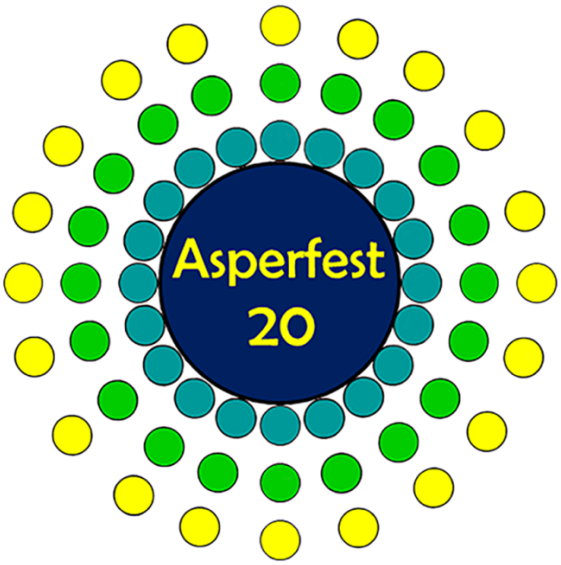
The 20th International Aspergillus Meeting

Asperfest20

March 11-12, 2024

Asilomar Conference Center, Pacific Grove CA, USA.



Aspergillus Genomes Research Policy Group (AGRPG)

An Aspergillus Genomics workshop was held at the March 2003 Asilomar Fungal Genetics meeting. From discussions in that workshop it was obvious that our community needed to organize to fully exploit genomics resources. A provisional Aspergillus Genomes Research Policy Committee (AGRPC) was conscripted and charged with creating a structure for community-wide coordination and organizing an annual meeting. The First Aspergillus Meeting was held in Copenhagen, April 21, 2004, as a satellite meeting of the European Congress on Fungal Genetics-7. In addition to scientific presentations, bylaws were approved, community research directions were discussed and the 2004 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).

2023 AGRPC

Michelle Momany, 2020-2023, University of Georgia, USA; mmomany@uga.edu
Gerhard Braus, 2020-2023, Georg-August-University Goettingen, Germany; gbraus@gwdg.de
David Cánovas, 2020-2023, University of Seville, Spain; davidc@us.es
Michael Bromley, 2020-2023, University of Manchester, UK. Mike.Bromley@manchester.ac.uk
Yainitza Hernandez-Rodriguez, 2022-2024, Florida Gulf Coast University, USA; yhernandez@fgcu.edu
Neta Shlezinger, 2022-2024, The Hebrew University of Jerusalem, Israel; neta.shlezinger1@mail.huji.ac.il
Eveline Snelders, 2022-2024, Wageningen University & Research, The Netherlands; eveline.snelders@wur.nl
Norio Takeshita, 2022-2024, University of Tsukuba, Japan; takeshita.norio.gf@u.tsukuba.ac.jp
Amelia Barber, 2023-2025, Friedrich Schiller University, Germany: amelia.barber@uni-jena.de
Fabio Gsaller, 2023-2025, Medical University of Innsbruck, fabio.gsaller@i-med.ac.at
Johanna Rhodes, 2023-2025, Radboudumc, The Netherlands, Johanna.Rhodes@radboudumc.nl
Richard Todd, 2023-2025, Chair and local organizer, Kansas State University, USA; rbtodd@ksu.edu

THANKS TO OUR MEETING SPONSOR!

novonesis

PROGRAM
The Twentieth International *Aspergillus* Meeting
Asperfest 20
Monday March 11 – Tuesday March 12, 2024
Merrill Hall, Asilomar Conference Center, Pacific Grove, CA.

Monday March 11

4:00PM **Poster hang up**

7:00PM - 10:00PM **Poster and Welcome Reception** (sponsored by Novonesis)

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

Judging for Novonesis Student Poster Prize, coordinated by David Canovas, University of Sevilla, Spain.

Tuesday March 12

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

8:40 **Session I** Richard Todd

Role of fungal transglutaminase domain-containing proteins in wound-related hyphal protection at the septal pore.
Jun-ichi Maruyama, *University of Tokyo, Japan.*

A multifaceted approach to improving outcomes of cerebral aspergillosis.
Sarah Beattie, *University of Iowa, USA.*

Diversity and functional characterization of filamentous fungal sugar transportomes.
Miia Mäkelä, *University of Helsinki, Finland.*

9:25 **Flash Talks from Abstracts** Michelle Momany
University of Georgia, USA
Mike Bromley
University of Manchester, UK

A Single Step Multi-Copy Integration System Based on Rolling-Circle Replication.
Martzel Antsotegi, *Technical University of Denmark (DTU), Denmark.*

The transcription factor Ndt80 is a negative regulator of virulence in *Aspergillus fumigatus*.
Vijendra Arya, *University of Iowa, USA.*

The *Aspergillus nidulans sarB* gene encodes a putative UDP-N-acetylglucosamine transporter involved in amino acid utilization.
Heather Forster, *Kansas State University, USA.*

A conserved oxylipin alarm blocks the fungicidal effects of echinocandins in pathogenic aspergilli.
Dante Calise, *University of Wisconsin-Madison, USA.*

The CakA kinase links the cell cycle with secondary metabolism in *Aspergillus nidulans*.
Zhiqiang Dong, *University of Macau, Macau SAR, China.*

Functional Characterization of a lncRNA in Stress Response and Pathogenesis of *Aspergillus fumigatus*.
Ritu Devkota, *Clemson University, USA.*

The role of mycotoxins in governing interactions between the maize colonists, *Aspergillus flavus* and *Fusarium verticillioides*.
Tim Satterlee, *Toxicology & Mycotoxin Research Unit, USDA-ARS, USA.*

Functional *in vitro* and physiological *in vivo* characterization of five new xylose transporters of *Aspergillus niger*.
Christina Lyra, *University of Helsinki, Finland.*

Uncovering important transcriptional regulations during conidiation and spore germination.
Pin Wu, *University of Macau, Macau SAR, China.*

10:00-10:30 Coffee Break and Posters

10:30 Session II

Mike Bromley
University of Manchester, UK

20 years of Asperfests
Michelle Momany, *University of Georgia, USA.*

What matters to Novonesis
Mikael Rørdam Andersen, *Novonesis, Denmark.*

11:00 Tools/Resources

David Canovas,
University of Sevilla, Spain

Aspergillus and Penicillium genus genome sequencing projects – an update.
Ronald de Vries, *Westerdijk, The Netherlands,*
Scott Baker, *Pacific Northwest National Laboratory, USA.*

A. fumigatus gene knockout library
Mike Bromley, *University of Manchester, UK.*

Fungal Genetics Stock Center: a status report.
Jaideep Mallick, *Kansas State University, USA.*

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

11:35 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 Yainitza Hernandez-Rodriguez
Florida Gulf Coast University, USA
Norio Takeshita
University of Tsukuba, Japan

Analysis of chronic host-aspergilloma interactions using a novel mouse model.
Masato Tashiro, *Nagasaki University, Japan.*

Life in the dark – new *Aspergillus* species isolated from the extreme habitat of a constantly dark, high temperature radon cave.
Monika Schmoll, *University of Vienna, Austria.*

Unveiling GRAsp, an online tool for the exploration of gene regulatory networks in *Aspergillus fumigatus* to gain insights into growth, development, and pathogenicity.
Cristobal Carrera Carriel, *University of Wisconsin-Madison, USA.*

Growth inhibition between filamentous fungal colonies of the same strain and its regulatory mechanism.
Yuya Hamanaka, *University of Tokyo, Japan.*

Exploring biosynthetic gene clusters in *Aspergillus fischeri*.
Karin Steffen, *Vanderbilt University, USA.*

2:15 Pontecorvo Lecture Richard Todd
Kansas State University, USA

Aspergillus niger: tools, genomic resource, and their uses.
Adrian Tsang,
Concordia University, Quebec, Canada.

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

Novonesis student poster prize presentation David Canovas,
University of Sevilla, Spain
Mikael Rørdam Andersen
Novonesis, Denmark

3:00PM Dismiss (Remove posters)

List of Posters

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

***1. Diversity and characterization of filamentous fungi isolated from sediments of Basque estuaries**
Ainara Otamendi, **Ziortza Agirrezabala Urkia**, Carla Perez-Cruz, Raquel Liébana, Laura Alonso-Sáez, Maria Teresa Dueñas, Anders Lanzén, Oier Etxebeste

2. A Single Step Multi-Copy Integration System Based on Rolling-Circle Replication
Martzel Antsotegi, Martí Morera Gomez, Zofia Jarczynska, Katherina García, Vasil D'Ambrosio, Jean-Marie Mouillon, Uffe H Mortensen

3. The transcription factor Ndt80 is a negative regulator of virulence in *Aspergillus fumigatus*
Vijendra Arya, Hong Liu, Mark A. Stamnes, Scott G. Filler, William Scott Moye-Rowley

4. The next dimension of CAZymes - Inferring the functional interplay between fungal carbohydrate-active enzymes for biomass conversion
Kristian Barrett, Lene Lange, Igor Grigoriev, Anne Meyer

5. A multifaceted approach to improving outcomes of cerebral aspergillosis
Martin Kelty, Aracely Miron-Ocampo, **Sarah Beattie**

6. Elevated mutation rates in multi-azole resistant *Aspergillus fumigatus* drive the rapid evolution of antifungal resistance
Michael J. Bottery, Norman van Rhijn, Harry Chown, Johanna L. Rhodes, Brandi N. Celia-Sanchez, Marin T. Brewer, Michelle Momany, Matthew C. Fisher, Christopher G. Knight, and Michael J. Bromley

***7. Deletion of core septin gene *aspB* in *Aspergillus fumigatus* results in fungicidal activity of caspofungin**
Rebecca J Busch, Carson Doty, Allie Mills, Flutur Latifi, Vjollca Konjufca, Laura Herring, José Vargas-Muñiz

8. Breaking down barriers: comprehensive functional analysis of the *Aspergillus niger* chitin synthase repertoire
Timothy Cairns, Lars Barthel, Sven Duda, Henri Müller, Sascha Jung, Heiko Briesen, Vera Meyer

***9. A conserved oxylipin alarm blocks the fungicidal effects of echinocandins in pathogenic aspergilli**
Dante G Calise, Sung Chul Park, Jin Woo Bok, Gustavo H Goldman, Nancy P Keller

10. Leverage of fungal growth data through modelling: applications to the study of antifungal drugs
David Canovas

***11. Unveiling GRAsp, An Online Tool for the Exploration of Gene Regulatory Networks in *Aspergillus fumigatus* to Gain Insights into Growth, Development, and Pathogenicity**
Cristobal Carrera Carriel, Spencer A. Halberg-Spencer, Saptarshi Pyne, Sung Chul Park, Hye-Won Seo, Aidan Schmidt, Dante Calise, Sushmita Roy, Jean-Michel Ané, Nancy P. Keller

***12. A global pangenome of *Aspergillus fumigatus* reveals the origin of azole resistance**
Johanna Rhodes, **Harry Chown**, Felicia Stanford, Rodrigo Leitao, Samuel Hemmings, Ali Abdolrasoul, Norman van Rhijn, Gillian Sigle-Hall, Zain Chaudhry, Iro Chatzidaki, Ben Simmons, Chuhan Qin, Darius Armstrong-James, Paul Verweij, Michael Bromley, Paul Dyer, Matthew Fisher

13. Modernizing high-throughput mycology with robotics and artificial intelligence
Johan V Christiansen, Søren D Petersen, Vilhelm K Møller, David Llorente, Parvathy Krishnan, Steen S Brewer, Katharina Steinert, Alexander R Brems, Sabrina M Pittroff, Mathilde Nordgaard, Vincent Wiebach, Niels Bjerg, Lars Jelsbak, Ling Ding, Jakob B Hoof, Jens C Frisvad, Rasmus J N Frandsen

14. Genetic and regulatory complexity in fungal primary carbon metabolism
Ronald P de Vries, Astrid Mueller, Jiajia LI, Li Xu, Mao Peng, Miia R Mäkelä

15. Functional Characterization of a lncRNA in Stress Response and Pathogenesis of *Aspergillus fumigatus
Ritu Devkota, Alexandra Randazza, Lela Lackey, Sourabh Dhingra

16. The CakA kinase links the cell cycle with secondary metabolism in *Aspergillus nidulans*
Zhiqiang Dong, Agnieszka Gacek-Matthews, Franz Zehetbauer, Niranjan Shirgaonkar, Kaeling Tan, Chris Koon Ho Wong, David Cánovas, Joseph Strauss

***17. Surprising strain-specific molecular determinants of *Aspergillus fumigatus* pathogenicity revealed by new cancer small molecule therapies**
Katherine E Doss, Matthew R James, Andrew Wishart, Tobias M Hohl, Michail S Lionakis, Robert A Cramer

18. Generation and characterization of serial deletion- and point-mutants within the 5´-UTR region of *brlA* allow the identification of promoter sequences required and dispensable for *Aspergillus nidulans* conidiation
Ainara Otamendi, Alotz Bereziartu, Ziortza Agirrezabala, Eduardo A. Espeso, **Oier Etxebeste**

***19. The *Aspergillus nidulans sarB* gene encodes a putative UDP-N-acetylglucosamine transporter involved in amino acid utilization**
Heather D Forster, Joel T Steyer, Sara M Hopkins, Richard B Todd

20. Are the type strains of Aspergillus oryzae and A. sojae truly domesticated?
Jens C Frisvad, Jos Houbraken, Giancarlo Perrone, Massimo Ferrara, Kristian Barrett, Jakob B Nielsen, Thomas O Larsen, Lene Lange

21. Starship elements drive genome evolution dynamics in a model eukaryotic microbe
Emile Gluck-Thaler, Adrian Forsythe, Charles Puerner, Jason E Stajich, Daniel Croll, Robert A Cramer, Aaron Vogan

22. Screening system based on growth defects due to unscheduled *brlA* expression to identify genes involved in the functional regulation of transcription factors in *Aspergilli*
Katsuya Gomi, Tomoko Shintani, Da-Min Jeong, Jikian Tokashiki

***23. Growth inhibition between filamentous fungal colonies of the same strain and its regulatory mechanism**
Yuya Hamanaka, Takuya Katayama, Hiroki Kuroda, Jun-ichi Maruyama

24. Regulation of sexual development by IndB and IndD, the physical interactors of the NsdD GATA factor in *Aspergillus nidulans*.
Sang-Cheol Jun, **Kap-Hoon Han**

***25. Developmental Specific Effects of Key Plant Essential Oils against *Aspergillus fumigatus* in Pre- & Post-Infection Plate Models**
William Holt, Arline Martinez, Eduarda Goncalves, Abby Welling, Riley Sheppard, Alma Slova & Yainitza Hernandez Rodriguez

26. Correlation among nuclear increase, enzyme production and hyphal morphology in *Aspergillus oryzae
Ayaka Itani, Naoki Takaya, Oda Ken, Hideyuki Yamashita, Kanae Sakai, Kenichi Kusumoto, Norio Takeshita

27. *Baf* against the wall: elucidating mechanisms of oxygen-driven adaptations in the human fungal pathogen *Aspergillus fumigatus
Angus Johnson, Nicole E Kordana, Kaesi A Morelli, Caitlin H Kowalski, Robert A Cramer

28. Second Alternative Oxidase Genes in Aspergillaceae: Genesis, Loss and Mutations
Levente Karaffa, Michel Flipphi, Alexandra Márton, Vivien Bíró, István Bakondi-Kovács, Viktória Ág-Rácz, Norbert Ág, Erzsébet Fekete

29. Transcriptome analysis of manganese(II) ion depletion during high-yield citric acid fermentation in *Aspergillus niger*
Levente Karaffa, Vivien Bíró, Alexandra Márton, István Bakondi-Kovács, Nicholas Geoffrion, Adrian Tsang, Christian P. Kubicek, Erzsébet Fekete

30. Identification of *A. fumigatus* virulence factors by *in vivo* RNA-seq analysis
Hong Liu, Vincent M Bruno, Quynh T Phan, Scott G Filler

31. The histone deacetylase HosA regulates host cell interactions, resistance to intracellular oxidative stress, and virulence in *A. fumigatus*
Hong Liu, Pamela Lee, Alice Vo, Quynh T Phan, Vincent M Bruno, Mark Stamnes, Scott G Filler

32. An essential telomere binding protein regulating the transition from primary to secondary metabolism in *Aspergillus nidulans
Shuhui Guo, **Xiaofeng Liu**, Lakhansing Pardeshi, Chris Koon Ho Wong

***33. Predicting culture conditions for secondary metabolite production based on binding targets of biosynthetic gene cluster-specific transcription factors**
Fan Lu, Shuhui Guo, Ruiwen Chen, Lakhansing Pardeshi, Chris Koon Ho Wong

34. Functional *in vitro* and physiological *in vivo* characterization of five new xylose transporters of *Aspergillus niger*
Christina Lyra, Aino-Elina Kuusimäki, Liinu Nummela, Robert Mans, Jack Pronk, Miia Mäkelä

35. Diversity and functional characterization of filamentous fungal sugar transportomes
Miia R. Mäkelä, Christina Lyra, Victor M. Gonzalez Ramos, Aino-Elina Kuusimäki, Liinu Nummela, Robert Mans, Jack Pronk, Li Xu, Ronald P. de Vries, Mao Peng

36. Fungal Genetics Stock Center: A Status Report
Jaideep Mallick

37. The putative translational repressor, SsdA, partially regulates carbon source-dependent roles of CotA signaling in *Aspergillus fumigatus*
Adela Martin-Vicente, Jinhong Xie, Harrison Thorn, Xabier Guruceaga, Ashley Nywening, Jarrod Fortwendel

38. Role of fungal transglutaminase domain-containing proteins in wound-related hyphal protection at the septal pore
Md Abdulla Al Mamun, **Jun-ichi Maruyama**

39. FACS-based method streamlines pooled transformations in *Aspergillus oryzae*
Sarah McFarland, Jonathan Pham, Sandeep Sharma Khatiwada, Eric Carter, Ceanne Brunton

***40. Is there localized mRNA translation at the hyphal tip?**
Domenico Modaffari, Edward W J Wallace, Kenneth E Sawin

41. High-throughput CAZyme production in *Aspergillus oryzae
Martí Morera, Martzel Antsotegi, Lucas Levassor, Bernard P Henrissat, Uffe H Mortensen

42. Chromatin structural changes alter *cyp51A* expression in TR34-containing mutant strains of *Aspergillus fumigatus*
Sanjoy Paul, Mark A. Stamnes, Abigail Deaven, Chandler Goldman, Zachary A. Lewis, **Scott Moye-Rowley**

***43. Study on environmental responses and peptidase genes transcriptional regulation in *Aspergillus oryzae* PrtR**
Rika Numamzawa, Yukako Tanaka, Sawako Nishioka, Ryotaro Tsuji, Hiroshi Maeda, Yoshiyuki Itoh, Michio Takeuchi, Mizuki Tanaka, Youhei Yamagata

44. Morphotype-specific fungal factors drive uptake and clearance of *Aspergillus fumigatus* by airway epithelial cells
Sebastien C Ortiz, Patrick J Dancer, Thomas Easter, Kayleigh Earle, Rachael Fortune-Grant, Mike Bromley, Sara Gago, Margherita Bertuzzi

***45. Exploring the role of alpha-1,3-glucan synthases on fungal cell wall integrity in Aspergillus niger**
Katharina J. Ost, Mark Arentshorst, Arthur F.J. Ram, Bruno M. Moerschbacher, Mareike E. Dirks-Hofmeister

***46. Deciphering the Regulatory Mechanisms Governing Recombinant Protein Secretion in Filamentous Fungi**
Everton Paschoal Antoniel, Jaqueline Gerhardt, Natália Wassano, Fernanda Lopes de Figueiredo, André Damasio

47. Network-based approach for discovering transcription factors associated with fungal plant biomass conversion
Mao Peng, Joanna E. Kowalczyk, Astrid E. Mueller, Ronald P. de Vries

48. Investigating the role of long non-coding RNA *afu-182* in azole response in opportunistic pathogen *Aspergillus fumigatus*
Nava R Poudyal, Sourabh Dhingra

***49. Metabolic Plasticity Contributes to Structure and Function of *Aspergillus fumigatus* Biofilms**
Katie Quinn, Charles Puerner, Sandeep Vellanki, Nicole E Kordana, Caitlin H Kowalski, Robert A Cramer

***50. Predicting fungal secondary metabolite activity from biosynthetic gene cluster data using machine learning**
Olivia Riedling, Allison S Walker, Antonis Rokas

51. Leveraging strain heterogeneity within the nonpathogenic fungus *Aspergillus fischeri* to highlight factors associated with virulence
David C Rinker, Karin Steffen, Thomas Sauters, Manuel Rangel-Grimaldo, Huzefa Raja, Camila Pinzan, Patrícia Alves de Castro, Gustavo E Goldman, Nicholas Oberlies, Antonis Rokas

52. FungiDB: Tools for Genomic-Scale Data Exploration, Analysis, Integration and Discovery
David S Roos … on behalf of the FungiDB team (a component of the VEuPathDB Bioinformatics Resource Center)

***53. A novel reporter system to identify arginoketides in soil that mediate cross-kingdom microbial interactions**
Maira Rosin, Mario K. C. Krespach, Maria C. Stroe, Nils Jaeger, Kirstin Scherlach, Volker Schroeckh, Thorsten Heinzel, Christian Hertweck, Axel A Brakhage

54. Fungi Unleashed – Rapid Ionomic Profiling with Laser-Induced Breakdown Spectroscopy
Tomas A Rush, Ann M Wymore, Miguel A Rodriguez, Sara A Jawdy, Rytas J Vilgalys, Madhavi Z Martin, Hunter B Andrews

55. Characterization of acid phosphatases in *Aspergillus oryzae* strain with reduced "umami" degradation activity
Kanae Sakai, Tadahiro Suzuki, Yuichiro Horii, Yutaka Wagu, Ken-Ichi Kusumoto

56. The role of mycotoxins in governing interactions between the maize colonists, *Aspergillus flavus* and *Fusarium verticillioides*
Tim Satterlee, Jaci A. Hawkins, Trevor R. Mitchell, Lincoln F. Adams, Anthony Pokoo-Aikins, Anthony E Glenn, Scott E. Gold

57. Learning from the negative: Studying pathogen evolution from the "non-pathogen" perspective
David Rinker, **Thomas J C Sauters**, Karin Steffen, Camila Figueiredo Pinzan, Huzefa Raja, Manuel Rangel Grimaldo, Thaila Reis, Patrícia Alves de Castro, Nicholas Oberlies, Gustavo Goldman, Antonis Rokas

58. Life in the dark – new *Aspergillus* species isolated from the extreme habitat of a constantly dark, high temperature radon cave
Wolfgang Hinterdobler, Miriam Schalamun and **Monika Schmoll**

59. New Regulators of Gliotoxin Synthesis, HsfA and RogA, Identified through the Systems Biology Network GRAsp
Hye-won Seo, Natalia Wassano, Nancy Keller

60. Investigating dormancy and its breaking in *Aspergillus fumigatus
Justina M Stanislaw, Michelle Momany

61. Exploring biosynthetic gene clusters in *Aspergillus fischeri*
Karin Steffen, David Rinker, Thomas Sauters, Adiyantara Gumilang, Manuel Rangel-Grimaldo, Huzefa Raja, Nicholas Oberlies, Gustavo H Goldman, Antonis Rokas

62. Expanding the fluorescent toolbox in *Aspergillus fumigatus
Isabelle S R Storer, Enrique V Sastré-Velásquez, Thomas J Easter, Birte Mertens, Michael J Bottery, Raveen Tank, Michael J Bromley, Fabio Gsaller, Norman van Rhijn

63. Regulated IRE1-dependent mRNA decay is induced by physiological ER stress associated with amylolytic enzyme production in *Aspergillus oryzae*
Mizuki Tanaka, Silai Zhang, Shun Sato, Jun-ichi Yokota, Yuko Sugiyama Sugiyama, Yasuaki Kawarasaki, Youhei Yamagata, Katsuya Gomi, Takahiro Shintani

64. Towards the development of a safeguarding CRISPR RNA-guided gene drive to mitigate the impacts of the non-native fungal pathogen *Sphaerulina musiva* on managed ecosystems
Joshua Sparks, Kelsey Sondreli, Cole Sawyer, Tomas Rush, Dana Carper, Wellington Muchero, Daniel A Jacobson, Carrie Eckert, Paul E Abraham, Jared LeBoldus, **Joanna Tannous**

65. Analysis of chronic host-aspergilloma interactions using a novel mouse model
Masato Tashiro, Ryosuke Hamashima, Yuichiro Nakano, Hotaka Namie, Yuya Ito, Tatsuro Hirayama, Kazuaki Takeda, Naoki Iwanaga, Kodai Nishi, Hong Liu, Takahiro Takazono, Takeshi Tanaka, Akira Watanabe, Yoshihiro Komohara, Akitsugu Furumoto, Katsunori Yanagihara, Hiroshi Mukae, Scott G Filler, Koichi Takayama & Koichi Izumikawa

***66. Conserved Regulators of the Septation Initiation Network are required for *Aspergillus fumigatus* Echinocandin Resistance and Virulence**
Harrison Thorn, Xabier Guruceaga, Adela Martin-Vicente, Ashley Nywening, Jinhong Xie, Wenbo Ge, Jarrod Fortwendel

***67. The proteomic response of Aspergillus fumigatus to Amphotericin B (AmB) reveals the involvement of the RTA-like protein RtaA in AmB resistance**
Sophie M. Tröger-Görler, Ammar Abou-Kandil, Annica Pschibul, Thomas Krüger, Maira Rosin, Franziska Schmidt, Parastoo Akbarimoghaddam, Arjun Sakar, Zoltán Cseresnyés, Yana Shadkchan, Thorsten Heinekamp, Markus Gräler, Marc T. Figge, Axel A. Brakhage, Nir Osherov, Olaf Kniemeyer

68. Deciphering the mechanistic basis of tolerance to olorofim in *Aspergillus fumigatus*
Clara Valero, Myles Mcmanus, Jamie Tindale, Ashvatti Anna, Sara Gago, Michael Bromley

69. Synthetic expression system enhances recombinant protein production in *Aspergillus oryzae
Casper R. B. van der Luijt, Vayu Maini Rekdal, Yan Chen, Christopher J. Petzold, Jay D. Keasling, Leonie J. Jahn, Morten O. A. Sommer

70. Exposure to agricultural DHODH inhibitors result in cross-resistance to the novel antifungal olorofim in *A. fumigatus*
Norman van Rhijn, Michael Bottery, Isabelle Storer, Johanna Rhodes, Mike Bromley

***71. Deacetylation by sirtuin E is important for *Aspergillus fumigatus* pathogenesis and virulence**
Natália S Wassano, Jaqueline Gerhardt, Everton P Antoniel, Gabriela B da Silva, Daniel Akiyama, Leandro Xavier Neves, Elton Vasconcelos, Patrícia Alves de Castro, Camila Figueiredo Pinzan, Gustavo H. Goldman, Adriana F. P. Leme, Taicia Pacheco Fill, Nilmar Moretti, Andre R. L. Damasio

***72. Uncovering important transcriptional regulations during conidiation and spore germination**
Pin Wu, Fang Wang, Winnie Weng In Chong, Chris Koon Ho Wong

***73. The sterol C-24 methyltransferase encoding gene, *erg6*, is essential for viability of *Aspergillus* species**
Jinhong Xie, Jeffrey M Rybak, Adela Martin-Vicente, Xabier Guruceaga, Harrison I Thorn, Ashley V Nywening, Wenbo Ge, Josie E Parker, Steven L Kelly, David Rogers, Jarrod R Fortwendel

74. Oryzapsin, orthologs of yeast yapsin in *Aspergillus oryzae*, are involved in ergosterol biosynthesis
Natsuno Shimizu, Tamaki Katagiri, Akira Matsumoto, Yoshihiko Matsuda, Hiroshi Arai, Nobumitsu Sasaki, Keietsu Abe, Toru Katase, Hiroki Ishida, Ken-Ichi Kusumoto, Michio Takeuchi, **Youhei Yamagata**

Presenting Authors

*(*Student presenters in bold)*

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Abstracts

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***1. Diversity and characterization of filamentous fungi isolated from sediments of Basque estuaries**

Ainara Otamendi[1], **Ziortza Agirrezabala Urkia**[2], Carla Perez-Cruz[3], Raquel Liébana[3], Laura Alonso-Sáez[3], Maria Teresa Dueñas[1], Anders Lanzén[4,5], Oier Etxebeste[1]
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Fungi and bacteria within marine ecosystems contribute to ecological balance by playing critical roles in nutrient cycles and by shaping food webs. In this context, marine microbes developed genetic mechanisms to adapt and survive in marine environments and stress conditions such as, *e.g.*, high salt concentrations and nutrient scarcity, or to degrade complex polymeric substrates. These features make marine microorganisms a valuable source for the development of new biotechnological tools. However, marine environments and mainly marine fungi are still underexplored. Research on marine microorganisms is mainly focused on bacteria, with a couple of hundreds of fungal species retrieved from marine environments, despite the fact that the kingdom fungi is composed of millions of species. Here, we focused on the isolation of filamentous fungi, using sediment samples collected in estuaries of the Basque Country, Bay of Biscay. Their phenotypic characterization led to the identification of strains potentially able to grow on minimal culture medium supplemented with recalcitrant algal polysaccharides or to produce secondary metabolites. Two isolates belonging to the order Hypocreales were selected for genome sequencing (Illumina and Nanopore technologies) and analysis: 1) *Marquandomyces marquandii* due to its ability to secrete a yellow pigment described in the literature as urea sorbicillin and 2) *Albophoma yamanashiensis* for its apparent ability to grow in minimal culture medium supplemented with commercial fucoidan. Analysis and comparison of their CAZyme and secondary metabolite gene cluster repertoires with those of other species of the order Hypocreales, in combination with RNA-seq results, suggest that these isolates could be used as a source of new enzymatic activities and secondary metabolites.

2. A Single Step Multi-Copy Integration System Based on Rolling-Circle Replication

Martzel Antsotegi[1], Martí Morera Gomez[1], Zofia Jarczynska[1], Katherina García[1], Vasil D'Ambrosio[2], Jean-Marie Mouillon[2], Uffe H Mortensen[1]
[1]DTU Bioengineering, Technical University of Denmark (DTU), [2]Novozymes R&D, Novozymes

Fungi are often used as cell factories for heterologous production of enzymes and metabolites. One strategy to obtain high yielding strains is to enhance the expression level of the gene(s) responsible for production of the product, which can be achieved by inserting multiple copies of the gene(s) of interest. Typically, this is achieved by transforming NHEJ proficient strains with large amounts of a DNA vector, which randomly integrates in multiple copies at different loci, or more often, into a single locus with copies arranged as direct- and inverted repeats (DRs and IRs). The drawback of this method is that the resulting strains are often unstable and difficult to characterize. We speculated that unstable multi-copy arrays may mostly be due to the presence of IRs as they are known to cause genomic instability; and that arrays which are solely formed by copies arranged as DRs are more stable. To test this idea, we have developed a method that allows an array of DRs to be integrated into a single genomic locus. In our method, the DRs are obtained by Rolling-Circle replication of a circular vector containing a sequence of interest, e.g. a gene, and a sequence matching the desired target site in the genome. Rolling-Circle replication is initiated by a 3' DNA-end liberated from the target site in the genome by a DNA double-strand-break induced by Cas9. After Rolling-Circle replication, the resulting DRs are integrated into the target site as the DNA double-strand-break is sealed by Homology Recombination (HR). Moreover, exploiting the *in-vivo* assembly toolbox developed in our lab[1], we demonstrated that plasmids assembled

from PCR fragments *in-vivo* after transformation can be used for Rolling-Circle replication allowing integration of DRs without any undesired *E. coli* sequences that would normally be part of a plasmid. To demonstrate the potential of the method, we took advantage of our DIVERSIFY gene expression system[2] that provides landing platforms for integration of expression cassettes by Cas9 induced HR. As proof of concept, we have used the method to insert an RFP expression cassette into A*spergillus nidulans*, *Aspergillus oryzae*, and *Aspergillus niger*. In all species, the transformants exhibited a spectrum of copy numbers, but as expected, all copies were integrated as DRs in all arrays examined. Importantly, even for strains with 12 copies of RFP, production levels appear stable in a colony with a diameter of 10 cm.

References:
1. Jarczynska, Z. D. *et al.* A Versatile in Vivo DNA Assembly Toolbox for Fungal Strain Engineering. *ACS Synth Biol* **11**, 3251–3263 (2022).
2. Jarczynska, Z. D. *et al.* DIVERSIFY: A Fungal Multispecies Gene Expression Platform. *ACS Synth Biol* **10**, 579–588 (2021).

3. The transcription factor Ndt80 is a negative regulator of virulence in *Aspergillus fumigatus*

Vijendra Arya[1], Hong Liu[2], Mark A. Stamnes[3], Scott G. Filler[2], William Scott Moye-Rowley[3]
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Aspergillus fumigatus is a ubiquitous filamentous fungus that is the leading cause of life-threatening invasive aspergillosis (IA) in patients. Using an overexpression approach, we identified a new *A. fumigatus* transcription factor that is a negative regulator of virulence. We designated this transcription factor Ndt80 on the basis of its structural similarity to its ortholog in *Candida albicans*. Using an immunosuppressed murine model of IA, we found that mice infected with a strain overproducing Ndt80 from the *gpdA* promoter (*gpdA-ndt80*) were avirulnet compared to mice infected with wild-type parent strain. By contrast, a *ndt80*Δ deletion mutant had wild-type virulence. To investigate the cause of the virulence defect of the *gpdA-ndt80* strain, we investigated its interactions with host cells vitro. These experiments revealed that the *gpdA-ndt80* strain had a 70% reduced capacity to invade pulmonary epithelial cells and caused 40% less damage to these cells, relative to the wild-type strain. By contrast, the *gpdA-ndt80* strain was as resistant as the wild-type strain to killing by bone marrow-derived macrophages. The reduced capacity of *gpdA-ndt80* strain to invade and damage pulmonary epithelial cells likely explains its negative role in mammalian virulence. To determine the suite of genes that are governed by Ndt80, we constructed a *gpdA-ndt80*-3X FLAG strain and performed a chromatin immunoprecipitation-highthroughput sequencing experiment. We found that Ndt80 binds to the promoter regions of more than 700 genes, with the largest number of these genes sharing a function as transcriptional regulators. RNA-seq analysis indicated that overproduction of Ndt80 led to the repression of genes involved in cell wall biogenesis. This effect on expression could help explain the observed defect in pulmonary cell invasion. Together these data provide the first evidence that Ndt80 has a direct role in negatively regulating virulence attributes in *A. fumigatus*.

4. The next dimension of CAZymes - Inferring the functional interplay between fungal carbohydrate-active enzymes for biomass conversion

Kristian Barrett[1,2], Lene Lange[3], Igor Grigoriev[4,5], Anne Meyer[1]
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Fungal biomass degradation is a huge collaboration exercise for the genome encoded enzymes. Particularly the secreted fiber-active enzymes have an incredibly complicated interplay during which some enzymes may have the direct catalytic effect on the polysaccharides whereas other enzymes may only assist in the conversion indirectly as accessory enzymes. Such accessory enzymes have been greatly overlooked, as the effect is not apparent with the enzyme alone but only in combination with one or more other enzymes. The aim of my work is to identify such putative enzymatic combinations, by systematic identification of co-occuring/co-regulated CAZymes through genome comparisons. Hence, unraveling enzyme interplay for fungal biomass conversion while understanding their deployed biological strategies. This method relies on our classification of all CAZy families into functional subgroupings powered by our

webserver CUPP.INFO [1].

[1] Kristian Barrett, Cameron J Hunt, Lene Lange, Igor V Grigoriev & Anne S Meyer. Conserved unique peptide patterns (CUPP) online platform 2.0: implementation of +1000 JGI fungal genomes, Nucleic Acids Research, gkad385 (2023).

5. A multifaceted approach to improving outcomes of cerebral aspergillosis

Martin Kelty[1], Aracely Miron-Ocampo[2], **Sarah Beattie**[1]
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Invasive mold infections are becoming more common with the introduction of novel immunomodulatory therapies and the emergence of new co-morbidities including COVID-19. The most common pathogenic mold is *Aspergillus fumigatus*, which is typically inhaled and causes invasive pulmonary aspergillosis (IPA). In a subset of patients with IPA, *A. fumigatus* disseminates to extrapulmonary organs including the brain, causing cerebral aspergillosis (CA). Once in the brain, *Aspergillus* is difficult to treat as most clinical antifungals do not readily penetrate the blood brain barrier (BBB). Thus, CA is one of the most fatal forms of invasive aspergillosis with mortality rates reaching 80-100%, even with treatment. Despite the high mortality rates of CA, the pathogenesis of *A. fumigatus* in the brain remains largely unexplored.
Our goal is to improve the treatment options and outcomes of this devastating disease using a multifaceted approach. First, we are developing mouse and cell culture models to identify fungal pathways that are essential for dissemination to the brain. We have demonstrated that C5-complement deficient mice develop robust cerebral infection with features of human disease and hallmarks of invasive aspergillosis. Using this model, we have shown that the pH responsive transcription factor, PacC, is required for dissemination to extrapulmonary organs including the brain. Work is ongoing to screen for additional transcription factors and kinases that are required for dissemination to or growth within the brain to elucidate mechanisms of pathogenesis in this niche.
Second, to directly address the need for central nervous system (CNS)-penetrant mold-active antifungals, we developed a luciferase-based, high throughput screening assay to screen directly against *A. fumigatus*. With this platform, we are screening a structurally diverse library of ~150,000 synthetic drug-like compounds, of which ~2/3 are predicted to penetrate the BBB. After screening through about half of this library, we have already been successful in identifying candidate scaffolds with desirable antifungal properties. These compounds are currently under investigation to identify the best candidates for lead development. Together, we hope our approaches will result in improved management of CA through better understanding of the pathogenesis within the brain and with the development of novel antifungals specifically targeted to treat CNS mold infections.

6. Elevated mutation rates in multi-azole resistant *Aspergillus fumigatus* drive the rapid evolution of antifungal resistance

Michael J. Bottery[1*], Norman van Rhijn[1], Harry Chown[1], Johanna L. Rhodes[2], Brandi N. Celia-Sanchez[3], Marin T. Brewer[4], Michelle Momany[3], Matthew C. Fisher[5], Christopher G. Knight[6], and Michael J. Bromley[1*]
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The evolution of antifungal resistance is a global problem. Of particular concern is the widespread emergence of azole resistance within *Aspergillus fumigatus*, a globally prevalent environmental mould that causes around 1 million life-threatening invasive infections in humans. It is becoming increasingly evident that the environmental use of azoles has led to selective sweeps across multiple genomic loci resulting in the rapid expansion of a genetically distinct lineage (clade A) that is resistant to clinically deployed azoles. Strains from this clade are more likely to be cross resistant to agricultural antifungals with unrelated modes of action suggesting they may be adapting rapidly to antifungal challenge. Here we show that this multi-azole resistant lineage is associated with increased mutation rates due to variants in

the mismatch repair system. A variant in *msh6* is found near exclusively within clade A, occurs in 88% of multi-azole resistant isolates harbouring the *cyp51A* azole resistance allelic variant TR_{34}/L98H and is globally distributed. Natural isolates with this variant display 4 to 9-times higher mutation rate. Through the pervasive anthropogenic use of azoles, a lineage of *A. fumigatus* has emerged that is not only multi-azole resistant but also displays increased adaptive capability. Compounding this, ipflufenoquin, a novel agricultural antifungal has been approved for use in crop protection and shares the same mechanism of action as olorofim, a next generation clinical antifungal. We show that ipflufenoquin can select for high-level cross-resistance to olorofim. The dual use of these novel classes of antifungal drugs coupled with increased mutation rates in azole resistant isolates increases the probability of the accumulation of multiple independent resistance mechanisms within clade A to both azole and novel antifungal compounds, which ultimately may lead to the evolution of a lineage with pan-drug resistance.

***7. Deletion of core septin gene *aspB* in *Aspergillus fumigatus* results in fungicidal activity of caspofungin**

Rebecca J Busch[1], Carson Doty[1], Allie Mills[2], Flutur Latifi[1], Vjollca Konjufca[1], Laura Herring[2], José Vargas-Muñiz[1]
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Septins are a family of GTP-binding proteins. Although highly conserved throughout many eukaryotes, their functions vary across species. In *Aspergillus fumigatus*, the etiological agent of invasive aspergillosis, septins participate in a variety of roles such as cell wall organization of conidia, septation, and response to anti-cell wall stress. Previous studies determined that the Δ*aspB* strain had a greater sensitivity to anti-cell wall drugs, especially the echinocandin caspofungin, yet mechanisms behind this augmented sensitivity are unknown. We performed cell viability staining post-caspofungin exposure and found that the Δ*aspA,* Δ*aspB*, and Δ*aspC* strains showed significant reduction in cell viability. Concomitant with the reduced viability, deletion strains are more susceptible to caspofungin on solid media. These results indicate that the septin cytoskeleton is important for *A. fumigatus* survival in the presence of caspofungin. Due to the potential of improved therapeutic outcome, we followed up using a neutropenic murine model of invasive aspergillosis. Deletion of the *aspB* gene resulted in improved survival, reduced pulmonary inflammation, and reduced fungal burden when treated with caspofungin when compared to the *akuB*^{KU80} wild-type or untreated Δ*aspB* strains. Quantitative proteomics analyses were used to find proteins involved in the septin-dependent adaptation to caspofungin. We identified four candidates with roles in cell wall integrity. Deletion of these candidate genes resulted in increase in susceptibility to caspofungin and moderate reduction in viability post-drug exposure. Taken together, these data suggest that septin AspB is essential in mediating the fungistatic response to caspofungin.

8. Breaking down barriers: comprehensive functional analysis of the *Aspergillus niger* chitin synthase repertoire

Timothy Cairns[1], Lars Barthel[1], Sven Duda[1], Henri Müller[2], Sascha Jung[1], Heiko Briesen[2], Vera Meyer[1]
[1]Technical University Berlin, [2]Technical University of Munich

A fungus is a heterotrophic eukaryote encased in a chitin containing cell wall. This polymer is vital for cell wall stiffness and thus cell shape. Systematic functional analysis of the full chitin synthase catalogue in a single species is rare, which limits fundamental understanding and potential applications of manipulating chitin synthesis across the fungal kingdom. We conducted *in silico* profiling and subsequently knocked out all predicted chitin synthase encoding genes in the multipurpose cell factory *Aspergillus niger*. Phylogenetic analysis, transcript profiling, and co-expression network construction revealed distinct evolutionary history and remarkably independent expression, strongly supporting specific role(s) for respective chitin synthases. Deletion mutants confirmed all genes were dispensable for germination, yet impacted colony spore titres, chitin content at hyphal septa, and internal architecture of submerged fungal pellets. We were also able to assign specific roles to individual chitin synthases, including those impacting colony growth rates (ChsE, ChsF), lateral cell wall chitin content (CsmA), chemical genetic interactions with therapeutics (ChsE), and those that modulated pellet diameter in liquid culture (ChsA, ChsB). From an applied perspective, we show *chsF* deletion increases total protein in culture supernatant over 3-fold compared to progenitor controls, indicating controlling filamentous fungal chitin content is a high priority strategy for strain engineering. In summary, we reveal universal and specific functional roles of chitin synthases in *A. niger*, thus improving fundamental understanding and opening new avenues for biotechnological applications in fungi.

***9. A conserved oxylipin alarm blocks the fungicidal effects of echinocandins in pathogenic aspergilli**

Dante G Calise[1], Sung Chul Park[1], Jin Woo Bok[1], Gustavo H Goldman[2], Nancy P Keller[1,3]
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Humans readily inhale spores of the ubiquitous mold *Aspergillus fumigatus*. Small enough to reach the alveoli of the lung, these spores are rapidly cleared by a healthy immune system without development of disease. However, in immunocompromised individuals, germination and tissue invasive hyphal growth can lead to a life-threatening infection termed invasive aspergillosis (IA). The recommended first line treatment of IA is with triazole antifungals, but in cases of poor clinical response to these membrane targeting drugs, salvage therapy with the cell wall active echinocandins is crucial for effective treatment. The echinocandin antifungals—caspofungin, micafungin, and anidulafungin—are limited in that they are only fungistatic against aspergilli due to their inability to kill established hyphae. However, *in vitro,* treatment of *A. fumigatus* with inhibitory concentrations of caspofungin results in the death of approximately fifty percent of germinating conidia by fungicidal lysis of their growing tips. Surviving germlings are further inhibited fungistatically and display severely stunted hyphal growth developing into highly branched chitin rich microcolonies. As they grow, caspofungin treated hyphae continue to undergo tip lysis, but the fungicidal effect is limited to the apical most hyphal compartment by the blocking of septal pores. Our lab recently found that the fungal oxylipin 5,8-diHODE, produced by *A. fumigatus* and related aspergilli, induces hyphal growth reminiscent of echinocandin treatment with increases in lateral branching, septation, and cell wall chitin. Here, we uncover an endogenous mechanism of antifungal tolerance in aspergilli whereby 5,8-diHODE activates echinocandin tolerant growth. We found that treatment of wild type *A. fumigatus* with echinocandins induced robust production of 5,8-diHODE by the enzyme PpoA. Further, we found that cotreatment with 5,8-diHODE blocked the fungicidal lysis of germinating conidia by caspofungin and micafungin. This protection against echinocandin tip lysis was also conserved in the related species *A. flavus* and *A. nidulans*. Lastly, we found that the transcription factor ZfpA was required for both induction of PpoA by caspofungin and full protection by 5,8-diHODE. Together, our findings reveal 5,8-diHODE to be an inducible and protective signal to activate echinocandin tolerant growth programs among pathogenic aspergilli.

10. Leverage of fungal growth data through modelling: applications to the study of antifungal drugs

David Canovas
Genetics, University of Sevilla

Microbial growth analysis serves as a valuable tool for extracting information from various strains growing in a variety of conditions, which is an established knowledge among microbiologists. However, it is common for such analyses to solely focus on the qualitative assessment of the quantitative data obtained experimentally. In order to fill this gap, in this study I tested different mathematical growth models using real experimental datasets. Among the tested models, one function emerged as the most cost-effective in fitting the growth datasets. To validate this model, I employed growth datasets obtained by growing *Aspergillus nidulans* under varying nitrogen concentrations and different inoculum sizes. This model yields four parameters delineating the growth curve characteristics. Altering nitrogen concentration impacted both maximum growth and growth rate, while inoculum size showed an inverse correlation with the inflection time. Once validated, the model was utilized to analyse the growth characteristics of the fungal human pathogen *Aspergillus fumigatus* in response to antifungal drugs. Voriconazole at subinhibitory concentrations primarily reduced the growth rate, leaving other parameters unaffected. Moreover, the Δ*nctA* and Δ*nctB* mutants, which have been reported to have an increased resistance to triazoles, exhibited an increased fitness in growth rate, but not in any other growth paramenter. This effect aligns with the primary effects of subinhibitory concentrations of voriconazole in the wild-type strain observed in this study. The method developed herein facilitates automated characterization of hundreds of samples simultaneously, presenting promising applications in high-throughput antibiotic drug screenings.

***11. Unveiling GRAsp, An Online Tool for the Exploration of Gene Regulatory Networks in *Aspergillus fumigatus* to Gain Insights into Growth, Development, and Pathogenicity**

Cristobal Carrera Carriel[1], Spencer A. Halberg-Spencer[2,3], Saptarshi Pyne[3], Sung Chul Park[4], Hye-Won Seo[4], Aidan Schmidt[4], Dante Calise[4], Sushmita Roy[2], Jean-Michel Ané[5,6], Nancy P. Keller[4,5]
[1]Department of Genetics, University of Wisconsin-Madison, [2]Department of Biostatistics and Medical Informatics, University of Wisconsin-Madison, [3]Wisconsin Institute for Discovery, [4]Medical Microbiology and Immunology, University of Wisconsin-Madison, [5]Department of Bacteriology, University of Wisconsin-Madison, [6]Department of Agronomy, University of Wisconsin-Madison

The notorious mold, *Aspergillus fumigatus*, is responsible for harmful and occasionally lethal respiratory conditions collectively known as aspergillosis. Understanding how genes fit into a regulatory pathway offers us valuable insight into the genetic determinants of this pathogen's growth and development. By leveraging expression datasets of *A. fumigatus*, we developed a comprehensive gene regulatory network we call GRAsp (**G**ene **R**egulation of ***Asp**ergillus fumigatus*). GRAsp successfully recapitulated previously characterized regulatory pathways related to hypoxia response, iron acquisition, and secondary metabolite synthesis. We also experimentally validated one of GRAsp's predictions that the transcription factor AtfA is required for the fungus' response to lipo-chitooligosaccharides, a chitin-based signaling molecule. We further unveil GRAsp as an online and user-friendly resource (grasp.wid.wisc.edu), enabling users to explore regulatory pathways of interest.

***12. A global pangenome of *Aspergillus fumigatus* reveals the origin of azole resistance**

Johanna Rhodes[1,2], **Harry Chown**[2,3], Felicia Stanford[4], Rodrigo Leitao[2], Samuel Hemmings[2], Ali Abdolrasouli[2], Norman van Rhijn[3], Gillian Sigle-Hall[2], Zain Chaudhry[2], Iro Chatzidaki[2], Ben Simmons[2], Chuhan Qin[2], Darius Armstrong-James[2], Paul Verweij[1], Michael Bromley[3], Paul Dyer[4], Matthew Fisher[2]
[1]Radboudumc, [2]Imperial College London, [3]University of Manchester, [4]University of Nottingham

Resistance to antifungal drugs in *Aspergillus fumigatus* infections is on the rise, posing a significant challenge in clinical settings. Despite this, our understanding of the temporal and spatial origins of drug-resistant genotypes remains limited. This study presents an investigation into the genomic dynamics of *A. fumigatus* through the creation of one of the largest fungal pangenomes, making it the largest for this species, to date.
Leveraging a diverse panel of over 1000 globally acquired isolates, we conducted a comprehensive assessment of the species' genomic plasticity. Employing clock-based phylogenetics, we traced the origins of azole drug resistance, shedding light on the anthropogenic drivers leading to resistance emergence. In addition to elucidating the temporal aspects, our analysis uncovered the contribution of accessory genes to novel resistance mechanisms and niche specificity through association tests. Furthermore, we test for evidence of horizontal gene transfer within the population, highlighting the ability of genetic exchange amongst *A. fumigatus*.
This research highlights the intricate interplay between antifungal usage and the development of drug resistance in *A. fumigatus.* Through analysis of a large-scale population pangenome we are able to obtain a nuanced understanding of the genetic repertoire that contributes to the species' resilience and adaptability in the presence of antifungals.

13. Modernizing high-throughput mycology with robotics and artificial intelligence

Johan V Christiansen, Søren D Petersen, Vilhelm K Møller, David Llorente, Parvathy Krishnan, Steen S Brewer, Katharina Steinert, Alexander R Brems, Sabrina M Pittroff, Mathilde Nordgaard, Vincent Wiebach, Niels Bjerg, Lars Jelsbak, Ling Ding, Jakob B Hoof, Jens C Frisvad, Rasmus J N Frandsen
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Filamentous fungi and their secondary metabolites have been proposed as solutions for many global crises, including as biocontrol agents of agricultural pests. However, high throughput functional screening of filamentous fungi is currently challenging due to the large morphological and physiological diversity, and as most mycological experimental methods have not been developed with a high throughput in mind. Therefore, our project has taken on the task of modernizing mycological methods to create a high throughput screening platform through robotic workflows, automatic data processing and machine learning. The goal is to be able to screen the extensive IBT fungal collection with 38,000+ isolates. To achieve this, we are transferring single-vial strain spore stocks to 96-well plates compatible with robotic workflows, such as cultivation on various defined and complex growth media, metabolite extraction, bioactivity assays and taxonomic profiling using genetic barcodes. We are systematically gathering high

resolution mass spectrometry metabolomics data for all isolates to allow for artificial intelligence-based data mining focused on identifying industrial relevant metabolites and predicting which metabolites and species carry bioactivity. For unbiased, fast, and automatic metabolomics data processing and analysis, we have developed a pipeline using state of the art tools wrapped in a Python framework. Additionally, we automated image analysis is used to score the outcome of fungal-fungal interactions and capture basic growth characteristics of the strains. The automated analysis workflow has proven extremely important as our current robotics workflow supports the analysis of 800 strains every two-to-three weeks.
We are currently using this platform to screen IBT isolates for their ability to in vitro inhibit growth of the plant pathogens *Fusarium graminearum* and *Zymoseptoria tritici*. The metabolomics database and taxonomic genotyping data allows for deselection of fungi that produce mycotoxins or are classified as pathogens. The best in vitro performing isolates are continuously tested in planta (green house and field trials) by our commercial partner, FMC. The combined dataset aims to identify fungal isolates that can be used as control agents in agriculture.

14. Genetic and regulatory complexity in fungal primary carbon metabolism

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Fungal primary carbon metabolism has been a topic of study for many decades, but the availability of fungal genome sequences and large omics datasets has provided an unprecedented view on the organization and diversity of this important biological system.
Starting from an *in silico* metabolic model for *Aspergillus niger*, we proceeded with experimental validation using individual and combined gene deletion strains, which resulted in the addition of novel genes to the pathways as well as removal of previously predicted genes. We then explored the reliability of this model when transferred to other species and showed that this can be done within the same phylum (although with reducing confidence when the taxonomic distance got larger), but it becomes questionable when transferred to other phyla.
To further explore the genomic and post-genomic diversity within and between species, we analyzed the presence and regulation of candidate sugar transporters in four ascomycete species and delved deeper into the paralogs of sugar reductases, which are present in several pathways. This demonstrated that the variation at the transcriptomic level is even higher than at the genomic level indicating a highly refined regulatory system that controls primary carbon metabolism, and which involves many transcriptional regulators, of which only the major ones have so far been identified.
Highlights of these studies will be presented.

15. Functional Characterization of a lncRNA in Stress Response and Pathogenesis of *Aspergillus fumigatus

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Aspergillus fumigatus is a saprophytic fungus that can cause a collection of diseases in an immunocompromised population termed aspergillosis; the most severe amongst them is invasive pulmonary aspergillosis (IPA). Azoles are the major classes of antifungal drugs used to treat invasive pulmonary aspergillosis. However, in recent years there has been an increase in fungal resistance to azole drugs exacerbating the problem. In addition, the fungal response to azole drugs is not entirely understood, resulting in poor disease outcomes associated with azole-susceptible strains.
It is becoming increasingly clear that lncRNA-mediated regulation is vital in stress response; however, their roles in fungi are lacking. Here, we have identified a lncRNA *afu-853*, which acts as a regulator of multiple stress response including azole response in *A. fumigatus*. Structural analysis showed that *afu-853* has flexible structure and can take many potential conformations *in vitro*. Thus, we aim to characterize the role(s) of ncRNAs in antifungal drug response and pathogenesis. This study aims to provide a novel genetic link between ncRNAs and stress regulation including azole response in *Aspergillus fumigatus*.

16. The CakA kinase links the cell cycle with secondary metabolism in *Aspergillus nidulans*

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Cell cycle control is indispensable for the growth and development of all organisms, governing many crucial cellular functions including cell proliferation, differentiation, and cell homeostasis.
In *Saccharomyces cerevisiae*, the mitotic and meiotic cell cycles are regulated by Cdc28, the catalytic subunit of the main cyclin-dependent kinase (CDK1). CDK1 is activated by cyclins and the Cyclin-dependent kinase-activating kinase (Cak1). The *CAK1* gene is conserved among fungi and is essential for *S. cerevisiae* (Espinoza *et al*., 1998). Interestingly, the *A. nidulans CAK1* ortholog (*cakA*) is not essential, suggesting the existence of redundant kinase(s) or a different function for CakA. Here, we study the global role(s) of CakA using a proteomic and functional genomics approach. BioID analysis (proximity-dependent biotin identification) showed that CakA interacts with the Cdc7 orthologue (AN3450), which is another essential kinase involved in the regulation of the cell cycle (De Souza *et al*., 2014). Consistent with this observation, transcription profiling analysis of the *cakA*Δ mutant showed that CakA affects expression of genes involving in growth, development, cell cycle and response to stresses. In addition, our result also revealed a role of CakA in regulation of secondary metabolite biosynthesis gene clusters (BGCs). We have found several downstream transcription factors of secondary metabolism upregulated in the *cakA*Δ mutant. To test whether interference with cell cycle progression generally affects secondary metabolism, we inhibited the cell cycle using a genetic approach with the temperature-sensitive mutant alleles of the cell cycle regulators *nimX*^{cdc28} and *bimE*^{apc1} and a pharmacological approach with hydroxyurea or torin 1. Transcriptional and metabolic HPLC MS/MS analysis of cell cycle mutants and HU/Torin1-treated wild type cells revealed drastic changes in SM profiles and associated BGC transcription. Moreover, cyclins are differentially expressed between BGC-silencing and BGC activating conditions and this transcriptional program changes in some tested kinase mutants. Therefore, our work demonstrates that the cell cycle can influence secondary metabolism in *A. nidulans*.

***17. Surprising strain-specific molecular determinants of *Aspergillus fumigatus* pathogenicity revealed by new cancer small molecule therapies**

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Clinical risk factors for diseases caused by *Aspergillus fumigatus* have expanded beyond neutropenia and high-dose corticosteroid therapy. As treatments move toward small molecule drugs that target specific host pathways, novel immune states that facilitate infection have emerged. An example of this precision therapy is the drug ibrutinib (IBT) that inhibits Bruton's tyrosine kinase (BTK) and is used for the treatment of B-cell malignancies like chronic lymphocytic leukemia. Patients receiving IBT are at a higher risk of *A. fumigatus* infection. Surprisingly, reference *A. fumigatus* strains AF293 and CEA10 are not pathogenic in the setting of IBT treatment or genetic BTK-/- deficiency in mice. Rather, only certain strains of *A. fumigatus* are pathogenic in IBT-treated and BTK-deficient mouse models. These data challenge the long-standing paradigm that any *A. fumigatus* strain can cause disease in an immune compromised host. Mechanistic studies of IBT-mediated susceptibility to *A. fumigatus* revealed an unexpected role of p40phox, a component of the neutrophil NADPH oxidase, and RAC2, which regulates NADPH oxidase. IBT treatment thus results in defective production of reactive oxygen species (ROS) that are a crucial aspect of host defense against *A. fumigatus*. We have tested the hypothesis that *A. fumigatus* strain-specific pathogenicity is ROS-mediated. To test this hypothesis, we are defining the genetic network that mediates *A. fumigatus* responses to NADPH oxidase-dependent host defense mechanisms. By utilizing the recently available protein kinase, phosphatase, and transcription factor null mutant collections, we are identifying key regulators of the fungal ROS response. In addition, to define the genetic and phenotypic variants associated with the strain-specific pathogenicity in the setting of BTK inhibition, we are utilizing both whole genome sequencing and ROS-related phenotyping of a unique collection of *A. fumigatus* isolates from patients on IBT. Preliminary results in both a biofilm and germling model suggest that five of seven isolates show reduced susceptibility to hydrogen peroxide. Using these approaches will allow us to define the cause-and-effect relationship between allelic variants in the *A. fumigatus* population

and murine model disease outcomes. Defining these mechanisms is expected to promote new insights into both fungal pathogenicity and host response in specific patient populations.

18. Generation and characterization of serial deletion- and point-mutants within the 5´-UTR region of *brlA* allow the identification of promoter sequences required and dispensable for *Aspergillus nidulans* conidiation

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In the genus *Aspergillus*, the transcription factor BrlA has a central role in the generation of the cell-types that form the conidiophore, multicellular structures each bearing thousands of conidia. Asexual development is induced in response to external and internal cues, and thus, it has been described that several transcriptional activators bind the promoter of *brlA* to determine its expression levels before and during development. Furthermore, transcriptional repressors also bind the 5´-UTR region of *brlA* 1) to inhibit expression at late stages of conidiophore development, and 2) to activate sexual development. In this work, we followed a mutagenesis procedure to generate 1) *brlA::HA$_{3x}$* strains that included serial deletions of the promoter of *brlA* (*brlA^{p}*; approximately 3 Kb from the start codon of *brlAβ*), strains in which the hypothetic binding sites for *brlA^{p}*-binding transcription factors were deleted and 3) strains bearing point mutations in the first 23 codons of *brlAβ* (those that differentiate *brlAβ* from the isoform *brlAα*). None of the strains generated showed the *fluffy* phenotype characteristic of the null-*brlA* mutant and only deletion of the *brlA^{p}* region that includes an upstream open reading frame caused a decrease in conidia production. There results raise new hypotheses on the mechanisms that control *brlA* expression and the role of BrlA.

***19. The *Aspergillus nidulans sarB* gene encodes a putative UDP-N-acetylglucosamine transporter involved in amino acid utilization**

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Nutrient acquisition is an essential and controlled process. In *Aspergillus nidulans*, the transcription factor AreA regulates nitrogen utilization genes. The *areA102* pleiotropic altered function mutation confers increased growth on histidine as a nitrogen source. The suppressor of *areA102* mutants *sarA* and *sarB* suppress this strong growth on histidine. The *sarA* gene (AN2350) is characterized and encodes an L-amino acid oxidase (LAAO), but *sarB* has remained unidentified. *sarB* was mapped to chromosome VII, 0.26 cM from *xprG*. As 1 cM usually represents ~5-10 kbp in *A. nidulans, sarB* was expected to lie within 2.6 kbp of *xprG*. This project aims to identify the *sarB* gene and understand its role in nitrogen acquisition. Nine candidate *sarB* genes adjacent to *xprG* failed to complement the *sarB7* phenotype in transformation experiments, suggesting that *sarB* is physically further away from *xprG* than predicted. We therefore adopted a Whole Genome Sequencing approach. The genomes of the *sarB7* mutant and its parent were sequenced and compared with the reference genome. Three SNPs on chromosome VII were unique to the *sarB7* mutant. The closest SNP to *xprG* was ~118 kbp away in the uncharacterized gene AN8875. AN8875 and *xprG* are close to, but separated by, the centromere, consistent with the larger physical distance than expected. The SNP introduces a stop codon about one-third of the way through the gene, producing a truncated protein. To investigate if AN8875 is *sarB*, the wild-type AN8875 gene was transformed into the *areA102 sarB7* strain and transformants were obtained by direct selection for growth on histidine, indicating that AN8875 complemented the *sarB7* phenotype. To determine the phenotype of complete loss of AN8875, the gene was deleted. In a wild-type background, the AN8875Δ mutation showed no phenotype on histidine, but in an *areA102* background, it phenocopied the *sarB7* phenotype of weak growth on histidine. These experiments provide strong evidence that AN8875 is *sarB*. AN8875 encodes a putative N-acetylglucosamine (GlcNAc) transporter conserved in fungi. Its orthologs in *Saccharomyces cerevisiae* and *Hansenula polymorpha* are involved in cell wall chitin biosynthesis, and the *Kluyveromyces lactis* ortholog is involved in N-glycosylation. Because mutations in *sarA* and *sarB* produce the same phenotype, we hypothesize SarB is necessary for SarA LAAO function.

20. Are the type strains of Aspergillus oryzae and A. sojae truly domesticated?

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Aspergillus oryzae is one of the most important species used in biotechnology, in R&D as transformation host and for industrial enzymes as production host; and in addition, as a production organism in use for production of soy sauce, miso, shoyu and many other foods and beverages. *A. oryzae* is regarded as a domesticated form of the producer of the mycotoxins aflatoxins, cyclopiazonic acid and 3-nitropropionic acid, *A. flavus*. Domesticated forms in the major genera *Aspergillus* and *Penicillium* have rarely been accepted as species, the few examples being *A. oryzae*, *A. sojae*, *Penicillium camemberti* and *Penicillium caseifulvum*. *A. oryzae* has often been separated from *A. flavus* by production of aflatoxins in the latter, and inability to produce aflatoxin in the former. Paradoxically, the ex-type culture of *A. flavus* does not produce aflatoxins, while the ex-type culture of *A. oryzae* can produce aflatoxins. The presently selected ex-neotype of *A. oryzae* produces sclerotia, indicating that the strain originates from nature and that it is not domesticated. A new ex-neotype of *A. oryzae* should thus be selected, one that is genuinely domesticated. We have compared cultures identified as *A. oryzae*, *A. flavus* and *A. aflatoxiformans*, regarding production of specialized metabolites and CAZymes, to find distinguishing characters between those three important taxa. The first genome sequenced strain of *A. oryzae*, RIB40 and an "industrial strain" IFO 4177 are also producing sclerotia, similar to *A. oryzae* as now neo-typified, while real domesticated *A. oryzae* strains do not produce sclerotia, aspergillic acid and aflatoxins. Nearly all strains of *A. flavus sensu stricto* and *A. aflatoxiformans* produce aspergillic acid, and often aflatoxins, while *A. flavus sensu stricto* produces the species-specific compound flavimin. Recently, a similar behavior has been observed also in the ex-type culture of *A. sojae* (CBS 100928) which resulted in detection of aflatoxin B1, also this domesticated species needs further insight and a possible retyping of the original ex type strain.

21. Starship elements drive genome evolution dynamics in a model eukaryotic microbe

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Microbial genomes are colonized by diverse mobile genetic elements (MGEs) whose activities shape their hosts' biology in profound ways. Yet for many of the microbes in which they are found, much remains unknown about the mode and tempo of MGE-mediated evolution. We recently described a new superfamily of fungal MGEs called Starships that are capable of mobilizing host genes but whose impact on their fungal hosts remains unquantified. Here, we investigate the extent to which Starships act as a mechanism of eukaryotic microbe evolution by systematically characterizing their activity and expression in the model organism *Aspergillus fumigatus*. Supplementing a global sample of 509 genomes with 12 newly sequenced long-read isolates, we find that *A. fumigatus* harbors 20 distinct Starships whose presence/absence varies in 154 regions distributed along all 8 chromosomes, including a biosynthetic gene cluster hotspot. At least 4.8% of genes in the *A. fumigatus* pangenome are Starship-mobilized and many are differentially expressed under antifungal- and infection-related conditions. Starships carry diverse molecular functions, including secondary metabolite and biofilm pathways previously known to contribute to stress tolerance and pathogenicity. Together, our results suggest Starship-mediated evolution must be taken into account when investigating ecologically- and clinically-relevant variation in fungi.

22. Screening system based on growth defects due to unscheduled *brlA* expression to identify genes involved in the functional regulation of transcription factors in *Aspergilli*

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Tohoku University

Pathway-specific transcription factors are involved in regulating the production of polysaccharide-degrading enzymes in filamentous fungi including *Aspergillus*, and to date a number of the Zn_2Cys_6 binuclear cluster-type transcription factors unique to fungi have been identified by many studies.

However, much remains to be elucidated about the transporters/sensors for inducing substrates, subsequent signal transduction, and regulation of transcription factor activation.
The aim of this study is to isolate unidentified genes involved in the functional regulation of transcription factors in *Aspergilli*. To this end, we first attempted to find the novel genes involved in the functional regulation of AmyR essential for amylolytic enzyme production as a model. We constructed an *Aspergillus nidulans* strain that overexpressed *brlA*, which is involved in conidiation, under the control of the α-amylase gene promoter, and this strain showed a significantly restricted growth in the presence of isomaltose, an inducer of amylase production. By using this strain as a parent, spontaneous mutant strains that recovered growth were isolated on isomaltose-containing agar medium. Consequently, we successfully identified a putative sugar transporter gene involved in isomaltose transport/sensing through next-generation sequencing of the spontaneous mutants (see Jeong's presentation for details). Further, we are currently applying the screening system to find unidentified genes involved in the functional regulation of the transcription factor XlnR essential for xylolytic enzyme production.

***23. Growth inhibition between filamentous fungal colonies of the same strain and its regulatory mechanism**

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Filamentous fungi form cell population as a colony and interact with adjacent cell populations. In particular, an interaction that inhibits growth between colonies is known as "antagonistic effect". The growth inhibition between different species/strains has been analyzed for a hundred years. A number of mechanisms such as secretion of antibiotic metabolites have been suggested for the growth inhibition. On the other hand, we found growth inhibition between colonies of the same strain in the filamentous fungus *Aspergillus oryzae*. Furthermore, growth inhibition between the same strain was observed in other filamentous fungal species. This phenomenon may reflect a fungal physiological interaction of self/nonself recognition among colonies of the same strain.
Based on RNA-seq and screening of transcription regulatory gene deletion library, we discovered that FlbA and LaeA were involved in the growth inhibition. To elucidate the regulatory mechanism, we first focused on RGS (Regulator of G protein signaling) protein FlbA, which accelerates GTPase activity of G protein α subunit FadA. As *flbA* deletion increases GTP-bound FadA, GTPase-deficient dominant active mutant of *fadA* was predicted to phenocopy Δ*flbA*. As expected, a dominant active FadA mutant did not show growth inhibition, suggesting the involvement of FadA-mediated G protein signaling in the growth inhibition. In addition, LaeA, another factor involved in the growth inhibition, is known to be positioned downstream of FadA-mediated G protein signaling, which regulates transition between vegetative growth and fungal development. Transcriptome analysis revealed that many downregulated genes at the confronted region of colonies overlapped in Δ*flbA* and Δ*laeA*, implying that FlbA and LaeA share a common signaling pathway. We will present the function of whole signaling pathway mediated by FadA G protein in the growth inhibition between colonies of the same strain.

24. Regulation of sexual development by IndB and IndD, the physical interactors of the NsdD GATA factor in *Aspergillus nidulans*.

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Woosuk University

IndB and IndD of *Aspergillus nidulans* are interactors with the NsdD, a GATA type transcription factor, and this interaction appears to regulate the sexual developmental process of this fungus. However, not only has the regulatory mechanism of sexual development by IndB and IndD not yet been confirmed, but the functional domains of the two proteins have also not been characterized to date. We analyzed the intracellular localization of the interaction between NsdD and Ind proteins in *A. nidulans* using the Split-YFP/BiFC method. YFP signals of NsdD-IndB, NsdD-IndD and IndB-IndD were mainly observed in the vesicle domes and metulae of the conidiophore, an asexual reproductive organ, and the fluorescence signals were also observed in the septum and cell wall of hyphae. Additionally, to determine whether the IndB and IndD have a redundant function, we constructed single and double knock-out mutants of *indB* and *indD* and analyzed the characteristics of sexual developmental process. Single mutations of *indB* or *indD* underwent normal sexual development similar to the wild type, but double mutants resulted in uncontrolled formation of mature and immature cleistothecium during sexual development.

Furthermore, the double mutant showed relative sensitivity to the reaction of cell wall lysis enzyme compared to the wild type or single mutants. These results suggest that the IndB/D proteins act as negative regulators in sexual development with redundant role, as well as the possibility that they are related to cell wall integrity signaling of *A. nidulans*.

***25. Developmental Specific Effects of Key Plant Essential Oils against *Aspergillus fumigatus* in Pre- & Post-Infection Plate Models**

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With the rise of drug-resistant fungi, specifically *Aspergillus fumigatus*, alternative treatment research is crucial as this devastating opportunistic pathogen adapts to modern treatments. Currently, Aspergillosis has surpassed other fungal diseases in hospital settings. For instance, Invasive Aspergillosis causes detrimental respiratory effects in immunocompromised patients and severely decreases respiratory function. *A. fumigatus* has demonstrated the ability to thrive under different immunological-defense environments and challenges the clearance and survival of infections. Alarmingly, there is a high incidence of resistance to the limited expensive treatments available, and depending on the patient's biology, toxic side effects have become an issue. Therefore, drug interactions, side effects, and the appearance of resistant strains urge the community to research additional therapeutic agents that can be used against these devastating fungal diseases. In the search for natural and safer resources able to inhibit the growth of *A. fumigatus*, we turned to the analysis and potential of Plant Essential Oils (PEOs) as possible antifungal agents. PEOs' potential as treatment can be a genuine and innovative way to treat fungal diseases. Previously, we analyzed the efficacy of 54 PEOs against *A. fumigatus* compared to the common antifungal Voriconazole by conducting Zone of Inhibition Assays in plate models. Assays were conducted at 37˚C (average human temperature). T-Test analysis of 54 different PEOs showed that 10 PEOs were able to outperform the preferred medical treatment Voriconazole. Here, we present the potential of these key PEOs as antifungals in different fundamental developmental stages: 1) before the spore breaks dormancy, pre-germ tube emergence, and pre-polarity establishment (or "pre-infection"), and 2) after polarity establishment, after fungal hyphal growth and development (or "post-infection"). We were able to identify PEOs that are effective at both developmental stages, in addition to PEOs that show developmental-stage specific inhibition. Here, we are excited to show preliminary results of PEOs as antifungal agents in key growth stages, their effect in the fungal cell wall and nucleus, as well as their ability to be fungistatic or fungicidal. This research opens the possibility of PEOs therapeutic use on their own or synergistically with current antifungals.

Key Words: *Aspergillus fumigatus*, Antifungal Agents, Plant Essential Oils (PEOs), Developmental-Inhibition, Germ Tube Emergence, Polarity

26. Correlation among nuclear increase, enzyme production and hyphal morphology in *Aspergillus oryzae

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Koji molds have long been used for fermentation and brewing due to their high secretion capacity of enzymes that break down carbohydrates, proteins, and other substances. We have discovered a phenomenon in which the number of nuclei of *Aspergillus oryzae* (RIB40) increases with the passage of incubation time. The increase in the number of nuclei is expected to increase the amount of transcription and translation. In fact, we found the correlation between the number of nuclei and enzyme activity. This phenotype was also observed in *Aspergillus sojae*, but not in the closely related *Aspergillus nidulans* or *Aspergillus flavus*. We also compared morphology and growth rates in hypha with and without increased nuclei. Live imaging showed that hypha with increased nuclei appeared by branching, grew very fast, and occupied the colony perimeter with incubation time. TEM imaging showed that hypha with increased nuclei had thicker cell wall and increased mitochondria. We searched for genes involved in the nuclear increase by comparing the genomes of *A. oryzae* strains with different phenotypes within the same clade. In addition, we collected hypha with and without increased nuclei by laser microdissection and analyzed changes in gene expression. In hypha with increased nuclei, the expression of genes

involved in morphology such as the cytoskeleton and cell wall, nuclear and cell division, and Ca^{2+} transport was specifically increased.

27. *Baf* against the wall: elucidating mechanisms of oxygen-driven adaptations in the human fungal pathogen *Aspergillus fumigatus

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The ability to grow during oxygen limitation (hypoxia) is a critical virulence determinant in the human fungal pathogen *Aspergillus fumigatus*. Yet, the mechanisms facilitating fungal growth and viability in hypoxic environments, such as those that arise during infection, remain to be fully defined. An experimental evolution approach, performed in a low oxygen atmosphere to elucidate such mechanisms, surprisingly linked filamentous fungal colony morphology with low oxygen fitness. The evolved, hypoxia-fit strain produced colonies with a broad rim of vegetative growth and furrows radiating from the center of the colony. This hypoxia-adapted morphology was termed H-MORPH. Importantly, H-MORPH isolates are often recovered from aspergillosis patient samples, but the implication of H-MORPH in the prognosis, diagnosis, and treatment of *Aspergillus* infections remains unclear. Both *in vitro*-generated and patient-derived H-MORPH strains are more fit in hypoxia, have altered biofilm architecture, decreased surface adherence, altered cell wall composition, and increased virulence in a murine invasive aspergillosis model. To define the implications of H-MORPH and why it emerges in clinical isolates, we are utilizing a family of recently evolved genes whose expression is sufficient to induce H-MORPH, the biofilm architecture factor (*baf*) genes. However, the mechanisms through which *baf* genes mediate the emergence of H-MORPH is unclear. By defining the genetic pathways that give rise to H-MORPH, we expect to delineate the mechanisms that contribute to the phenotypes that H-MORPH strains exhibit. We are currently conducting co-immunoprecipitation experiments with epitope tagged Baf proteins to identify potential interaction partners. Additionally, we are performing random mutagenesis experiments to identify mutations that suppress H-MORPH, and targeted genetic approaches based on gene candidates identified in clinical H-MORPH isolates to define possible downstream pathways. By defining the genetic pathways that give rise to H-MORPH via *baf* function, we expect to delineate the mechanisms that contribute to the phenotypes H-MORPH strains exhibit and explain the emergence of this morphotype in clinical *A. fumigatus* isolates.

28. Second Alternative Oxidase Genes in Aspergillaceae: Genesis, Loss and Mutations

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Alternative oxidase (Aox) is a terminal oxidase in branched mitochondrial electron transport that provides a non-electrogenic alternative to canonical cytochrome-mediated electron flow, bypassing the proton-pumping complexes III and IV. The consequence of the direct transfer of electrons from ubiquinol to oxygen without concomitant build up of proton motive force is the uncoupling of ATP synthesis via oxidative phosporylation from NADH reoxidation, to allow carbon catabolism to continue unabated even when ATP demand is low or when non-carbon nutrients become limiting. Thus, Aox plays an important role in the energetics of overflow metabolism-based bioprocesses such as *Aspergillus niger* citric acid fermentation and *Aspergillus terreus* itaconate production.
Aox (*aoxA* gene) is near ubiquitous in the fungal kingdom, but coexistence of multiple *aox* genes is rare. However, a second *aox* gene (*aoxB*) is present in some taxa of *Aspergillaceae*. Paralogous genes generally originate from duplication and inherit vertically; we provide evidence for four independent duplication events at different points in evolution that resulted in *aoxB* paralogs in contemporary Aspergilli and Penicillia. The paralog in *A. niger* has a different origin than the paralog in *A. terreus*, while a third independently formed paralog is found in *A. wentii*. All paralogous clades arise from original *aoxA* parent genes but never replace them. Few species have accumulated three co-expressed *aox* genes. Therefore, loss of once acquired paralogs co-determines contemporary *aox* gene content in individual species. For instance, section *Fumigati* has lost all its transient paralogs. In the subgenus *Nidulantes*, we identified seven independent occasions of *aoxB* gene loss and two gains. In *A. calidoustus*, both more ancient *aoxB* paralogs present in the last common ancestor of the subgenus have been substituted by two other *aoxB* genes of completely distinct origins.

We found that the paralogous *aoxB* gene in some 75 genome-sequenced *A. niger* strains features variation at a level not detected for the ubiquitous *aoxA* gene. Five mutations were identified that plausibly affect transcription, function, or terminally modify the gene product. A full-length AoxB is encoded in the acid producer ATCC 1015. Hence, the *A. niger sensu stricto* complex can be subdivided into six taxa according to the resident *aoxB* allele. To date, confident separation could only be accomplished after comparative analyses of whole genome sequences.

29. Transcriptome analysis of manganese(II) ion depletion during high-yield citric acid fermentation in *Aspergillus niger*

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Citric acid (citrate) is amongst the most important bulk products of industrial biotechnology. The discovery of its accumulation by the filamentous fungus *Aspergillus niger* led to the development of a large-scale submerged fermentation process employing stirred tank reactors, which today accounts for 95% of the world's production. High-yield citric acid production requires a combination of unusual nutritional conditions, of which establishing a low (<5 ppb) manganese(II) ion concentration at the onset of the fermentation is a key feature.

Changes in the metabolism of *A. niger* prompted by Mn(II)-deficiency has not been investigated on a functional genomics level so far. In order to get an insight into how Mn(II) deficiency triggers citric acid accumulation, we compared the transcriptome of the citric acid hyperproducer *A. niger* NRRL 2270 strain at three time points (24-hour, 48-hour, and 72-hour) at Mn(II)-deficient (=5 ppb) and Mn(II)-sufficient (=100 ppb) fermentation conditions. This experimental design emanates from our previous observation that the effect of Mn(II) ions on citric acid accumulation is dependent on the cultivation time: it has the strongest effect at the onset of the cultivation and then gradually decreases (Fekete et al., 2022).

Comparison of the transcriptomes of Mn(II)-deficient and Mn(II)-sufficient fermentation conditions revealed that 1436 transcripts are differentially regulated (log2> 2 at p <0.05). Of these, 629 transcripts are upregulated and 807 transcripts are downregulated under Mn(II)-deficient condition. Of the transcripts that displayed differential regulation only at the 24-hour timepoint, 101 transcripts were upregulated and 101 were downregulated. Among the 133 transcripts that were upregulated at all three timepoints under Mn(II)-deficient condition, the majority (= 97) transcripts are predicted to encode secreted or membrane-bound proteins. Notably, *cexA*, encoding the major citrate exporter in *A. niger* (Reinfurt et al., 2023), was upregulated 75-, 15- and 2-fold at the three respective timepoints of cultivation under Mn(II)-deficient condition.

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30. Identification of *A. fumigatus* virulence factors by *in vivo* RNA-seq analysis

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Background. Growth in the mammalian lung exposes *A. fumigatus* to a unique environment with multiple stressors. However, the transcriptional response of this organism during IA is incompletely understood. Our goal was to use a fungal RNA enrichment approach to identify *A. fumigatus* genes that encode GPI-anchored proteins that are highly expressed *in vivo* and determine their role in the host-pathogen interaction and virulence.

Methods. The transcriptional response of *A. fumigatus* Af293 in two mouse models of IA was analyzed using a fungal RNA enrichment technique followed by RNA-seq. Mice were immunosuppressed with cortisone acetate only (non-neutropenic) or cortisone acetate and cyclophosphamide (neutropenic) and then infected with *A. fumigatus*. After 2, 4, and 6 days of infection, the mice were sacrificed and RNA was

isolated from the lungs for RNA-seq. Gene deletion mutants were constructed using CRISPR-Cas9 and tested for virulence in mice.
Results. The *A. fumigatus* transcriptomes in both neutropenic and non-neutropenic mice were very similar, with an R^2 value of 0.79. Gene Ontology enrichment analysis of these transcriptomes indicated that 138 genes involved in oxidative stress, pH response, lipid metabolic process, and ion homeostasis were highly expressed in neutropenic mice; 339 genes involved in nitrogen metabolic process were highly expressed in non-neutropenic mice. Of the 86 genes in the *A. fumigatus* genome that are annotated as encoding GPI-anchored proteins, 18 were found to be highly expressed during IA. From these 18 genes, 7 were selected for mutant construction and virulence testing. Two of the 7 mutants, Δ*gapA* and Δ*gapB,* had significantly attenuated virulence in non-neutropenic mice. Because the Δ*gapA* mutant had the greatest virulence defect, it was selected for further study. *In vitro*, the Δ*gapA* mutant had impaired invasion of A549 alveolar epithelial cells and HSAEC1-KT small airway epithelial cells. It also had increased susceptibility to BMDM killing, but wild type susceptibility to H_2O_2 and menadione.
Conclusions. Growth in the mammalian lung induces a unique transcriptional response in *A. fumigatus*. Although the fungal transcriptional responses in both neutropenic and non-neutropenic immunosuppressed mice are generally similar, growth in neutropenic mice induces a more diverse stress response relative to growth in non-neutropenic mice. A subset of genes predicted to encode GPI-anchored proteins are highly expressed *in vivo* and are likely to function in virulence. Of these genes, *gapA* encodes a virulence factor that is required for maximal *A. fumigatus* invasion of pulmonary epithelial cells and resistance to macrophage killing. Thus, determining the *in vivo* transcriptome of *A. fumigatus* yields novel insights into pathogenicity.

31. The histone deacetylase HosA regulates host cell interactions, resistance to intracellular oxidative stress, and virulence in *A. fumigatus*
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Background. Invasive aspergillosis is a leading cause of morbidity and mortality in immunosuppressed patients. Epigenetic modifications in *A. fumigatus* can be induced by environmental changes and stresses such as those induced by interaction with host cells. However, very little is known about the role of epigenetics in the pathogenicity of *A. fumigatus*. *hosA* (Afu2g03810) encodes a class 1 histone deacetylase. Our aim was to investigate the role of HosA in the host cell interactions and virulence of *A. fumigatus*.
Methods. Δ*hosA* deletion and Δ*hosA*+*hosA* complemented strains were constructed in *A. fumigatus* Af293. Growth and conidiation were investigated in Sabouraud dextrose agar plates. Susceptibility to Congo red, calcofluor white, H_2O_2, menadione, and protamine was determined in organisms grown on Aspergillus Minimum Medium (AMM) plates. The capacity of these strains to adhere to, invade, and damage A549 alveolar epithelial cells and HSAEC1-KT (HSAE) small airway epithelial cells was analyzed. The virulence of the various strains in triamcinolone immunosuppressed mice was evaluated by mouse survival and pulmonary fungal burden. The host inflammatory response was measured by Luminex multiplex cytokine array. For RNA-seq analysis, *A. fumigatus* strains were cultured in AMM with low zinc and low iron.
Results. The Δ*hosA* mutant had normal growth, morphology and conidiation when grown on Sabouraud dextrose agar media, but had a mild growth defect on AMM. The mutant had wild-type susceptibility to Congo red, calcofluor white, H_2O_2, and protamine. However, the Δ*hosA* mutant was sensitive to menadione, suggesting that HosA may induce resistance to intracellular oxidative stress. Although germlings of the Δ*hosA* mutant had normal adherence to A549 and HSAEC1-KT cells, they had 85% less invasion of A549 cells and 49% less invasion of HSAE cells. They also caused 55% and 25% less cell damage to A549 cells and HSAE cells, respectively. Mice infected with the Δ*hosA* mutant had significant reduced mortality compared to mice infected with the wild type or Δ*hosA*+*hosA* complemented strains. Surprisingly, the pulmonary fungal burden of mice infected with the Δ*hosA* mutant was similar to that of mice infected with the wild-type strain. Infection with the Δ*hosA* mutant induced lower levels of CXCL1, CXCL2, CCL2, IL-1α, and IL-6 in the lungs relative to the wild-type strain. RNA-seq data suggested that HosA may govern toxin production or antigen expression on the cell surface, which stimulates excessive inflammation and leads to increased host tissue damage.

Conclusions. The HosA histone deacetylase governs *A. fumigatus* pathogenicity. It is required for resistance to intracellular oxidative stress and for maximal invasion of and damage to pulmonary epithelial cells *in vitro*. HosA also contributes to virulence by inducing a pathogenic inflammatory response during invasive aspergillosis *in vivo*.

32. An essential telomere binding protein regulating the transition from primary to secondary metabolism in *Aspergillus nidulans

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Fungi can produce a wide range of diverse secondary metabolites (SMs). These SMs are synthesized by specialized enzymes encoded by genes that are often organized into clusters located near the telomeres of chromosomes. The sub-telomeric arrangement is believed to facilitate gene silencing, preventing secondary metabolism during the active growth stage when the cell primarily focuses on primary metabolism for energy and macromolecule biosynthesis. Under nutrient limitation or during the stationary growth phase, the cell undergoes a transition from primary to secondary metabolism, producing SMs that are crucial for fungal survival in specific environmental niches. Although various global regulators of secondary metabolism and SM cluster-specific transcription factors have been identified, the molecular mechanism underlying the coordination between primary and secondary metabolisms remains unclear. In this study, we have identified a hitherto uncharacterized protein that binds to telomeres as well as numerous secondary metabolism genes of different SM biosynthetic gene clusters in *Aspergillus nidulans*. Transcription profiling analysis reveals the protein's function as a transcriptional activator for the secondary metabolism genes. Interestingly, this protein also negatively regulates gene functions that are essential for the active growth stage, such as primary carbon and nitrogen metabolism, energy production, and nucleotide metabolism. Based on these findings, we propose that the telomere binding protein acts as a molecular switch, regulating the transition from primary to secondary metabolism.

***33. Predicting culture conditions for secondary metabolite production based on binding targets of biosynthetic gene cluster-specific transcription factors**

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Fungi possess remarkable capabilities for synthesizing diverse secondary metabolites (SMs) with significant application potential in medicine, agriculture, and industry. SM biosynthetic genes are often organized in clusters on fungal genomes, where each cluster is responsible for synthesizing a specific SM. Genome sequencing has identified many novel SM biosynthetic gene clusters (BGCs) in fungi, representing a rich resource for drug discovery. However, most SM BGCs remain transcriptionally silent under standard laboratory conditions, presenting challenges for their identification. In many BGCs, a transcription factor gene is embedded within the cluster, playing a crucial role in activating other biosynthetic genes within the same cluster. However, whether these cluster-specific transcription factors can bind and regulate genes outside their cluster is not clear. We hypothesize that BGC transcription factors may regulate non-BGC genes with functions essential or favorable for the biosynthesis of the intended SM, such as metabolic reprogramming to produce necessary metabolic precursors, response to specific environmental cues, or physiologies specific for developmental stages. If this were true, it would be possible to deduce the conditions favorable for SM production based on the genome-wide targets of the BGC transcription factor for a given SM. To demonstrate this, we conducted Chromatin Immuno-Precipitation followed by Sequencing (ChIP-Seq) to map the genome-wide binding sites of AflR – the well-studied transcription factor of sterigmatocystin (ST) biosynthesis in *Aspergillus nidulans*. Our results revealed extensive AflR binding to genomic regions beyond the ST BGC, exerting control over numerous physiological processes. More importantly, we successfully devised specific growth conditions that promote ST production based on the AflR binding target information. Taken together, this work provides valuable insights into the regulation of ST and introduces a novel approach to activate cryptic SM BGCs, enabling the discovery of novel secondary metabolites.

34. Functional *in vitro* and physiological *in vivo* characterization of five new xylose transporters of *Aspergillus niger*

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Plant biomass degrading fungi are important organisms for bio-based economy. Fungi can convert a pentose sugar D-xylose, abundantly present in plant biomass, through the pentose catabolic pathway (PCP). Recently, an extensive *in silico* identification showed that *Aspergillus niger* has a wide array of putative xylose transporters[1] of which only two, XltA and XltB, have been functionally characterized in *Saccharomyces cerevisiae*[2].

To characterize the *in vitro* functional properties of five of the *in silico* identified *A. niger* xylose transporter candidates, we heterologously expressed them in the yeast *S. cerevisiae* devoid of all hexose and disaccharide transporters, and disaccharide hydrolases[3]. *A. niger* XltA was included as a control. The growth of the recombinant yeast strains was analysed on different sugars both by spot assays on agar medium and in liquid cultures. All five transporters and XltA were able to uptake xylose when endogenous xylose pathway in *S. cerevisiae* was induced with a low glucose concentration. Two out of the five new transporter candidates were also able to uptake hexoses.

In addition, we characterized the xylose transporters *in vitro* in *A. niger* using deletion mutant strains generated by CRISPR/Cas9 method. The deletion strains were analyzed for their ability to uptake xylose through growth and sugar consumption experiments. To determine whether the xylose transporter deletions induce the expression of additional putative xylose transporter genes in *A. niger*, the deletion mutants were also assayed with qPCR. Highlights of these experiments will be presented. Our ultimate aim is to connect the xylose uptake from the exogeneous environment to endogenous processes, to provide a more comprehensive view of plant biomass conversion by *A. niger* and thus advance the transition from our current fossil-based economy to a bio-based economy.

[1]Xu *et al. Bioresource Technology* 391: 130006 (2024)
[2] Sloothaak *et al. Biotechnology for Biofuels and Bioproducts* 9: 148 (2016)
[3]de Valk SC *et al. Biotechnoogy for Biofuels and Bioproducts* 15: 47 (2022)

35. Diversity and functional characterization of filamentous fungal sugar transportomes

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Filamentous fungi play a crucial role in the degradation and modification of plant biomass, contributing significantly to terrestrial carbon cycling. Their abilities are also widely exploited in biotechnological applications. One of the key aspects of the fungal plant biomass conversion process is the uptake of the biomass-derived small sugar compounds into the fungal cells where they are metabolized as carbon and energy sources. Surprisingly, the knowledge of filamentous fungal sugar transporters (STs) is limited, despite of their significant biological role and biotechnological potential as targets of genetic engineering to improve fungal biomass conversion.

To shed more light on filamentous fungal sugar transportomes, we analyzed the genomic and transcriptomic diversity of four ascomycete fungi, i.e., *Aspergillus niger*, *Aspergillus nidulans*, *Penicillium subrubescens* and *Trichoderma reesei*. Phylogenetic analysis divided the predicted STs into ten subfamilies to which putative sugar specificities were assigned based on the available functional data. Interestingly, the STs within each of the subfamilies showed diverse expression profiles on a broad set of monosaccharides even for orthologs of different fungal species. This suggests the existence of a sophisticated regulatory mechanism for sugar uptake in filamentous fungi.

To systematically investigate the overall sugar transport ability of a filamentous fungus, we are characterizing the identified 90 candidate STs of *A. niger* both physiologically and biochemically. Determination of the *in vivo* roles of the transporters is facilitated by *A. niger* ST deletion strains, whereas their *in vitro* functions are studied in *Saccharomyces cerevisiae*. The comprehensive data aims not only to provide information of the role of individual STs in plant biomass conversion by *A. niger*, but also identify novel candidate genes for engineering of industrial fungi at the level of sugar transport. Highlights of these studies will be presented.

36. Fungal Genetics Stock Center: A Status Report

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The Fungal Genetics Stock Center has expanded from 400 strains in 1960 to well over 90,000 strains from 40 species and almost a thousand plasmids. Our project with the K-State library to make all of the FGSC strain deposit sheets available through an on-line archive is nearly complete and will enable researchers to have access to strain details previously available only to FGSC staff. The most recent species addition is *Candida auris*, an emerging health risk. Orders are for about 500 strains per year and the revenue generated funds the salary for a half-time technician and operating supplies.
With Kevin McCluskey's departure, FGSC now has a new curator, Dr. Jaideep Mallick, who took up the position in the 4th quarter of 2022. Support for the curator is partially from K-State, a grant from Open Philanthropy, and a DoD grant that began in October 2022 and has a five-year term. The DoD project focuses on *Agrobacterium tumefaciens* and plasmids that can be used with it to transform fungi with genes that can be used to degrade wastes found in post-military environmental settings. The goal is to optimize transformation protocols that use *A. tumefaciens* for fungal transformations and to make strains, plasmids and protocols readily available to the fungal research community. Please continue to send your materials (and deposit sheets!) to us so that we can expand the collection further and keep it relevant!

37. The putative translational repressor, SsdA, partially regulates carbon source-dependent roles of CotA signaling in *Aspergillus fumigatus*

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Aspergillus fumigatus SsdA is a putative translational repressor regulating growth, cell wall integrity, and virulence. Orthologs of SsdA are known to be negatively regulated by the conserved Nuclear Dbf2-Related (NDR) kinases Cbk1 and COT-1 in *Saccharomyces cerevisiae* and *Neurospora crassa*, respectively. We previously identified the *A. fumigatus* Cbk1 ortholog, CotA, as an important virulence component in mouse models of invasive aspergillosis. Although the underlying virulence mechanism is unknown, we found CotA to regulate invasive hyphal growth in response to host-relevant carbon sources. Strains carrying *cotA* gene disruptions (*cotA*-1) fail to grow on non-sugar carbon sources that are readily available in the host lung environment, such as acetate and amino acids. The main objective of this work was to determine if CotA orchestrates growth in non-preferred carbon sources through the conserved downstream effector, SsdA.
In vitro media supplementation assays showed that loss of *ssdA* in the wild type strain (CEA10) (Δ*ssdA*) caused significant growth defects in both repressing and de-repressing conditions, reducing colony diameter of mature cultures by 30-50% depending on the carbon source. In contrast, deletion of *ssdA* in the *cotA*-1 disruption mutant (Δ*ssdA*/*cotA*-1) caused only a minor growth reduction in the presence of glucose. Strikingly, when compared to the parental *cotA*-1 disruption mutant, this double mutation resulted in significant growth recovery in non-sugar carbon sources. These results correlated with biomass accumulation observations, where the Δ*ssdA*/*cotA*-1 mutant displayed a significant increase in biomass in comparison to the *cotA*-1 parental strain under similar conditions. Therefore, loss of *ssdA* partially restored growth to the *cotA*-1 mutant in alternative carbon sources. As it is a putative translational repressor, we next sought to test if SsdA might function in a feedback loop to regulate CotA protein abundance in response to carbon source. In the wild type, we observed that culture in acetate resulted in only mild reductions in CotA protein abundance when compared to glucose culture conditions. Loss of *ssdA* did not impact CotA abundance in glucose, when compared to the wild type. In contrast, we observed a significant reduction of CotA abundance in Δ*ssdA* under acetate versus glucose culture. Together, our findings support the hypothesis that the conserved translational repressor, SsdA, operates downstream of CotA to partially regulate the carbon source-dependent roles of CotA signaling in *A. fumigatus*. However, this regulatory mechanism is unlikely to be through direct feedback regulation of carbon source-responsive CotA translation.

38. Role of fungal transglutaminase domain-containing proteins in wound-related hyphal protection at the septal pore

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Filamentous fungi under the multicellular subdivision, Pezizomycotina of Ascomycota, possess a primitive morphological structure known as the septal pore, which mediates cell-to-cell connectivity between flanking cells. An interconnected array of cells in fungal hyphae are highly vulnerable to the risk of excessive cytoplasmic bleeding via the septal pore upon hyphal wounding. The Woronin body is a fungal-specific organelle that plugs the septal pore upon hyphal wounding, thereby protecting the flanking cells from excessive cytoplasmic loss via septal pores. We previously identified a series of septal pore plugging (SPP) proteins[1)], one of which, SppB, contains the transglutaminase domain. Transglutaminase, an enzyme that crosslinks substrates via the isopeptide bond formation, is known to participate in blood clotting and wound healing in humans, but the related functions of microbial transglutaminases are unknown.

In this study, we performed a functional characterization of the transglutaminase domain-containing proteins in the filamentous fungus *Aspergillus oryzae*[2)]. Here, the cytokinesis-related protein Cyk3 and peptide *N*-glycanase Png1 were also analyzed as the transglutaminase domain-containing proteins. SppB and AoPng1 accumulated at the septal pore upon wounding, whereas AoCyk3 and AoPng1 normally localized around the septal pore. All these proteins exhibited functional importance in wound-related hyphal protection at the septal pore. The putative catalytic triads of SppB and AoCyk3 were involved in the septal pore-related functions. Similar to typical transglutaminases, SppB was cleaved in response to wounding to remove the N-terminal region, which is required for its hyphal protective function. Finally, using a fluorescent-labeled artificial substrate, transglutaminase activity was detected *in vivo* at the septal pore of wounded hyphae, which functionally involves SppB and its putative catalytic triad. Our study suggests a conserved role for transglutaminase domain-containing proteins in wound-related cellular protection in fungi, similar to humans.

1) Mamun *et al.* (2023) *Nat. Commun.* 14:1418.
2) Mamun and Maruyama (2023) *Mol. Biol. Cell* 34:ar127.

39. FACS-based method streamlines pooled transformations in *Aspergillus oryzae*

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Through precision fermentation, we use genetically engineered microbes including *Aspergillus oryzae* to produce enzymes, proteins, or other compounds in a controlled, sustainable, and animal-free manner. If we can make more protein/enzymes production from our production organisms for the same amount of input costs, we can make our biosolutions increasing cost competitive. Therefore, we often look for ways to improve the productivity of our production strains. Signal peptides are an important contributor to the secretion potential of a candidate protein and a possible route to increasing production strain performance. Here we discuss the how we tested a library of 100+ signal peptides and developed a high throughput method for pooled transformation, FACS (Fluorescence-activated cell sorting), and automated screening to rank *Aspergillus oryzae* strains based on productivity.

***40. Is there localized mRNA translation at the hyphal tip?**

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Hyphal growth is driven by vesicle fusion at the cell tip. In many fungal species, a vesicle organizing center called the Spitzenkörper (SPK) forms at the cell tip. Electron micrographs show ribosomes at the base of the SPK. However, the molecular components of the SPK remain largely uncharacterized. The possible regulation of local protein translation at the SPK and hyphal cell tip has not been yet investigated.

Using the model mold *Aspergillus nidulans*, we show that the RNA-binding protein SsdA travels towards the hyphal tip on microtubules. SsdA is the ortholog of *S. cerevisiae* translational-repressor protein Ssd1 and *N. crassa* GUL-1. Ssd1 recognizes a conserved RNA motif. The motif is enriched on genes encoding cell wall proteins which localize at the hyphal tip. Overall, SsdA could be part of a greater system that regulates local protein production at the hyphal tip.

We also report a straightforward CRISPR-Cas9 system for scarless genetic engineering of *Aspergillus nidulans* and *Aspergillus* codon-optimized latest-generation fluorescent protein tags.

41. High-throughput CAZyme production in *Aspergillus oryzae

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To support the transition to a green and sustainable economy, novel industrial enzymes are necessary. Enzymes of particular interest in this context are Carbohydrate-Active EnZymes (CAZymes) where novel CAZymes targeting specific substrates are in great demand to address specific challenges in industrial applications. Fortunately, eukaryotic genome sequences show that there is a vast repertoire of uncharacterized CAZyme genes, which may deliver the desired activities. As our understanding of enzyme structure-function relationships advances, the engineering and discovery of new CAZymes hold immense potential for driving progress in various sectors of the bioeconomy, but for many of the novel genes it is still difficult to predict their function and to elucidate their substrate specificity, therefore it is necessary to characterize their gene products *in vitro*.

A successful strategy has been to heterologously produce the new enzymes in bacterial hosts followed by purification and characterization. However, this strategy has been less successful for the analysis of eukaryotic genes where enzyme yields have been low or absent. We hypothesize that a strategy for elucidating eukaryotic CAZymes based on fungal cell factories will result in a higher success rate, since fungi are better suited for eukaryotic enzymes, due to a dedicated secretory pathway that offers folding control and post translational modifications.

So far, this strategy has been hampered by the lack of specific tools for high-throughput strain engineering; and the goal of this project is to establish an automated setup that allows heterologous expression and characterization of new CAZyme genes. This system allows for mid-throughput when performed by hand, and high-throughput by using different liquid handlers (i.e. Tecan and Opentrons) and robotic equipment (i.e. QPix). To date, we have validated the method with a subset of 22 targets. We aim at automating all the fungal genetic engineering steps, which will allow us to express, produce and purify any desired target of enzymes in a high-throughput manner, with a vision to cover unexplored territory in the eukaryotic CAZyme map for novel enzyme discovery.

42. Chromatin structural changes alter *cyp51A* expression in TR34-containing mutant strains of *Aspergillus fumigatus*

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Aspergillus fumigatus is the primary human filamentous fungal pathogen. Disease associated with this organism is complicated by the increasing incidence of resistance to the primary antifungal drugs used to treat aspergillosis, the azole compounds. Aspergillosis associated with azole resistance organisms has a mortality of ~30%, even with the best standard of care. The principal route to azole resistance involves the duplication of a short element in the *cyp51A* promoter region, either 34 or 46 bp. Together, these duplications are present in as many as 80% of resistant clinical isolates. These alleles are referred to as TR34 (tandem repeat of 34 bp) or TR46. The presence of TR34 or TR46 has been shown by several labs to drive elevated transcription of *cyp51A* and is required for the observed increase in azole resistance. We have found that the transcription factor AtrR binds to a short element contained in the 34 bp repeat called the AtrR response element (ATRE) and is required for function of this 34 bp region, both as a single copy and in the TR34 context. To identify factors that work with AtrR to control expression of *cyp51A* and other ATRE-containing target genes, we used a biochemical approach to identify proteins that co-purify with a tandem affinity purification (TAP)-tagged form of AtrR (AtrR-TAP). Mass spectrometric analysis of these co-purifying factors identified several as chromatin remodeling proteins including the Arp4 protein, Ash1 histone methyltransferase, Ngg1 (component of the SAGA histone acetylase complex) and a RSC complex subunit (RscE). The abundance of these chromatin remodeling factors led us to examine the chromatin structure of both TR34 and TR46 versions of the *cyp51A* promoter using Assay for transposase-accessible chromatin (ATAC)-seq. We found that the presence of either the TR34 or TR46 duplication in the *cyp51A* promoter was sufficient to lead to an

increase in chromatin accessibility for this gene, even in the absence of the known azole drug induction seen for *cyp51A*. Our data argue that the duplication of a small region in the *cyp51A* promoter, either 34 or 46 bp, is sufficient to trigger increased accessibility to this critical region, with subsequent transcriptional induction of the *cyp51A* gene and accompanying azole resistance.

***43. Study on environmental responses and peptidase genes transcriptional regulation in *Aspergillus oryzae* PrtR**

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Aspergillus oryzae is used as an enzyme source for producing traditional Japanese fermented foods. *A. oryzae* has a strong ability to secrete many hydrolytic enzymes, mainly amylases and peptidases. Although the regulation of amylase production has been well studied, little is known about the regulation of peptidase production. It is thought that *A. oryzae* would recognize the external environment and produce peptidases as needed. However, the mechanism from recognition of external environmental signals to peptidase production is not clear. If this signaling network is elucidated, it may be possible to produce peptidases selectively from many peptidase genes in *A. oryzae*. In this study, we focused on PrtR, an ortholog of the transcription factor PrtT, which positively regulates the transcription of extracellular peptidase genes in some *Aspergillus.* We aimed to elucidate the role of PrtR in the network from the recognition of environmental signals to peptidase production.
First, we identified peptidase genes regulated by PrtR using *prtR* gene-deficient strain. It was shown that PrtR was involved in the transcription of almost all extracellular peptidase genes. Furthermore, PrtR optimizes transcription of peptidase genes in response to culture conditions.
Next, localization analysis was performed using GFP-PrtR-expressing strain. The results showed that PrtR localized to the nucleus when protein was used as a nitrogen source. Meanwhile, PrtR was generally localized to the cytoplasm when NH_4Cl was used. This result was consistent with the *prtR* mRNA levels when cultured with each nitrogen sources. This suggests that PrtR is controlled in mRNA levels depending on the nitrogen source. It was also shown that the excess amount of *prtR* mRNA was degraded depending on the nitrogen source.
Furthermore, LC-MS/MS analysis identified the phosphorylated amino acids of PrtR. Transcription of the peptidase gene was also enhanced in PrtR with these amino acid replaced with alanine. This suggested that PrtR would be activated form in the dephosphorylated state.
In summary, *A. oryzae* recognizes environmental nitrogen sources and would dephosphorylate PrtR to the activated state. The activated PrtR localizes to the nucleus and optimizes peptidase gene transcription in response to the nitrogen source. PrtR, itself would be also regulated at the mRNA level in response to the nitrogen source.

44. Morphotype-specific fungal factors drive uptake and clearance of *Aspergillus fumigatus* by airway epithelial cells

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Aspergillus fumigatus (*Af*) affects over 3,000,000 individuals annually, with invasive aspergillosis having mortality rates of over 50%. Airway epithelial cells (AECs), which cover the entire alveolar surface and comprise 24% of all cells in the human lung parenchyma, have instant, extensive, and likely prolonged contact with *Af* conidia upon inhalation. Recent evidence from our lab demonstrates that AECs provide a potent means of antifungal defense against *Af in vivo*, and that dysfunctional epithelial antifungal activity in at-risk patients may provide an opportunity for *Af* to exploit AECs as a safe haven to reside intracellularly. Relatively little is known about the fungal and host factors controlling *Af* uptake and clearance by AECs and the dependency of these processes on the morphotype-specific changes associated with fungal germination. To characterize how morphotype-specific fungal factors shape AEC-*Af* interactions, we locked *Af* into specific morphotypes using fluorescent auxotrophic *pyrG*$^-$ strains, evaluated internalization using imaging flow cytometry, and determined that swollen conidia, locked at 3 and 6 hours, are 2-fold more readily internalized than resting conidia locked at 0 hours. Using a

combination of fluorescent lectins and cell wall mutants, we are now systematically evaluating morphotype-specific factors on *Af* surface for their role in mediating fungal uptake and clearance by AECs and determined that surface mannose likely dictates these interactions. Supporting this, mannose and the mannose-binding lectin Concanavalin A were able to reduce (by 88%) and abolish (100%) *Af* internalization, respectively. Through the evaluation of candidate receptors, we have identified a receptor (Rc1) with known mannose binding affinity, as a key receptor in these interactions. When Rc1 is knocked out, there is a 68% decrease in *Af* internalization. Our work is now focused on systematically evaluating the role of Rc1 and related polymorphisms in Af-AEC interactions, and their importance in disease. Understanding how AECs contribute to antifungal clearance could provide novel avenues for the prevention and treatment of fungal diseases.

***45. Exploring the role of alpha-1,3-glucan synthases on fungal cell wall integrity in Aspergillus niger**

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Alpha-glucan synthases (ags) play a key role in the synthesis of alpha-1,3-glucan, a crucial component of the fungal cell wall that is (i) contributing to its structural integrity and is (ii) involved in cross-linking of different polymers, so that it influences the composition of both: the outer shell and inner membrane of fungal cells. In the filamentous fungi Aspergillus niger five ags genes are annotated, of which agsA and agsE were shown to be the most highly expressed genes during different stages of development. In addition, an agsE deletion mutant caused smaller micro-colonies as well as a shift in the secretome composition of A. niger, without affecting biomass production. Concurrently, intracellular cross-linking between chitin and beta-glucan is primarily mediated by the seven-membered cell wall-related transglycosylase gene family (crh). Although an impact on cell wall integrity has been expected when deleting the entire crh gene cluster in A. niger, significant alterations in cell wall integrity became only evident when the crh gene cluster deletion was combined with the reduction of alpha-glucan and galactomannan by deleting respective agsE or ugmA. With this background, we aimed to further explore the impact of ags gene deletions – both individually and as an entire family – on fungal cell wall integrity of A. niger. Using targeted CRISPR/Cas9 technology, we engineered various ags-deficient strains, including the deletion of the entire gene family (ΔagsA-E) in a mutant strain lacking all chitin-glucan cross-linking enzymes (Δcrh, TLF39). Subsequent morphological and biochemical characterization of these mutants pinpoint the importance of agsE for maintaining cell wall stability and suggesting its potential influence on protein production and/or secretion. These findings therefor provide not only new insights into fungal biology but also potential targets for biotechnological applications.

***46. Deciphering the Regulatory Mechanisms Governing Recombinant Protein Secretion in Filamentous Fungi**

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University of Campinas

Filamentous fungi represent promising hosts for the production of industrially important enzymes, owing to their robust secretory machinery and capacity to yield complex proteins at high levels. These attributes are particularly significant in producing biofuels and various other bioproducts. However, despite their exceptional capacity for protein secretion, there is still room for improvement in recombinant protein production. Thus, comprehending the intricate regulatory mechanisms that underlie the secretion of these proteins is paramount for enhancing its biotechnological applications. In this context, we propose a systems biology approach, involving the analysis of high-throughput data from various fungi, such as A. nidulans, A. oryzae, and A. niger, all of which overexpressing recombinant enzymes. Our systematic approach entailed the filtration of up- and down-regulated genes, followed by the selection of their respective promoter regions for thorough motif analysis. Subsequently, these promoter regions were analyzed to identify specific binding motifs, from which the top ten candidate motifs for each fungal species were selected. To gain further insights, we compared these identified motifs with a collection of known motifs from yeast, facilitating the identification of corresponding transcription factors in S. cerevisiae. Finally, we obtained the transcription factors from the analysis in S. cerevisiae and conducted

alignments against the A. nidulans database to search for potential orthologs. Through this intricate process, we successfully identified five common transcription factors in the three Aspergillus species, showcasing significant potential to influence recombinant protein production positively. Subsequent genetic manipulation of these transcription factors resulted in a substantial 2.8-fold and 2.6-fold increase in the secretion of recombinant enzymes from two distinct strains of A. nidulans. Through these investigations, we aim to enhance our understanding of the mechanisms governing protein secretion in filamentous fungi and develop novel strategies to improve the efficiency and yield of recombinant protein secretion in filamentous fungi.

47. Network-based approach for discovering transcription factors associated with fungal plant biomass conversion

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Fungal plant biomass conversion (FPBC) is an important component of the global carbon cycle and has been widely applied for the production of biofuel, enzymes and biochemicals. Identification of transcription factors (TFs) governing the FPBC process is important for genetic engineering of fungi towards sustainable production of high-value bioproducts from renewable lignocellulose. However, the functional characterization of new TFs is challenging due to the difficulties in computational prediction and labor consuming experimental validation.

Here, we developed a bioinformatics framework for screening of FPBC related TFs based on reconstructing gene regulatory networks from transcriptome data and enrichment analysis of manually curated FPBC gene sets. Applying this method on model fungi *Aspergillus niger* and *Neurospora crassa,* and the less-studied Basidiomycete *Dichomitus squalens*, we successfully identified both known TFs and promising candidates. The function of one identified TF, HapX, has been experimentally validated, and several candidates were supported by literatures and transcriptome data. Our new method will accelerate the identification of novel TFs involved in FPBC, and facilitate the further improvement of fungal cell factories.

48. Investigating the role of long non-coding RNA *afu-182* in azole response in opportunistic pathogen *Aspergillus fumigatus*

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Aspergillus fumigatus (AF) is a leading cause of aspergillosis in immunocompromised patients, and drug resistance has exacerbated the problem, with mortality rates reaching >90% for infections caused by drug-resistant isolates. Only 5-7% of invasive aspergillosis cases are caused by drug-resistant isolates; however, mortality rates still reach 50% for infections caused by drug-sensitive AF isolates for infections caused by drug-sensitive AF isolates. Thus, there is a knowledge gap in understanding fungal azole response. Here, we characterized long non-coding RNA, *afu-182*, as a negative regulator of azole drug response in Aspergilli. Our data show that *afu-182* controls fungal pan-azole response without a change in the minimum inhibitory concentration. Interestingly, clinically relevant biofilm of the D*afu-182* strain is recalcitrant to azole drugs, whereas overexpression of *afu-182* makes fungus more susceptible to azole drugs. Importantly, *afu-182* is indispensable for azole-mediated fungal clearance in a murine model of invasive pulmonary aspergillosis, highlighting a role of *afu-182* in virulence and providing a novel genetic link between low rates of successful treatment outcomes for infections caused by azole-susceptible isolates.

***49. Metabolic Plasticity Contributes to Structure and Function of *Aspergillus fumigatus* Biofilms**

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Aspergillus fumigatus can cause invasive aspergillosis (IA) in immunocompromised individuals by forming complex, multinucleated biofilms. These biofilms, a known virulence factor, contribute to the chronic nature of these infections in part through mediating substantial resistance to contemporary antifungal drugs. Adaptations to the infection environment, specifically to reduced oxygen availability, contribute to the phenotypic heterogeneity of these biofilms. Strikingly, *A. fumigatus* experimentally evolved in a low-oxygen environment forms a distinct biofilm morphology termed H-MORPH. Intriguingly, H-MORPH

strains are recovered from patient samples. H-MORPH strains possess increased growth in low-oxygen environments and virulence in murine models of IA. How H-MORPH mediates these important phenotypes is ill-defined.
H-MORPH strains have increased growth on alternative carbon sources found at the site of infection including acetate, lactate, and ethanol, all products of eukaryotic cell low oxygen metabolism. These data suggest metabolic rewiring contributes to their altered biofilm structure and function. As such, we hypothesize that these H-MORPH strains are partially carbon catabolite de-repressed.
To test this hypothesis, we utilized the CRISPR Cas-9 system to generate alcohol (*alcA*) and aldehyde (*aldA*) dehydrogenase null mutants in our wild-type and isogenic H-MORPH strain background. As expected, all null mutants have a significant growth defect when ethanol is the sole carbon source. Interestingly, H-MORPH biofilm architecture is altered with the loss of *alcA* and *aldA*, but not in wild-type. Consistent with an important role for ethanol catabolism in H-MORPH biofilm form and function, null mutants also display adherence defects predicted to impact the host-fungal interaction *in vivo.* Ongoing experiments include testing how these alcohol and aldehyde dehydrogenase mutants alter disease initiation and progression in murine models of IA. Given the clinical relevance of HMORPH, understanding the role of carbon catabolism regulation in virulence is important for understanding disease initiation and progression.

***50. Predicting fungal secondary metabolite activity from biosynthetic gene cluster data using machine learning**

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Fungal secondary metabolites (SMs) contribute to the diversity of fungal ecological communities, niches, and lifestyles. Many fungal SMs have one or more medically and industrially important activities (e.g., antifungal, antibacterial, and antitumor). The genes necessary for fungal SM biosynthesis are typically located right next to each other in the genome and are known as biosynthetic gene clusters (BGCs). However, whether fungal SM bioactivity can be predicted from specific attributes of genes in BGCs remains an open question. We adapted machine learning models that predicted SM bioactivity from bacterial BGC data with accuracies as high as 80% to fungal BGC data. We trained our models to predict antibacterial, antifungal, and cytotoxic/antitumor bioactivity of fungal SMs on two datasets: 1) fungal BGCs (dataset comprised of 314 BGCs), and 2) fungal (314 BGCs) and bacterial BGCs (1,003 BGCs). We found that models trained on fungal BGCs had balanced accuracies between 51-68%, whereas training on bacterial and fungal BGCs had balanced accuracies between 56-68%. The low prediction accuracy of fungal SM bioactivities likely stems from the small size of the dataset; this lack of data, coupled with our finding that including bacterial BGC data in the training data did not substantially change accuracies, currently limits application of machine learning approaches to fungal SM studies. With >15,000 characterized fungal SMs, millions of putative BGCs in fungal genomes, and increased demand for novel drugs, efforts that systematically link fungal SM bioactivity to BGCs are urgently needed.

51. Leveraging strain heterogeneity within the nonpathogenic fungus *Aspergillus fischeri* to highlight factors associated with virulence

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Strain heterogeneity within the human fungal pathogen *Aspergillus fumigatus* is appreciated to be a complicating factor when addressing questions related to the detection, control, treatment, and prognosis of acute aspergillosis cases. The variable pathogenic potentials exhibited between conspecific fungal isolates hints at the myriad and dynamic evolutionary processes which underlie this phenotype. This range in pathogenicity also suggests the surprising hypothesis that even strains of an *Aspergillus* species considered as being “nonpathogenic” may show similar heterogeneity in their pathogenic potential.
To explore this possibility, we comprehensively characterized the genomics, metabolomics, and pathogenic phenotypes of 16 strains of a widely distributed, “nonpathogenic” sister species to *A. fumigatus, A. fischeri. In vitro* and *in vivo* assays measuring the pathogenic potential of these strains demonstrated there to be a wide range of variation across these *A. fischeri* isolates. Genomic, transcriptomic and metabolomic profiling suggested several pathways and metabolites that may contribute to the observed intraspecific variation in virulence. Notably, pangenome analysis showed that

our strains likely did not capture the complete breadth of genomic diversity within *A. fischeri*, holding open the possibility that the range of phenotypic variation may be even greater than we observed.
In summary, we employed a multidisciplinary research strategy to provide a novel perspective on some of the factors underlying the evolution of virulence within *Aspergillus* section Fumigati. Importantly, our results reinforce the contribution of strain heterogeneity to phenotypes, particularly within rapidly evolving species.

52. FungiDB: Tools for Genomic-Scale Data Exploration, Analysis, Integration and Discovery
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Biomedical research is increasingly driven by Big Data: genome sequences and population-level diversity data, all manner of multi-Omics datasets, genome-scale phenotypic analyses, *etc*. How can we effectively collect, store, maintain and integrate this information to ensure FAIR (**F**indable, **A**ccessible, **I**nteroperable, **R**eusable) data access, advancing biological understanding and defining targets for further study in the lab, field & clinic? The Eukaryotic Pathogen & Vector Genomics Resource (VEuPathDB.org) – including FungiDB.org – provides a robust, sustainable, data-mining resource, accessed by thousands of researchers daily to inform and expedite discovery research and translational applications involving diverse eukaryotic microbes (fungi & protists). VEuPathDB staff will be available throughout Asperfest (and the main Fungal Genetics Conference) to demonstrate database functionality, answer questions, and discuss topics of community interest, including:

- How to access and interpret information on genes, gene models, automated & curated annotation, genomes, population diversity, comparative genomics, epigenetics, transcriptomes, proteomes, DNA & protein motifs, protein structures, interactomes, subcellular localization, metabolomics, pathways, phenotypic characterization, orthology-based functional inference, *etc*
- Strategies for integrating & interrogating diverse datasets (*in silico* experiments) … and analyzing & sharing the results obtained
- Assessing and improving the quality and accuracy of available annotation … capturing expert knowledge from the community
- Analyzing *your own* (or any public) datasets using the free, private, easy-to-use, cloud-based VEuPathDB Galaxy instance … and integrating/querying these results in the context of other data in FungiDB
- Identification and prioritization of new *Aspergillus* (and other) datasets for future integration into FungiDB; options for accommodating clinical & field datasets (with complex metadata)
- Recently-added database features, including alpha-fold structures, long-read RNA-seq, support for single-cell RNA-seq data, expression summaries, improved orthology detection, *etc*) … and what to expect in future
- Database usage statistics, suggestions on how/when to cite; funding status and prospects for the future
- How/where to get additional help & assistance with database mining & FAIR data access/sharing

***53. A novel reporter system to identify arginoketides in soil that mediate cross-kingdom microbial interactions**
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In all habitats on earth microorganisms form consortia with many different species closely living together in the soil. Interspecies communication in these communities are decisive for function of microbial communities and further lead to the induction of otherwise silent natural product biosynthesis gene clusters. One prominent example is the interaction of the fungus *Aspergillus nidulans* and the bacterium *Streptomyces rapamycinicus*. Upon co-cultivation, the streptomycete is able to reprogram the epigenetic machinery of the fungus by activation of the histone acetyltransferase GcnE which leads to the induction of the otherwise silent *ors* biosynthesis gene cluster in *A. nidulans* [1,2]. By inhibitor studies with

the pan-sirtuin inhibitor nicotinamide and analyses of several histone deacetylase mutants, we identified the silent information regulator SirE as the histone deacetylase terminating the induction of the *ors* BGC by *S. rapamycinicus* [3]. Furthermore, we discovered that the compound family of arginoketides including azalomycin F produced by *S. iranensis* and *S. rapamycinicus* serve as the long sought-after bacterial signals for this induction [4]. To estimate the induction of silent gene clusters, we developed a fungal reporter system encoding the gene for the green fluorescence protein (GFP) coupled to the nanoluciferase gene and the gene of interest. Thus, enabling the qualitative and quantitative measurement of the transcriptional activation of genes. Here, this construct was translationally fused to the *orsA* gene of the orsellinic acid biosynthesis gene cluster of *A. nidulans.* Transformants showed fluorescence and luciferase activity upon addition of *S. iranensis*, azalomycin F or the pan-sirtuin inhibitor nicotinamide to the culture. Interestingly, extracted soil also led to an increased nanoluciferase activity and green fluorescence indicating that arginoketides are indeed present in the soil. Further, with this reporter we were able to identify several bacterial strains, isolated from a random soil sample, that induce green fluorescence in the fungus [4]. This indicates that arginoketides can be found around the world and playing an important role in mediating microbial interactions in the soil.
1. Schroeckh V, *et al*. PNAS 2009; 2. Fischer J, *et al*. eLife 2018; 3. Jäger *et al.* BioRxiv; 4. Krespach MKC, Stroe MC, *et al.* Nat. Microbiol. 2023

54. Fungi Unleashed – Rapid Ionomic Profiling with Laser-Induced Breakdown Spectroscopy
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Nutrient acquisition, delivery, and availability dictates microbes' phenotype, lifestyle, and survival. One of the best microbes to study the influence of substrate availability is fungi, because they are heterotrophic, meaning they cannot produce their food from environmental nutrients. Tapping into this biological process by manipulating the substrate available has led to discoveries in identifying how fungi interact with a host or environment or biological products like alternative sources of protein and natural products. Despite the scientific interest and multifaceted roles that nutrient acquisition plays in controlling fungal behavior and producing biological products, the elements that are obtained and how they differentiate across fungal species have remained a largely unexplored area of research. To address this knowledge gap, we used laser-induced breakdown spectroscopy (LIBS) to identify the fungal ionomic profiles of two genetically different fungal species, *Hyaloscypha finlandica* and *Mucor hiemalis*, grown on defined and undefined substrate media. Through Pearson correlation coefficients, we had identified strong positive correlations with the emissions from carbon, zinc, phosphorus, manganese, and magnesium. The positive correlations seen with these elements in both species indicates their vital role in fungi propagation and survival. When the Pearson correlation coefficients of each fungi species are compared to one another a few noticeable differences are seen. Firstly, *H. finlandica* exhibits strong positive correlations between sodium, hydrogen, and the essential element group. This indicates *H. finlandica* has a reliance on sodium that *M. hiemalis* does not exhibit. A similar behavior is seen with potassium in *H. finlandica*, but generally a medium positive correlation exists between the essential elements and potassium. Interestingly, *M. hiemalis* shows a strong negative correlation between potassium and the essential elements. *M. hiemalis* shows stronger positive correlations between silicon, iron, and the essential elements; although, *H. finlandica* shows a positive correlation between silicon and calcium that *M. hiemalis* does not. Taken together, we provide data for the building blocks of what elements are needed for fungal growth and sustainability and how they differ across genetically diverse fungi.

55. Characterization of acid phosphatases in *Aspergillus oryzae* strain with reduced "umami" degradation activity
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Miso, fermented soy bean paste, is a traditional Japanese seasoning. It is made from soybeans, salt, water, and *koji* (solid-state culture of *Aspergillus oryzae* on rice, soybean, or barley). To fit the demand of modern busy lifestyle, production of dashi (broth) containing miso has been increasing in recent decades.

In the manufacturing process of dashi containing miso, heat treatment of miso is needed before adding dashi. Since acid phosphatase secreted by *A. oryzae* degrades one of the dashi component ribonucleotides yielding no taste ribonucleosides and phosphoric acid, acid phosphatase should be inactivated by heat treatment. However, heat treatment requires energy and special equipment and it reduces the quality of miso. In this study, we attempted to obtain a strain with low acid phosphatase activity to avoid heat treatment, and analyzed the characteristics of acid phosphatase for breeding purpose.
Through the screening of 503 practical *A. oryzae* strains stocked in Bio'c Co., we found a strain with greatly reduced acid phosphatase activity while maintaining protease and amylase activities sufficient for miso fermentation and named KBN-p [Food Sci. Technol. Res. (2012) 83-90]. Among 13 putative extracellular acid phosphatase genes (*aphA-M*) in *A. oryzae* genome, AphC was considered to be one of the main causes of low acid phosphatase activity in KBN-p strain based on the results of transcriptional analysis and activity test. When AphC amino acid sequence of KBN-p was compared to RIB40 and practical strain for miso koji (No.6020), 5 and 1 amino acid substitutions were found, respectively. So, we analyzed the properties of AphC with three different amino acid sequences. At first, 3 kinds of *aphC* genes were expressed under the *tef1* promoter in each *A. oryzae* strain and found that AphC (KBN-p) have some problem in secretion. However, it was difficult to determine the reason whether the secretion defect of AphC (KBN-p) in KBN-p host lies in the AphC sequence or the host strain itself. Three kinds of AphC were expressed in a unified *aphC* null mutant host and tested the properties of AphC activity in response to heat and NaCl which thought to be related to dashi containing miso making process. As a result, it was found that AphC (KBN-p) was less stable than the other AphCs (RIB40, No.6020). This property is thought to be advantageous for producing dashi containing miso without heat treatment.

56. The role of mycotoxins in governing interactions between the maize colonists, *Aspergillus flavus* and *Fusarium verticillioides*
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The mycotoxigenic fungi, *Aspergillus flavus* and *Fusarium verticillioides,* commonly co-colonize maize in the field, yet their direct interactions at the chemical communication level have not been well characterized. Here we examined if and how the two most infamous mycotoxins produced by these species, aflatoxin and fumonisin, respectively, govern interspecies growth and mycotoxin production. We showed that fumonisin producing strains of *F. verticillioides* suppressed the growth of *A. flavus* while non-producers did not. However, while aflatoxin did not inhibit *F. verticillioides* growth, it did suppress fumonisin production. No fumonisin was detectable when *F. verticillioides* was challenged with a high dose of aflatoxin. With these findings, expression of the respective biosynthetic gene clusters was investigated for these two fungi. While no strong effect was seen on genes in the aflatoxin gene cluster when exposed to fumonisin, in preliminary analysis the key fumonisin biosynthetic cluster gene, *FUM1,* was unexpectedly induced when *F. verticillioides* was challenged with aflatoxin but, consistent with suppressed fumonisin production, so was the recently identified repressor of fumonisin synthesis, *ZBD1,* laying directly adjacent to the cluster. We also assessed the expression of *veA* and *laeA,* global regulators of fungal secondary metabolism, and found that expression of both is altered in *A. flavus* and *F. verticillioides* when exposed to their competitor's mycotoxin. Based on this, we initiated exploration into the roles of other mycotoxins produced by *A. flavus* and *F. verticillioides* in their interactions. This work gives insights into the ecological roles of mycotoxins and why these fungi may produce them as weapons in the interspecies battle for resource acquisition.

57. Learning from the negative: Studying pathogen evolution from the "non-pathogen" perspective

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Microbial trait evolution is an ever-evolving topic in biology. The canonical method of study involves direct investigation of the species that display the trait of interest. While great strides are made using this method, there is fantastic potential for defining trait evolution by studying closely related sister taxa that do not display the trait of interest. The repeated, independent evolution of pathogenesis in *Aspergillus* section *Fumigati* presents the opportunity to study the origins and building blocks of fungal pathogenesis. In this study, we define a phenotypic, genotypic, and transcriptional profile of the non-pathogenic fungus, *Aspergillus fischeri*, using 16 strains and contrast these findings with similar work in the pathogen *Aspergillus fumigatus* using 14 strains. We find low genomic variation between strains in *A. fischeri*, in contrast to the high variation across *A. fumigatus*. Constructing an *A. fischeri* pangenome, we find a large degree of core proteome conservation from *A. fumigatus* to *A. fischeri,* including 204/207 of the verified virulence genes. We also find a large degree of transcriptional heterogeneity at both 30°C and 37°C in *A. fischeri* prompting gene regulatory network comparisons within and between species. The transcriptional heterogeneity is mirrored in virulence assays as there is a great deal of intraspecific variation. Our work raises questions about the drivers of phenotypic breadth and highlights the grey area found between the categories of pathogen and non-pathogen when studying intraspecific strain diversity.

58. Life in the dark – new *Aspergillus* species isolated from the extreme habitat of a constantly dark, high temperature radon cave

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Light is among the most important environmental cues for life on earth and besides triggering multiple responses, it serves as zeitgeber for the circadian clock. Also microbes and especially fungi adapt their physiology to light and darkness. Therefore we were interested, if and how fungi adapt if the light cue is missing for at least decades.

The dark environment of a cave like in the tunnels close to Bad Gastein, Austria, presents ideal conditions for investigation of evolution if adaptation to light is not needed. Additionally, the concentration of Rn222 is elevated, causing increased probability of mutagenesis. Such habitats are known to comprise a complex microbiome with bacteria, archea and fungi. The low nutrient availability triggers fierce competition, involving production of defensive secondary metabolites, but also development of resistance mechanisms is to be expected.

We were able to isolate more than 20 strains from the rock surface of the cave, which grew slowly as expected. Three fungi were identified by sequencing of ITS2, which revealed two likely new species of the genus Aspergilli with only 90 % identity to the closest *Aspergillus* species of the section Polypaecilum (BG A1-1 and BG F1-1) and one closely related to *Penicillium rubrum* isolated from a mining site (BG D2-2). Growth tests in daylight and darkness showed a pale phenotype and rather slow growth for the tested isolates on two different media (malt extract and glucose medium). In many cases, we observed formation of conidiation/growth rings reflecting reactions to light. Interestingly, BG F1-1 could not grow in light, although it did grow in darkness on both media. We also saw indications for altered secondary metabolite production upon growth in darkness or in light.

In order to assess interaction or antagonism of isolates, we performed confrontation assays with two isolates growing on the same plate. Between BG O-1 and BG A1-1 antagonism is clearly visible with formation of a clear zone between the growing colonies. Additionally, BG O-1 reacts to the presence of BG A1-1 with altered secondary metabolite production.

Genome sequencing of three isolates revealed that their sequences were not closely related to the genomes of species indicated by ITS-identification. Hence we conclude that the isolated strains suffered considerable mutation rates due to the radiation in their habitat or that they belong to previously uncharacterized species.

59. New Regulators of Gliotoxin Synthesis, HsfA and RogA, Identified through the Systems Biology Network GRAsp

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Aspergillus fumigatus, a pathogenic fungus, is a significant medical threat and causal agent of invasive aspergillosis. The fungus employs toxic secondary metabolites, notably gliotoxin, as key virulence factors to attack the host's immune cells. Gliotoxin also exhibits antifungal properties and *A. fumigatus* has evolved self-protection strategies against gliotoxin including GliT affecting disulfide bridge closure in the gliotoxin molecule and GtmA generating the less toxic bisdethiobis(methylthio)gliotoxin. Although the regulation of the *gli* biosynthetic genes is well known and orchestrated by the transcription factor GliZ, GliZ does not regulate *gliT* or *gtmA.* Here we utilized our recently developed gene regulatory network named GRAsp (**G**ene **R**egulation of ***Asp****ergillus fumigatus*) to explore the regulatory mechanisms governing gliotoxin self-protection in *A. fumigatus* . GRAsp analysis pinpointed 2 genes, AFUA_5G01900 and AFUA_3G11990, potentially involved in gliotoxin regulation. AFUA5G01900 encodes the heat shock protein HsfA and AFUA3G11990 encodes a C6 transcription factor we termed RogA (Regulator of Gliotoxin). GRAsp predicted that RogA regulated all of the biosynthetic gene cluster (BGC) genes responsible for gliotoxin synthesis as well as both *gliT* and *gtmA*. HsfA also was predicted to regulate *gliT* and *gtmA.* Gene expression data showed that RogA and HsfA negatively regulated all *gli* biosynthetic genes as well as both *gliT* and *gtmA.* Overexpression of both genes increased gliotoxin synthesis simultaneously with *gliT* and *gtmA* expression. By creation of single and double RogA and HsfA mutants, we present a model where HsfA regulates *gli* and *gtmA* expression through induction of RogA expression. Our work highlights the use of computational modeling to provide insight into previously unknown regulatory systems in self-protection mechanisms against endogenous toxins.

60. Investigating dormancy and its breaking in *Aspergillus fumigatus

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The initiation, maintenance, and breaking of dormancy across fungi is understudied and the biological mechanisms are ill defined. In the case of *Aspergillus fumigatus*, it is intriguing that a process so crucial to the survival of the conidium, the infectious propagule of invasive aspergillosis, remains unclear. In the present study, we investigate the germination process by analyzing *A. fumigatus* RNA-seq transcriptome data. We hypothesize that factors important in inhibiting germination will be proteins of unknown function that are highly differentially expressed in conidia. Transcripts that were found to be High In Conidia (HIC) relative to hyphae were selected as candidates for creation of a knockout collection, and HIC mutants were screened for their roles in dormancy and its breaking. Here we show data on HIC mutant Δ*hicA* suggesting that *hicA* may be an inhibitor of germination. Future work with this group of candidate genes will include phenotypic characterization of their roles in dormancy, germination and other aspects of development.

61. Exploring biosynthetic gene clusters in *Aspergillus fischeri*

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Biosynthetic gene clusters (BGCs) are groups of genes in close physical proximity that produce specialized metabolites (SMs) and other natural products. Many BGCs and their SMs are considered virulence factors in pathogens. Therefore, BGCs are typically studied in pathogens such as *Aspergillus fumigatus*, the causal agent of aspergillosis. However, in addition to this pivotal role in pathogens BGCs are also found in non-pathogens indicating BGC and SM functions beyond virulence in nature. Our aim was to gain a better understanding of the true diversity of BGCs and SMs, their presence and prevalence in other *Aspergillus* species. To that end, we studied *Aspergillus fischeri*, a close relative of *A. fumigatus* investigating BGC content and SM repertoire across 16 strains. We mined whole genome assemblies with antiSMASH and initially detected 37 known and 35 unknown potential BGCs. This corroborates that the non-pathogen has a higher number of BGCs detected by antiSMASH than its pathogenic relative. We combined the genomics approach with metabolomics to verify predictions via the

detections of corresponding SMs when possible. However, we emphasize the importance of manual curation as we rejected the prediction of several (false positive) BGCs while also recovering additional BGCs (false negatives). This richness in BGCs in *A. fischeri* indicates the versatility and wide range of BGC and SM functions in the ubiquitous non-pathogenic soil fungus. Beyond this overall assessment of known BGCs some of the hypothetical new BGCs may represent new avenues for future natural product discovery.

62. Expanding the fluorescent toolbox in *Aspergillus fumigatus

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Fluorescent proteins are indispensable tools used to understand the spatio-temporal dynamics of molecular processes in living cells. Even though *Aspergillus fumigatus* causes more deaths globally than any other fungal disease, we lack a well-characterised tool kit of next-generation fluorophores, limiting our ability to probe fundamental biological processes of this critical human fungal pathogen. In this work, we chromosomally transform *A. fumigatus* with 18 fluorescent proteins with emissions covering the visible light spectrum and characterise their practical brightness during the different morphological stages. Through live cell imaging using fluorescence confocal microscopy and imaging flow cytometry, the relative intensity of each fluorophore was measured during hyphal growth and in spores. The fluorescent proteins mTagBFP2, mNeonGreen, Citrine, mKO2, mApple, and Katushka2S - green, yellow, orange, red and far-red respectively - displayed the highest relative fluorescent intensity in germlings. We demonstrate the utility of these reporters as inducible promoter systems, protein tagging, and pathogenicity. Finally, we generate a 4-colour strain by exploiting counter-selectable markers of the pyrimidine salvage pathway. This strain visualises the mitochondria, vacuoles, peroxisomes, and cell membrane to understand the dynamics of these subcellular structures in response to antifungal agents. This new resource will enable the community to conduct advanced live-cell imaging to gain a deeper understanding of subcellular localisation, quantify protein-protein interactions, elucidate novel druggable targets, and visualise host-pathogen interaction models.

63. Regulated IRE1-dependent mRNA decay is induced by physiological ER stress associated with amylolytic enzyme production in *Aspergillus oryzae*

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Regulated IRE1-dependent mRNA decay (RIDD) is a feedback mechanism in which the endoribonuclease, IRE1, cleaves endoplasmic reticulum (ER)-localized mRNAs encoding secretory and membrane proteins in eukaryotic cells under ER stress. RIDD is artificially induced by chemicals that generate ER stress; however, its importance under physiological conditions remains unclear. In this study, we found that RIDD is induced not only by chemicals but also upon induction of the production of endogenous secretory hydrolases in *Aspergillus oryzae*. α-Amylase mRNA was rapidly degraded by IreA, an Ire1 ortholog, when mycelia were treated with dithiothreitol (DTT), an ER-stress inducer. In *A. oryzae*, maltose uptake by the maltose permease, MalP, is a prerequisite for the activation of AmyR, a transcriptional activator of amylolytic gene expression. Notably, even without DTT, *malP* transcripts underwent RIDD when *A. oryzae* was grown in a maltose medium. Loss of the superkiller (Ski) complex (involved in 3′–5′ mRNA degradation) resulted in marked accumulation of short fragments of the *malP* mRNA resulting from cleavage by IreA and, to a lesser extent, of short fragments of the α-amylase mRNA. The decrease in the abundance of the full-length *malP* mRNA and appearance of its short fragments were suppressed in Δ*amyR* strain. These results indicate that RIDD occurs under physiological ER stress caused by the production of amylolytic enzymes. In addition, *amyR* deletion rescued the growth defect of *ski* mutants on maltose medium. Overall, these findings suggest that RIDD contributes to the maintenance of cellular homeostasis in *A. oryzae* under conditions that produce amylolytic enzymes.

64. Towards the development of a safeguarding CRISPR RNA-guided gene drive to mitigate the impacts of the non-native fungal pathogen *Sphaerulina musiva* on managed ecosystems

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Invasion of non-native fungal species is acknowledged as one of the major external drivers altering the structure, biodiversity, and function of ecosystems. Understanding the mechanisms of establishment of these invaders and developing mitigation approaches to manage them is a critical aspect of sustaining native biodiversity and normal ecosystem functions. The fungal pathogen *Sphaerulina musiva* is a well-characterized example of an invasive species spread unintentionally by human activities. Originally native to Eastern North America, *S. musiva* was only recently introduced and established into the Pacific Northwest of North America resulting in deleterious effects on susceptible *Populus* species/genotypes, a foundational bioenergy crop, and a keystone tree species in forested ecosystems.
In our efforts to establish a safeguarding CRISPR RNA-guided gene drive to mitigate *S. musiva*'s diseases on *Populus* plantations, we recently developed a CRISPR-Cas9 system to genetically manipulate this non-model fungal species. This tool has been used to identify and validate genetic determinants of establishment and pathogenicity in *S. musiva* including effector genes and secondary metabolite biosynthetic genes. Those target genes will guide engineering risk mitigation strategies such as gene drives for more sustainable and productive ecosystems.

65. Analysis of chronic host-aspergilloma interactions using a novel mouse model

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Chronic aspergillosis poses a formidable challenge as an infection caused by *Aspergillus* spp. Hemoptysis, which occurs in approximately half of patients, adds complexity and occasionally leads to fatal outcomes. Despite its grim prognosis, with reported 5-year mortality rates ranging from 38% to 52%, chronic aspergillosis remains less recognized than invasive aspergillosis. Unlike the invasive form, chronic aspergillosis manifests in immunocompetent patients without *Aspergillus* tissue invasion and presents with unique features such as persistent aspergilloma within air-filled cavities for more than 3 months. We investigated aspergilloma, a critical element of chronic aspergillosis, using a novel mouse model. Implanted in our model was an *A. fumigatus* fungus ball into an air-filled subcutaneous cavity. Initially, a live fungus ball was introduced into the cavity of a healthy mouse, expecting no tissue invasion due to the immunocompetent nature of the mice. Unexpectedly, however, *Aspergillus* invaded the tissues. Based on earlier clinical findings showing dead hyphae in aspergilloma, we attempted to implant an autoclaved, killed fungus ball. Remarkably, a fungus ball of entirely dead hyphae persisted in the mouse cavity for over 3 months without clearance. Cellular analysis revealed an initial predominance of neutrophils around the fungus ball, later transitioning to foamy macrophages accumulating lipids.

Aspergillus fragments were detected within the cells of these foamy macrophages. In vitro experiments further confirmed macrophage damage induced by dead hyphae, suggesting a potential barrier to aspergilloma clearance. In addition, elevated levels of vascular endothelial growth factor in the dead fungus ball and increased vascularity around it were observed in our mouse model. Even in the scenario where all *Aspergillus* within the aspergilloma is deceased, the persistent presence of a substantial number of fungal bodies could contribute to hemoptysis. Our findings emphasize the need for innovative treatments that target fungal clearance and challenge the limited efficacy of antifungal agents against deceased fungal bodies. This research marks a substantial advancement in our comprehension of chronic aspergillosis, particularly in unraveling the interactions between dead hyphae and host cells.

***66. Conserved Regulators of the Septation Initiation Network are required for *Aspergillus fumigatus* Echinocandin Resistance and Virulence**

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Aspergillus fumigatus is a major invasive mold pathogen and the most frequent etiologic agent of invasive aspergillosis. The currently available treatments for invasive aspergillosis are limited in both number and efficacy. Our recent work has uncovered that the β-glucan synthase inhibitors, the echinocandins, are fungicidal against strains of *A. fumigatus* with defects in Septation Initiation Network (SIN) kinase activity. These drugs are known to be fungistatic against strains with normal septation. Surprisingly, SIN kinase mutant strains also failed to invade lung tissue and were significantly less virulent in immunosuppressed mouse models. Inhibiting septation in filamentous fungi is therefore an exciting therapeutic prospect to both reduce virulence and improve current antifungal therapy. However, the SIN remains understudied in pathogenic fungi. To address this knowledge gap, we characterized the putative regulatory components of the *A. fumigatus* SIN. These included the GTPase, SpgA, it's two-component GAP, ByrA/BubA, and the kinase activators, SepM and MobA. Deletion of *spgA*, *byrA* or *bubA* resulted in no overt septation or echinocandin susceptibility phenotypes. In contrast, our data show that deletion of *sepM* or *mobA* largely phenocopies disruption of their SIN kinase binding partners, *sepL* and *sidB*, respectively. Reduced septum formation, echinocandin hypersusceptibility, and reduced virulence were generated by loss of either gene. These findings provide strong supporting evidence that septa are essential not only for withstanding the cell wall disrupting effects of echinocandins, but are also critical for the establishment of invasive disease. Therefore, pharmacological SIN blockage may be an exciting strategy for future antifungal drug development.

***67. The proteomic response of Aspergillus fumigatus to Amphotericin B (AmB) reveals the involvement of the RTA-like protein RtaA in AmB resistance**

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The opportunistic human pathogen *Aspergillus fumigatus* poses a significant threat by causing mycoses, which can be fatal especially in immunocompromised individuals. Due to the increase of azole resistance in *A. fumigatus*, treatment options are often limited to amphotericin B (AmB), a member of the polyene family of antifungals that has well known side effects. A rising number of resistant isolates against AmB as well as limited knowledge about resistance and compensatory mechanisms give rise to concerns. To elucidate the effects of AMB on the fungal proteome, we conducted liquid chromatography-tandem mass spectrometry analyses to identify changes in the proteomic profiles of *A. fumigatus* treated with

sublethal concentrations of AmB and its liposomal formulation. Selected proteins with significant increase in abundance upon AmB exposure were then characterized.
By comparison of the proteomic response of AmB-treated samples and untreated controls, we found significant increases in the abundance of proteins belonging to secondary metabolite biosynthesis gene clusters, proteins anchored to the membrane, involved in catabolic processes or aromatic acid degradation. One of the proteins with the highest increase in abundance was RtaA, a fungal Rta1-like family protein. While deletion of *rtaA* led to increased sensitivity against AmB, overexpression resulted in a two-fold increase of resistance. Interestingly, only treatment with AmB and nystatin resulted in a rise of *rtaA* transcript levels, which hints towards a specific protection mechanism against polyenes. Deletion of *rtaA* did not significantly change the ergosterol content and intracellular lipid droplets of *A. fumigatus.* While not being crucial for the virulence of *A. fumigatus* itself, RtaA is most likely involved in the resistance against AmB by maintaining lipid homeostasis and membrane stability. These findings reveal a novel polyene resistance mechanism.

68. Deciphering the mechanistic basis of tolerance to olorofim in *Aspergillus fumigatus*
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Respiratory infections caused by the mould pathogen *Aspergillus fumigatus* annually kill as many as tuberculosis or malaria. These infections occur in a scenario with limited therapeutic options and resistance to azoles, the main antifungal agents to treat and prevent aspergillosis, is globally increasing. The development of new antifungals with novel mechanisms of action has been proposed as the most promising intervention to stop and contain the emergence of antifungal resistance. Olorofim acts by inhibiting the *de novo* synthesis of pyrimidines in a fungal-specific manner and will reach the clinic in the following years. However, we have recently demonstrated that *A. fumigatus* exhibits tolerance to olorofim that relies on increased survival in the presence of lethal concentrations of the drug. By screening *A. fumigatus* genome-wide mutant libraries for resistance to olorofim killing, we have identified the genetic factors contributing to olorofim tolerance. Understanding the mechanistic basis of *A. fumigatus* tolerance to olorofim will increase our knowledge on its contribution to resistance allowing the development of strategies to suppress it before this promising antifungal reaches clinical implementation.

69. Synthetic expression system enhances recombinant protein production in *Aspergillus oryzae
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For centuries, the koji mold *Aspergillus oryzae* has played a crucial role in East Asian food fermentations, producing culinary staples such as soy sauce, miso, and sake. More recently, its proficiency in secreting enzymes has positioned it as a cornerstone in industrial biotechnology. This makes *A. oryzae* uniquely suitable for the heterologous production of food proteins for use in novel meat and dairy alternatives. However, there is a critical lack of well-characterized strong constitutive promoters for *A. oryzae*.
To address this, we established a synthetic expression system for *A. oryzae*. This modular system involves a synthetic transcription factor in one locus and a gene of interest under control of a core promoter and upstream activating sequence (UAS) in the other. These elements interact with the synthetic transcription factor, allowing precise control over gene expression.
Using the synthetic expression system, we screened a curated library of thirteen core promoters, derived from *A. oryzae* genes with high expression levels. This revealed a wide dynamic range, enabling the fine-tuning of gene expression levels. The most potent core promoter, in conjunction with six repeats of the UAS, enabled a remarkable sixfold increase in mycelial fluorescent protein levels compared to the strong native alpha-amylase promoter (PamyB) under PamyB-inducing conditions, and was equally effective on minimal glucose media. To our knowledge, this makes it the strongest promoter for *A. oryzae* published to date.

Furthermore, by combining UASs with two core promoters oriented in opposing directions, we engineered synthetic bidirectional promoters. These substantially reduce the time it takes to optimize production strains by enabling multiplex gene integrations in a single transformation.
Collectively, these results will contribute to the establishment of *A. oryzae* as a reliable platform for more efficient and sustainable recombinant protein production and help pave the way for novel precision fermentation processes.

70. Exposure to agricultural DHODH inhibitors result in cross-resistance to the novel antifungal olorofim in *A. fumigatus*
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Pesticides, including fungicides, are extensively used in agricultural practice to protect plants from unwanted growth of weeds, plant pathogens and other pests. Dual use of antifungals in the environment and in the clinic, with similar mode of actions, has been shown to drive the development of resistance. Although not a plant pathogen, *A. fumigatus*is ubiquitous in the environment and therefore exposed to agricultural fungicides. Extensive use of triazoles in the environment has led to high rates of resistance found in clinical *A. fumigatus* isolates. The development of novel antifungals is paramount to be able to treat azole-resistant aspergillosis. Olorofim is a novel antifungal for clinical use, targeting the essential protein DHODH, for which resistance is rare. Recently, several agricultural DHODH inhibitors, including ipflufenoquin, quinofumelin and tetflupyrolimet, have gone through the approval process. We show that these DHODH inhibitors are active against *A. fumigatus*, and have the same mode of action as olorofim. Spontaneous mutation analysis revealed we can select for ipflufenoquin resistant *A. fumigatus* isolates. These ipflufenoquin resistant mutants show cross-resistance to olorofim. Furthermore, other agricultural DHODH inhibitors recently approved as herbicide have the potential to result in cross-resistance to olorofim. Lastly, we show that *A. fumigatus* isolates which are multi-drug resistant to a range of agricultural fungicides and clinically used antifungals are more fit under exposure to sub-inhibitory concentrations of ipflufenoquin and olorofim. Our results highlight the potential dangers of using DHODH inhibitors in agriculture and the future threat of resistance development to novel antifungals by selection in the environment.

***71. Deacetylation by sirtuin E is important for *Aspergillus fumigatus* pathogenesis and virulence**
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Protein acetylation is a crucial post-translational modification that controls gene expression and a variety of biological processes. Sirtuins, a prominent class of NAD+-dependent lysine deacetylases, serve as key regulators of protein acetylation and gene expression in eukaryotes. In this study, six single knockout strains of fungal pathogen *Aspergillus fumigatus* were constructed, in addition to a strain lacking all predicted sirtuins (SIRTKO). Phenotypic assays suggest that sirtuins are involved in cell wall integrity, secondary metabolite production, thermotolerance, and virulence. AfsirE deletion resulted in attenuation of virulence, as demonstrated in murine and *Galleria* infection models. The absence of AfSirE leads to altered acetylation status of proteins, including histones and non-histones, resulting in significant changes in the expression of genes associated with secondary metabolism, cell wall biosynthesis, and virulence factors. These findings encourage testing sirtuin inhibitors as potential therapeutic strategies to combat *A. fumigatus* infections or in combination therapy with available antifungals.

***72. Uncovering important transcriptional regulations during conidiation and spore germination**
Pin Wu, Fang Wang, Winnie Weng In Chong, Chris Koon Ho Wong
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Filamentous fungi have the remarkable ability to undergo asexual reproduction, generating an enormous quantity of spores (a.k.a. conidia). These spores serve as the primary means of dissemination, acting as infectious propagules for human and plant pathogens, as well as significant contributors to food spoilage.

The germination of spores and their subsequent growth as hyphae under favorable conditions are critical processes for infection and food spoilage. Therefore, understanding the mechanisms underlying conidiation and spore germination holds not only biological importance but also clinical and agricultural implications. To gain insight into the molecular mechanisms of conidiation and spore germination, we performed active transcription profiling by ChIP-seq and transcriptome profiling by RNA-seq on *Aspergillus nidulans* at distinct stages of conidiation and germination with a high temporal resolution. Our results unveiled dynamic transcriptional and post-transcriptional changes throughout different stages of germination and conidiation. Each stage exhibited enrichment of distinct gene sets with specialized functions. For some of these gene sets, we have identified potential candidate transcriptional regulators that could serve as valuable drug targets for impeding conidiation and spore germination. Further characterization of these regulators holds great promise for the development of novel antifungal drugs for preventing human and plant fungal infections.

***73. The sterol C-24 methyltransferase encoding gene, *erg6*, is essential for viability of *Aspergillus* species**

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Ergosterol is a critical component of fungal plasma membranes. Triazoles, the most widely used antifungal drug class in the world, inhibit ergosterol biosynthesis for antifungal effect. Global increases in triazole resistance have threatened the continued use of these drugs and highlight the need for novel antifungals. Recent studies identified the fungus-specific sterol C-24 methyltransferase enzyme, Erg6, as a bona fide novel antifungal target in human pathogenic yeast. Unfortunately, Erg6 enzymes are largely unstudied in filamentous fungal pathogens like *Aspergillus fumigatus*. Here, we show for the first time that the lipid droplet-associated sterol C-24 methyltransferase, Erg6, is essential for *A. fumigatus* viability. We further show that this essentiality extends to additional *Aspergillus* species, including *A. lentulus, A. terreus,* and *A. nidulans*. Downregulation of *erg6* causes loss of sterol-rich membrane domains required for apical extension of hyphae and altered sterol profiles consistent with the Erg6 enzyme functioning upstream of the triazole drug target, *cyp51*A / *cyp51*B. Unexpectedly, *erg6* repressed strains demonstrate wild-type susceptibility against the ergosterol-active triazole and polyene antifungals.
Finally, *erg6* repression reduces fungal burden accumulation in a murine model of invasive aspergillosis. Taken together with recent studies, our work supports Erg6 as an attractive and potentially pan-fungal novel drug target.

74. Oryzapsin, orthologs of yeast yapsin in *Aspergillus oryzae*, are involved in ergosterol biosynthesis

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The oryzapsin genes *opsA* and *opsB* in *Aspergillus oryzae* encoding glycosylphosphatidylinositol (GPI)-anchored aspartic endopeptidase are homologs of *Saccharomyces cerevisiae* yapsins. However, the profiles and roles of the proteins encoded by these genes have not yet been clarified. First, we produced *opsA* and *opsB*-overexpression strains and performed enzymatic analyses, revealing that OpsA and OpsB can attack sites other than the carboxyl-terminal peptide bonds of basic amino acids. Second, *opsA* and *opsB* single-deletion and double-deletion strains (ΔopsA, ΔopsB, and ΔopsAΔopsB) were constructed to explore the expected roles of oryzapsins in cell wall synthesis, similar to the role of yapsins. The transcription level of *mpkA* in the cell wall integrity pathway was increased in ΔopsB and ΔopsAΔopsB strains, suggesting that OpsB might be involved in processing cell wall synthesis-related proteins. Treatment with an ergosterol biosynthesis inhibitor reduced the growth of the ΔopsAΔopsB strain. Moreover, the mRNA levels of Ao*erg1*, Ao*erg3-1*, Ao*erg3-2*, Ao*erg7b*, Ao*erg11*, and Ao*hmg1,2* showed a decreasing tendency in the ΔopsAΔopsB strain, and the ergosterol content in the membrane was reduced in the ΔopsAΔopsB strain. These results suggest that oryzapsins play roles in the formation of cell walls and cell membranes, especially ergosterol biosynthesis.

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