The 18th International Aspergillus Meeting

Asperfest 18

March 14-15, 2022

Asilomar Conference Center, Pacific Grove, CA.
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 AGPRC 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).

2021 AGRPC

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David Cánovas, 2020-2023, University of Seville, Spain; davidc@us.es
Michael Bromley, 2020-2023, University of Manchester, UK. Mike.Bromley@manchester.ac.uk

THANKS TO OUR MEETING SPONSORS!
Program
The Eighteenth International Aspergillus Meeting
Asperfest 18
Monday March 14 – Tuesday March 15, 2022
Chapel, Asilomar Conference Center, Pacific Grove, CA.

Monday March 14

4:00PM  Poster hang up

7:00PM - 10:00PM  Poster and Welcome Reception (sponsored by Novozymes, Inc.)

7:00 - 8:30  Even-numbered posters
8:30 -10:00  Odd-numbered posters

Judging for Novozymes Student Poster Prize, coordinated by Norio Takeshita, University of Tsukuba, Japan.

Tuesday March 15

8:30AM  Welcome, introductions and announcements  Richard Todd
Kansas State University, USA

8:40  Session I  Richard Todd

A new role in G2-M control revealed by a reciprocal translocation involving the snxA shuttling mRNA-binding protein and a GYF-domain protein in Aspergillus nidulans.
Steven James, Gettysburg College, USA.

New Aspergillus labs 1  Robb Cramer
Dartmouth College, USA

The significance of virus infection in Aspergillus fumigatus strain AF293.
Ioly Kotta-Loizou, Imperial College London, UK.

Exploring the unfolded protein response as a target for treating fungal corneal infections.
Kevin Fuller, University of Oklahoma Health Sciences Center, USA.

Distinct type I interferon (IFN) signaling is involved in regulating host damage and fungal clearance during Aspergillus pulmonary infection.
Kelly Shepardson, Montana State University/University of California-Merced, USA.

9:30  Flash Talks from Abstracts  Michelle Momany
University of Georgia, USA
Nancy Keller
University of Wisconsin-Madison, USA

The molecular resistance mechanisms and population structure of azole-resistant Aspergillus fumigatus present on commercial agricultural products in the United States.
Caroline Burks, University of Georgia, USA.
Complex and critical roles for the AtrR transcription factor in control of cyp51A expression in Aspergillus fumigatus.
Sanjoy Paul, The University of Iowa, USA.

Distribution of non-canonical septins in fungi.
Brent Shuman, University of Georgia, USA.

CRISPR/Cas9-based engineering of Aspergillus oryzae mycelium for meat-like flavor and appearance.
Vayu Maini Rekdal, UC Berkeley, USA.

The fungal sexual revolution continues: indications of sexuality in the citric acid producing fungus Aspergillus niger.
Valeria Ellena, TU Wien, Austria.

Olorofim and the azoles are antagonistic in Aspergillus fumigatus and functional genomic screens reveal mechanisms of cross resistance.
Norman van Rhijn, Manchester Fungal Infection Group, UK.

Adela Martin-Vincente, University of Tennessee Health Science Center, USA.

Oxygen mediated cell-cell heterogeneity and antifungal drug susceptibility in Aspergillus fumigatus biofilms.
Kaesi Morelli, Dartmouth College, USA.

Program the Future with Ginkgo's Cell Development Kit.
Jesse Dill, Ginkgo Bioworks, USA.

10:00-10:30 Coffee Break and Posters

10:30 Session II
New Aspergillus labs (cont.)
Robb Cramer
Dartmouth College, USA

Aspergillus fumigatus hexameric septin complex is involved in spore cell wall organization and immune evasion.
Jose Vargas-Muniz, Southern Illinois University, USA.

Antifungal screening of 54 single plant essential oils against Aspergillus fumigatus.
Yainitza Hernandez-Rodriguez, Florida Gulf Coast University, USA.

ncRNAs – novel regulatory links in fungal azole tolerance?
Sourabh Dhingra, Clemson University, USA.
11:00 Tools/Resources
Norio Takeshita, University of Tsukuba, Japan

Structural determination of fungal secondary metabolites using cryo-EM method microcrystal electron diffraction.
Clay Wang, University of Southern California, USA.

Enzyme Profile Relatedness in Aspergillus
Lene Lange, BioEconomy, Research & Advisory, Denmark.

COFUN: Final report on the construction of the genome wide-knockout library in A. fumigatus.
Michael Bromley, University of Manchester, UK.

FungiDB: what's new and planned at FungiDB.
David Roos FungiDB

11:40 Community directions: Discussion; Elections.
Richard Todd
Kansas State University, USA

Discussion; Elections

12:00PM - 1:15PM Lunch
Crocker Dining Hall.

1:15PM Session III: Talks from Abstracts
Michelle Momany
University of Georgia, USA

tRNA-ome of the human fungal pathogen Aspergillus fumigatus: high-throughput functional analysis reveals a valine tRNA isodecoder involved in Azole sensitivity
Lauren Dineen, University of Manchester, UK.

Duplication, Redundancy, and Divergence: roles of the Aspergillus nidulans paralogous transcription factors LeuR and LeuB in leucine biosynthesis, nitrogen assimilation, and transcriptome regulation.
Joel T. Steyer, Kansas State University, USA.

Flotillin-dependent membrane microdomains are required for functional phagolysosomes against fungal infections.
Franziska Schmidt, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute & Friedrich Schiller University Jena, Germany.

Microevolution in the pan-secondary metabolome of Aspergillus flavus has macroevolutionary implications for filamentous fungi.
Milton T. Drott, University of Wisconsin-Madison, USA.

Quantifying fungal pellets during submerged cultivation: from 3D X-ray microtomography imaging to diffusive mass transport.
Lars Barthel, Technische Universität Berlin, Germany.
2:15 Pontecorvo Lecture (sponsored by Zymergen, Inc.)
Richard Todd
Kansas State University, USA
Brandon Pfannenstiel
Zymergen, Inc., USA

Ad astra per Aspergillus, from mitosis to natural products chemistry.
Berl Oakley, Irving S. Johnson Distinguished Professor,
University of Kansas, Lawrence, KS, USA.

2:45PM Election results; other discussion items
Richard Todd
Kansas State University, USA

Novozymes student poster prize presentation
Norio Takeshita,
University of Tsukuba, Japan

3:00PM Dismiss (Remove posters)
List of Posters

Presenter indicated in bold type, * denotes a student poster presenter

*1. The narrow footprint of ancient balancing selection surrounding nonself recognition genes in *Aspergillus fumigatus*
Ben Auxier, Jianhua Zhang, Eveline Snelders, Alfons Debets.

2. Meiosis in the human pathogenic fungus *Aspergillus fumigatus* produces the highest known number of crossovers
Ben Auxier, Alfons Debets, Joost van den Heuvel, Eveline Snelders.

3. Parasexual recombination enables *Aspergillus fumigatus* to persist in cystic fibrosis

*4. The C2H2 transcription factor SltA is involved in conidial germination and hyphal elongation in *Aspergillus fumigatus*
Tim Baltussen, Norman van Rhijn, Paul Verweij, Michael Bromley, Willem Melchers.

5. *Aspergillus fumigatus* pan-genome analysis identifies genetic variants associated with human infection
Amelia Barber, Tongta Sae-Ong, Kang Kang, Bastian Seelbinder, Jun Li, Grit Walthier, Gianni Panagiotou, Oliver Kurzai.

*6. Quantifying fungal pellets during submerged cultivation: from 3D X-ray microtomography imaging to diffusive mass transport*
Lars Barthel, Stefan Schmideder, Henri Müller, Heiko Briesen, Vera Meyer.

7. COFUN: Final report on the construction of the genome wide-knockout library in A. fumigatus
Michael Bromley, Can Zhao, Lauren Dineen, Isabelle Storer, Thorsten Heinekamp, Axel Brakhage, Daniela Delneri, Paul Bowyer, Ressa Sinaia Lebedinec.

*8. The molecular resistance mechanisms and population structure of azole-resistant *Aspergillus fumigatus* present on commercial agricultural products in the United States*
Caroline Burks, Natalie Miller, Douglas Vines, Michelle Momany, Paul Severs, Marin Brewer.

*9. MERLIN unlocks the secrets to chitin signaling: Using gene-network inference to predict mediators of fungal response to lipo-chitooligosaccharides*
Cristobal Carrera Carriel, Spencer Halberg-Spencer, Saptarshi Pyne, Jean-Michel Ané, Nancy P. Keller, Sushmita Roy.

*10. Analysis of 439 Cyp51 protein sequences shows 5 major Cyp51 gene family groups across Fungi*
Brandi Celia, Michelle Momany, Marin Brewer.

*11. Uncovering long non-coding RNA associated with drug response in *Aspergillus fumigatus*
Danielle Weaver, Harry Chown, Takanori Furukawa, Fabio Gsaller, Daniella Delneri, Paul Bowyer, Michael J. Bromley.

*12. tRNA-ome of the human fungal pathogen *Aspergillus fumigatus*: high-throughput functional analysis reveals a valine tRNA isodecoder involved in Azole sensitivity*
Lauren Dineen, Ressa Lebedinec, Marcin Fraczek, Can Zhao, Daniela Delneri, Paul Bowyer, Mike Bromley.
13. Microevolution in the pan-secondary metabolome of *Aspergillus flavus* has macroevolutionary implications for filamentous fungi

14. The fungal sexual revolution continues: indications of sexuality in the citric acid producing fungus *Aspergillus niger*.
Valeria Ellena, Matthias Steiger.

15. Primary, secondary and tertiary metabolites, proteins and carbohydrates
Jens Frisvad.

*16. *Aspergillus niger* conidial germination: 3D live cell exploration
Susanne Fritsche, Matthias Steiger.

17. *Aspergillus fumigatus* Septation Initiation Network (SIN) kinases contribute to fungal pathogenesis, cell wall construction, and rRNA metabolism.
Xabier Guruceaga, Adela Martin-Vicente, Ana Camila Oliveira Souza, Ashley V. Nywening, Harrison Thorn, Jinhong Xie, Wenbo Ge, Brian M. Peters, Jarrod R. Fortwendel.

*18. Targeting *Aspergillus fumigatus* hypoxia response pathways to potentiate contemporary antifungal therapies
Cecilia Gutierrez Perez, Sourabh Dhingra, Steven M Kwasy, Timothy J Opperman, Robert A Cramer.

19. Transcriptome investigation of *mpkB-mkkB* mutants related to secondary metabolism and sexual development of *Aspergillus nidulans*
Sang-Cheol Jun, Jong-Hwa Kim, Kap-Hoon Han.

20. FungiDB: Free online informatic tools for fungal and oomycete biologists
Evelina Basenko, Omar Harb, David Roos, presenting on behalf of the entire VEuPathDB Bioinformatics Resource Center.

21. Antifungal Screening of 54 Single Plant Essential Oils Against *Aspergillus fumigatus*
Matthew Swearingen, Elizabeth Myers, Yainitza Hernandez-Rodriguez.

22. A new role in G2-M control revealed by a reciprocal translocation involving the *snxA* shuttling mRNA-binding protein and a GYF-domain protein in *Aspergillus nidulans*
Steven James, Jonathan Palmer, Nancy Keller, Sarah Lea Anglin.

*23. The role of cytochrome c in leukocyte induced *Aspergillus fumigatus* cell death.
Matthew James, Ko-Wei Liu, Elisa Vesley, Tobias Hohl, Robert Cramer.

24. Human p11-mediated re-direction of phagosomes to the recycling endosome-expulsion pathway induced by fungal pathogen
Leijie Jia, Muhammad Rafiq, Lukáš Radosa, Peter Hortschansky, Thomas Krüger, Franziska Schmidt, Thorsten Heinekamp, Maria Stassbruger, Olaf Kniemeyer, Axel Brakhage.

25. Fungi to the rescue – revolutionizing food production through biotechnology
Britta Winterberg, Bastian Joehnk.

*26. Unfolded protein response is critical for the corneal pathogenesis of *Aspergillus fumigatus*
Manali Kamath, Jorge Lightfoot, Emily Adams, Kevin Fuller.
27. The ndrC gene, which is regulated by nsdD, controls sexual development in *Aspergillus nidulans*
Yu Kyung Kim, Kap-Hoon Han.

28. Aspergillus as model for analyzing the fungal digestive enzyme profile -to be included in species description and classification?

29. CRISPR/Cas9-based engineering of *Aspergillus oryzae* mycelium for meat-like flavor and appearance
Vayu Maini Rekdal, Jay Keasling.

30. The *Aspergillus fumigatus* morphogenesis-related kinase, CotA, orchestrates hyphal growth in response to carbon source quality
Adela Martin-Vicente, Xabier Guruceaga, Ashley V Nywening, Jinhong Xie, Harrison I Thorn, Wenbo Ge, Jarrod R Fortwendel.

*31. Oxygen mediated cell-cell heterogeneity and antifungal drug susceptibility in *Aspergillus fumigatus* biofilms
Kaesl Morelli, Caitlin Kowalski, Robert Cramer.

32. Pathogenic fungi in Norwegian barns - first survey of *Aspergillus fumigatus* azole resistance in Norway
Erik Magnus Nedland Henriksen, Hege Divon, Elin Rolén, Lonny Margrethe Klovfjell, Ellen Christensen, Ida Skaar.

*33. Computational advances in the discovery of a new class of fungal natural products
Grant Nickles, Milton Drott, Nancy Keller.

*34. The *Aspergillus fumigatus* Spindle Assembly Checkpoint components, sldA and sldb, play roles in maintenance of triazole susceptibility
Ashley Nywening, Adela Martin-Vicente, Wenbo Ge, Xabier Guruceaga Sierra, Jarrod Fortwendel.

35. Computer-Aided, Resistance-Gene-Assisted Genome Mining for Proteasome and HMG-CoA Reductase Inhibitors
Cory Jenkinson, Adam Podgorny, Cooncong Zhong, Berl Oakley.

36. Nanoparticles and pathogenic fungi: a non-uptake delivery
Thomas Orasch, Gauri Gangapurwala, Katherine Gonzalez, Julien Alex, Alicia De San Luis, Antje Vollrath, Christine Weber, Stephanie Hoeppener, Zoltan Cseresnyes, Marc Thilo Figge, Ulrich S. Schubert, Axel A. Brakhage.

37. Complex and critical roles for the AtrR transcription factor in control of cyp51A expression in *Aspergillus fumigatus*

38. Functional analysis of the bZIP transcription factors AtfA and AtfB in *Aspergillus nidulans*
Beatrix Kocsis, Mi-Kyung Lee, Jae-Hyuk Yu, Istvan Pocsi, Eva Leiter.

39. Interaction of the bZIP-type transcription factors NapA and RsmA in the regulation of oxidative stress defence and sterigmatocystin production of *Aspergillus nidulans*
Bernadett Bákány, Yin Wen-Bing, Beatrix Dienes, Tibor Nagy, Éva Leiter, Tamás Emri, Nancy P. Keller, István Pócsi.
40. Genome-wide analysis of AtfA/AtfB-mediated menadione stress response in Aspergillus nidulans
Beatrix Kocsis, Mi-Kyung Lee, Jae-Hyuk Yu, István Pócsi, Éva Leiter, Tamás Emri.

*41. cexA and its regulatory processes – a closer look into the citric acid production mechanism of Aspergillus niger
Aline Reinfurt, Valeria Ellenia, Matthias Steiger.

42. Flotillin-dependent membrane microdomains are required for functional phagolysosomes against fungal infections
Franziska Schmidt, Andreas Thywißen, Marie Goldmann, Cristina Cunha, Zoltán Cseresnyés, Hella Schmidt Schmidt, Muhammad Rafiq, Silvia Galiani, Markus H. Gräler, Georgios Chamilos, João F. Lacerda, António Campos Jr., Christian Eggeling, Marc Thilo Figge, Thorsten Heinekamp, Scott G. Filler, Agostinho Carvalho, Axel A. Brakhage.

*43. Methionine synthase as a target for antifungal drug development
Jennifer Scott, Benjamin Thornton, Jonathan Fowler, Rachael Fortune-Grant, Riba Thomas, Lydia Tabernero, Elaine Bignell, Jorge Amich.

*44. Distribution of non-canonical septins in fungi
Brent Shuman, Michelle Momany.

*45. Investigating germination initiation in the pathogenic fungus Aspergillus fumigatus
Justina Stanislaw, Michelle Momany.

*46. Duplication, Redundancy, and Divergence: roles of the Aspergillus nidulans paralogous transcription factors LeuR and LeuB in leucine biosynthesis, nitrogen assimilation, and transcriptome regulation

*47. Identification of a protein-protein interaction site essential for mitotic entry to guide antifungal drug design in Aspergillus fumigatus
Isabelle Storer, Benjamin Thornton, Lydia Tabernero, Michael Bromley.

48. A versatile selection free CRISPR-Cas9 transformation system for A. fumigatus
Norman van Rhijn, Takanori Furukawa, Lauren Dineen, Tim Baltussen, Jochem Buil, Paul Verweij, Willem Melchers, Michael Bromley.

49. Olorofim and the azoles are antagonistic in Aspergillus fumigatus and functional genomic screens reveal mechanisms of cross resistance.
Norman van Rhijn, Sam Hemmings, Clara Valero, Jorge Amich, Mike Bromley.

50. Aspergillus fumigatus hexameric septin complex is involved in spore cell wall organization and immune evasion
Alban Sinani, Wyatt Boyer, José Vargas-Muñiz.

51. Structural characterization of secondary metabolites from filamentous fungi.
**Presenting Authors**

*(Alphabetical; Student presenters in bold type with asterisk)*

<table>
<thead>
<tr>
<th>Presenter</th>
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## Student Presenting Authors

(Alphabetical)

### 7:00-8:30 Even

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**Abstracts**

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* denotes a student poster presenter

**1. The narrow footprint of ancient balancing selection surrounding nonself recognition genes in Aspergillus fumigatus**

**Ben Auxier**, Jianhua Zhang, Eveline Snelders, Alfons Debets. *Laboratory of Genetics, Wageningen University, NL*

Fusion within a fungal individual is necessary for many life processes, yet is restricted between individuals. The genetics of this have been worked out particularly in *Neurospora* and *Podospora*. The similarity of the genetics, if any, was unknown in any *Aspergillus*. To address this, we used heterokaryotic complementation of auxotrophic nitrate assimilation mutants in *Aspergillus fumigatus*. We assessed compatibility between ~150 progeny of a sexual cross between two environmental strains. We recovered ~3% of offspring were compatible, consistent with segregation of 5 genes. 4 of these loci had a strong effect, while the fifth only delayed, not prevented, heterokaryon formation. Using whole-genome sequences of each offspring, we identified 5 candidate het loci, 4 of which could be identified to a single gene. Heterologous expression of candidate alleles from autonomous plasmids confirmed the causal role of 3 of these loci, and validation of the remaining is ongoing. The 4 identified strong effect genes include het gene mechanisms known from other species, including NLR and patatin-like proteins. One gene, *hetB*, appears to involve a protease containing a CARD domain known to interact in mammalian apoptosis which fungi do not use. Phylogenetic analysis of these genes shows strong trans-species polymorphisms, with alleles being shared between *A. fumigatus, A. lentulus, A. fisheri,* and *A. udagawae,* spanning >10 million years of evolution. Surprisingly, the diversity surrounding these identified *het* genes is restricted to only +/- 200bp of the coding sequence, potentially related to the high recombination rate in this species. Knowledge of the *het* gene complement in this species presents an opportunity to investigate the downstream cell death pathways, which may have clinically relevant opportunities.

**2. Meiosis in the human pathogenic fungus Aspergillus fumigatus produces the highest known number of crossovers**

**Ben Auxier, Alfons Debets, Joost van den Heuvel, Eveline Snelders. Wageningen University**

The decade-old discovery of a sexual cycle, combined with population genetic data, indicates that sex is common in the fungus *Aspergillus fumigatus*. However, the impact of sex remained unclear. Here we show that meiosis in *A. fumigatus* produces the highest known number of crossovers between chromosomes. Using genome-wide markers, we observed 29 crossovers per chromosome on average. This remains the highest known crossover rate after correcting for genome size or chromosome number. We show that this calculated high recombination rate, combined with abundant sexual progeny, can explain the origin of highly antifungal resistant haplotypes from individual tightly linked antifungal resistance mutations. Understanding the consequences of this unparalleled crossover rate not only enriches our population-level understanding of this emergent human pathogen, but of meiosis in general.

**3. Parasexual recombination enables Aspergillus fumigatus to persist in cystic fibrosis**

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*Aspergillus fumigatus* is a saprobic fungus that causes a range of pulmonary diseases, some of which are characterised by fungal persistence such as is observed in cystic fibrosis (CF) patients. Creation of genetic variation is critical for *A. fumigatus* to adapt to the lung environment, but biofilm formation, especially in CF patients, may preclude mutational supply in *A. fumigatus* due to its confinement to the hyphal morphotype. We tested our hypothesis that genetic variation is created through parasexual recombination in chronic biofilms by phenotypic and genetic analysis of *A. fumigatus* isolates cultured from different origins. As diploids are the hallmark of parasex, we screened 799 *A. fumigatus* isolates obtained from patients with CF, chronic pulmonary lung disease and acute invasive aspergillosis, and from the environment for spore size. Benomyl sensitivity, nuclear content measurements through fluorescence-activated cell sorting and scanning electron microscopy were used to confirm the diploid
state of large size spores. Whole genome sequencing was used to characterise diploid-associated genetic variation. We identified 11 diploids in isolates recovered from six of 11 (55%) CF patients and from one of 24 (4%) chronic aspergillosis patients, but not in 368 isolates from patients with acute Aspergillus infection and the environment. Diploid formation was associated with accumulation of mutations and variable haploid offspring including a voriconazole-resistant isolate. Parasexual recombination allows A. fumigatus to adapt and persist in CF patients, and plays a role in azole resistance development. Our findings are highly significant for understanding the genetics and biology of A. fumigatus in the human lung.

*4. The C_{2}H_{2} transcription factor SltA is involved in conidial germination and hyphal elongation in Aspergillus fumigatus*

Tim Baltussen\(^1\), Norman van Rhijn\(^2\), Paul Verweij\(^1\), Michael Bromley\(^2\), Willem Melchers\(^1\). 1) Department of Medical Microbiology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, the Netherlands; 2) Manchester Fungal Infection Group, Division of Infection, Immunity and Respiratory Medicine, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Aspergillus fumigatus is a filamentous saprophytic fungus that produces multicellular tubular cells termed hyphae. Hyphal tip extension occurs through the synthesis and addition of new cell wall and membrane at the apical plasma membrane. This highly polar extension of the tip helps A. fumigatus to penetrate and invade blood vessels and tissue which results in invasive aspergillosis (IA). Before the fungus grows in a highly polarized manner, the conidium breaks dormancy and the reactivated cell expands isotropically before it undergoes localized expansion of the cell membrane which leads to a tubular outgrowth. Potential regulators of germination and early growth remain largely unexplored. We selected fourteen transcription factors (TFs) upregulated during isotropic and/or polarized growth using transcriptomic data from our previous study. TF knock out mutants were generated in the parental strain MFIG001 (WT), which is deficient in non-homologous end joining. We utilized bright-field and fluorescence microscopy to examine conidial germination of the TF null mutants and WT temporally (0 to 12h). We observed a markedly distorted hyphal elongation morphology in the ΔsltA mutant that is not apparent in WT strain and other TF null mutants used in this study. The ΔsltA mutant had a germination rate almost two times higher after 6 h compared with the WT and ΔsltArec strain. Germination rate was similar after 8 h of growth and reached around 95% in all strains. Hyphae of the ΔsltA mutant hyperbranched and some of the branched hyphae annihilated tubular elongation in RPMI-1640 medium. After 72 h the ΔsltA strain showed reduced colony growth on Aspergillus Minimal Medium when compared with the WT and ΔsltArec strains. However, when exposed to cell wall stress agents (calco fluor white, congo red and caspofungin) the relative colony size increased in the ΔsltA strain compared with WT and ΔsltArec strain. This suggests a role for sltA in cell wall biosynthesis and membrane stability. Altogether, we identified a role for the transcription factor sltA in germination and tubular growth of the hyphal tip. Additional experiments will be performed to analyze the molecular mechanisms underlying the distorted hyphal elongation phenotype in the ΔsltA strain.

5. Aspergillus fumigatus pan-genome analysis identifies genetic variants associated with human infection

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Aspergillus fumigatus is an environmental saprobe and opportunistic human fungal pathogen. Despite an estimated annual occurrence of more than 300,000 cases of invasive disease worldwide, a comprehensive survey of the genomic diversity present in A. fumigatus—including the relationship between clinical and environmental isolates and how this genetic diversity contributes to virulence and antifungal drug resistance—has been lacking. In this study, we define the pan-genome of A. fumigatus using a collection of 300 globally sampled genomes (83 clinical and 217 environmental isolates). We found that 7,563 of the 10,907 unique orthogroups (69%) are core and present in all isolates and the remaining 3,344 show presence/absence of variation, representing 16–22% of the genome of each isolate. Using this large genomic dataset of environmental and clinical samples, we found an enrichment for clinical isolates in a genetic cluster whose genomes also contain more accessory genes, including genes coding for transmembrane transporters and proteins with iron-binding activity, and genes involved
in both carbohydrate and amino-acid metabolism. Finally, we leverage the power of genome-wide association studies to identify genomic variation associated with clinical isolates and triazole resistance as well as characterize genetic variation in known virulence factors. This characterization of the genomic diversity of A. fumigatus allows us to move away from a single reference genome that does not necessarily represent the species as a whole and better understand its pathogenic versatility, ultimately leading to better management of these infections.

*6. Quantifying fungal pellets during submerged cultivation: from 3D X-ray microtomography imaging to diffusive mass transport
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Filamentous fungal cell factories are of great importance in modern biotechnology and for the creation of a sustainable circular bioeconomy. They are broadly applied for the industrial production of many crucial biomolecules of everyday life, including enzymes or organic acids. Fungal (macro)morphology is strongly interlinked with product titers. In this study, X-ray microcomputed tomography (µCT) was proven to be a powerful tool for non-destructive, three-dimensional structural analysis of pellet-forming filamentous fungi in utmost detail on the example of Aspergillus niger. The morphological data obtained from µCT measurements of individual pellets were used to calculate the effective diffusion factor (k_{eff}) in representative cubic subvolumes and subsequently correlate them to the hyphal fraction (c_{h}) equal to solid fraction of these cubes. This analysis revealed the relation k_{eff} = (1- c_{h})^a, with only one fitting parameter a. To prove the universal validity of this law for diffusive mass transport through fungal mycelium, four morphologically different fungal strains as well as a total number of 3125 simulated fungal structures, covering a very broad field of theoretically possible morphological properties of filamentous fungi, were analysed. This investigation validated the universality of the discovered law and determined the fitting parameter a = 1.76. Being able to calculate mass transport in fungal structures based on the profile of hyphal fraction constitutes a significant step towards the prediction of metabolite and nutrient concentration inside pellets.

7. COFUN: Final report on the construction of the genome wide-knockout library in A. fumigatus
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Genome-wide knockout (KO) libraries have been used to great effect to establish an in depth understanding of microbial functional genomics. Despite their obvious value, no KO collection is available in a pathogenic filamentous fungus. To address this, in 2017 we initiated the COFUN project to generate a genome-wide collection of KO mutants in the leading mould pathogen Aspergillus fumigatus. Here we will present our final report, updating on our progress and highlighting how the libraries can be accessed. We will also describe how the libraries can be used in competitive fitness studies to elucidate interconnected networks of genes that are critical for stress adaptation in A. fumigatus.

*8. The molecular resistance mechanisms and population structure of azole-resistant Aspergillus fumigatus present on commercial agricultural products in the United States
Caroline Burks, Natalie Miller, Douglas Vines, Michelle Momany, Paul Severns, Marin Brewer. University of Georgia

Aspergillus fumigatus is a common environmental saprophyte as well as a human-pathogenic fungus that can infect patients with a variety of immune function; however, the disease aspergillosis can be deadly in immunocompromised individuals. Since the late 1990’s resistance to azole antifungals - the first line of defense against aspergillosis - has been documented in A. fumigatus. This resistance was found in patients without prior exposure to azoles, leading investigators to believe that the azole resistance had developed in agricultural environments where azoles are frequently used against plant-pathogenic fungi. Previous studies in the United States have documented azole-resistant A. fumigatus across different agricultural environments but none have looked at commercial plant products. Five hundred twenty-five isolates were collected from produce and garden products and screened for tebuconazole and itraconazole resistance; and 130 isolates were chosen for further azole-resistance phenotyping. Twenty-four isolates from compost, soil, flower bulbs, and peanuts were pan-azole resistant. The primary genetic mechanisms underlying theazole resistance in the pan-azole-resistant isolates were cyp51A-based
(TR34/L98H, TR46/Y121F/T289A, and H147Y), although some isolates had alternative mechanisms. To determine the genetic relatedness of azole-resistant and sensitive commercial isolates with isolates from agriculture and clinical samples, 95 isolates from this study and 80 clinical and environmental isolates from a previous study were selected for population genetics analysis. Minimum spanning networks and discriminate analysis of principal components (DAPC) were used to identify if populations were structured. The minimum spanning networks both showed a large amount of diversity in the population and had 2 primary branches distinguished from the other isolates that contained isolates with a tandem repeat in the promoter of the cyp51A allele and isolates collected from commercial products with a T248N/E255D allele. Three primary clusters were identified with DAPC, which appeared to separate out based on cyp51A genotype. This is consistent with previous studies that have found that panazole-resistant isolates are found in a unique clade. Overall, panazole resistance can be found in lawn and garden products in the United States. Further surveying is necessary to determine the extent to which azole-resistant A. fumigatus is present in these products and others.

*9. MERLIN unlocks the secrets to chitin signaling: Using gene-network inference to predict mediators of fungal response to lipo-chitooligosaccharides
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Chitin is a naturally occurring polymer composed of N-acetylglucosamine and is synthesized by many organisms, including fungi. Although chitin is mainly considered a structural component, derivatives can also serve as signaling molecules. A 2020 study found that all fungi produce a lipid-containing derivative of chitin called a lipo-chitooligosaccharide (LCO), and that treatment of the filamentous fungus Aspergillus fumigatus with LCOs increases germination and reduces hyphal branching. To investigate the gene networks important for LCO response, we used the MERLIN algorithm to infer regulatory gene networks for A. fumigatus using publicly available RNA-seq datasets. MERLIN implicated transcription factor AtfA as an important regulator of many genes that were differentially regulated in response to LCOs response, and we hypothesized that atfA is important for mediating Aspergillus phenotypic response to LCOs. Studies of atfA deletion and overexpression mutants reveal that atfA is required for A. fumigatus germination and hypo-branching responses to LCOs. Our work here is the first to uncover, using gene-network predictions, a transcription factor responsible for a fungus regulatory response to LCOs. Future work will investigate if LCO perception and response occurs through the high-osmolarity glycerol (HOG) pathway as further predicted by MERLIN.

*10. Analysis of 439 Cyp51 protein sequences shows 5 major Cyp51 gene family groups across Fungi
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Azole drugs target fungal sterol biosynthesis and are used to treat millions of human fungal infections each year. Resistance to azole drugs has emerged in multiple fungal pathogens including Candida albicans, Cryptococcus neoformans, Histoplasma capsulatum, and Aspergillus fumigatus. The most well-studied resistance in A. fumigatus arises from missense mutations in the coding sequence combined with a tandem repeat in the promoter of Cyp51A, a cytochrome P450 enzyme in the fungal sterol biosynthesis pathway. Filamentous Ascomycetes such as A. fumigatus have three paralogs of Cyp51 (Cyp51A, Cyp51B, and Cyp51C) with most previous research focused on Cyp51A due to its role in panazole resistance. We used the A. fumigatus Cyp51A protein sequence as the query in database searches to identify Cyp51 proteins across Fungi. We found 439 Cyp51 proteins in 297 species spanning from early-diverging fungi (Zygomycetes) to late-diverging fungi (filamentous Ascomycetes). We found these sequences formed 5 major Cyp51 groups: Cyp51, Cyp51 Erg11, Cyp51A, Cyp51B, and Cyp51C. Surprisingly, we found all filamentous Ascomycetes had a Cyp51B paralog, while only 50% of species had a Cyp51A paralog.
11. Uncovering long non-coding RNA associated with drug response in *Aspergillus fumigatus*
Danielle Weaver, Harry Chown, Takanori Furukawa, Fabio Gsaller, Daniella Delneri, Paul Bowyer, Michael J. Bromley
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Our understanding of the non-coding RNA (ncRNA) repertoire in the pathogenic fungus *Aspergillus fumigatus* is limited. Excluding housekeeping ncRNA, less than 20 ncRNAs have been identified in the sequenced type strain AF293, with the majority of these being small ncRNA. Long non-coding RNAs (lncRNAs) have emerged as important regulatory elements in many organisms and we hypothesised that they could influence the way *A. fumigatus* responds to antifungal drugs. RNAseq data from 6 drug exposure experiments were used to generate a novel *A. fumigatus* transcriptome assembly and identify lncRNA candidates. Using this assembly, we performed differential expression analysis to discover over 200 candidates which are associated with response to the antifungal, Itraconazole. Of these, we show that some lncRNA are found flanking protein-coding genes in the genome, hinting towards similar azole-resistant roles. This study has revealed novel putative lncRNA in *A. fumigatus* which may contribute to, and inform our understanding of, the mechanisms of drug resistance in this pathogen.

*12. tRNA-ome of the human fungal pathogen *Aspergillus fumigatus*: high-throughput functional analysis reveals a valine tRNA isodecoder involved in Azole sensitivity*
Lauren Dineen, Ressa Lebedinec, Marcin Fraczek, Can Zhao, Daniela Delneri, Paul Bowyer, Mike Bromley. University of Manchester
*Aspergillus fumigatus* is an opportunistic human fungal pathogen responsible for an alarming number of life-threatening infections worldwide. There are limited antifungal treatments currently available to clinicians, and rates of resistance of *A. fumigatus* to several key antifungals is increasing. A growing number of transcriptional and post translational factors have defined roles in antifungal drug resistance however little is known about the role of translational factors. Transfer RNAs (tRNAs) are ancient RNA molecules with an integral role in translation. Recently, tRNAs have been implicated in complex stress responses and adaptive translation (Torrent et al 2018, Thompson et al 2008, Begley et al 2007). To investigate the significance of tRNAs in drug resistance, a genome wide barcoded tRNA knock out library has been generated in *A. fumigatus*. Through library generation we have identified 5 tRNA genes that are essential for viability. We further show that under optimal growth conditions, *A. fumigatus* displays robustness to tRNA gene deletion. By employing a competitive fitness (bar-seq) screening approach we have identify a set of tRNA deletion mutants displaying both sensitivity and resistance to antifungals including a unique valine tRNA isodecoder that plays a role in Azole sensitivity.

13. Microevolution in the pan-secondary metabolome of *Aspergillus flavus* has macroevolutionary implications for filamentous fungi
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Fungi produce a wealth of pharmacologically bioactive secondary metabolites (SMs) from biosynthetic gene clusters (BGCs). It is common practice for drug discovery efforts to treat species’ secondary metabolomes as being well represented by a single or a small number of representative genomes. However, this approach misses the possibility that intraspecific population dynamics, such as adaptation to environmental conditions or local microbiomes, may harbor novel BGCs that contribute to the overall niche breadth of species. Using 94 isolates of *Aspergillus flavus*, a cosmopolitan model fungus, sampled from seven states in the United States, we dereplicate 7,821 BGCs into 92 unique BGCs. We find that more than 25% of pangenomic BGCs show population-specific patterns of presence/absence or protein divergence. Population-specific BGCs make up most of the accessory-genome BGCs, suggesting that different ecological forces that maintain accessory genomes may be partially mediated by population-specific differences in secondary metabolism. We use ultra-high-performance high-resolution mass spectrometry to confirm that these genetic differences in BGCs also result in chemotypic differences in SM production in different populations, which could mediate ecological interactions and be acted on by selection. Thus, our results suggest a paradigm shift that previously unrealized population-level reservoirs of SM diversity may be of significant evolutionary, ecological, and pharmacological importance. Last, we find that several population-specific BGCs from *A. flavus* are present in *Aspergillus parasiticus* and *Aspergillus miniscerotigenes* and discuss how the microevolutionary patterns we uncover inform macroevolutionary inferences and help to align fungal secondary metabolism with existing evolutionary theory.

14. The fungal sexual revolution continues: indications of sexuality in the citric acid producing fungus *Aspergillus niger*.

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Sexual reproduction is an important proliferation mechanism which provides advantages to a species by promoting genetic variation. Interestingly, for a large group of fungal species, including the industrially relevant *Aspergillus niger*, this mode of reproduction has not been described yet. In recent years, strong indications of the sexual potential of *A. niger* have accumulated1. These include the ability of certain strains to produce pre-mature sexual structures (sclerotia) and the presence of a mating type MAT1-1 locus in the commonly used *A. niger* strains, such as ATCC 1015 and CBS 513.88. Here we show additional evidence of the sexual potential of *A. niger*. Formation of sclerotia could be induced in the progenitor of the industrial citric acid producing strains of *A. niger*, ATCC 1015, and in *pyrG* mutants derived from it2. The capability of ATCC 1015 to form sclerotia, known to act as sexual structures in related organisms, represents a step forward towards the discovery of a sexual cycle in this important industrial species. To find the second mating type locus, we performed genome sequencing of the *A. niger* neotype strain CBS 554.65 and identified the missing MAT1-2 locus3. A nucleotide alignment showed a different orientation of the MAT1-1 locus of ATCC 1015 compared to the MAT1-2 locus of CBS 554.65. While the genomic context of the MAT1-2 locus in CBS 554.65 is similar to the one of other MAT1-2 *A. niger* strains and other *Aspergillus* species, the region comprising the MAT1-1 locus is inverted in all sequenced strains of *A. niger*. These observations suggest the occurrence of genetic flipping or switching events at the MAT1-1 locus of *A. niger*, which might have a direct impact on its sexuality. These results provide new insights in the mating system of *A. niger* and pave the way for the discovery of a sexual cycle in a species long thought to be asexual.


15. Primary, secondary and tertiary metabolites, proteins and carbohydrates

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Many words have been suggested to denote small molecules (metabolites) and few words have been used to denote different kinds of macromolecules such as proteins and polysaccharides. Primary metabolites, primary proteins, and primary polysaccharides are present in nearly all kinds of organisms, or at least in large groups of organisms, such as bacteria, archaea, plants, fungi, algae, animals etc., but certain groups of organisms such as anaerobic fungi have adapted their primary metabolism to function under anoxic condition. Examples of primary metabolites in fungi are oxaloacetate present in the
mitochondria of all aerobic fungi and tryptophan. Secondary metabolites (SMs), secondary proteins, secondary polysaccharides are the results of conspicuous outwards directed differentiation, and they are taxonomically restricted, often being only produced by few species (for example only 1-30 species of filamentous fungi) in major taxonomic groups. They are accumulated products, often secreted (the secretome) or deposited on the cell wall or in the cell membrane (the depositome). Metabolites, proteins, polysaccharides that are produced by one species, but used by another species are tertiary (one example of a tertiary metabolite is the milkweed toxin used by the monarch butterfly to protect it against being eaten by certain birds). Other words used to denote primary metabolites are general metabolites and other words to denote secondary metabolites are specialized metabolites, special metabolites, small molecules, idiolites, natural products, natural compounds, while such expressions have not been coined for proteins and polysaccharides. Examples of secreted fungal SMs are citrinin and penicillin while deposited SMs include penicillin A and aflavinin. Examples of secondary proteins are PAF (Penicillium rubens antifungal protein) and the bubble protein from Penicillium brevicompactum, and exoenzymes and hydrophobins. Examples of fungal secondary polysaccharides are pullulan and mannocarolose. A common word for all secondary metabolites, secondary proteins and secondary carbohydrates is extrolites. Ruderal selected fungi, such as most yeasts, Mucoromycetes, Neurospora, produce few and simple SMs (for example carotenoids and simple amino acid derived SMs), stress selected fungi also produce few SMs, while competition selected fungi can often (potentially) produce a large number of simple and very complex SMs.

*16. Aspergillus niger conidial germination: 3D live cell exploration
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Conidial germination describes the transition of dormant spores to hyphal structures. Breaking dormancy is followed by isotropic and polarized growth with a cell wall constantly being remodeled. To explore and understand the rearrangement and timing of key events in A. niger, we use the Nanolive stain-free live cell imaging system - a combination of holography and tomography. A temperature-controlled growth chamber is used for incubating the fungus on-stage of the microscope. With the 3D Nanoscopy technique, videos of the germination process can be recorded and morphological structures distinguished based on different refractive indices (RI). Images of germinating conidia and digital cell reconstruction in 3D based upon the cell’s inherent physical properties revealed a ring formation that might direct germ tube formation.

This knowledge is critical to the development of future approaches to manipulate fungal growth for medical, agricultural or industrial purposes.

17. Aspergillus fumigatus Septation Initiation Network (SIN) kinases contribute to fungal pathogenesis, cell wall reconstruction, and rRNA metabolism.
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Aspergillus fumigatus is the main causative agent of invasive aspergillosis and the therapeutic armamentarium to fight against this disease is limited. Therefore, we sought to identify genetic pathways that might serve as novel therapeutic targets through generating and studying an A. fumigatus protein kinase disruption mutant library. Our previous work found the SIN kinase genes, sepH, sepL and sidB, to be essential for hyphal septation and survival under cell wall stress. We also found that loss of hyphal septation resulted in almost complete avirulence characterized by lack of tissue invasion. Interestingly, co-culture of mutant and wild type conidia with a macrophage cell line revealed reduced ability of the SIN kinase mutants to elicit pro-inflammatory signaling evidenced by reduced IL-1β and TNFα release. To see if the cell wall hypersusceptibility and reduced immunogenicity phenotypes of the SIN kinase mutants were due to altered cell wall formation or hyphal development, we performed in vitro phenotypic characterization of the mutant strains coupled with RNAseq profiling. Interestingly, conidia of the mutant strains were found to initiate germination earlier than wild type and hyphae formed by the mutant.
germlings were significantly longer than the wild type at the same developmental timepoint. In contrast, the mitotic rate was similar between all the strains, suggesting that loss of SIN activity uncoupled the processes of mitosis polarized morphogenesis. Hyphal staining revealed altered distribution of chitin cell wall deposition and reduced β-glucan levels in the Sin kinase mutants. RNAseq analyses identified 997, 625 and 337 genes from the ΔsepH, sepL-1, and sidB-1 mutant strains, respectively, that were differentially expressed when compared to the wild type. Of these genes, only 28 were downregulated in common between the three mutant strains. Gene Ontology enrichment analyses revealed that 45% of these genes were related with transport processes, 34% with RNA metabolic process and 28% with ribosome biogenesis. Our results suggest that SIN kinase activity is essential for normal germination and mitosis and for proper cell wall formation. Although no direct transcriptional link to cell wall biosynthesis was uncovered, RNAseq analyses suggest hyphal septation in A. fumigatus is linked to cellular transport, RNA metabolism and ribosome biogenesis.

18. Targeting Aspergillus fumigatus hypoxia response pathways to potentiate contemporary antifungal therapies
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Aspergillus fumigatus is a ubiquitous airborne filamentous fungus that is estimated to contribute to 600,000 deaths each year. There are currently only three contemporary antifungal therapies to treat invasive Aspergillus infections. Rapidly increasing resistance to first line therapy voriconazole highlights a significant need to develop novel antifungals with innovative mechanisms of action. Research from our lab has shown that the hypoxia response, mediated by the transcriptional regulator SrbA, is necessary for virulence andazole resistance in Aspergillus fumigatus. Therefore, identifying a compound that inhibits the SrbA mediated hypoxia response pathway would introduce a potentially novel antifungal that could potentiate azoles activity in vivo. We designed and performed a high-throughput screen by adapting a gpdA-luciferase reporter system to screen over 200,000 small molecule compounds for antifungal activity in the presence of fluconazole or hypoxic conditions. Using a secondary screen measuring enhanced fluconazole sensitivity and hypoxia specificity, we confirmed 50 compounds that fit all parameters to date. We then prioritized compounds that show limited human toxicity and an MIC≤10 µM for further investigation. Increased SrbA expression through expression of the N terminus bHLH transcription reduces susceptibility to several of these molecules. These data suggest that the compounds may act on the SrbA dependent hypoxia response. Since SrbA pathway inhibition increasesazole sensitivity, we next tested the compounds against voriconazole-resistant clinical isolates and determined that combination therapy increases voriconazole efficacy. Using this high-throughput screen and follow up secondary screens we have identified compounds that are hypoxia specific and potentiateazole therapy with minimal human toxicity. Additionally, preliminary data suggests that several compounds are acting through the SrbA dependent hypoxia response pathway, a well characterized virulence factor. Through this work we are finding that we can identify novel antifungal compounds that act through innovative and well characterized biological mechanisms. One potential application of these findings is that these compounds can be used in combination therapy to potentiate azoles and combat antifungal resistance.

19. Transcriptome investigation of mpkB-mkkB mutants related to secondary metabolism and sexual development of Aspergillus nidulans
Sang-Cheol Jun, Jong-Hwa Kim, Kap-Hoon Han. Woosuk University
The MpkB MAP kinase plays a prominent role in the sexual differentiation signaling and secondary metabolism pathway, acting as the terminal MAPK activating expression of SteA and VeA. This MAP kinase is expected to balance the expression of genes encoding cell fusion, anastomosis, and secondary metabolism. Deletion of the mpkB gene causes conidiophore morphological abnormalities, a decrease in asexual spore production, and inhibition of homo-/heterothallic mating. This mutation has been reported
to inhibit the production of sterigmatocystin (ST), a secondary metabolite, but our experiments did not find any evidence that ST production was inhibited in the mpkB mutant. In an effort to find other connections not yet known for this pathway, we constructed aspartic acid or alanine residues that could mimic the phosphorylation effect in amino acids 218 and 222 of MkkB MAPK kinase (upstream MAPK kinase of MpkB) to produce mutations that either constitutively phosphorylate or not phosphorylate MpkB MAPK. These mutants were analyzed for transcripts using the RNA-seq method under 16 hours of asexual stage. A total of 4 strains (wild type, mkkB knock-out mutant, MkkB constitutively activation mutant and MkkB constitutively inactivation mutant) generated more than 6 Gbp data by pair-end sequencing. When mutants were compared to the control strain, markedly altered expression levels of the relevant genes were identified, suggesting that MpkB signaling is involved with asexual sporulation, sexual differentiation and ST biosynthesis pathway, among others.

20. FungiDB: Free online informatic tools for fungal and oomycete biologists
Evelina Basenko1, Omar Harb1, David Roos1, presenting on behalf of the entire VEuPathDB Bioinformatics Resource Center. 1) University of Pennsylvania; 2) University of Liverpool
FungiDB (https://FungiDB.org) is a free online resource enabling browsing, querying, and sophisticated mining of genomic-scale datasets. FungiDB is component of The Eukaryotic Pathogen & Vector Bioinformatics Resource Center (VEUPathDB.org) that integrates genomic, transcriptomic, proteomic, epigenetic, population diversity, phenotypic, and other types of data for eukaryotic microbes (pathogenic & nonpathogenic, free-living & parasitic), as well as the hosts and vectors of human disease. As one of the largest VEuPathDB databases, FungiDB encompasses hundreds of genomes including yeast, filamentous fungi and oomycetes. A user-friendly web interface with embedded bioinformatics tools supports in silico experiments leveraging public data, along with private datasets uploaded by the user into My Workspace via the VEuPathDB Galaxy interface. With FungiDB, you can browse genomes and examine gene record pages, create searches to mine Omics-scale datasets, annotation, and the results of automated analyses (protein domains, orthology predictions from OrthoMCL.org, metabolic pathways, etc.) Expert knowledge of genes, phenotypes, publications, and other gene-centric data may be captured as User Comments, via bulk data submission, or through the integrated Apollo annotation platform. New datasets can be nominated for integration by emailing the FungiDB help desk or via social media. FungiDB also offers a range of tutorials and webinars for novice and expert users, and will host a Help Desk (in-person and virtual) during all poster sessions at FGC, to answer any questions that meeting participants may have.

21. Antifungal Screening of 54 Single Plant Essential Oils Against Aspergillus fumigatus
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One bioprospective approach towards antimicrobial resistance is to source and research antimicrobial compounds from natural sources. Plant essential oils conceivably represent a safe and natural alternative to fight against microbial infections. These volatile oils have been used in medicine for years, and current research explores their possible efficacy towards diseases and infections that have been challenging to treat, or more significantly, in cases where current medicine is being challenged by antimicrobial resistance. For instance, some clinical isolates of Aspergillus fumigatus, an opportunistic pathogen of the respiratory system, have shown resistance to currentazole treatments against aspergillosis. In addition, some studies have shown that prolonged azole treatments can result in negative implications for patients. In order to explore alternative methods for inhibiting the growth of A. fumigatus, we conducted a screen of 54 single plant essential oils (PEOs) from a single vendor and measured their inhibitory activity. We conducted disc diffusion assays of the PEOs and voriconazole against A. fumigatus (AF293) and measured zones of inhibition. We found that 21/54 PEOs had average ZOIs ranging from 10 – 30 mm, 16/54 with ≥ 30 mm, and cilantro, lemongrass, Melissa (lemon balm), thyme, and oregano were fully inhibitory. In addition, 30/54 PEOs efficacy was greater than the inhibitory effects of voriconazole (1µg/ml). The majority of the PEOs tested displayed some inhibitory effect against A. fumigatus. Interestingly, fully inhibitory PEOs contain a variation in their major constituents, with the presence of different monoterpenes being common for all. Lemongrass and Melissa (lemon balm) contain geranial and neral as common major constituents which are monoterpene aldehydes shown to work as antimicrobial, anti-inflammatory, analgesic, and chemo-preventives. In addition, thyme and oregano both contain thymol a monoterpenic phenol with antimicrobial, antifungal, anti-inflammatory, and anesthetic
activities. Moreover, cilantro contains linalool a monoterpane alcohol shown to have antibacterial, antifungal, anti-inflammatory, and anti-cancer activities. The present study shows that some PEOs may serve as an alternative for inhibiting fungal growth and raises the possibility for their use in combination with current treatments. In addition, PEOs efficacy variation may help indicate components in the oils that could be used as possible treatments against resistant microbes.

22. A new role in G2-M control revealed by a reciprocal translocation involving the snxA shuttling mRNA-binding protein and a GYF-domain protein in Aspergillus nidulans

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Aspergillus nidulans snxA, an ortholog of Saccharomyces cerevisiae Hrb1/Gbp2 messenger RNA shuttle proteins, is – in contrast to budding yeast – involved in cell cycle regulation, in which snxA1 and snxA2 mutations as well as a snxA deletion suppress mutations in regulators of the CDK1 mitotic induction pathway (James et al. 2014. doi.org/10.1534/genetics.114.167445). snxA mutations confer cold-sensitivity, and snxA1/A2 mRNA and protein expression are strongly repressed at permissive temperature. Genetic studies demonstrated linkage of snxA1/A2 cold-sensitivity to markers on two chromosomes, suggesting a chromosomal rearrangement. Whole-genome sequencing revealed a chromosome I – II reciprocal translocation with breakpoints in the snxA first intron and in the fourth exon of a GYF-domain gene that we have named gyfA. Surprisingly, a complete deletion of gyfA and a reconstructed gyfA translocation allele suppressed the heat-sensitivity of CDK1 pathway mutants in a snxA1 background, indicating that the reciprocal translocation simultaneously disrupted two unrelated genes, snxA and gyfA, each of which act through the CDK1-CyclinB axis to restrain the G2-M transition, and for the first time identifying a role in G2-M regulation for a GYF-domain protein.

The translocation breakpoint in the snxA first intron eliminated an 11-exon transcript and allowed only weak expression of a wild-type 9-exon transcript. To better understand the basis for snxA1/A2 reduced expression, we generated suppressors of snxA1/A2 cold-sensitivity, and discovered that loss of setB2cold histone H3 lysine36 (H3K36) methyltransferase rescued the mutants by restoring full transcriptional proficiency. These phenotypes were largely mirrored in a non-methylatable histone H3K36L mutant, indicating that methylation of H3K36 acts to restrain 9-exon snxA expression. These observations are in line with known SET2 functions in preventing excessive and cryptic transcription of active genes, and suggests that restoration of snxA1/A2 expression may occur by reactivating a strong transcription startsite(s) (TSS) within intron 2, proximal to the 9-exon start codon, or by activating more distal TSS(s) mapped to intron 1.

23. The role of cytochrome c in leukocyte induced Aspergillus fumigatus cell death.

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Aspergillus fumigatus is a ubiquitous environmental mold that can cause a life-threatening infection known as invasive aspergillosis (IA). IA is caused by defects in innate immune system function that result in failed clearance of inhaled conidia from the lung. While it is known that innate immune function, particularly NADPH oxidase activity, is responsible for clearance of these conidia from the lung, the mechanism by which these conidia are killed by leukocytes remains unknown. While processes of cell death in have been identified in animals, these processes remain largely unknown in outside of metazoa. Contributing to this question regarding non-metazoan cell death, many canonical components of regulated cell death are not conserved outside of metazoa. Here we investigate the role of A. fumigatus cytochrome c (cycA) in both hydrogen peroxide-induced and leukocyte-induced fungal cell death.

Cytochrome c is a canonical cell death effector in higher order metazoa that functions in programmed and regulated forms of cell death and is conserved across eukaryotes. Using a flow cytometry approach, we observe that a cycA strain displays altered cell death phenotypes including reduced histone fragmentation, reduced caspase-like activity, and reduced sytox blue staining after 6hr and 8hr exposure to 10mM H2O2. However, using a germination assay to monitor growth 30hrs after acute 2.5hr exposure to 10mM H2O2, we observe that loss of cycA results in no observable growth after treatment, suggesting a loss of viability as compared to the WT and complement strains. Using in vivo FLorescent Aspergillus REporter (FLARE) technology, we observe that loss of cycA results in lower in viability in leukocyte.
subsets including neutrophils and total macrophages as compared to WT and complement strains. However, the ΔcycA strain displayed higher viability in specifically the alveolar macrophage subset as compared to other leukocyte subsets. Taken together, these data suggest cytochrome c presence in A. fumigatus contributes to cell survival under death inducing conditions and future studies will seek to define the underlying mechanisms.

24. Human p11-mediated re-direction of phagosomes to the recycling endosome-expulsion pathway induced by fungal pathogen

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The saprotrophic fungus Aspergillus fumigatus is an opportunistic fungal pathogen, which causes invasive pneumonia and disseminated infections in immunocompromised patients. The fungus produces hydrophobic spores (conidia) that are released into the air and are continuously inhaled. Because of their small size of 2–3 μm, conidia can reach the alveoli, where they are eliminated by phagocytes and intracellularly by phagolysosomal killing. Without an effective immune response, inhaled conidia invade pulmonary epithelial cells by induced phagocytosis which can lead to the onset of life-threatening infection.

Although dihydroxynaphthalene melanin on fungal conidia has been shown to inhibit phagosome maturation, a proportion of melanin-lacking mutant conidia still escaped killing by phagosomes, implying that additional mechanisms must be in place. The analysis of host pathogen interactions bears the potential to discover novel pathogenicity mechanisms and also to obtain novel insights into fundamental mechanisms of cell biology. In this study, we obtained unprecedented insight under both aspects. We discovered a novel function for the so far uncharacterized fungal surface protein HscA: it binds to the host p11 protein and acts as a fungal effector protein that induces expulsion of conidia. Also, we found that the human p11 protein is a decisive factor for targeting phagosomes either to the degradative or secretory pathway and that this factor is manipulated by HscA. Specifically, after phagocytosis of pathogens, phagosomes undergo a series of maturation steps to develop into phagolysosomes. A key step is the recruitment of Rab7 to phagosomal membranes. A. fumigatus induces accumulation of the p11-Annexin A2 tetramer (A2t) on phagocytic cups. The conidial surface effector protein HscA anchors the A2t complex to the membranes of phagocytic cups and phagosomes. This excludes Rab7 recruitment but rather triggers recruitment of Rab11, which is a marker for recycling endosome, and thereby interferes with phagosome maturation. As a consequence, conidia escape phagolysosomal killing by germinating inside of Rab7-negative phagosome or these conidia-containing phagosomes are translocated to the surface of host cells and thereby released to the medium or even transferred to other cells.

25. Fungi to the rescue – revolutionizing food production through biotechnology

Britta Winterberg, Bastian Joehnk. Formo Bio GmbH

The current food system is causing colossal problems. From climate change to food security and public health, the issues are manifold. Over 4% of global greenhouse gas (GHG) emissions come from dairy cattle alone. That’s as much as the GHG as is produced by all airplanes and ships combined worldwide shipping and aviation combined. The UN Food and Agriculture Organization predicts there’ll be a 50-100% increase in demand for animal protein by 2050. If we source this through traditional methods, we will continue causing serious environmental damage and resource scarcity. Animal agriculture is the number one source of zoonotic disease outbreaks. Almost 90% of the world’s animal species will lose their habitat to agriculture by 2050 if we continue on the current path. It’s also a key contributor to antibiotic resistance. We need a safer, more sustainable, and more efficient way of producing food. Formo’s approach of combining biotechnology and traditional cheese production is revolutionizing the food sector. To this end, Formo uses various microbial hosts and precision fermentation to recombinantly express the milk proteins. These form the basis for dairy products such as animal-free cheese. Caseins, the most prominent proteins in bovine milk, are responsible for texture and organoleptic properties of cheese. These proteins exhibit no secondary structure and form large protein aggregates (micelles) in milk. Expressing these proteins in microbial cell factories poses a major challenge due to their molecular
structure. Fungi are ideal hosts due to their ability to secrete recombinant proteins with high yield and capability for post-translational modification.

Life-cycle assessments have shown that milk protein production through precision fermentation causes 93% less GHG emission, uses 98% less water and 84% less energy. Microorganisms, especially fungi, carry enormous potential to help us achieve the UN Sustainability Development Goals. They have a long history in the production of enzymes and food additives. Producing the main ingredient of food through precision fermentation is the next logical step on the path to a more sustainable food system.

*26. Unfolded protein response is critical for the corneal pathogenesis of Aspergillus fumigatus
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Aspergillus fumigatus is a predominant agent of fungal keratitis (FK), an ocular infection resulting in long-term visual impairment and blindness worldwide. The high treatment failure rate for FK underscores the need to identify fungal pathways that could serve as targets for novel antifungals. The site of infection for FK, the avascular cornea, is comprised of a dense collagen matrix that is ostensibly poor in free soluble nutrients. We, therefore, hypothesized that fungal experience a nutrient limitation in the cornea and must utilize collagen (protein) catabolic pathways to support infection. To test this, we infected murine cornea with WT A. fumigatus using the corticosteroid epithelial abrasion model of keratitis and found that genes encoding various secreted proteases as well as protein chaperones were upregulated 48h post-infection relative to baseline in vitro conditions (glucose minimal media). These results support a model in which A. fumigatus upregulates secreted hydrolases in the nutrient-poor cornea, which in turn places the fungus under endoplasmic reticulum (ER) stress. As it is known that the unfolded protein response (UPR) is critical for maintaining the secretory capacity and growth of A. fumigatus during ER stress, we next hypothesized that UPR is essential for corneal virulence. To test this, we first deleted the A. fumigatus hacA gene, encoding the bZIP transcription factor that is critical for UPR function. Consistent with previous reports, the A. fumigatus hacA KO displayed hypersensitivity to stresses that induce protein misfolding, including increased temperature, DTT, and tunicamycin. The mutant was also deficient in secreted collagenase activity and had severe growth defect on biological tissue ex vivo. We then tested the virulence of this mutant in the above-described corneal infection model. In contrast to WT-infected corneas, those infected with the hacA-KO failed to develop signs of corneal disease (opacification), displayed normal corneal structure (optical coherence tomography), and did not harbor viable fungus (CFUs). This corresponded with the histopathology and flow cytometry analyses which demonstrated reduced inflammation in hacA-KO infected corneas. Our results are consistent with our hypothesis that the UPR is essential for A. fumigatus nutrient acquisition on protein-rich substrates as well as virulence in a murine model of FK. These data suggest that the fungal UPR could serve as a target for antifungal intervention.

27. The ndrC gene, which is regulated by nsdD, controls sexual development in Aspergillus nidulans
Yu Kyung Kim, Kap-Hoon Han. Woosuk University

Aspergillus nidulans is a filamentous model fungus that has both asexual and sexual life cycles, which depend on environmental factors such as nutritional conditions and stresses. The nsdD gene is a well-known GATA type transcription factor responsible for the regulation of sexual and asexual development. In this study, we identified a gene, named ndrC (nsdD-dependent regulation) by using RNA-seq experiment followed by DEG analysis. The NdrC protein encoded by the ndrC gene is conserved in some Aspergillus species but not in other organisms. It has no known domain except DUF4267 and reported as hypothetical protein. To characterize the function of the gene, deletion mutants were generated, and the phenotypes under the various differentiation induction conditions were observed. The colony size of the mutant was similar to the host strains and the control strains, but more conidia were produced compared to the control strains, suggesting that the gene is negatively regulate asexual development or conidia production. Microscopic observations showed that there was no cleistothecium or hüll cell formed after incubation of sexual induction condition. In wilt-type strain the ndrC gene expression was not detected in cleistothecia whereas the gene was expressed in conidia. Taken together, the ndrC gene is responsible for the sexual development and repression of asexual development in A. nidulans.
28. Aspergillus as model for analyzing the fungal digestive enzyme profile -to be included in species description and classification?
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Species of Aspergillus are rich in carbohydrate active enzymes (CAZymes) and also efficient secreters; array of CAZymes is basis for invasive power and reflects specialized habitat and substrate affinity. Aspergillus was chosen as model for analyzing evolution of the fungal secretome. Despite its importance for fitness, enzyme secretome is generally not used, neither in description of fungal species nor fungal classification, since overarching relationship between CAZyme profiles and fungal phylogeny/taxonomy has not been established. For robust, high precision/high sensitivity prediction of function, we used CUPP, Conserved Unique Peptide Pattern, a new, automated and validated method. Evolutionary pressure selects for having i) the needed functions, ii) found in the optimal type of proteins; the latter being important for steric accessibility, protein stability, pH & temperature optimum. Thus, to annotate, closely mimicking fitness relevance, we based our annotation on recognizing integrated “Function;Family observations”. Our hypothesis, that “F;F”-observation-based annotation of digestive Aspergillus secretome is congruent with the phylogeny of the species was confirmed. The phylogenetic tree of Aspergillus is a stunning match to the dendrogram of a Yule dissimilarity calculation of “F;F” observations of the secretomes! Identifying Global CAZyme Hotspots: For each genome a summing-up was done of number of F;F observations, in order to rank species according to capacity and diversity of digestive enzymes. Of approx. 2000 genome sequenced fungi, the 103 Aspergillus species/strains ranked from topmost, A. latus #5, to the species with the weakest digestive secretome, A. cejpii, ranking #1109. For comparison, Penicillium ranked a bit lower, highest of 48 Penicillium species, was P. sp. #61 (unidentified, unfortunately); lowest ranking in biomass degrading capacity, P. decumbens, #1258. The F;F-observations-based Hotspot analysis of genome-sequenced Aspergillus, distinguishes the species with highest total capacity in digestive enzymes and the ones with the richest function specificity diversity. And it identifies top-degraders of cellulose, xylan, lignin or pectin. Thus, providing a short cut for enzyme discovery! Next step is to analyze the secretome of Aspergillus (and Penicillium) for both mycotoxins and enzymes. Hypothesis: Patterns of integrated evolution exist between the two, major fungal secretome components, mycotoxins and digestive enzymes.

29. CRISPR/Cas9-based engineering of Aspergillus oryzae mycelium for meat-like flavor and appearance
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Filamentous fungi are predicted to play key roles in a more sustainable food system, including the transition from resource-intensive animal agriculture towards sustainable meat alternatives. Although their nutritional value and filamentous textures make fungi promising meat replacements, most products based on fungal biomass require processing and additives to mimic meat in flavor, texture, and appearance. Genetic engineering could reduce the need for processing fungal biomass and has the potential to both decrease cost and increase the meat-like properties of fungal foods. Here we develop a CRISPR/Cas9-based method to genetically engineer Aspergillus oryzae, an edible fungus that is involved in traditional fermentations and is currently used in alternative meat products available on the market. The CRISPR/Cas9 method involves protoplast transformation with Cas9-sgRNA Ribonucleotide (RNP) complexes and linear fixing templates, followed by selection and locus-specific loop-out of a pyrG marker. The method is precise and efficient, does not necessitate the use of a ku70 mutant, and allows for unlimited repeatable engineering. In addition to validating the method for both targeted mutagenesis and protein overexpression, we identify 8 neutral chromosomal integration loci that enable high expression of proteins of interest. Finally, we apply this method to the production of heme, a key flavor compound in red meat. By overexpressing both heme biosynthetic enzymes and heme-binding hemoglobin, we engineered a strain that has deeply red mycelia and produces heme at more than 10-fold higher levels than the wild-type. Moving forward, the method will be used to overexpress additional flavor pathways.
and to alter fungal morphology to change texture, laying the foundation for a new generation of fungal meat substitutes.

30. The Aspergillus fumigatus morphogenesis-related kinase, CotA, orchestrates hyphal growth in response to carbon source quality

Adela Martin-Vicente, Xabier Guruceaga, Ashley V Nywening, Jinhong Xie, Harrison I Thorn, Wenbo Ge, Jarrod R Fortwendel. University of Tennessee Health Science Center

Fungal pathogens must exhibit strong nutritional plasticity, effectively sensing and utilizing varying nutrient sources, for development of invasive disease. How the molecular signals generated by nutritional sensing are efficiently translated to the cellular morphogenetic machinery for optimal orchestration of growth under nutritional stress remains incompletely understood. Here, we sought to identify and characterize protein kinases required for pathogenic growth in the opportunistic fungal pathogen, Aspergillus fumigatus. To identify molecular mechanisms supporting A. fumigatus growth in the mammalian lung environment, we first screened a protein kinase disruption library for growth on a mouse lung agar. This screen identified the disruption of only the conserved morphogenesis-related protein kinase gene, cotA, as essential. Further in vitro studies revealed that, although the cotA disruption mutant (cotA-1) displayed only a slight growth defect compared to the parental strain when grown on glucose, hyphal growth was almost completely abolished when grown on lung agar, as well as on lung explants. Employing a mouse model of invasive pulmonary aspergillosis, we also found that the cotA-1 mutant displayed reduced virulence, characterized by the inability to form tissue-invasive hyphae in vivo. Additional in vitro studies revealed that, unlike the parental and the complemented strains, the cotA-1 mutant formed only compact colonies when provided non-sugars as the sole carbon source. However, we show that the CotA protein does not regulate, nor is cotA gene expression regulated by, the carbon catabolite repression system. Using a novel CotA-specific antibody, we instead uncovered that wild type A. fumigatus produces two CotA protein isoforms, long and short, whereas the cotA-1 disruption mutant produces only the short isoform. Culture on non-sugar carbon sources caused complete loss of CotA protein in the cotA-1 mutant. Additional mutational analyses showed that conserved phospho-regulatory sites on the CotA protein were required for CotA support of hyphal growth, independent of carbon source quality. Taken together, our data show that the A. fumigatus cotA gene encodes a conserved morphogenesis-related kinase that is produced as two protein isoforms, long and short. The presence of the long isoform is required for growth under host mimicking in vitro conditions and for in vivo virulence in a manner dependent on carbon source quality.

*31. Oxygen mediated cell-cell heterogeneity and antifungal drug susceptibility in Aspergillus fumigatus biofilms

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Aspergillus fumigatus is a filamentous fungus commonly found in compost and soil which can cause invasive disease in immunocompromised individuals. Infections with strains whose conidia are susceptible to antifungals in vitro frequently fail to respond to treatment in vivo. This is in part due to A. fumigatus adopting a biofilm mode of growth in vivo. As A. fumigatus biofilms mature, steep oxygen gradients form due to increased fungal oxygen consumption. These zones of low oxygen correlate with regions of hyphae with reduced translational activity and subsequent antifungal drug resistance. However, treatment of A. fumigatus biofilms increases oxygen availability in the biofilm and subsequently increases antifungal susceptibility of fungal biofilms. Moreover, reoxygenation of the biofilm exogenously also increases antifungal susceptibility. However, the mechanisms underlying reactivation of antifungal drug susceptibility upon reoxygenation remain ill defined. To address this question, we utilized RNA sequencing to identify transcripts responsive to reoxygenation in A. fumigatus biofilms. Transcripts highly differentially expressed in reoxygenated biofilms include genes involved in active transmembrane transport, positive regulation of transcription, and nitrogen metabolism. Genes of interest that we have selected for characterization include genes with domains predicted to be involved in both heme-binding or metabolite-binding as well as phosphorelay or transcription factor domains. CRISPR mediated gene replacement of candidate genes and characterization of reoxygenation phenotypes has revealed promising candidates critical for oxygen mediated biofilm antifungal drug susceptibility. Collectively, these data report a critical role for oxygen in the antifungal drug response and highlight the importance of defining mechanisms of cell-to-cell heterogeneity in complex filamentous fungal biofilms.
32. Pathogenic fungi in Norwegian barns - first survey of *Aspergillus fumigatus* azole resistance in Norway

**Erik Magnus Nedland Henriksen**, Hege Divon, Elin Rolén, Lonny Margrethe Kløvbjell, Ellen Christensen, Ida Skaar. *Norwegian Veterinary Institute, Ås, Norway*

Azoles are efficient fungicides commonly used to treat and prevent fungal diseases in humans and animals. Moreover, the azole class of fungicides is widely used in agriculture to ensure healthy plants and high yield in food production, horticulture and wood industry. Unfortunately, several international studies indicate that residual azoles in the environment act as potential drivers for development of azole resistance in human pathogenic fungi, such as *Aspergillus fumigatus*.

*A. fumigatus* is an opportunistic human pathogen and the causal agent for aspergillosis, one of the most common fungal diseases in human medicine world wide. The increasing number of reports about azole resistance development in *A. fumigatus* is alarming. With the spread of resistance, treatments of such fungal diseases will be progressively more ineffective, with fatal consequences. This is a growing global concern.

The goal of the BARNS project was to provide preliminary data on how widespread *A. fumigatus* azole resistance is in Norwegian farms. Citizen science was used to sample *A. fumigatus* from indoor environments in animal housing and storage rooms on farms across the country. Adhesive PCR plate foiles were used as spore traps, and trapped spores were cultivated and identified both morphologically and by calmodulin sequencing as *A. fumigatus*. Pure isolates were further screened for resistance against itraconazole, voriconazole, and posaconazole using VIPCheck™ and E-tests. Resistant isolates were in addition characterized by sequencing of the *cyp51A* gene.

Out of a total of 108 isolates tested, three (2.8%) were found resistant, exhibiting cross-resistance to all three azoles. Two of the resistant isolates had documented mutations in the *cyp51A* gene, one with the TR34/L98H mutation and the other with the TR46/Y121F/T289A mutation. The third isolate did not have mutations in the *cyp51A* gene and has gained resistance by a, so far, unknown mechanism. While the percentage of resistant isolates are in line with data from other countries, a larger study is in progress to more accurately reflect the occurrence of azole-resistant *A. fumigatus* in Norway.

*33. Computational advances in the discovery of a new class of fungal natural products*

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Fungal secondary metabolites (SMs) are major sources of antimicrobial (e.g. penicillin, griseofulvin) and therapeutic (e.g. cyclosporine, mycophenolate) compounds. Ecologically, they provide important fitness adaptations that are finely tailored to the niche of an organism. The fungal biosynthetic genes responsible for SM synthesis and transportation are uniquely arranged in contiguous clusters within the genome, termed biosynthetic gene clusters (BGCs). Current genome mining algorithms capable of identifying putative BGCs are limited to what is considered 'canonical' (BGCs defined by biochemically characterized synthetases and synthases, i.e. nonribosomal peptide synthetases or polyketide synthases). SMs synthesized by BGCs lacking canonical structure are difficult to be incorporated into current predictive software (i.e. AntiSmash) and thus preclude informative analysis such as extensive phylogenetic studies. One such example of a noncanonical BGC class blind to existing genome mining software is the isocyanide (N≡C−) metabolite producing BGCs. Isocyanides have been a major interest of organic and synthetic chemists since the 1920s due to their unique divalent carbon, and high reactivity. While numerous bioactive isocyanide metabolites have been extracted from bacteria and fungi, the genes responsible for their synthesis were largely unknown prior to our laboratory publishing the first examples of isocyanide synthase (ICS) containing BGCs in the fungus *A. fumigatus*. We have developed paradigm-shifting software and a computational approach that allows detection of diverse ICS BGCs across the fungal kingdom, and offer initial predictions of functionality in specific ecological settings.
34. The *Aspergillus fumigatus* Spindle Assembly Checkpoint components, sldA and sldB, play roles in maintenance of triazole susceptibility

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*Aspergillus fumigatus* is the most common cause of invasive mold infections in susceptible human populations. Invasive aspergillosis is characterized by high mortality ranging from 30-90%. The recent rise of antifungal resistance in *A. fumigatus* is of increasing concern as infection with resistant isolates is associated with increased treatment failure. Much remains unknown concerning adaptation to antifungal stress and development of antifungal resistance, threatening the future use of triazole antifungals. Protein kinase activity is involved in mediating many cellular processes in fungi. *A. fumigatus* is predicted to encode 147 protein kinases and the influence of these kinases on triazole susceptibility and adaptation to triazole drugs remains largely unknown. Here, we sought to reveal the impact of each of the predicted protein kinases on susceptibility to medical triazoles. CRISPR/Cas9 gene editing was used to generate a library of 118 protein kinase disruption mutants in a wild type genetic background and voriconazole minimum inhibitory concentration (MIC) for each of disruption strain was determined by broth microdilution assays. Initial screening of the protein kinase disruption library uncovered only two disruption mutants with altered voriconazole susceptibility, both with a 4-fold increase in MIC when compared to the parent strain. One of these mutants possessed a disruption of AFUB_074100, an uncharacterized ortholog of the *Aspergillus nidulans* SldA kinase. This kinase is a vital component of the mitotic Spindle Assembly Checkpoint (SAC). To confirm our screen results, we next deleted the entire gene sequence encoding either sldA (ΔsldA), or that of the predicted SldA-protein binding partner, sldB (ΔsldB). These mutants were re-examined for susceptibilities to a panel of triazoles, as well as the spindle poison benomyl. Both ΔsldA and ΔsldB displayed increased triazole MICs, mimicking the sldA disruption mutant. Moreover, both deletion mutants exhibited markedly increased sensitivity to benomyl, a phenotype characteristic of SAC dysfunction. Although loss of sldA in *A. nidulans* is associated with moderate growth defects, the *A. fumigatus* ΔsldA and ΔsldB mutants displayed normal growth. Therefore, loss of sldA or sldB generate reduced susceptibility to triazole antifungals and play conserved roles in regulation of the SAC. Future studies will focus on delineating connections between SAC dysfunction and triazole resistance in *A. fumigatus*.

35. Computer-Aided, Resistance-Gene-Assisted Genome Mining for Proteasome and HMG-CoA Reductase Inhibitors

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Fungi produce a plethora of biologically active small molecules, called secondary metabolites (SMs), many of which are medically valuable. The genes that encode particular SM biosynthetic pathways are usually clustered together in the genome, forming biosynthetic gene clusters (BGCs). Genome sequencing reveals that the number of SM BGCs vastly exceeds the number of known SMs, and, thus, that huge numbers of potentially valuable SMs are yet to be discovered. Resistance-gene-assisted genome mining is a strategy to exploit the greater fungal secondary metabolome efficiently, by identifying SM BGCs that are likely to make useful products. It takes advantage of the fact that some SM BGCs contain a gene encoding a resistant version of the protein targeted by the compound produced by the BGC. This allows the producing organism to survive while its competitors are inhibited. The bioinformatic signature of such SM BGCs is that they contain an allele of an essential gene with no SM biosynthetic function, and there is a second allele elsewhere in the genome. Manually applying this approach to thousands of sequenced genomes is daunting, so we have developed a computer-assisted approach that allows users to query large databases for SM BGCs that putatively make compounds that have particular targets of therapeutic interest. Working with the MycoCosm genome database, we create databases of the genomic coordinates of all core SM biosynthetic genes. We next generate genomic coordinates for all alleles of our target gene. A computer script then determines if an allele of our target gene is located within a user-defined distance of a core SM biosynthetic gene AND at least one other allele of the target gene is present elsewhere in the genome. We have applied this approach to look for SM BGCs that target the proteasome β6 subunit, the target of the proteasome inhibitor fellutamide B, or HMG-CoA reductase.
(HMGCR), a key enzyme in sterol biosynthesis and the target of cholesterol reducing therapeutics such as lovastatin. Our approach proved effective, finding known fellutamide and lovastatin SM BGCs as well as fellutamide- and lovastatin-related BGCs with variations in the SM genes that suggest they may produce structural variants of fellutamides and lovastatin. Gratifyingly, we also found SM BGCs that are not closely related to lovastatin BGCs but putatively produce novel HMGCR inhibitors. Supported by the KU Endowment and NIAID Grant R21AI156320.

36. Nanoparticles and pathogenic fungi: a non-uptake delivery
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Newly discovered antifungal substances often display pharmacological problems, like low solubility or high toxicity. Several studies showed that nanoparticles (NPs) can be used to overcome these problems of antimicrobials. Liposomal Amphotericin B is an example for such a nanoformulated antifungal drug already being on the market. The mechanism, how polymeric NPs deliver encapsulated substances into pathogenic fungi, was suggested to be either via endocytosis or an endocytosis-independent uptake of the whole NP. However, the mechanism of uptake remains to be understood. Therefore, we investigated the interaction of different NPs with several human pathogenic fungi to elucidate the uptake mechanism irrespective of the polymer or fungal species.

NPs were prepared by utilizing 4 different polymers and were labelled with 3 different covalently attached fluorescent dyes and/or a fluorescent dye or antifungal drug encapsulated. The interaction of the fluorescently labelled NPs with the filamentous fungi Aspergillus fumigatus, A. nidulans, A. terreus, and A. oryzae, and the yeasts Cryptococcus neoformans and Candida albicans was investigated by confocal laser scanning microscopy and transmission electron microscopy. The efficacy of itraconazole-loaded NPs on these species was determined by MIC-testing following the respective EUCAST methodology. Irrespective of the applied condition (such as pH value or salt concentration in the medium, polymers used, size of the NPs, incubation time up to 24h, culture medium used), none of the used NPs reached the fungal cytosol, but adhered to the fungal surface. Investigations on the exact localization of NPs revealed their appearance in the interspace between cell wall and membrane of the fungi. Nevertheless, encapsulation of a fluorescent dye or itraconazole led to an accumulation of the fluorescent dye in the fungal lumen or a lower MIC compared to the pristine drug, respectively.

In conclusion, polymeric NPs are not taken up by pathogenic fungi. Nevertheless, the delivery of hydrophobic substances like antifungals into these fungi with the help of NPs is possible and effective, making NPs a promising tool for antifungal treatment.

37. Complex and critical roles for the AtrR transcription factor in control of cyp51A expression in Aspergillus fumigatus
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Aspergillus fumigatus is the major filamentous fungal pathogens in humans, causing invasive aspergillosis in immunocompromised patients that can often be fatal. Unfortunately, there is a limited repertoire of antifungal drugs available to treat this fungus. The most popular and only oral antifungal drug used to treat invasive aspergillosis is theazole class of drugs that target an important step in the fungal specific ergosterol pathway. However, persistent and prolonged use of azoles, not only in chronic aspergillosis treatment but also in agriculture as fungicides, have resulted in the appearance of azole-resistant isolates at an alarming rate. Arguably the most commonazole-resistant mutations found are associated with the gene that encodes the enzymatic target of azole drugs, referred to as cyp51A. Azole-resistant alleles of cyp51A often have the unusual promoter containing a duplication of a 34 bp region in the promoter that leads to enhanced gene transcription. This 34 bp duplication is called TR34 and contains binding sites for the SrbA and AtrR transcription factors that play an important role in regulating
expression of cyp51A. In this study, we provide genetic evidence that the presence of the atrR gene is essential for clinical strains containing the TR34 or its related TR46 (46 bp repeat) versions of the cyp51A genes to confer enhanced azole resistance. Using site-directed mutagenesis, we demonstrate that both the binding site for SrbA (sterol response element: SRE) and the AtrR binding site (AtrR response element: ATRE) are required for normal expression of the cyp51A gene. In the context of the TR34 promoter, loss of either the SRE or ATRE from the distal 34 bp repeat (further 5' from the transcription start site), caused loss of expression of cyp51A and decreased voriconazole resistance. Surprisingly, loss of these same binding sites from the proximal 34 bp repeat led to increased cyp51A expression and voriconazole resistance. A similar increase in gene expression and drug resistance was also observed at the TR46 promoter upon alteration of either SRE or ATRE elements in the 46 bp repeat proximal to the cyp51A gene. These data dissect the molecular inputs to cyp51A transcriptional regulation and reveal a complicated function of the promoter of this gene that is critical in azole resistance.

38. Functional analysis of the bZIP transcription factors AtfA and AtfB in Aspergillus nidulans
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Basic leucine zipper (bZIP) transcription factors (TFs) are conserved regulators found in all eukaryotic organisms and play a crucial role in many cellular processes including development and stress responses in fungi. We have been studying the two key bZIP TFs AtfA and AtfB in fungi. AtfA orchestrates the stress defense system of filamentous fungi by globally regulating expression of genes associated oxidative/osmotic stress responses, conidial heat stress tolerance, and secondary metabolism and asexual sporulation in various filamentous fungi. AtfB governs resistance to oxidative stress, controls gene expression during conidial development, and plays a role in the regulation of carbon and amino acid metabolisms in the Aspergilli.

To analyze further the physiological functions of these key TFs in Aspergillus nidulans, we constructed gene deletion and overexpression mutants in all combinations including the ΔatfA, ΔatfB, ΔatfAΔatfB, ΔatfAatfBOE, ΔatfBatfAOE, atfAOE, atfBOE and atfAOEatfBOE strains. The following phenotypes were observed on stress agar plates in the presence of oxidative, osmotic, heavy metal and cell wall stress generating agents: The ΔatfA mutant showed an oxidative stress sensitive phenotype in the presence of 0.8 mM H2O2, 2 mM diamide and 0.08 mM menadione meanwhile the oxidative stress sensitivity of the ΔatfB mutant was comparable to that of a control strain. The ΔatfAΔatfB mutant was moderately sensitive to 0.8 mM H2O2 and highly sensitive to 2 mM diamide. The overexpression (OE) of neither atfA nor atfB compensated for the negative effects of iBOOH in the null mutants. However, OE of atfB alone protected the fungus against iBOOH stress. Moreover, OE of atfB either alone or together with atfA as well as the deletion of atfA resulted in heavy metal stress sensitive phenotypes while the ΔatfB mutant was moderately tolerant to CdCl2. In addition, the ΔatfB mutant showed reduced growth on solid medium containing 1.5 M NaCl meanwhile this mutant was the most tolerant to 2 M sorbitol. The atfAOEatfBOE mutant showed an increased tolerance to NaCl. Only the ΔatfA mutant displayed moderate tolerance to the cell wall stress inducing agent, Congo Red. In summary, AtfA and AtfB play distinct roles in governing the fungal responses to various stresses.

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39. Interaction of the bZIP-type transcription factors NapA and RsmA in the regulation of oxidative stress defence and sterigmatocystin production of Aspergillus nidulans

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Basic leucine zipper (bZIP) transcription factors are of critical importance in the environmental stress response of eukaryotes. In this work, we studied the effect of gene deletions and overexpressions of two bZIP transcription factors, NapA and RsmA, in the oxidative stress response and sterigmatocystin production of Aspergillus nidulans.

We found that NapA is crucial in the neutralization of oxidative stress by negatively regulating the intracellular reactive species production and positively modulating catalase activities, meanwhile RsmA affected catalase activities slightly negatively.

With respect to sterigmatocystin production, the highest level was determined in the ΔrsmAΔnapA double deletion mutant but increased sterigmatocystin production was also found in the OErsmAOneapA strain. Our results indicate that NapA coordinated sterigmatocystin production via regulating oxidative species level while RsmA modulated toxin production independently of the redox regulation of the cells. We also examined the effect of rsmA on the expression of wild type napA and vice versa. The pairwise comparison of ΔrsmA, control and OEsrmA strains showed that overexpression of rsmA increased the transcription of napA while overexpression of napA resulted in increased rsmA expression. Deletion of either rsmA or napA had no significant effect on the transcription of the other gene.

Considering the complex regulatory network of NapA and RsmA on the oxidative stress response and secondary metabolite production of A. nidulans as well as their observed influence on each other’s expressions we can assume that NapA and RsmA may interact with each other either genetically or even physically to orchestrate ST production.

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40. Genome-wide analysis of AtfA/AtfB-mediated menadione stress response in Aspergillus nidulans

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The bZIP transcription factors (TFs) are important regulators of the oxidative stress response in filamentous fungi. In this work we carried out genome-wide expression studies employing Illumina RNAseq to understand the roles of the two bZIP TFs AtfA and AtfB in Aspergillus nidulans. Specifically, comparative analyses of transcriptomes of vegetative mycelium and conidiospores obtained from the surface cultures of the control, ΔatfA, ΔatfB, ΔatfAΔatfB mutant strains with/without menadione sodium bisulfite (MSB, a superoxide generating agent) treatment were carried out. Evaluation of transcriptomics data was carried out via Venn diagram comparisons of the global gene expression patterns gained for the four strains.

As both the presence of MSB and ΔatfA downregulated expression of atfB, AtfA can affect the AtfB-dependent genes via activating atfB expression. In untreated mycelia we have found a higher number of differentially expressed genes (DEGs) in ΔatfA than in ΔatfB.

Compared to untreated ones, MSB treated mycelia showed a lower number of DEGs in ΔatfA, or ΔatfB
vs. control. Only 9 AtfB-dependent genes were found in MSB treated mycelia and most of these were also AtfA-dependent. The observed differences in the responsive gene sets of the MSB treated and untreated cultures suggest that AtfA regulates distinct genes under different culture conditions. In conidia, more DEGs were found in ΔatfA or ΔatfB, supporting the idea that AtfA and AtfB play more global regulatory roles in conidiospores than in vegetative tissues. Interestingly, MSB treatment of conidia did not lower atfB mRNA levels, and most of the atfB deletion DEGs were also found in ΔatfA. In general, DEGs in conidia showed low overlap with DEGs found in the corresponding mycelial samples. Functional category analyses of DEGs have revealed that AtfA affects some glycolytic genes and iron-sulfur cluster assembly genes in general. Phosphorelay response regulator genes were enriched in all AtfA-dependent gene sets except the untreated mycelial samples. Genes that were regulated by AtfA under all experimental conditions include those encoding catalase, histidine-containing phosphotransfer proteins.

Only 23 genes were solely dependent on AtfB considering all transcriptomics data sets, including a putative α-glucosidase (agdB), a putative α-amylase, calA involved in early conidial germination and an alternative oxidase.

In summary, our genome-wide expression studies have revealed cell-type dependent distinct regulatory roles of AtfA and AtfB in A. nidulans.

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*41. cexA and its regulatory processes – a closer look into the citric acid production mechanism of Aspergillus niger*

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Aspergillus niger is an important filamentous fungus used for the industrial production of citric acid. One of the main contributors to high citric acid accumulation by the fungus is the citrate transporter CexA. It belongs to the major facilitatory superfamily subclass DHA1 which act as drug-H⁺ antiporters. Since cexA and its regulators are essential within the citric acid production process, it is important to study their regulatory mechanism, which is the focus of this work. LaeA for one is known to be a major regulator of the cexA gene. It regulates the expression via methylation levels of the histones H3K4 and H3K9. There are indications that other transcriptional regulators such as AmyR and XlnR are also involved in the regulation of cexA on a transcriptional level. Another factor that affects citric acid production is the amount of manganese that is present during the production process. Observations showed that the fungus develops a certain pellet-like morphology under manganese limitation conditions and that this limitation is decisive for high citric acid accumulation by A. niger34. However, the exact mode of action of manganese in the cell is not clear. The transcriptional influence of manganese on cexA and laeA is investigated in order to find out more about the connection between manganese limitation and citric acid production.

42. Flotillin-dependent membrane microdomains are required for functional phagolysosomes against fungal infections

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Aspergillus fumigatus represents the most important air-borne fungal pathogen. In the lung, resident alveolar macrophages belong to the first line of defense against inhaled conidia. Dihydroxynaphthalene (DHN) melanin on the conidial surface is crucial to inhibit phagolysosomal acidification and to cause increased damage to macrophages compared to non-pigmented pksP mutant conidia. Here, we discovered the importance of lipid rafts for maturation of functional phagosomes and as target of DHN-melanin that leads to an impaired formation of lipid-raft microdomains in the membrane of phagolysosomes. As a result, the assembly of vATPase is prevented and as a consequence, phagolysosomal acidification is inhibited. We thus report an unprecedented mechanism of the interference of fungal spores with immune cells. The conidia-containing phagolysosomal membrane is characterized by a high content of cholesterol and sphingolipids. Further analysis showed that flotillin-enriched lipid rafts co-localize with sphingolipids on phagolysosomal membranes containing pksP conidia but not wild-type conidia. In human and mouse, two flotillins, Flot-1 and 2, are present. As chaperons, they stabilize lipid-raft microdomains and thus represent marker proteins.

Optimization of a protocol for isolation of phagolysosomes from macrophages allowed us to monitor the colocalization of microdomains and receptor/enzyme complexes by high resolution fluorescence microscopy. In addition, bone marrow-derived macrophages (BMDMs) of Flot-1/2 knockout mice were compared with C57BL7/6 wild-type macrophages. In line with our previous results, in flotillin-1/2 knockout BMDMs acidification of phagolysosomes containing pksP conidia was drastically reduced as well as vATPase assembly and phagocytosis. Infection of Flot-1/2 knockout mice with pksP conidia resulted in increased cell damage and killing compared to wild-type conidia in a mouse infection model. Furthermore, we identified an SNP for flotillin-1 in human donors of stem cells, which is associated with an increased susceptibility for invasive aspergillosis. Taken together, these data indicate that lipid raft microdomains provide important platforms for signaling and defense of immune cells and can be manipulated by A. fumigatus.

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*43. Methionine synthase as a target for antifungal drug development

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Mycoses pose an urgent threat to human health and are responsible for approximately 1.6 million deaths annually. Current therapeutic options for life threatening fungal infections, such as those caused by Aspergillus fumigatus, Candida albicans and Cryptococcus neoformans, are severely limited. Even under antifungal treatment these infections have mortality rates of ~50% and rising antifungal resistance exacerbates the need for the development of novel drugs.

Fungal pathogenic potential is strongly influenced by their metabolic versatility. Therefore, pathways required for cellular metabolism and nutrient homeostasis in host tissues are fundamental for infection and may represent exciting targets for antifungal drug discovery. As the conditions encountered throughout the course of infection, and consequently the fungal metabolic requirements, may vary significantly, it is crucial to validate targets in established infections.

We recently optimised a genetic model to allow characterisation and validation of prospective antifungal drug targets in growing A. fumigatus hyphae and established in vivo infections. We used the model to investigate methionine synthase (MetH), which has been described as essential for viability or virulence in A. fumigatus, C. albicans and C. neoformans. We showed that downregulation of metH expression triggered a complex metabolic imbalance, beyond methionine auxotrophy, which inhibited growth and thus virulence in two in vivo models of established infection. In addition, a structure-based virtual screening predicted differential druggability between the human and fungal enzymes, which could guide the design of novel specific inhibitors with reduced off target binding.

Following the validation of MetH as a promising antifungal target this work initiates the early stages of drug development. We have optimised the expression and purification of soluble MetH and designed a simple, cost-effective enzymatic assay suitable for high throughput screening. From our virtual screening, we have identified fragments which have a high probability to specifically bind to regions crucial for MetH’s enzymatic activity.

Taken together our results support methionine synthase’s potential as a druggable, specific and broad-spectrum antifungal drug target. We provide initial results that will permit hit identification using complementary approaches: high throughput screening of compound libraries and directed design of binding fragments.

*44. Distribution of non-canonical septins in fungi

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Septins are the fourth component of the cytoskeleton. Three or four different septin proteins assemble to form nonpolar hetero-hexamers and hetero-octamers, respectively, which perform a variety of functions in fungi, most notably forming the septin ring necessary for cytokinesis. While four septin clades are conserved from yeast to humans, some fungi possess a fifth group of septins that is not evolutionarily similar any other septin group, and does not perform canonical functions. Present even in basal clades of the holomyzota, likely this group was present in the opisthokont ancestor and lost in the holozoan ancestor. Deletion of a Group 5 septin in filamentous fungi causes mislocalization of hexamer septins and vice versa, but no distinct morphological phenotypes are observed. We are working to understand the evolutionary distribution of Group 5 septins in fungi and how that has selected for their non-canonical function.

*45. Investigating germination initiation in the pathogenic fungus Aspergillus fumigatus

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Aspergillus fumigatus is a ubiquitous fungus that can cause human infection, especially in immunocompromised individuals. Examining the development of A. fumigatus may uncover important mechanisms that could be essential for organisinal growth, and may potentially be used in uncovering new drug targets for treatment of human hosts. In Aspergillus species, germination is well-characterized. The fungus produces small dormant spores that can be dispersed by wind, and when they contact a carbon source they break dormancy, swell, and extend germ tubes. If a susceptible host inhales the spores, they can break dormancy and grow in the lungs causing invasive disease. Although the
In the present study, we investigate the germination process by analyzing *A. fumigatus* RNA-seq transcriptome data to identify the most highly differentially expressed genes in dormant conidia versus hyphae. We hypothesize that potential factors inhibiting germination will be fungal specific genes with unknown function that are highly differentially expressed in conidia over hyphae. Future work with this group of candidate genes will include phenotypic characterization of their roles in germination and development, drug resistance, and pathogenicity.

*46. Duplication, Redundancy, and Divergence: roles of the *Aspergillus nidulans* paralogous transcription factors LeuR and LeuB in leucine biosynthesis, nitrogen assimilation, and transcriptome regulation*

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Numerous fungal species, including *Aspergillus, Candida*, and *Saccharomyces*, can synthesize branched chain amino acids (BCAAs) while animals cannot. Proper regulation of BCAA metabolism is important for protein synthesis, growth, secondary metabolite production and virulence. In *Aspergillus nidulans*, the Zn(II)2Cys6 transcription factor LeuB is modulated from repressor to activator by the biosynthetic intermediate α-isopropyl malate (α-IPM) to regulate the leucine biosynthesis pathway. LeuB also regulates expression of *gdhA*, which encodes the key nitrogen assimilation enzyme NADP-glutamate dehydrogenase, NADP-GDH. We have identified a paralog of LeuB named LeuR and examined the intersection of LeuB and LeuR in leucine biosynthesis and *gdhA* regulation. Phylogenetic analysis shows that while LeuB is conserved in Ascomycetes, LeuR is conserved only within Eurotiomycetes. The *leuBΔ* mutant is a leaky leucine auxotroph. We deleted *leuR* and found the *leuRΔ* mutant to be a prototroph. However, the *leuBΔ leuRΔ* double mutant is a tight leucine auxotroph, indicating a role for LeuR in regulating leucine biosynthesis. Using a *gdhA-lacZ* translational fusion reporter gene and exogenous leucine, we show that LeuR also regulates *gdhA* expression. By artificially altering the levels of α-IPM through loss of function mutants, we are working to determine if α-IPM also modulates LeuR activity. Previously, we used a series of promoter deletions in *gdhA-lacZ* to identify two sites of action for LeuB in the *gdhA* promoter. We have now used these promoter deletions to compare the wild type strain to *leuBΔ*, *leuRΔ*, and *leuBΔ leuRΔ* mutants and identified the site of action for LeuR. Additionally, we performed RNA-Seq with the wild type, *leuBΔ*, *leuRΔ*, and *leuBΔ leuRΔ* mutants to determine the genome-wide direct and indirect targets and overall physiological roles of LeuB and LeuR in *A. nidulans*. Our experiments show that the transcription factors LeuB and LeuR overlap in regulating nitrogen assimilation and leucine biosynthesis. However, LeuB and LeuR diverge in the total number of genes regulated and play distinct roles in the regulation of metabolic pathways for iron metabolism, metabolism of other amino acids, and ribosomal RNA biogenesis and processing.

*47. Identification of a protein-protein interaction site essential for mitotic entry to guide antifungal drug design in *Aspergillus fumigatus*

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Invasive fungal diseases have high associated mortality and there are limited antifungal agents to combat infections. Resistance mechanisms are rapidly emerging against current antifungal drugs that mainly target the cell wall and cell membrane; therefore, there is an urgent need to develop drugs with novel modes of action. Protein kinases and phosphatases regulate critical processes including metabolism, signalling, and the cell cycle, making them promising drug targets. However, high homology with human proteins causes issues with selectivity and specificity when considering them as antifungal targets. During the *Aspergillus fumigatus* cell cycle, a signalling cascade initiated by the protein phosphatase NimT mediates progression from G2 phase to mitosis. Although these processes are highly conserved, we hypothesise that drugs can be developed to selectively target the interaction of the fungal enzymes. Here we characterise domains within the essential protein phosphatase NimT that mediate its interaction with its target, the protein kinase NimX. The interaction interface we describe is physically distant from the highly conserved catalytic domain of NimT and NimX and is poorly conserved in the orthologous human enzymes CDC25 and Cdk2. Using our virtual screening pipeline (VSpipe) we have identified a druggable
pocket at the NimT-NimX interface. We hypothesise that drug-like molecules occupying this space will inhibit the interaction between NimT and NimX, arresting the cell before mitosis can occur.

48. A versatile selection free CRISPR-Cas9 transformation system for A. fumigatus
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Improvements in methods that facilitate genetic modifications in fungi are required to aid research in this area. Aspergillus fumigatus is a sapphytic fungus that is the cause of more than 300,000 life-threatening infections annually. The development of rapid and versatile gene editing methodologies for A. fumigatus is essential. Unlike to model yeast Saccharomyces cerevisiae, targeted allele replacement in Aspergillus fumigatus is complicated by low rates of homologous recombination and the fact that replacement cassettes require long homology arms of c. 1kb. CRISPR-Cas9 mediated transformation has been widely using as a genome editing tool to overcome some of these issues. However, successful editing normally relies on time consuming multi-step cloning procedures paired with the use of selection markers, which can result in a metabolic burden for the host and unintended transcripational modifications at the site of integration. Recently we published data showing that an in vitro CRISPR-Cas9 assembly methodology could be used to perform genome editing without the need for selectable markers. Here we show how the method can be used to perform epitope-tagging, site-directed mutagenesis and insertion of genetic constructs. We have introduced a functional GFP-epitope tag to the N- and C-terminus of the pacC and srbA protein. In addition, we generate targeted point mutations in the pyrG and pyrE genes. Lastly, we use a batch screening method to rapidly evaluate over 500 viable colonies from selection free CRISPR-mediated transformations. This enables rapid identification of transfomed colonies. Overall, our selection free method decreases the time required for complex construct synthesis and can potentially be translated to other fungi.

49. Olorofim and the azoles are antagonistic in Aspergillus fumigatus and functional genomic screens reveal mechanisms of cross resistance.
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Aspergillosis, is a major cause of morbidity and mortality. Very few classes of antifungals have been approved for clinical use to treat these diseases and resistance to the first line treatment, the triazoles, is increasing. Resistance to the azoles is thought to arise from long term clinical use and its use in agri- and horticulture. A new class of antifungals, the orotomides, are currently in Phase II of clinical trials. The first compound in this class, orolofim, shows efficacy against a large range of filamentous fungi, including Aspergillus fumigatus. In this project, we screen a genome wide collection of transcription factor null mutants, for changes in susceptibility to orolofim. We identify 4 transcription factor mutants with altered susceptibility, including the previously characterised HapB, AreA and DevR. We show that, unlike the azoles, changes in susceptibility are only minor (up to 4-fold). Through phenotypic characterisation and RNA-seq we show that these transcription factors modulate expression of genes involved in the production of pyrimidine biosynthetic precursors. Interestingly, two of the transcription factor mutants that have reduced susceptibility to orolofim, HapB and AreA, are also resistant to theazole class of antifungals. In addition, we show the azoles and orolofim have an antagonistic effect.

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50. *Aspergillus fumigatus* hexameric septin complex is involved in spore cell wall organization and immune evasion

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*Aspergillus fumigatus* is the major etiology of invasive aspergillosis, a leading cause of death in immunocompromised patients. Septins are conserved GTPases involved in septation, conidiation, and cell wall organization. The requirement of septins for tissue invasion and virulence has been demonstrated in the human pathogenic yeasts *Candida albicans* and *Cryptococcus neoformans*. *Aspergillus* spp. contain five genes encoding for septins (AspA-E), *Aspergillus* septins can interact to form a hexameric complex (AspA, AspB, and AspC) and octameric complex (AspA, AspB, AspC, and AspD). Previous studies showed that the octameric complex is required for full conidiation. Furthermore, the electron micrograph of ∆aspB strain spores shows that the spores lack the characteristic electron-dense outer layer. Based on their role in conidiation, we hypothesized that septins are also required for conidia cell wall organization. To test this hypothesis, we are using a combination of microscopy techniques and cell culture-based methods. We utilized Atomic Force Microscopy to determine the organization of the spore surface. Using this technique, we found that spores from the ∆aspB strain have a disorganized rodlet layer compared to the parent strain. Concomitant with a disorganized rodlet layer, spores from the ∆aspA, ∆aspB, ∆aspC strains have more exposed chitin. However, ∆aspD chitin exposure is similar to those of the wild-type, indicating that the octameric complex is dispensable for conidia surface organization. Previously, it was reported that *Galleria* infected with these strains showed rapid melanization, which is a response of the immune system to pathogens. This points out a possible role of the septin hexamer in regulating spore-host interaction via the assembly of the conidia cell wall. To test this, we first exposed macrophage-like cells (J744.1) to septin deletion strain’s spores and measured TNF-α production to determine spore immunogenicity. As expected, only the deletion of any gene involved in the hexameric complex leads to a significant increase in TNF-α. Taken together, these results suggest the septin hexameric complex is at least sufficient for *A. fumigatus* spore cell wall organization and immune evasion.

51. Structural characterization of secondary metabolites from filamentous fungi.

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Genome sequencing has revealed that filamentous fungi contain many secondary metabolite gene clusters. Our labs have been actively engaged in chemically characterizing the metabolites produced by *Aspergillus* species. A time-consuming part of our research has been on the scale-up and purification of metabolites to isolate sufficient products for NMR characterization, typically several milligrams of purified compounds. In this presentation, I would like to present our efforts to structurally determine secondary metabolites using a sub-milligram of material.

Virtual

*52. (FGC# 784V) Exploring the Divergence of Interactions between Fungi and Bacteria*

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Fungi and bacteria comprise a large fraction of biomass in the soil and since they interact with each other, bacterial-fungal interactions are crucial for understanding the microbial ecosystem which is closely related to agriculture, medicine, and the environment. It is apparent that microbial interactions promote the activation of cryptic biosynthetic pathways leading to the production of various secondary metabolites and other bioactive compounds that confer defense functions, cell to cell communication and other interactive dynamics. Now the majority of the studies based on the dynamics of microbiota employ coculturing which is proven to be an effective method to mimic the conditions existing among the microbial interactions in the natural environment, which in turn could potentially facilitate the production of novel bioactive compounds like antimicrobials.

Our recent study characterized the mutualistic relationship between the filamentous fungus *Aspergillus nidulans* and gram-positive bacterium *Bacillus subtilis* providing evidence to show their spatial and metabolic interaction that facilitates the communication in between species to explore untraveled environmental niches and obtain nutrients. Addressing this interactive nature, the current study comprised of coculturing of 35 environmental fungal species and 20 bacterial species to investigate their interaction...
dynamics in the cocultures. Parameters such as the effect on the fungal growth, the affinity of the bacterial cells to the fungal hyphae, bacterial cell dispersal distance and the velocity of movement of bacteria were analyzed to define the interaction specificity. Depending on the nature of interactions, the combinations were then classified as positive, negative, and neutral. Selected combinations were then subjected to LC-MS analysis and subsequent transcriptomic analysis to visualize their genomic potential and expression in coexistence compared to their monoculture state. This study impart insights to the ecological context of interactions of the environmental microbiota and utilization of the metabolic capacity of the chemically prolific microorganisms.

*53. (FGC# 861V) Population Structure and Genomic Analysis of Aspergillus sojae and Aspergillus parasiticus

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Domestication is the genetic modification of a species by breeding it in isolation from its ancestral population in an effort to enhance its utility to humans, involving different evolutionary mechanisms such as selections, bottlenecks, and gene flow. Most domestication research focuses on the plant and animal kingdoms, leaving microorganisms understudied. Here, we phylogenetically distinguish Aspergillus sojae and Aspergillus parasiticus as two distinct populations, based on their genomic differences. Among isolates collected by the USDA, a single domestication event of A. sojae, followed by a clonal expansion, was observed. A. sojae, a putative domestic filamentous fungus which combines flavor-enhancing and enzymatic proteins to ferment several Asian foods, such as: soy sauce, miso, and mirin. A. parasiticus, a close relative, is a wild fungus that produces aflatoxins, making it pathogenic to plants and animals. Using whole-genome assemblies of 12 A. sojae, and nine A. parasiticus isolates, 658,329 SNPs were identified using Freebayes1.3.5. Using principal component analysis, and linkage disequilibrium less nucleotide diversity and recombination was observed in the A. sojae isolates as compared to the A. parasiticus population. Structural variation using copy number variation (CNV) analysis was done to assess presence or absence of secondary metabolite genes amongst the A. sojae and A. parasiticus isolates. CNV profiles were generated for each sample to investigate the quantity and function of these genes in A. parasiticus. A total of 12,370 genes were annotated, of these, 8% represented the coding sequence for secondary metabolites. High-throughput screening of metabolites and liquid chromatography-mass spectrometry (LC-MS) was done to evaluate the potential wealth of useful secondary metabolites in Aspergillus sojae species. In detail, there were 13 A. sojae strains selected based on our previous phylogenetic tree and these strains with A. flavus NRRL3357 were grown in various culture conditions, including rice-koji and soy sauce-koji. The SMs from the extracted fractions were analyzed by adopting a tandem-approach of direct-infusion high-resolution mass spectrometry (DI-HRMS) based metabolomics for efficient, high-throughput screening of metabolites and liquid chromatography quadrupole time-of-flight mass spectrometry LC-QTOF-MS (MS/MS) for further metabolite validation. In the first approach, DI-HRMS analysis focused on 21 mycotoxins regulated by Joint FAO/WHO Expert Committee on Food Additives (JECFA). Aflatoxin B2 putatively detected in soy sauce-koji condition, aflatoxin G2 in corn and citrinin, ergot alkaloids among others detected in different conditions were further validated by LC-QTOF-MS (MS/MS). Results revealed no significant traces of 21 mycotoxins found in all 13 A. sojae strains grown in various conditions. In the second approach, DI-HRMS analysis detected putative SMs which were further subjected to multivariate analysis to determine the SM production pattern resulting from diverse responses among the species. Distinct SM pattern was observed among
the strains particularly in *A. oryzae* RIB40, RIB128, RIB915, RIB1172 grown in rice-koji and in RIB301, RIB915, RIB1108 grown in soy sauce-koji conditions possibly contributed by varying putative production of useful known and nonelucidated SMs. Furthermore, it was observed that *A. flavus* was clearly separated among the *A. oryzae* when grown in corn, YES and CYA suggesting the production of aflatoxins as well as other metabolites likely induced by plant material and nutrient-rich culture media under laboratory conditions. 

Taken together, the efficient tandem-approach of metabolomic analysis in various growth conditions provides a plethora of candidate metabolites such as possible novel biomarkers useful for rapid discrimination between *A. oryzae* and the aflatoxigenic *A. flavus* as well as the interesting SM candidates produced by the dependable *A. oryzae* for promising pharmaceutical and other bio-industrial uses.

*55. (FGC# 863V) Comparative genomics of *Aspergillus oryzae* genomes from different clades reveals signatures of artificial selection in primary and secondary metabolism in domesticate environments*

Katherine Chacon-Vargas, John Gibbons. *University of Massachusetts Amherst*

Humans domesticated different species by selecting for desired traits to enhance their benefits. Domestication is not limited to plants and animals. In parallel, microbes (bacteria, yeasts, and molds) were also domesticated for their roles in food preservation, nutrition and flavors. *Aspergillus oryzae* is a domesticated filamentous fungal species used during the fermentation of traditional Asian foods and beverages such as sake, soy sauce, and miso. The artisanal practice of continuous passage of *A. oryzae* on food substrates over thousands of years resulted in adaptation to the food environment along with genetic differentiation from its wild relative *A. flavus*, a toxin producing agricultural pest. Here, we analyzed 300 isolates of *A. oryzae* and *A. flavus* to understand how the history of domestication and how this process shaped patterns of genomic variation. Using population structure and phylogenetic analysis we identified 2 major population of *A. oryzae* and two major lineages of *A. flavus*. Next, we used two population genomic metrics to identify regions of the *A. oryzae* genome possessing signatures of artificial selection. We identified 30 candidate genes possessing strong signatures of artificial selection, several of which have functional annotations directly related to fermentation. Specifically, alcohol dehydrogenase, fructose transmembrane transporters and glutathione metabolism genes. Additionally, we examined differences in gene copy number variation between *A. oryzae* and *A. flavus*. Most strikingly, we found significantly more copies of the α-amylase encoding genes in *A. oryzae* compared to *A. flavus*, suggesting selection for increased carbohydrate metabolism during fermentation. Further, gene absences in *A. oryzae* compared to *A. flavus* were enriched for secondary metabolism function, suggesting selection for loss of toxicity in *A. oryzae*. Taken together, our results show the *A. oryzae* genome was significantly reshaped as a result of domestication.

*56. (FGC# 415V) Cell wall dynamics in fast growing fungal hyphal cells*

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Tip growth is a highly polarized cellular process used by walled cells of fungi, plants, or bacteria to colonize space, reproduce or infect. Tip growing cells are encased in a rigid cell wall that ensures surface integrity and limits cell growth, yet these cells can elongate at unusually high speeds of up to few mm/hrs. These considerations raise the fundamental question of how the cell wall may be dynamically assembled at cell tips to safeguard integrity while allowing rapid surface shape changes. We implemented a sub-resolution imaging approach to map cell wall thickness spatio-temporal dynamics, cell wall elasticity, and turgor pressure in very rapid growing hyphal cells of the filamentous fungus *Aspergillus nidulans*. We found that hyphal cells grow with a near homogenous cell wall thickness of about 80nm, and a marked gradient in cell wall bulk elastic modulus, with hyphal tips being twice softer than cell sides. By co-imaging cell wall thickness dynamics and secretory vesicle accumulations that deliver new cell wall material to cell tips, we found that both fluctuated with typical amplitudes of up to 150% and periods of 1-2 min during growth. Affecting secretory vesicle transport or fusion caused a rapid loss of polarity, growth arrest, and rapid thickening of the cell wall at cell tips. These data provide unprecedented details on cell wall dynamics, from synthesis to assembly and deformation, and suggest important dynamic coupling mechanisms between surface material synthesis and deformation rates, likely essential to support rapid growth and cell viability.
57. (FGC# 322V) Deciphering new compound pathways in non-engineered Aspergilli using a CRISPR toolbox: Aspergillus californicus as a starting point

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The growing number of filamentous fungi genome sequences published since 2000s shows a myriad of gene clusters that have not been investigated so far, potentially hiding new bioactive compounds. One important reason for this is the lack of genetic tools for pathway investigation. However, with the successful implementation and evolution of CRISPR technologies for fungal genetic engineering, metabolic pathways can now be elucidated in quite exotic fungi never engineered previously.

Nevertheless, since the complete elucidation often requires both deletion and overexpression of gene(s) of interest, the construction of a platform strain for heterologous production is necessary. To complement genetic pathway elucidation in the natural host, we have recently developed a gene-expression platform, DIVERSIFY, including in an Aspergillus oryzae strain, which can be used for heterologous production of secondary metabolites. A. oryzae is an excellent host for pathway elucidation as it has a very low background of endogenous secondary metabolite production. Our A. oryzae strain contains a target expression site that harbors an uidA marker gene. Insertion of novel genes into the target site eliminates the marker gene and corrects transformants containing the relevant gene at the target expression site can therefore be easily identified. To use our setup, we chose the fungus Aspergillus californicus that has never been engineered previously to investigate the pathways behind production of the newly identified compounds. After a chemical investigation, we isolated a polyketide-nonribosomal peptide hybrid calipyridone A. Its biosynthetic gene cluster cpd was next discovered by genome mining. Initially a CRISPR/Cas9 multiplex technology was applied to generate a non-homologous end joining and pyrG deficient strain by using different guides to cleave DNA using a silent 4 kb cassette for DNA repair. The compound biosynthesis was elucidated by multiple gene deletion experiments in the host strain as well as the heterologous expression of the hybrid gene cpdA in A. oryzae. The results indicate that the 2-pyridone moiety of calipyridone A is formed directly from the nucleophilic attack of the amide nitrogen to the carbonyl group, which is different from the generation of other fungal 2-pyridone products via P450-catalyzed ring expansions. These findings open door for elucidation of new metabolic pathways in non-engineered fungi.

58. (FGC# 440V) Identification and functional characterization of the putative cyclin FlpA as a regulator of the metula-to-phialide transition during conidiophore development of Aspergillus nidulans

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The genus Aspergillus includes industrially, medically and agriculturally important species. All of them, as do fungi in general, disperse to new niches and hosts principally by means of asexual spores. When it comes to the study of the genetic/molecular mechanisms controlling asexual development, Aspergillus nidulans is the main reference. Two main pathways control in A. nidulans the production of conidiophores, asexual structures containing thousands of asexual spores known as conidia. The UDA pathway transduces environmental signals, determining whether the CDP pathway and thus the required morphological changes are induced. The transcriptional regulator BrlA links both pathways, while loss-of-function mutations in flb (UDA) genes block brlA transcription and, consequently, conidiation. However, the aconidial phenotype of some flb mutants can be reverted under salt-stress conditions. A library of ΔflbB mutants unable to conidiate on medium supplemented with NaH₂PO₄ (0.65M) (FLIP, fluffy in phosphate mutants) was generated by us. Mutants were grouped according to their phenotypical features. Here, we have identified a Gly347Stop mutation within flpA as responsible for the mutant phenotype FLIP57. Deletion of the gene causes a significant reduction in germination, radial extension, quantity of cleistothecia and conidia production. Functional characterization of the putative cyclin FlpA suggests that it is necessary in the transition from metulae to phialides during conidiophore development.
The ortholog of FlpA in *Schizosaccharomyces pombe* has been described to form a complex regulating RNA pol II activity together with orthologs of *A. nidulans* Stk47 and FlpB. Here, it is shown that the corresponding single-null mutants show the same phenotype, the three proteins are localized into nuclei and this localization is based on specific functional dependencies. Overall, this work adds new elements to the complex networks of proteins coordinating growth with sexual and asexual developmental programs.

59. (FGC# 287V) The emerging role of a cyclase gene in the biosynthesis of ochratoxin A: The case study of *Aspergillus carbonarius*

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Ochratoxin A (OTA) is a well-known mycotoxin with wide distribution in food and feed, including cereal products, grapes and by-products, coffee, beverages, cocoa, nuts, dried fruits, and cured meat. Ochratoxin A is produced by many species of the genus *Aspergillus* and *Penicillium*. One of the main OTA producing species is *Aspergillus carbonarius*, known not only for its high capacity for producing OTA and its high percentage of toxigenic strains but also because it is considered the main species for OTA contamination of grapes in the vineyard worldwide. Fungal genome sequencing has great utility for identifying secondary metabolites gene clusters for known and novel compounds. A comparative genomic analysis of the OTA-biosynthetic cluster in 21 OTA-producing species has revealed a high synteny in OTA cluster organization in five structural genes (*otaA, otaB, ota, otaR1*, and *otaD*). Moreover, a cyclase gene, *otaY*, located in the OTA cluster between the *otaA* and *otaB* genes, was identified. This gene encodes for a predicted protein with high similarity to SnoaLs domain containing proteins. Similar proteins have been shown in *Streptomyces* to catalyze cyclization steps in the biosynthesis of polyketide antibiotics and we hypothesized it to be involved also in OTA biosynthesis. The expression level of the cyclase gene has been investigated to demonstrate its correlation to the kinetics of OTA accumulation and the expression profile of the other OTA biosynthetic genes under permissive OTA conditions. We demonstrated the role of the *otaY* gene by complete gene deletion using the CRISPR/Cas9 approach. The deletion of *otaY* gene stopped the biosynthesis of OTA and giving the first functional evidence of the involvement of a new gene in the biosynthetic pathway of OTA in *A. carbonarius*. Our findings represent a knowledge advancement in the molecular basis of OTA biosynthesis.

60. (FGC# 903V) Structure-activity predictions from computational mining of protein databases to assist modular design of antimicrobial peptides

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Antimicrobial peptides (AMPs) are promising alternatives to antibiotics to fight multidrug resistant microorganisms. However, despite thousands of AMP entries in respective databases, predictions about their structure-activity relationships are still limited. Similarly, common or dissimilar properties of AMPs that have evolved in different taxonomic groups, e.g., fungi, bacteria, mammals, are nearly unknown. We leveraged data entries for 10,987 peptides currently listed in the three antimicrobial peptide databases APD, DRAMP and DBAASP to aid structure-activity predictions. However, this number reduced to 3,828 AMPs that we could use for computational analyses, due to our stringent quality control criteria. The analysis uncovered a strong bias towards AMPs isolated from amphibians (1,391), whereas only 35 AMPs originate from fungi (0.9%), hindering evolutionary analyses on the origin and phylogenetic relationship of AMPs. The majority (62%) of the 3,828 AMPs consist of less than 40 amino acids but with a molecular weight higher than 2.5 kDa, have a net positive charge and share a hydrophobic character. They are enriched in glycine, lysine and cysteine but are depleted in glutamate, aspartate and methionine. Remarkably, the γ-core motif claimed so far as an ancient unifying structural signature in cysteine-stabilised AMPs is absent in nearly 90% of the peptides. However, this motif appears to be of high relevance with respect to fungi. Firstly, antifungal AMPs show an increased presence of the γ-core motif, and, secondly, almost 50% of AMPs originate from fungi contain the γ-core motif. The disclosure of AMPs pattern and their variation in producing organism groups extends our knowledge of the structural diversity of AMPs and will assist future peptide screens in unexplored microorganisms.
Structural design of peptide antibiotic drugs will benefit using natural AMPs as lead compounds. However, a reliable and statistically balanced database is missing which leads to a large knowledge gap in the AMP field. Particularly, AMPs from fungi depict a minority in current AMP databases and, furthermore, testing available AMPs for their antifungal activity is significantly neglected compared to antibacterial activities. Thus, thorough evaluation of the available data, mitigation of biases and standardised experimental setups need to be implemented to leverage the full potential of AMPs for drug development programs in the clinics and agriculture.

61. (FGC# 659V) Identification of novel transcription factors involved in Aspergillus fumigatus adherence

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Background: Adherence to cells is a key step in fungal pathogenesis. In Aspergillus fumigatus, hyphal adherence to host cells is mediated by the exopolysaccharide galactosaminogalactan (GAG). While several studies have identified genes whose product is required for GAG biosynthesis, little is known about the genetic regulation of GAG production.

Methods: A library of 400 A. fumigatus transcription factor knock-outs was screened for their capacity to form adherent biofilms using the crystal violet assay. Transcription factor mutants with impaired biofilm-forming capacity were re-constructed to conﬁrm the role of each candidate gene in the regulation of adherence. Mutants were then tested for potential growth defects by visual observation and XTT metabolic activity. GAG synthesis was quantiﬁed by ELISA and immunofluorescence microscopy. Cell wall composition was assessed by gas chromatography/mass spectrometry.

Results: Out of 400 transcription factor knockouts, 9 strains exhibited a reduction of > 50% in biofilm formation as compared with the parent strain Ku80. After reconstruction of the 9 deletions, the simultaneous study of biofilm adherence and growth allowed the classiﬁcation of these mutant strains into 4 categories: 1 mutant had no growth defect and exhibited impaired formation of adherent biofilms throughout the growth period, 4 mutants had no growth defect, and reduced biofilm formation that could be restored with longer incubation, 3 mutants exhibited both a growth defect and a defect in biofilm formation that persisted despite prolonged incubation. Finally, 1 mutant displayed a severe germination defect and was excluded from further study. Interestingly, all strains except one produced both cell wall-associated or secreted GAG. Further studies of the cell wall polysaccharides in these mutants suggested a wider dysregulation of cell wall biosynthesis.

Conclusion: This study highlights the role of several novel transcription factors in the regulation of A. fumigatus adherence and cell wall synthesis. Further, the inability of several of these strains to form adherent biofilms despite the production of GAG may provide insights into other GAG-interacting or independent factors required for fungal adhesion and biofilm formation. The identiﬁcation of these new adherence actors and their precise role may identify new therapeutic targets to prevent the development of A. fumigatus biofilms.

62. (FGC# 280V) Beyond the symbiosis: Novel modulating roles of lipochitoooligosaccharides and chitoooligosaccharides in the development of fungi and nearby microbes.

Tomas Rush1, Ivan Villalobos Solís1, Joanna Tannous1, Muralikrishnan Gopalakrishnan Meena1, Matthew Lane2, Nancy Engle3, Alyssa Carrell1, Margaret Spangler1, Jean-Michel Ané3, Nancy Keller3, Sylvain Cottaz2, Sébastien Fort5, Daniel Jacobson1, David Kainer1, Dale Pelletier1, Timothy Tschaplinski1, Robert Hettich1, Richard Giannone1, Paul Abraham1, Jesse Labbé1. 1) Oak Ridge National Laboratory, Oak Ridge, TN, USA; 2) University of Tennessee, Knoxville, TN, USA; 3) University of Wisconsin-Madison, Madison, WI, USA; 4) Université Grenoble Alpes, CNRS, CERMAV, Grenoble, France

Lipochitoooligosaccharides (LCOs) and chitoooligosaccharides (COs) are chemical signaling molecules produced by rhizobia bacteria and fungi. Both signals have been well characterized for their role as symbiotic molecules and their interactions with host plants. They are perceived by a lysin motif receptor-like kinases in various plants, which can elicit the Common Symbiosis Pathway. Some observed phenotypes are oscillations in nuclear calcium or, as shown in some legumes, will prompt root hair
branching or root hair curling, observable phenotypes in developing roots. However, the role of LCOs and COs outside of symbiosis is still in its infancy. Recently, LCOs and COs were shown to be produced in most fungi in the absence of a host, regulating the fungal physiology and transcriptomic. However, it remains unknown why LCO- and CO- producing organisms use these molecules. We test for alternative roles of LCOs and COs, outside symbiosis with a host plant, to address this question. We first determined that these molecules could control secreted metabolites to modulate nearby microbial growth. In addition, we demonstrate that observed fungal physiological changes may be attributed to differences in proteomic and metabolomic outputs. Our results showed that various types of LCOs and COs would differentially regulate the production of known secondary metabolites in Aspergillus fumigatus and Laccaria bicolor. Moreover, through network analysis, we determined specific types of LCOs, or COs to promote the production of several unknown analytes. These metabolites negatively or positively influenced the growth behavior of soilborne bacteria across five different phyla. Finally, we provide evidence that LCOs produced in abundance by a fungus, will have in return, the most significant effect on its behavior. Overall, we speculate that LCOs could be a fungistatic compound produced and used by the fungus to organize microbial communities.

63. (FGC# 883V) Development of CRISPR-Cas editing tools in Sphaerulina musiva towards controlling its establishment and pathogenicity in the model ecosystem, Populus
Joanna Tannous1, Cole Sawyer1,2, David Kainer1, Alyssa Carrel1, Mindy Clark1, Jesse Labbe1,3, Wellington Muchero1, Melissa Cregger1, Carrie Eckert1, Paul Abraham1. 1) Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA; 2) Graduate School of Genome Science and Technology, University of Tennessee, Knoxville, TN, USA; 3) Invaio Sciences, Cambridge, MA, USA
The genus Populus are economically important biofuel crops cultivated worldwide, but mainly in the Northern hemisphere to fulfill the demands for bioenergy and fiber production. Poplars and their hybrids’ widespread distribution and usage are limited by their instability to various diseases, of which the leaf spot and canker disease caused by the fungal pathogen Sphaerulina musiva is the most detrimental one. Breeding and cultivation of resistant plant species have been the primary approaches adopted to control the damage caused by this pathogen.

In our effort to mitigate Poplar disease caused by S. musiva we focused on advancing both the fundamental knowledge on the S. musiva -Populus pathosystem and the applied research by developing genetic engineering tools on the pathogen as a strategy to control the fungal abundance and disease severity. The above-ground part of the S. musiva’s lifecycle is relatively well studied; however, the underground establishment of S. musiva’s spores, from fallen infected leaves, in the presence of Populus soil microbiota had remained obscure. Therefore, using RNA-sequencing, we first aimed to identify genetic markers implicated in the establishment of S. musiva within native Populus soil microbial communities. Later we developed and validated the first CRISPR-Cas9 gene-editing tool to successfully transform S. musiva. The development of this genetic tool, along with the molecular markers identified from this study would allow for specific gene targeting to disrupt S. musiva’s establishment in soil. Lastly, we are also in the process of leveraging from this established tool to develop a self-propagating synthetic gene drive designed to suppress leaf spot disease caused by S. musiva in Populus.

*64. (FGC# 430V) GAG, a polysaccharide cytotoxin?
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Aspergillus fumigatus is a ubiquitous filamentous mold that causes necrotizing pneumonia in immunosuppressed individuals. One of the virulence factors of A. fumigatus is the synthesis of the adhesive, cationic exopolysaccharide GAG, which is mediated by the products of a five gene cluster. One of these genes, ega3 encodes a glycosyl hydrolase that is specific for deacetylated GAG that is anchored to the cell membrane of A. fumigatus. We hypothesized that Ega3 is necessary for GAG synthesis, and to test this hypothesis, we sought to disrupt ega3 in A. fumigatus.

Multiple attempts to disrupt ega3 by conventional methods were unsuccessful. Switching to CRISPR/Cas9 generated a single Δega3 clone. As with other mutations in the GAG cluster, this mutant
did not produce deacetylated GAG. Complementation with an ega3 allele failed to restore deacetylated GAG production, suggesting the presence of a secondary mutation in this strain. Analysis of the expression of the rest of the GAG cluster genes revealed that agd3, encoding the GAG deacetylase, was not expressed in the ∆ega3 mutant. We therefore hypothesized that ega3 is conditionally essential in the presence of deacetylated GAG production. To test this, agd3 was expressed in the ega3 null mutant under the control of a tetracycline-inducible promoter (∆ega3-Tet ON-aggd3). Under agd3-expressing conditions, GAG production was restored, however fungal growth was inhibited. We hypothesized that GAG may be toxic to the cell membrane of A. fumigatus and that Ega3 is necessary for degrading GAG near the membrane. To test this, ATP was measured in fungal culture supernatants as a proxy for cell leakage. Induction of agd3 expression in the ∆ega3-Tet ON-aggd3 mutant causes increased ATP release, suggesting that deacetylated GAG disrupts the fungal cell membrane. Since GAG causes membrane permeability in ∆ega3, we hypothesized that it may cause host cell injury. This was tested in a cell damage assay where epithelial cells were loaded with radioactive chromium and exposed to culture supernatants of wild-type A. fumigatus with and without recombinant Ega3. A. fumigatus-induced epithelial cell damage was nearly abolished in the presence of Ega3. Similar findings were observed using propidium iodide staining of bone marrow-derived macrophages exposed to GAG with or without Ega3. This data suggests that cationic GAG is cytotoxic to both fungal and host cells, and that Ega3 can serve as an antitoxin to mitigate these effects.

65. (FGC# 905V) Flower bulb waste material is a natural niche for the sexual cycle in Aspergillus fumigatus

Jianhua Zhang1, Paul Verweij2, Antonius Rijs2, Alfons Debets1, Eveline Snelders1. 1) Wageningen University; 2) Radboud University Medical Center, Nijmegen, the Netherlands

With population genetic evidence of recombination ongoing in the natural Aspergillus fumigatus population and a sexual cycle demonstrated in the laboratory the question remained what the natural niche for A. fumigatus sex is. Composting plant-waste material is a known substrate of A. fumigatus to thrive and withstand temperatures even up to 70°C. Previous studies have shown indirect evidence for sexual reproduction in these heaps but never directly demonstrated the sexual structures due to technical limitations. Here, we show that flower bulb waste material from stockpiles undergoing composting can provide the conditions for sexual reproduction. Direct detection of ascospore structures was shown in agricultural flower bulb waste material by using a grid-based detection assay. Furthermore, we demonstrate that ascospores can germinate after exposure to 70°C for up to several days in contrast to asexual conidia that are unable to survive a two-hour heat shock. This indicates a sufficient time frame for ascospores to survive and escape composting stockpiles. Finally, sexual crosses with cleistothecium and viable ascospore formation could successfully be performed on flower bulb waste material. Recombination of A. fumigatus can now be explained by active sexual reproduction in nature as we show in this study that flower bulb waste material provides an environmental niche for sex.
New Aspergillus labs talk abstracts.
The significance of virus infection in Aspergillus fumigatus strain AF293
Ioly Kotta-Loizou
Molecular Microbiology, Imperial College London.
Fungi, similar to all species, are susceptible to viral infection. Aspergillus is arguably the most well studied fungal genus because of its medical, ecological and economical significance. Mycoviruses were initially detected in Aspergillus species almost 50 years ago and the field continues to be active today with ground-breaking discoveries. The prototype member of the novel virus family Polymoviridae, Aspergillus fumigatus tetramycovirus-1 (AfuTmV-1), was discovered in A. fumigatus AF293 and related viruses have been reported in other Aspergilli. AfuTmV1 is a non-conventionally encapsidated virus with four double-stranded (ds) RNA segments as its genome. Each segment is monocistronic, encoding respectively an RNA-dependent RNA polymerase, a putative scaffold protein, a methyl transferase and an intrinsically disordered, RNA-binding, proline-alanine-serine rich protein that coats the viral dsRNA. AfuTmV1 is a target of the A. fumigatus antiviral RNA silencing machinery and individual AfuTmV1 strains may affect morphology, growth and virulence of their fungal host. AfuTmV1 also influences the interactions between A. fumigatus and Pseudomonas aeruginosa, the most common fungus and bacterium respectively in immunocompromised individuals. P. aeruginosa inhibits growth of AfuTmV-1-infected A. fumigatus as compared to a virus-free isogenic line, in liquid culture and in biofilms. This effect is iron-dependent and is mediated via pyoverdin and Pseudomonas quinolone signal, two important P. aeruginosa exoproducts with iron-binding properties. AfuTmV-1 infection interferes with A. fumigatus iron metabolism, affecting siderophores responsible for iron uptake and storage, thus weakening AfuTmV-1-infected A. fumigatus in its competition with P. aeruginosa for iron. This phenomenon may be significant in a clinical setting and merits further investigation.

Exploring the unfolded protein response as a target for treating fungal corneal infections.
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Fungal keratitis (FK) is a leading cause of ocular morbidity and unilateral blindness worldwide. The polyene natamycin is the only FDA-approved drug to treat these infections and, even with its use, long-term vision loss occurs in 75% of all cases. Our group is therefore interested in identifying novel drug targets, which we predict are fungal metabolic or stress pathways that promote fungal growth within the corneal environment. Given its prevalence as an agent of FK and its genetic tractability, Aspergillus fumigatus serves as our model pathogen. We began with a simple prediction that the cornea represents a nutrient-poor environment for the fungus due its avascularity and collagen-rich composition. Indeed, we identified an upregulation of secreted proteases and alternative carbon utilization pathways in Aspergillus isolated from infected mouse corneas, relative to standard in vitro culture conditions. We next reasoned that the increased secretory activity in vivo placed the fungus under endoplasmic reticulum stress, which would render the unfolded protein response (UPR) critical for virulence. This was confirmed as a UPR-deficient mutant, ΔhacA, was unable to establish infection in our murine model. From a translational perspective, HacA may not represent an ideal drug target as it is a transcription factor against which no inhibitory compounds have been described. By contrast, several compounds are known to inhibit the mammalian ortholog of the regulatory kinase/ribonuclease, IreA, that activates HacA. One such compound, 4u8C, was recently shown to also block the ribonuclease activity of A. fumigatus IreA. Interestingly, we found that the compound displays antifungal activity at concentrations that inhibit IreA function (hacA splicing), suggesting that the protein is essential for growth. Indeed, attempts to isolate an ireA knockout were unsuccessful and repression of ireA expression with a tetracycline-repressible promoter almost completely blocked hyphal proliferation. Taken together, these data not only suggest that A. fumigatus IreA harbors hacA-independent functions that are essential for growth, but they further suggest that IreA inhibitors may serve as novel antifungals to treat FK. We are currently testing the clinical utility of these compounds in our murine.
Distinct type I interferon (IFN) signaling is involved in regulating host damage and fungal clearance during *Aspergillus* pulmonary infection
Kelly M. Shepardson1,2, Julia Roemer1, Agnieszka Rynda-Apple1;
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Recently, there has been an increased incidence of invasive pulmonary aspergillosis (IPA), caused by the human fungal pathogen *Aspergillus fumigatus* (*Af*), occurring in patients infected with influenza or SARS-CoV-2. Along with the recently described involvement of type I interferon (IFN) signaling in increased *Af* susceptibility during viral infection in mice, this strongly indicates that anti-viral immune responses, such as type I IFNs, create an environment permissive to fungal infection. Supporting this, we found that type I IFN signaling, via the type I IFN receptor 2 (IFNAR2) of IFNAR1/2, contributes to regulation of susceptibility to and damage from influenza in mice, while others have found that IFNAR2 expression correlates with SARS-CoV-2 infection severity. As clinical outcome to *Af* is associated with host tissue damage, this suggests that IFNAR2’s regulation of the damage response during pulmonary infection may control the immune status of the lung, via tissue damage, allowing for fungal infection to occur. We found that absence of IFNAR2 (*Ifnar2*−/− mice) resulted in increased damage, weight loss, and morbidity early during infection with *Af* strain CEA10 compared to WT and *Ifnar1*−/− mice. Additionally, we also found that both WT and *Ifnar1*−/− mice had decreased *Af* clearance early during infection compared to *Ifnar2*−/− mice and that this difference in killing of *Af* required *in vivo* interactions/signaling. However, as *Af* infection progressed we found that although *Ifnar2*−/− mice cleared *Af* early, this did not prevent invasive hyphal growth from occurring. This invasive growth in the *Ifnar2*−/− mice was found to be associated with increased damage and cell death in the *Af* lesions within the lung. Importantly, our results suggest that this IFNAR2 damage response is being mediated by distinct type I IFNs, specifically IFNα.

Our preliminary data also suggest that this damage is *Af* strain specific, as infection with AF293, a less aggressive strain, did not result in increased morbidity of the *Ifnar2*−/− mice. Together, our results begin to establish a role for IFNAR2 in regulation of the host damage response to *Af* and suggests that the type of type I IFN signaling may contribute to a permissive environment allowing for *Af* infection to occur. Understanding the mechanisms involved in IFNAR regulation of damage and anti-fungal immunity could inform design of better treatments aimed at minimizing damage in patients with IPA or controlling lung tissue damage.

Funding: NIH/NIGMS INBRE P20GM103474, Francis Family Foundation, Parker B. Francis Fellowship.

*Aspergillus fumigatus* hexameric septin complex is involved in spore cell wall organization and immune evasion
Alban Sinani, Wyatt Boyer, José Vargas-Muñiz. Microbiology Program, School of Biological Sciences, Southern Illinois University at Carbondale
See Poster abstract 51.

Antifungal Screening of 54 Single Plant Essential Oils Against *Aspergillus fumigatus*
Matthew Swearingen1, Elizabeth Myers2, Yainitza Hernandez-Rodriguez2. 1) Heartland Food Products Group; 2) Florida Gulf Coast University
See Poster abstract 21.

ncRNAs – novel regulatory links in fungal azole tolerance?
Sourabh Dhingra
Saprophytic mold *Aspergillus fumigatus* causes a spectrum of diseases known as aspergillosis. Drug resistance has exacerbated the problem and has increased from less than 1% to >10% in the last 20 years. Recent studies show that different isolates of *A. fumigatus* have diverse virulence and drug resistance profile; however, the mechanisms of this response are enigmatic. We hypothesize that the variable expression of non-coding RNAs allows the fungus to adapt to environmental stresses impacting its virulence and drug response. In support of that, our preliminary data indicate that the ncRNA profile in commonly used laboratory strains, CEA10 and AF293, and clinical isolates are significantly different. Additionally, we have identified a role for long ncRNA, *afu-182*, in azole drug tolerance. Our data show that *afu-182* genetically controls tolerance to multiple azole drugs.
Significance – Azole-sensitive *Aspergillus fumigatus* strains cause about 90% of infections; however, about 50% of those infections do not respond to azole drugs. Our preliminary data show that long ncRNA, *afu-182*, increases azole fungal tolerance without impacting its minimum inhibitory concentration. A better understanding of fungal drug tolerance will improve the dichotomous resistant/sensitive classification, thus providing treatment interventions for tolerant strains.
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