCA stress. The genes underlying this unique protection response remain mostly unknown. To investigate the genes that *P. edwinii* utilizes to sequester and reduce PCA, an axenic mariner-based transposon mutagenesis assay was performed. Here, transposon mutants are plated onto a pH adjusted agar plate containing PCA, mimicking the presence of the fungus, which triggers the bacterial protection response. On this medium, the wild-type bacteria appear yellow, indicating the reduction of PCA into its hydroquinone form, which produces a yellow pigment. Transposon mutant colonies that appear as pale-yellow/white serve as an indicator of deficiencies in PCA sequestration or reduction. The transposon mutants that show deficiencies in sequestration or reduction are then subjected to a co-colony protection assay with *A. calidoustus* to test the bacterium's ability to protect the fungus from PCA toxicity. The candidate mutants that show phenotypic differences in the protection of *A. calidoustus* are then subjected to whole genome sequencing to identify and analyze the disrupted genes. This study aims to identify the bacterial genes that are responsible for the sequestration and reduction of PCA. Preliminary results have identified genes involved in metabolic, membrane, and transport functions as being critical to PCA reduction and sequestration. The knowledge gained from this research will aid in understanding bacterial-fungal interactions in competitive microbial environments.

37. FluG confers conidial heterogeneity in *Aspergillus fumigatus*

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Although conidia (asexual spores) are genetically homogeneous, their germination timing varies. In the human pathogenic fungus *Aspergillus fumigatus*, we found that D-galactosamine (D-GalN) more effectively promoted dormancy breaking than D-glucose (D-Glc). To identify genes involved in D-GalN utilization, we constructed several gene disruptants based on D-GalN-induced transcripts identified by RNA-seq. Contrary to our expectation of finding a mutant unresponsive to D-GalN, the *fluG* disruptant ($\Delta fluG$) displayed notable phenotypes: its growth began earlier than that of the parental strain in minimal medium with D-Glc, resembling the D-GalN condition. Thus, FluG appears to negatively regulate dormancy breaking, mirroring the effect of D-GalN. While FluG is essential for conidiation in *A. nidulans*, it is dispensable in *A. fumigatus*. We therefore investigated the role of FluG in conidial dormancy breaking in *A. fumigatus*. Although $\Delta fluG$ conidia were morphologically similar to the parental strain, they germinated earlier in all tested carbon sources. Dormancy breaking was quantified by β -1,3-glucan exposure. At 6 h in minimal medium, ~90% of $\Delta fluG$ conidia showed dormancy breaking compared to ~40% of conidia of the parental strain. These results indicate that conidial germination is heterogeneously regulated under nutrient-limited conditions, and that FluG plays a key role in establishing this heterogeneity in *A. fumigatus*.

38. A Novel Fungal Association Characterizes Crohn's Disease in a South Korean Cohort

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Alterations in the gastrointestinal (GI) microbiota have been linked to a multitude of human health outcomes, including Inflammatory Bowel Disease (IBD), and improper recognition of fungal antigens is associated with exacerbated GI tract inflammation in models of IBD. However, bacteria remain the predominant focus for studies of the IBD-associated microbiota. Additionally, while recognition of the fungal microbiota has increased in recent years, inconsistencies in the direction of fungal diversity changes and in which specific fungal taxa may be implicated in IBD

remain.

In this study, we characterized the bacterial and fungal GI microbiota of healthy South Koreans (n=52) and South Korean patients with Crohn's disease (CD, n=34) to determine if any specific fungal species are associated with CD in this population. Stool samples were collected from this cohort, and 16S rRNA V4 and ITS1 sequencing were performed using the extracted DNA to profile the bacterial and fungal compositions of these groups. QIIME1 and DADA2 were utilized to process the resulting data and profile bacterial and fungal community composition. Differential taxa and correlations between bacterial and fungal species were determined using a variant sparse partial least squares discriminant analysis (sPLS-DA) framework using mixOmics.

We observed that the fungal species *Candida albicans* and *Aspergillus ruber* were overrepresented in patients with CD compared to healthy controls. We found that *A. ruber* was the most well-connected species in a cross-kingdom correlational analysis and was often correlated with bacterial species that are associated with GI health. Importantly, we also found that *A. ruber* can inhibit permeabilization of GI epithelial monolayers by *C. albicans*.

Overall, this study highlights the fungus *A. ruber*, thus far undescribed in the GI microbiota, as being associated with CD in a human cohort with naturally limited genetic and dietary variability. We demonstrate here the need for a greater focus on cross-kingdom approaches in future studies of the microbiota in human health. Additionally, this study implicates *A. ruber* as a potential modulator of disease in CD.

39. *Aspergillus fumigatus* Af293/CEA10 community sequencing initiative

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The model *Aspergillus fumigatus* isolates Af293 and CEA10 have been the workhorses of fungal research for decades. As these strains have been independently cultured in laboratories across the globe, they have likely accumulated unique, unstudied mutations. Understanding the extent of this "lab-to-lab" genomic drift is crucial for reliably comparing findings on physiology, pathogenicity, and genetics across different research groups.

While community sequencing projects have been conducted for other model organisms, this effort is the first of its kind for a filamentous fungus. Our goal is to quantify the genetic divergence between parental Af293 and CEA10 strains currently in use globally and to create a foundational genomic resource for the entire *Aspergillus* community. All raw and processed sequencing data will be provided back to the contributing labs. This presentation will provide updates on the initiative's progress and details on how the community can participate by submitting their own Af293 and CEA10 isolates for sequencing.

40. Global Proteomic and Phosphoproteomic Analysis Reveals Potential Calcineurin-Dependent Effectors in Cell Wall and Membrane Organization in *Aspergillus fumigatus*

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Calcineurin phosphatase (CN) orchestrates growth and virulence of *Aspergillus fumigatus*, a critical human fungal pathogen responsible for life-threatening infections in immunocompromised patients making it an attractive antifungal target. Understanding the CN network of proteins in *A. fumigatus* and the mechanism of how this phosphatase regulates important fungal-specific effectors involved in cell wall and membrane biosynthesis and organization will lead to designing novel therapeutic approaches for the treatment of invasive aspergillosis. Here we utilized whole proteomic/phosphoproteomic and lipidomic approaches to define CN-dependent regulation of various cell wall- and cell membrane-related effectors. Our two-pronged proteomic approach included: (1) Quantitative LC-MS/MS analysis to identify CN interactors via CN-tagged GFP using GFP-Trap[®] affinity purification; and (2) Comparative whole proteomic/phosphoproteomic analysis between the wild-type versus the CN-deletion mutant to identify CN-dependent effectors. Additionally, we systematically assessed the influence of CN on membrane organization via quantitative lipidome profiling by comparing the wild-type versus the CN mutant to define CN-dependent regulation. Our whole proteomic and lipidomic results from this holistic approach will provide a mechanistic understanding of how the CN network drives cell wall and membrane organization during growth and antifungal response in *A. fumigatus*.

41. AtrR Interaction Network and Its Role in Azole Resistance in *Aspergillus fumigatus*

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Aspergillus fumigatus is an ubiquitous environmental mold and a major opportunistic pathogen responsible for invasive aspergillosis, particularly in immunocompromised patients. Development of antifungal drug resistance, especially to azoles, poses a serious challenge to treatment and limits therapeutic options. AtrR, a fungal-specific Zn₂-Cys₆ transcription factor, plays a critical role in azole resistance by directly regulating the expression of *cyp51A* (encoding the azole drug target) and *abcG1/cdr1B* (an ABC transporter involved in drug efflux). To better understand how AtrR modulates azole resistance, protein extracts from an AtrR-TAP tagged strain were purified under native conditions followed by mass spectrometry. Several proteins co-purified with AtrR and were confirmed as interactors by co-immunoprecipitation, including chromatin assembly factors (Ngg1, Arp4), a proteasomal degradation component (FbdA), and a nuclear-localized protein of unknown function (NcaA). Single, double, and triple deletions of *ngg1*, *fbdA*, and *ncaA* resulted in progressively increased azole sensitivity. Interestingly, these mutants exhibited

azole susceptibility without altering expression of known AtrR targets such as *cyp51A* or *abcG1*. To identify additional AtrR-dependent genes contributing to azole resistance, RNA-seq analysis was performed comparing wild-type and the triple deletion strain in the presence and absence of azole drug. Transcriptomic analysis revealed novel AtrR-regulated genes, including one involved in sphingolipid biosynthesis, that may play a role in azole resistance. We have also developed an inducible protein degradation system that can proteolyze fusion proteins between *A. fumigatus* genes-of-interest and green fluorescent protein (GFP). We will discuss progress in using this system to degrade GFP fusion proteins, including AtrR-GFP, in vitro and in vivo. These findings highlight AtrR as a central regulator of azole resistance and suggest that its interaction network extends beyond canonical drug resistance genes, implicating chromatin remodeling, protein turnover, and lipid metabolism in antifungal resistance mechanisms.

42. The *Aspergillus fumigatus* growth determinant Afu6g07200 is the first granulin described in fungi

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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 defects in maintaining cell morphology, decreased proliferation and conidiation, increased septa and branch formation, and disorganized polarity. The zigzag phenotype of the hyphae was stronger under osmotic pressure and was completely rescued by the deletion of the polarity marker TeaC. At the molecular level, the deletion strain was unable to activate the two major mitogen-activated cascades, cell wall integrity and the high-osmolarity pathway, even after the addition of stressors. In addition, a higher sensitivity of the deletion strain to cell wall stressors, disorganized walls, and increased periplasmic spaces were detected. Finally, functional homology between human granulin A and Afu6g07200 gene was confirmed by the complementation of the deletion strain with human granulin A. The endogenous expression of human granulin rescued the phenotypic defects at the morphological and molecular level.

***43. A new reference graph-pangenome for *Aspergillus fumigatus* recovers gene expression of accessory genes absent from the reference strain**

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Fungi harbour an incredible amount of genomics and phenotypic diversity, even within the same species. An example of this is the ubiquitous saprotrophic fungus *Aspergillus fumigatus*, which is responsible for the pulmonary infection aspergillosis, causing about 1.5 million deaths per year. Approximately 31% of the whole gene content of this species is considered accessory, exhibiting a presence-absence variation among isolates. This diversity also extends to phenotypes, with isolates displaying marked variation in their ability to withstand stresses such as iron starvation, hypoxia, and oxidative stress, and potentially making them more prone to infecting humans and/or more resistant to antifungals. Currently, the function of these accessory genes and their potential role in these phenotypic differences are not well understood. Part of this is due to the extensive use of a linear reference during bioinformatics analysis: a single strain of the species -here, Af293- is used as a guide for the mapping of genomics and transcriptomics reads, preventing the analysis of genes absent from this reference.

We propose here a new reference for *A. fumigatus*, called a reference graph-pangenome. Built using 26 genetically diverse long-read assemblies from a global distribution and environmental and clinical origin, the new reference graph-pangenome is almost the double of the current linear reference (55.6 Mb). It contains a total of 14,016 protein-coding genes, including 4,176 absent from the existing Af293 reference. To better characterize these accessory genes, we performed an RNA sequencing of seven different isolates across six different stresses. Using the graph-pangenome as a reference, we were able to recover up to 10% more reads aligned, leading to 984 additional genes expressed absent from the strain Af293. Furthermore, we identified up to 8% more differentially expressed genes in response to these stresses. In future work, we will perform a co-expression network analysis to assign putative functions to these non-functionally characterized genes. Altogether, this new tool provides not only a novel reference for the *A. fumigatus* community, but also establishes a reference pangenome framework for other fungal species, while offering novel insights into the function of accessory genes in virulence or antifungal resistance.

44. AI-driven optimization of growth factor manufacturing from waste by *Aspergillus oryzae*

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As natural decomposers, filamentous fungi hold tremendous potential for circular biomanufacturing, but this potential is limited by their relatively underdeveloped synthetic toolkit. Specifically, *Aspergillus oryzae* is a major workhorse in the enzyme industry thanks to its well-developed secretion mechanisms for potent endogenous amylases. However, it is rarely used to valorize starchy waste streams into heterologous proteins, which are often lost through endoplasmic reticulum-associated degradation after being recognized as misfolded. We aim to bridge this gap by optimizing the transcription and translation rates of bovine IGF, as a model protein, through AI-based machine learning of codon-usage patterns in the fungal host while it grows on waste sidestreams. Proteomic and transcriptomic data were collected from submerged cultures grown on broken rice, wheat bran, and apple pomace. Position-specific Chimera Map (PScMAP) and tRNA Adaptation Index Calculator models, trained on the collected data, predicted protein abundance from codon usage and tRNA index with a Pearson coefficient of up to 0.52 and a p value of $4.7 \cdot 10^{-104}$. Transcript variants generated by the trained models were tailored to ensure optimized transcription and translation when expressed under the top four promoters mined from the omics data. Constructs were integrated into the fungal genome using CRISPR Cas9-based transformation, and the resulting transformants were cultured on waste sidestreams and screened for product yield. Purified IGF was identified by LC-MS/MS following dual-protease digestion and quantitatively determined by ELISA. This AI-assisted optimization of heterologous protein production from complex substrates represents a significant step toward unlocking the full potential of *A. oryzae* in circular biomanufacturing.

***45. Strain-specific redox balancing for *Aspergillus fumigatus* pathoadaptation and antifungal drug susceptibility**

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Significant genotypic and phenotypic diversity exists in the *Aspergillus fumigatus* population whose significance for pathogenesis and drug susceptibility is only beginning to emerge. The complexity of this diversity poses both challenges and opportunities to define mechanisms of pathogenicity, virulence, and antifungal drug susceptibility. Previously, we identified *A. fumigatus* isolates with an increased ability to grow in low oxygen conditions found at sites of infection and with enhanced virulence in murine models of invasive pulmonary aspergillosis. However, the underlying mechanism(s) for increased low oxygen fitness and virulence remain ill-defined. An experimental evolution experiment with the reference strain AF293 revealed a novel gene cluster located on a moveable Starship element necessary and sufficient for low oxygen fitness and virulence. These strains provide a basis for defining mechanisms of low oxygen adaptation in a host-relevant context. With these strains, we discover that an enhanced ability to reduce nitrate and catabolize ethanol provides increased biomass generation in low oxygen conditions and the ability to form more robust biofilms. In contrast to strains less fit in low oxygen conditions, the ability to reduce nitrate and catabolize ethanol is necessary for the low oxygen growth of these biofilm-fit strains. Loss of this ability impacts pathogenesis and significantly impacts biofilm antifungal drug susceptibility. These data suggest important redox balancing metabolism that also provides key substrates for cell wall and ergosterol biosynthesis under oxygen limited conditions. These observations have potential implications for the emergence of antifungal drug resistance in the environment and the ability of a given strain to persist and progress disease in the dynamic lung infection microenvironment. The extent to which this metabolic rewiring and flexibility is observed across the *A. fumigatus* phylogeny is under further investigation.

***46. Global patterns of diversity and distribution in *Aspergillus* fungi are driven by human and environmental influences**

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Aspergillus fungi are key producers of pharmaceuticals, enzymes, and food products and exhibit diverse lifestyles, from saprophytes to opportunistic pathogens. The genus is divided into 28 taxonomic sections sharing traits (e.g., natural products) and lifestyles (e.g., xerophily, pathogenicity). Understanding global patterns of *Aspergillus* occurrence and diversity would deepen our ecological understanding and help monitor pathogens and producers of mycotoxins and diverse natural products. To identify key environmental factors influencing their geographic distributions and estimate the impact of future climate change, we trained a random forest machine learning classifier on 19,105 terrestrial occurrence records for 27 taxonomic sections and 96 environmental variables to predict distributions of each section. We found that regions with high section diversity are concentrated in temperate forests, suggesting that areas with mild seasonal variation serve as diversity hotspots. Section range estimates revealed extensive variability, and low range overlap suggests distinct niches. The top predictors of average section richness were the index of cumulative human impact, ecosystem productivity, and forested vs non-forested ecoregions. Our future climate analyses revealed considerable variation in section range estimates in response to changing climates; suitable habitats for some sections are predicted to expand (e.g., section *Restricti*), and others to contract (e.g., section *Nigri*) or remain stable (e.g., section *Fumigati*) in the next few decades. Our findings reveal that both natural and human factors influence the macroecology of *Aspergillus* fungi and highlight their ecological diversity,

including the diversity of their responses to changing climates, which is of relevance to pathogen and mycotoxin risk assessment.

***47. Construction of the *Aspergillus oryzae* strain producing high levels of kojic acid by CRISPR/Cas9-mediated multicopy gene integration**

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Background and Objective: *Aspergillus oryzae* is widely used as a host for productions of recombinant proteins as well as secondary metabolites. Recently, we have successfully integrated multiple xylanase genes into transposon-like sequences on chromosomes of *A. oryzae* by a CRISPR/Cas9-mediated gene editing to achieve high production (3 g/L) of xylanase in flask culture. However, it remains undetermined whether this multicopy gene integration system is applicable to genes other than xylanase. Therefore, we aimed to construct an *A. oryzae* strain inserted with multiple copies of *kojA*, the gene encoding a key enzyme in biosynthesis of kojic acid, a secondary metabolite of *A. oryzae*, and to evaluate kojic acid production.

Methods: The *kojA* gene, driven by the strong promoter *PglaA142* was cloned into a transposon-targeting plasmid, which was subsequently used for transformation of *A. oryzae*. Gene insertion was confirmed by PCR after an induction of Cas9 expression.

Results: We successfully obtained a strain containing 13 copies of the *kojA* gene (hereafter referred to as the multicopy strain). We compared the kojic acid production levels, as well as the transcription and translation products of *kojA*, between the multicopy strain and a single-copy (1-copy) strain. The multicopy strain produced 5.5-fold more kojic acid than the 1-copy strain. The qRT-PCR analysis demonstrated that the *kojA* expression levels were significantly increased in the multicopy strain compared to the 1-copy strain. SDS-PAGE analysis of the intracellular proteins revealed that a protein with the estimated molecular weight of KojA was detected as the most abundant protein in the multicopy strain, suggesting that the technique is also effective for high-level production of intracellular proteins. In conclusion, we have successfully developed the *A. oryzae* strain capable of high-level kojic acid production, demonstrating that our multicopy gene integration method is applicable to various genes, including those involved in the biosynthesis of secondary metabolites.

48. Acyl-coA binding protein *AcbdA* regulates peroxisome movement via hitchhiking on early endosomes in *Aspergillus nidulans*

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Motor-driven transport on microtubules is critical for distributing organelles throughout the cell. Most commonly, organelle movement is mediated by cargo adaptors, proteins on the surface of an organelle that directly recruit microtubule-based motors. An alternative mechanism called hitchhiking was recently discovered: some organelles move, not by recruiting the motors directly, but instead by using membrane contact sites (MCS) to attach to motor-driven vesicles and hitchhike along microtubules. Organelle hitchhiking is observed across fungi and animals. In filamentous fungi, nearly all peroxisomes move by hitchhiking on early endosomes (EE). In the fungus *Aspergillus nidulans*, EE-associated linker proteins PxdA and DipA are critical for establishing EE-peroxisome MCS required for peroxisome movement. Whether peroxisome-membrane proteins exist that regulate peroxisome hitchhiking on EEs is not known. Through a forward mutagenesis screen, we discovered an acyl-CoA binding (ACB) domain-containing protein *AcbdA/AN1062* that localizes to peroxisomes via its tail-anchored transmembrane domain (TMD). Deleting the *AcbdA* gene or only its N-terminal ACB domain perturbs the movement and distribution of peroxisomes. Importantly, *AcbdA* is not required for the movement of EEs or for the recruitment of PxdA and DipA on EEs. Fatty acid (FA)-induced increases in peroxisome movement require *AcbdA*, suggesting that peroxisome hitchhiking on EEs is coupled to FA metabolism. Mutating a conserved FFAT motif, predicted to interact with the endoplasmic reticulum (ER), has no effect on peroxisome movement. Taken together, our data indicate that *AcbdA* is a peroxisome-membrane protein required for peroxisome hitchhiking on EEs. *AcbdA*'s involvement in peroxisome hitchhiking represents a divergence from known functions of *Acbd4/5* proteins and adds layers to our understanding of the functionality of the *Acbd4/5* family of proteins.

49. Investigating the repeated evolution of pathogenesis in *Aspergillus* section *Fumigati*

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Opportunistic fungal pathogens repeatedly evolve across all major clades of fungi. The ability of these fungi to cause disease is the culmination of multiple exaptations resulting in the convergent trait, pathogenicity. In clades like *Aspergillus* section *Fumigati*, pathogenicity evolved independently over relatively short evolutionary time scales. We utilized 36 closely related species of pathogens and non-pathogens from within *Aspergillus* section *Fumigati* to ask whether the independent evolution of pathogenicity is species-specific or conserved through a combination of growth and immunological assays and high-quality *de novo* assemblies. Growth at four different temperatures (30°C, 37°C, 40°C, 45°C) underlines the section's thermotolerance, with many species growing better at 37°C than 30°C.

Conidiation and conidia germination, important ecological factors, display large outliers and no clear signal for pathogenic species. Similarly, survival with murine macrophages and the production of pro and anti-inflammatory cytokines support the independent evolution of pathogenesis across the section. The genomic data tells a similar story. Genome size, gene presence-absence, TE cargo, gene functional conservation, and gene gain/ loss rate all support the species-specific model of evolution. Finally, we consider potential ecological roles using predicted species occupation ranges and overlaps that may influence phenotypic and genotypic differentiation while still resulting in pathogenic potential

50. *Tribolium castaneum*: a novel, ecologically-relevant arthropod model for human fungal pathogens

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Advances in human fungal pathogenesis are increasingly revealing a need for understanding the ecological context pathogens evolve in. Current *in vitro* models for fungal pathogen research are invaluable tools; however, they lack ecological relevance, suffer from limited sample sizes, and some even suffer from confounding batch effects. We present the flower beetle, *Tribolium castaneum*, as a novel model for studying fungal pathogenesis. *T. castaneum* naturally interacts with saprophytic species of fungi, including the fungal pathogens, *Aspergillus fumigatus* and *Aspergillus flavus*. We demonstrate that *T. castaneum* surmounts the shortcomings of current models, using three species of *Aspergillus*. *T. castaneum* infections display dose-dependent infections plus inter- and intra-specific responses to infection. Furthermore, we find *T. castaneum* survival correlates with mouse survival both between and within species. Additionally, we track fungal infection progress and report the transcriptional immune response of *T. castaneum*, relating it back to mammalian immune responses

51. Substantial overlap in virulence-related genes and traits across strains of two *Aspergillus* species with differing clinical relevance

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Pathogenicity has evolved multiple times across the genus *Aspergillus*; however, the identification of discrete, causal genomic elements remains elusive. Most efforts to identify pathogenic elements have concentrated on the highly prevalent clinical pathogen *A. fumigatus*, resulting in the identification of dozens to hundreds of genomic features associated with pathogenic potential. These elements, however, are present in both pathogenic and non-pathogenic species. This complication stems from the fact that "pathogenicity" is not a measurable trait outside of the host-pathogen dynamic. For organisms like *A. fumigatus* that are not obligate pathogens, the genetic determinants of virulence are likely exaptations, and therefore refractory to tidy summarization. We previously reported that the conceptualization of "pathogenicity" as a binary trait at the species level is misguided, as evidenced by the pathogenic heterogeneity observed among strains of the "nonpathogenic" species *A. fischeri*.

Now, we extend this examination by comparing *in vivo* and *in vitro* measures of virulence among 16 strains each of *A. fumigatus* and *A. fischeri*. We find that strains exhibit species-specific growth characteristics in isolation, but that this distinction breaks down when strains are cultured in the presence of murine macrophages. Moreover, interspecific overlaps in virulence occur in a murine model of pulmonary aspergillosis, where three strains of the "non-pathogen" effected lethality rates of >50% while three strains of the "pathogen" were among the least virulent of all 32 strains tested. Thus, while certain traits appear species-distinct, strain heterogeneity can result in overlapping pathogenic potentials. Importantly, we could not statistically attribute these differences in pathogenic potential to specific genomic elements or secondary metabolites. These results further support the notion that the pathogenic potential of an opportunistic pathogen cannot be viewed either as a binary trait or as one that is strictly species-specific. Rather, the pathogenicity of any strain likely involves complex interactions among myriad genomic and ecological factors that only coincidentally converge to result in pathogenicity

52. Chromatin dynamics of ergosterol biosynthetic gene regulation in wild-type and azole-resistant *Aspergillus fumigatus

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Aspergillus fumigatus is a ubiquitous saprophyte responsible for chronic pulmonary infections and life-threatening invasive aspergillosis. Recently, *A. fumigatus* was classified as a critical priority pathogen by the World Health Organization in part due to rapid emergence of antifungal resistance. The first-line therapy for *A. fumigatus* infections is the azole drug voriconazole, which targets Cyp51A to inhibit ergosterol biosynthesis. Transcription of *cyp51A* and other ergosterol biosynthesis genes is controlled by multiple transcription factors, including SrbA, AtrR, and others, yet how these factors modulate chromatin structure to regulate gene expression is unknown. To determine how chromatin changes in response to ergosterol limitation, cells were grown in the presence or absence of voriconazole and examined by ATAC-seq. Following voriconazole exposure, a subset of ergosterol biosynthetic genes displayed enhanced promoter accessibility. We will present findings from our ongoing work to: (1) quantify differential accessibility in voriconazole-exposed cells, (2) identify enriched motifs within these regions, and (3) integrate chromatin accessibility data with histone modification profiles. We also examined chromatin structure in two azole-resistant *A. fumigatus* strains. The most prevalent azole-resistant alleles of *cyp51A* contain short promoter

duplications of 34 and 46 base pairs. Both duplications include binding sites for the global regulators of ergosterol biosynthesis: SrbA and AtrR. In both alleles, the *cyp51A* promoter was hyperaccessible, even in the absence of voriconazole. Future studies will determine if DNA sequence, SrbA, or AtrR is responsible for increased promoter accessibility in the tandem repeat mutants.

53. Delineation of the sterigmatocystin biosynthetic gene cluster in *Aspergillus nidulans* through functional analysis of *stcX*

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Production of sterigmatocystin (ST) in *Aspergillus nidulans* involves the ST biosynthesis gene cluster, which has been considered to comprise 26 genes. However, functions of several genes within this cluster remain unclear. In this study, we investigated the role of the *stcX* gene in ST biosynthesis. As the open reading frame of *stcX* has been undefined, we deleted three putative genes adjacent to *stcW* simultaneously. Deletion of the *region* did not significantly impact growth rate across various growth conditions compared to the control strain. Chemical analyses revealed no significant change in ST production in the deletion strain compared to the control. Furthermore, the three transcripts were largely undetectable during ST biosynthesis, and the expression of other ST cluster genes was unaffected by the deletion. Our results suggest that these genes are not essential for ST biosynthesis in *A. nidulans*, indicating that the cluster should be delineated to exclude *stcX*.

54. The increase in cell volume and nuclear number of the koji-fungus *Aspergillus oryzae* contributes to its high enzyme productivity.

Norio Takeshita

University of Tsukuba

While the ratio of nuclei to cell volume is well regulated, it remains largely unexplored in multinucleate organisms. The koji-fungus *Aspergillus oryzae*, traditionally used in Japanese brewing and fermentation for over a thousand years, is now widely utilized in modern biotechnology as a host for enzyme production. We discovered that, over time in culture, hyphae become thicker, resulting in a tenfold increase in cell volume, and the number of nuclei in hyphal cells also increases tenfold, exceeding 200. The increase in cell volume and nuclear number is unique among the investigated *Aspergillus* species and correlates with its high enzyme production capabilities. Since nuclear number and cell volume are correlated, both must increase simultaneously for either to expand. Our analyses identified genetic factors and nutritional environmental signals involved in each of these increases. Increases in nuclear number and cell volume were also observed in other fungi bred for industrial use. This study not only deepens our understanding of the evolutionary processes that promote high enzyme productivity through fungal breeding, but also provides insights into the molecular mechanisms regulating cell volume and nuclear number in multinucleate organisms.

***55. Characterizing genetic parts in fungal mycelium using large particle flow cytometry**

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Characterizing genetic parts in filamentous fungi is challenging as they form mycelial networks that complicate high-throughput single-cell analysis. Here, we design and characterize promoters within the mycelium using encapsulation and large-particle flow cytometry (COPAS). COPAS enabled the quantification of over 4,000 individual mycelial growths under each condition, enabling a detailed analysis of population-level dynamics. The promoters were introduced into nine fungal strains, including *Fusarium oxysporum*, *Aspergillus awamori*, *Cladosporium cladosporioides*, *Trichoderma reesei*, and *Aureobasidium pullulans*. The performance of two constitutive promoters and a doxycycline-inducible system was assessed. Flow cytometry of mycelium revealed that the fluorescence signal in transformants was 6-times higher under the control of *gpdA* promoter compared to wild-type strains in *A. awamori* and *C. cladosporioides*. COPAS enabled direct measurement of gene expression in encapsulated fungal mycelium providing data for cell-to-cell heterogeneity. Combining *Agrobacterium*-mediated delivery with large-particle flow cytometry could allow for engineering reliable genetic circuits in filamentous fungi, enabling advances in biomanufacturing, biomaterials, and synthetic biology applications.

***56 Identification of *Aspergillus fumigatus* Septation Effectors through Proximity-labeling with TurboID**

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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, as loss of septation generates avirulence characterized by the loss of tissue-invasive hyphal growth. We have shown that the terminal Septation Initiation Network (SIN) signaling complex, composed of the kinase SidB and its activator MobA, is essential for proper septation, virulence, and echinocandin resistance. However, the downstream effectors of this complex remain largely unknown. As proteins that interact with the SIN are likely important effectors for septation and virulence, we employed proximity-based labeling of the SidB/MobA kinase module using TurboID for the first time in *A. fumigatus*. TurboID can identify transient protein-protein interactions exhibited by kinases such as SidB, making

it a putatively useful tool to study this pathway. LC-MS/MS analyses of streptavidin-precipitated triplicate lysates indicated that SidB-TurboID and MobA-TurboID strains shared significantly overlapping interaction profiles, as expected based on our previous genetic analysis revealing that MobA is essential for SidB's role in septation. Further validating the dataset, both SidB and MobA were among the significantly enriched streptavidin-precipitated proteins commonly identified in both tagged strains. After prioritization of the remaining enriched proteins, 18 candidate proteins were selected for further characterization based on predicted roles in cell wall synthesis or cytoskeletal dynamics. Genes encoding candidate proteins were deleted and initially scored for vegetative growth defects, septation and cell wall stress susceptibility. Most notably, our analysis identified multiple putative components of the STRIPAK complex which has been shown to downregulate SIN signaling in other fungi. Deletion of the gene encoding one component, *pabA*, resulted in hyperseptation and hyperbranching consistent with loss of negative SIN regulation. Among the prioritized genes was also the *A. fumigatus* ortholog of the IQGAP, *sepG*, which functions in scaffolding at the contractile actin ring during cytokinesis in *A. nidulans*. Repression of *sepG* resulted in loss of septation. 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.

***57. Saturation mutagenesis of fungal *pyrE* to identify variants that endow resistance to DHODH inhibitors without impacting fitness**

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The use of demethylation inhibitor fungicides as plant protective products have driven the expansion of azole-resistant populations in the human fungal pathogen, *Aspergillus fumigatus*, reducing the effectiveness of first-line therapeutics. In the wake of this problem, new antifungals with novel modes of action have been developed. The dihydroorotate dehydrogenase (DHODH) inhibitor olorofim is in the late stages of clinical development. However, in parallel, an analogous DHODH inhibitor, ipflufenquin, has been developed as a fungicide and has been approved for use in several territories, including the USA. This puts at risk decades of clinical antifungal development. We have recently shown that mutations at several sites in *pyrE*, the gene that encodes DHODH, can provide cross-resistance to both olorofim and ipflufenquin in *A. fumigatus*. The G119 codon position is one of these key sites. Here we used a rapid *in vivo* saturation mutagenesis approach coupled with competitive fitness assays to understand which G119 variants are most important for cross resistance.

We deployed selection-free CRISPR-Cas9 to generate a pooled library of all 64 possible codon variant strains at the G119 position, achieving a transformation rate of between 8 and 32%. Competitive profiling of the library in the absence of drug revealed that high fitness correlated with substitution of glycine with other small, uncharged amino acids. Variant fitness was modulated in the presence of DHODH inhibitors, with some strains exhibiting high fitness in either olorofim, ipflufenquin or both. Worryingly, several variants, including G119C, G119A and G119S showed cross resistance to both inhibitors while displaying no fitness defect in the absence of drug. We also demonstrate that this approach enables evaluation of fitness in complex growth substrates, including soil microcosms, allowing us to determine minimum selective concentrations of ipflufenquin. Furthermore, we reveal that selection for ipflufenquin resistance in soils can be mitigated by exogenous pyrimidine supplementation.

58. Genetic characterization of N-acetylglucosamine utilization in *Aspergillus nidulans*

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The *Aspergillus nidulans* transcription factor XprG plays important roles in regulation of autolysis and nutrient acquisition, including catabolism of N-acetylglucosamine (GlcNAc). We identified the putative genes of the GlcNAc catabolic pathway in *A. nidulans*, most of which are clustered adjacent to the *xprG* gene on chromosome VII. Extracellular GlcNAc is predicted to be imported into the cell by the GlcNAc transporter NgtA, phosphorylated by the hexokinase HxkC to GlcNAc-6-phosphate, then deacetylated by DacA to glucosamine-6-phosphate, and subsequently deaminated by DamA to ammonium and fructose-6-phosphate, which can enter nitrogen and carbon metabolism, respectively. Deletion of the *hxkC* gene was previously shown to confer reduced GlcNAc utilization. We deleted the *ngtA*, *dacA* and *damA* genes by gene replacement. Each deletion mutant showed impaired growth on media containing GlcNAc as a sole nitrogen, carbon, or nitrogen and carbon source. We also identified a putative GlcNAc sensor and histone deacetylase, NgsA, expected to interact with XprG and regulate GlcNAc utilization, based on the phenotypes and interactions of their orthologs in *Candida albicans* and *Trichoderma reesei*. Deletion of the *ngsA* gene confers decreased ability to utilize GlcNAc. Furthermore, we show by construction of double mutants that *xprG* and *ngsA* exhibit genetic interaction. We identified a second potential GlcNAc transporter in *A. nidulans*, NgtB. The *ngtB* gene is located on chromosome II. We deleted the *ngtB* gene and the resulting mutant showed slightly reduced growth on GlcNAc as a nitrogen or carbon source. Therefore, NgtB acts in the GlcNAc utilization pathway. The GlcNAc utilization phenotypes of the *ngtA* Δ and *ngtB* Δ single mutants are less severe than those of the catabolic gene mutants. To determine if deletion of both transporter genes prevents growth on GlcNAc, we attempted to construct a *ngtA* Δ *ngtB* Δ double mutant. Putative diploid and heterokaryotic transformants were obtained. A heterokaryon rescue test indicated that simultaneous deletion of both transporter genes is lethal, suggesting that transport of extracellular GlcNAc into the cell is necessary for hyphal growth.

59. Role of pyrimidines transport in the adaptation to the novel antifungal olorofim in *Aspergillus fumigatus*

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Aspergillus fumigatus is an environmental fungus that can infect humans and cause life-threatening or debilitating lung diseases. Azole drugs are the first-line treatment, but the number of human infections caused by azole-resistant *A. fumigatus* have increased over last years. The development of new antifungals with novel mechanisms of action has been proposed as the most promising intervention to stop and contain the emergence of antifungal resistance. Olorofim acts by inhibiting the de novo synthesis of pyrimidines in a fungal-specific manner and will reach the clinic in the following years. Therefore, a thorough understanding of processes impacting olorofim's effectiveness is urgently needed. Supplementing exogenous pyrimidines (uracil and uridine) upon olorofim exposure completely restores *A. fumigatus* growth. However, when decoupling them, uracil alone is enough to reverse olorofim's activity in all *A. fumigatus* strains tested in the study while uridine only restores growth in a subset of them. By using functional genomics, genome association analyses and transcriptomics we have identified the amino acid starvation-responsive transcription factor CpcA as a regulator of uridine uptake in *A. fumigatus*. These findings indicate a pre-existent genomic variability in uridine uptake regulation in natural populations of *A. fumigatus* strains that might impact olorofim's performance upon clinical deployment.

60. Epigenetic rewiring of the low-oxygen response by an Inhibitor of Growth (ING) family Protein drives *Aspergillus fumigatus* disease progression

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Adaptation to dynamic oxygen microenvironments in the lungs is critical for *Aspergillus fumigatus* disease progression. Higher oxygen levels are available in the large airways, but decline progressively in the alveoli, eventually leading to severe hypoxia in deep tissues. While epigenetic mechanisms are known to play an essential role in low-oxygen adaptation in mammals, their function in fungi is ill-defined. To address this gap in knowledge, we screened mutants in chromatin regulatory genes from the *Neurospora crassa* deletion library and identified an Inhibitor of Growth domain-containing Protein (IngA) required for low oxygen growth. The IngA ortholog in *A. nidulans* is part of the RpdA-L histone deacetylase complex. Loss of *ingA* in *A. fumigatus* resulted in stunted biofilms at both 21% and 10% atmospheric oxygen levels. A significant reduction in the Δ *ingA* submerged biofilm biomass at 10% oxygen was observed compared to wild-type and complemented strains.

Under hypoxic conditions (0.2% oxygen), both shaking batch cultures and submerged biofilms of Δ *ingA* exhibited a severe growth defect. Our results suggest that Δ *ingA* is metabolically limited, as it cannot grow on plates containing ethanol and 2-deoxy-D-glucose under normoxic conditions. Moreover, transcripts of genes encoding proteins involved in ribosome biogenesis and protein translation are significantly increased in Δ *ingA* in hypoxic conditions. We are currently testing the hypothesis that the growth failure of Δ *ingA* in hypoxic conditions arises from its inability to sense oxygen changes and adjust protein translation.

We also observe that IngA is a positive regulator of ergosterol biosynthesis, and Δ *ingA* is less susceptible to voriconazole. Critically, the loss of *ingA* results in a striking reduction in fungal burden and attenuated virulence in the triamcinolone murine model of invasive aspergillosis. Histological analysis reveals that the mutant can grow in the larger airways but cannot grow in deep tissues, where oxygen levels are much lower. Therefore, while IngA is not required for disease initiation, it is essential for disease progression in low-oxygen environments. As antifungals are typically administered after disease establishment in patients, identification of novel disease progression factors is critical for developing new antifungals to treat this deadly disease.

61. Mycotechnology to the rescue: Biosustainable Production of secondary metabolites by filamentous fungi using carbon sourced from Polystyrene and Polyethylene Plastic Waste

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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 economical contribution. Consequently, long-term plastic sustainability is an urgent matter that requires new approaches to achieve, such as the utilization of microorganisms. We previously have 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. In this talk I will present our groups efforts to produce various useful secondary metabolites using our hybrid chemical biological approach. The talk will demonstrate the power of mycotechnology to address issues in plastic waste and sustainability.

***62. Bacterial partner protects *Aspergillus calidoustus* from Voriconazole**

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Microbial communities are made of interacting and competing microorganisms that play a critical role in human, plant, and environmental health. These communities are partially shaped by the production of natural antimicrobial compounds (AMCs) that exclude the growth of susceptible bacteria and fungi. Phenazines are one major AMC that most fungi are susceptible to. Our lab uses a co-isolated bacterial-fungal pairing where the bacterium, *Paraburkholderia edwinii* can protect its partner fungus, *Aspergillus calidoustus*, from the oxidative stress

of phenazine-1-carboxylic acid. To determine if the same protection occurs in the presence of synthetic antifungal compounds, we have conducted a co-colony experiment using our model organisms in the presence of voriconazole. Voriconazole is a frontline clinical antifungal drug used to treat serious *Aspergillus* and *Candida* infections. The co-colonies confirmed the protection of *Aspergillus calidoustus* by *Paraburkholderia edwinii* in the presence of Voriconazole. Bacterial genes previously associated with protection of *A. calidoustus* from phenazines were found to be unassociated with protection from voriconazole, suggesting a different mode of protection. We are working to identify the bacterial genes responsible for voriconazole protection by conducting mariner transposon mutagenesis of the bacterium, and screening co-colonies for a lack of fungal growth in the presence of Voriconazole. Preliminary analyses will focus on the pathways that may implicate the sharing of sterol-like lipids or their precursors with the fungus due to their physical association, secreted proteins, and surface-exposed carbohydrates on the bacterial surface. Understanding how bacteria protect fungi from synthetic antifungal assault can provide insight on overcoming both clinical and agricultural concerns such as antifungal resistance in both humans and crops.

63. Regulation of the mannanase genes by multiple transcription factors in *Aspergillus nidulans

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Aspergillus species produce cellulolytic and mannanolytic enzymes, which are utilized in a wide range of industrial fields. Generally, orthologs of a single specific transcription factor ClrB (e.g., ManR in *A. oryzae*, ClrB in *A. niger*) regulate the expression of both enzymes. However, *A. nidulans* possesses ManS, a paralog of ClrB. While ClrB primarily regulates cellulase genes and is also involved in regulating some mannanase genes, ManS significantly affects the expression of all mannanase genes. To elucidate the regulatory mechanisms of mannanase genes by ManS and ClrB, we have been analyzing the DNA recognition by these paralogous transcription factors. In the previous conference, we reported the results of DNA binding analysis by EMSA for the transcription factors in *manE*, regulated solely by ManS, and *manB*, regulated by both ManS and ClrB. We proposed that the CGGN₁₆CCG sequence within the *manE* and *manB* promoters constitutes the ManS binding site, while the CCG/CGG-rich region within the *manB* promoter enables simultaneous binding by ManS and ClrB. In this study, the activity of the identified motifs was assessed by a reporter assay. Furthermore, we investigated whether McmA, another cellulase gene regulator that acts cooperatively with ClrB, is involved in the expression of *manB*.

In the reporter assay, when galactomannan was used as an inducer, the approximately 250-bp DNA fragments containing the CGGN₁₆CCG sequence derived from the *manB* and *manE* promoters exhibited high promoter activity. For *manE*, this galactomannan-responsive expression was lost in the *manS*-deficient strain. This suggests that CGGN₁₆CCG is a *cis*-element involved in ManS-dependent expression. For *manB*, the 250-bp also contained the CCG/CGG-rich region alongside CGGN₁₆CCG. Currently, we are analyzing the activity of each motif by introducing specific mutations.

Previous EMSA studies have demonstrated that His-ManS₁₆₄ and FLAG-ClrB₁₁₈ bind as a heterocomplex to the CCG-rich region within the *manB* promoter. This region contains cellulose responsive element (CeRE, CCGN₂CCN₆GG), a *cis*-element in the cellulase gene promoters, where ClrB and McmA bind cooperatively. Hence, we analyzed the binding of FLAG-ClrB₁₁₈ and His-McmA to the CCG-rich region by EMSA. The results showed that the ClrB-McmA complex bound more strongly than the ManS-ClrB complex. This result suggests that, similar to the cellulase genes, binding of the ClrB-McmA complex to CeRE may regulate the *manB* expression.

***64. A tunable and strict gene expression system with a high dynamic range in filamentous fungi**

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Filamentous fungi are widely used for the bioproduction of valuable compounds, owing to their strong secretory capacity, metabolic diversity, and low cultivation cost. Among them, *Aspergillus oryzae* is recognized as a safe and versatile host because of its long history of use in food industry. These features highlight the need for flexible gene expression systems applicable to synthetic biology in filamentous fungi. However, existing inducible expression systems rely highly on nutrient-dependent promoters, which limits flexibility of culture and induction conditions. In this study, we developed a versatile drug-inducible gene expression system for filamentous fungi. We introduced an artificial transcription factor (LexA-ER-VP16; XEV) composed of the *Escherichia coli* LexA DNA-binding domain, the human estrogen receptor ligand-binding domain (ER), and the VP16 activation domain into *A. oryzae*. A GUS reporter gene (*uidA*), which encodes the enzyme β -glucuronidase, under the control of *lexO* and the *CYC1* core promoter derived from *Saccharomyces cerevisiae* showed strict β -estradiol-dependent induction with minimal basal expression. Notably, induction levels of this system reached those of the strong *amyB* promoter, while expression remained unaffected by the choice of major carbon sources. We further applied this system to control transcript level regulation of the endogenous transcription factor AmyR. β -Estradiol-dependent regulation of *amyR* expression led to dose-dependent increases in the production of multiple amyolytic enzymes, demonstrating that endogenous pathways can be precisely controlled with this system. We next optimized the system to achieve a broader dynamic range. Expressing XEV under a stronger promoter increased the overall induction level with fewer inducers. Furthermore, replacing the *CYC1* core promoter downstream of *lexO* with the *A. oryzae* *gpdA* (GAPDH) or *hhfA* (Histone H4) core promoters resulted in higher gene expression. The system was also functional in *Aspergillus nidulans*, demonstrating its versatility to *Aspergilli*.

Overall, we established a nutrient-independent inducible expression platform that offers low leakage, tunable induction, and a wide dynamic range, providing a highly useful tool for synthetic biology and industrial applications in filamentous fungi.

65. Pseudouridine modification regulates development, azole resistance and genome stability in *Aspergillus fumigatus*

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Aspergillus fumigatus is a major opportunistic fungal pathogen that poses a growing global health threat due to the expanding population of immunocompromised individuals and the increasing prevalence of antifungal resistance. Understanding the molecular mechanisms underlying fungal development and drug resistance is crucial for developing effective control strategies. RNA modifications have recently emerged as important epigenetic regulatory mechanisms in eukaryotes, yet their roles in pathogenic fungi remain poorly understood. Here, we identify RNA pseudouridylation as a previously unrecognized regulatory layer governing fungal growth, differentiation, and antifungal resistance. Systematic functional analyses of pseudouridine synthases revealed their essential contributions to hyphal development, sporulation, and genome stability. Perturbation of pseudouridylation altered global translation efficiency, as demonstrated by polysome profiling, and induced adaptive responses associated with enhanced antifungal resistance. These findings indicate that RNA pseudouridylation modulates translational homeostasis to fine-tune fungal stress adaptation. Collectively, our study uncovers a fundamental role of RNA modification in fungal physiology and drug resistance, providing new mechanistic insights into antifungal tolerance and potential directions for therapeutic intervention.

66. CRISPR/Cas9-Mediated Multicopy Integration into 13 Chromosomal Loci Enables Hyperproduction of Xylanase in *Aspergillus oryzae*

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Background and Purpose : *Aspergillus oryzae* is widely used as a host for recombinant protein production.

Increasing gene copy number is an effective strategy to improve protein yields. However, in *A. oryzae*, multicopy integration typically occurs as tandem repeats at a single chromosomal locus, which may in turn lead to the loss of repeat sequences through homologous recombination. In this study, we aimed to establish a genome engineering method that enables the simultaneous insertion of exogenous DNA fragments into multiple chromosomal loci in a single transformation procedure.

Methods and Results: *A. oryzae* RIB40 strain contains 13 highly conserved DDE1 transposon-like sequences on its chromosomes. We examined whether CRISPR/Cas9-mediated cleavage of these sites could facilitate multicopy gene integration. A guide RNA was designed to target a conserved region near the center of the transposon-like sequence, allowing Cas9 to introduce a double-strand break at all homologous loci. The donor DNA consisted of an *xynF1* (xylanase F1) expression cassette flanked by ~300-bp homology arms corresponding to the regions upstream and downstream of the Cas9 target sites. All elements required for multicopy integration, including xylose-inducible Cas9, the gRNA cassette, the donor DNA, the AMA1 replicon, and a selectable marker, were assembled into a single plasmid and then introduced into the *A. oryzae* Δ *ligD* strain. Transformants were initially maintained under Cas9-repressive conditions (glucose) and then transferred to xylose plates to induce genome editing. Conidial PCR verified successful integration of the *xynF1* cassette at all 13 targeted loci. Furthermore, evaluation of the enzyme productivity of the constructed multicopy strains revealed that the edited strain exhibited an approximately 10-fold increase in xylanase production compared with the single-copy strain, reaching a high titer of over 3 g/L.

Aspergillus talks at FGC

In assigning talks for Asperfest, the AGRPC selects speakers to ensure a diversity of themes are covered and to promote exposure of research activities beyond those already being discussed at the FGC meeting.

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