The 14th International *Aspergillus* Meeting

Asperfest 14

March 13-14, 2017

Asilomar Conference Center, Pacific Grove, CA

Aspergillus Genomes Research Policy Group (AGRPG)

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

2016 AGRPC

Gerhard Braus, 2014-2016, Georg-August-University Goettingen, Germany; gbraus@gwdg.de
Elaine Bignell, 2014-2016, University of Manchester, UK; elaine.bignell@manchester.ac.uk
Vera Meyer, 2014-2016, Berlin University of Technology, Germany; vera.meyer@tu-berlin.de
David Cánovas, 2014-2016, University of Seville, Spain; davidc@us.es
Michelle Momany, Chair, 2015-2017, University of Georgia, USA; mmomany@uga.edu
Isabelle Benoit-Gelber, 2015-2017, Concordia University, Canada; isabelle.benoit@concordia.ca
Nancy Keller, 2015-2017, University of Wisconsin Madison, USA, npkeller@wisc.edu
Nick Read, 2015-2017, University of Manchester, UK, nick.read@manchester.ac.uk
Mikael R. Andersen, 2016-2018, Technical University of Denmark (DTU); mr@bio.dtu.dk
Robert Cramer, 2016-2018, Dartmouth, USA; robert.a.cramer.jr@dartmouth.edu
Ling Lu, 2016-2018, Nanjing Normal University, China; linglu@njnu.edu.cn
Richard Todd, 2016-2018, Kansas State University, USA; rbtodd@ksu.edu
Kevin McCluskey (Ex officio), Curator, Fungal Genetics Stock Center; mccluskeyk@ksu.edu

THANKS TO OUR MEETING SPONSORS!

novozymes
Rethink Tomorrow

FUNGAL GENETICS STOCK CENTER

GSA
Genetics Society of America

zymergen

The Fourteenth International *Aspergillus* Meeting
(Asperfest14)
March 13-14, 2017
Merrill Hall, Asilomar Conference Center

March 13 (Monday)

3:00PM Registration and poster hang up
7:00PM - 10:00PM Poster and Welcome Reception (sponsored by Novozymes, Inc.)

7:00 - 8:30 Students with even-numbered posters
8:30 -10:00 Students with odd-numbered posters

Judging for Novozymes Student Poster Prize coordinated by David Cánovas, University of Seville, Spain

March 14 (Tuesday)

8:45 Welcome, introductions and announcements Robb Cramer
Geisel School of Medicine at Dartmouth, USA

8:50 Session I

A. fumigatus clonal spores are not homogeneous
Michelle Momany, University of Georgia, USA

Using KO libraries to explore the regulation of drug resistance in *Aspergillus fumigatus* / Update on the genome-wide knockout project
Mike Bromley, University of Manchester, UK

Genome wide consequences of the deletion of the Aspergilli non-homologous end joining (NHEJ) DNA repair mechanism
David Cánovas, University of Seville, Spain

From Antimicrobial peptides to moonlighting proteins
Sascha Jung, Berlin University of Technology, Germany

10:10-10:30 Coffee Break

10:30 Session II: Genomic Resources Mikael Andersen
Technical University of Denmark, Denmark

Gold-standard genome for *Aspergillus*
Jean-Paul Ouedraogo, Concordia University, Canada

Comparative genomics reveals high biological diversity and specific adaptations in the industrially and medically important fungal genus *Aspergillus*
Ronald P. de Vries, CBS-KNAW Fungal Biodiversity Centre, Netherlands

From Eurofung to Standfun – a mission possible?
Vera Meyer, Berlin University of Technology, Germany

FungiDB: What can you do, what's new? What do you need, what to expect?
David Roos, University of Pennsylvania, USA

11:30 Pontecorvo Lecture (sponsored by Zymergen, Inc.) Patrick Westfall
Zymergen, Inc., USA

Classifying *Aspergillus*: from microscope to full genome
Rob Samson, CBS Fungal Biodiversity Centre in the Netherlands

12:00PM - 1:15PM Lunch

Students and postdocs interested in industry, look for the tables with Novozymes and Zymergen reps!

1:15PM **Community directions discussion; Elections** — Michelle Momany, University of Georgia, USA

1:45PM **Session III: Talks from Abstracts** — Isabelle Benoit-Gelber, Concordia University, Canada

Aspergillus nidulans Protein kinase C and SepA physically interact at sites of polarized growth
Loretta Jackson-Hayes, Rhodes College, USA

Expression data integration in an *Aspergillus niger* genome-scale metabolic model
Maria-Victoria Aguilar Pontes, CBS-KNAW Fungal Biodiversity Centre & Utrecht University, NL

Understanding cell tropisms in *Aspergillus fumigatus* hyphae
Pavlos Geranios, University of Manchester, UK

An experimental approach to studying the role of Horizontal Gene Transfer in shaping fungal secondary metabolism
Elise de Reus, Technical University of Denmark, Denmark

2:45PM **Election results; other discussion items** — Michelle Momany, University of Georgia, USA

Novozymes student poster prize presentation — Amanda Fischer, Novozymes Inc., USA

3:00PM **Dismiss** (Remove posters)

List of Posters

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

1. Adsorption kinetics and self assembled structures of *Aspergillus oryzae* hydrophobin RolA on chemically modified solid surfaces.
Y. Terauchi, M. Nagayama, T. Tanaka, H. Tanabe, T. Arita, H. Higuchi, F. Hasegawa and **K. Abe**

2*. Expression data integration in an *Aspergillus niger* genome-scale metabolic model
M. V. Aguilar-Pontes, E. McDonnell, K. Strasser, D. Fulton, A. Tsang and R. P. de Vries

3. Components of the *Aspergillus fumigatus* Ras Post-translational modification pathway regulate conidial viability, hyphal growth, and virulence
Q. Al Abdallah, TA Norton and JR Fortwendel

4*. Evidence for inducer function of 2-keto-3-deoxy-L-galactonate in D-galacturonic acid induced gene expression in *Aspergillus niger*
E. Alazi, C. Khosravi, T.G. Homan, M. Arentshorst, M. Di Falco, M. Peng, M.V. Aguilar Pontes, A. Tsang, J. Visser, R.P. de Vries and A.F.J. Ram

5*. Parallel fitness using barcode sequencing in *A. fumigatus*: Assessment of the role of Aspergillus Kinases in azole resistance and as Targets for Antifungal Drug Discovery
N. ALFURAIJI, J. Mabey, M. Bromley and P. Bowyer

6. Expansions and reductions in fungal primary metabolism studied across 100 fungal species
Julian Brandl, Jane Nybo, Tammi C. Vesth and **Mikael Rørdam Andersen**

7. Mutations in *set2*, the Histone H3-K36 methyltransferase, rescue transcriptional down-regulation in *snxA1* and *snxA2* mutants of *Aspergillus nidulans*.
SW James, JM. Palmer, A Soukup, NP Keller, L Ciraku, M Dunworth, S Francisco, and **SL Anglin**.

8. FungiDB: An integrated functional genomics database for fungi and oomycetes.
Evelina Basenko, Omar Harb, Achchuthan Shanmugasundram and David Roos

9. The Negative Cofactor 2 complex mediates azole resistance through transcriptional regulation of an efflux transporter and ergosterol biosynthesis in *Aspergillus fumigatus.*
Takanori Furukawa, Fabio Gsaller, Josie Parker, Steve Kelly, Scott Moye-Rowley, Paul Bowyer and **Mike Bromley**

10. Adapting filamentous fungi to an automated environment for the application of a Design-Build-Test-Analyze-Learn approach to strain optimization
K.S. Bruno, E. Szewczyk, K Rothschild-Mancinelli and P.J. Westfall

11. Genome wide consequences of the deletion of the Aspergilli non-homologous end joining (NHEJ) DNA repair mechanism
I. Álvarez-Escribano, C. Sasse, J. Woo Bok, A. Lipzen, W. Schackwitz, J. Marin, K. Barry, I. Grigoriev, A.T. Marcos, N.P. Keller, G.H. Braus and **D. Canovas**

12. Role of the urea cycle in the synthesis of nitric oxide
D. Canovas, A.T. Marcos, J.F. Marcos, T. Schinko and J. Strauss

13. Identification and characterization of the *Aspergillus flavus* aspergillic acid gene cluster
J. Cary, M. Lebar, C. Carter-Wientjes, B. Mack, R. Majumdar, J. Diana Di Mavungu and S. De Saeger

14. Global Analysis of CreA Regulatory Network in *Aspergillus nidulans*
Yingying CHEN, Liguo DONG, Md Ashiqul Alam, Fang Wang, Joan Kelly and Koon Ho Wong

15*. An experimental approach to studying the role of Horizontal Gene Transfer in shaping fungal secondary metabolism
P.E. de Reus, K.F.N. Nielsen and R.J.N. Frandsen

16*. Understanding the role of septin AspD in the early vegetative growth of *Aspergillus nidulans*
I. Dorter and M. Momany

17. Pivotal role for the alternatively spliced, spliceosomal twin intron in one-bp intron drift
E. Fekete, M. Flipphi, N. Ág, N. Kavalecz, G.C. Cerqueira, C. Scazzocchio and L. Karaffa

18. Using intra-species fungal diversity to get different epigenetic imprints on fungal exometabolite production
J. Frisvad

19*. *Aspergillus niger versus Aspergillus oryzae*: Expression platforms for heterologous secondary metabolite production
E. Geib and M. Brock

20*. Sexual fertility in *Aspergillus flavus*: understanding genetic exchange and phenotypic inheritance through analysis of F1 progeny
R.M. Gell and I. Carbone

21*. Understanding cell tropisms in *Aspergillus fumigatus* hyphae
P. Geranios, N. Al-Furaji, K. Lord, M. Bromley and N. Read

22. ZtfA is a novel regulator of asexual development, secondary metabolism and oxidative stress defense in *Aspergillus nidulans*
K.G. Thieme, **J. Gerke**, C. Sasse, S. Thieme, O. Valerius and G.H. Braus

23. Functional and subcellular localization analyses of transcription factors, AtrR and SrbA, involved in azole drug resistance in *Aspergillus oryzae*
K. Sugiyama, A. Ohba-Tanaka, D. Hagiwara, S. Kawamoto, M. Tanaka, T. Shintani and **K. Gomi**

24*. Elucidating the biosynthetic pathway of the anticancer secondary metabolite calbistrin in *Penicillium decumbens*
Sietske Grijseels, Carsten Pohl, Zahida Wasil, Jens Christian Nielsen, Yvonne Nygård, Jens Nielsen, Jens C. Frisvad, Kristian Fog Nielsen, Mhairi Workman, Thomas Ostenfeld Larsen, Arnold Driessen and Rasmus John Normand Frandsen

25. The opposing roles of SrbA and the HapX/CCAAT binding complex in the regulation of sterol biosynthesis and azole tolerance in *Aspergillus fumigatus*
F. Gsaller, P. Hortschansky, T. Furukawa, P.D. Carr, B. Rash, J. Capilla, C. Müller, F. Bracher, H. Haas, A.A Brakhage and M.J. Bromley

26. Unveiling a novel regulatory role of the pH regulator PacC and the CCAAT-Binding-Complex in *Aspergillus fumigatus* 5-Flucytosine resistance
F. Gsaller, T. Furukawa, P.D. Carr and M.J. Bromley

27. Developmental regulation by constitutive activation and inactivation of MpkB MAPK pathway in *Aspergillus nidulans*
Sang-Cheol Jun, Kwang-Yeop Jahng, Jong-Hwa Kim and **Kap-Hoon Han**

28. Ascospore-specific gene expression analysis in *Aspergillus nidulans*
Mi-Kyoung Lee, Jong-Hwa Kim, Jae-Hyuk Yu and **Kap-Hoon Han**

29. Biosynthesis of acurin A and B in *Aspergillus aculeatus*
M.L. Nielsen, P.P. Wolff, L.M. Petersen, L.N. Andersen, T.I. Petersen, D.K. Holm, U.H. Mortensen, C.S. Nødvig, T.O. Larsen and **J.B. Hoof**

30*. Nuclear accumulation of the *Aspergillus nidulans* GATA transcription factor AreA is independent of DNA binding.
C.C. Hunter, M.J. Hynes, J.A. Fraser, D.F. Clarke, M.A. Davis and R.B. Todd

31*. Subcellular localization and stability of deubiquitinase CreB involved in carbon catabolite repression in *Aspergillus oryzae*
S. Ichinose, M. Tanaka, T. Shintani and K. Gomi

32*. Intron within 5′ untranslated region enhances transcription of the enolase-encoding gene (*enoA*) in *Aspergillus oryzae*
Taishi Inoue, Hiroki Toji, Mitsuru Takama, Mizuki Tanaka, Takahiro Shintani and Katsuya Gomi

33. *Aspergillus nidulans* Protein kinase C and SepA physically interact at sites of polarized growth.
Loretta Jackson-Hayes, Z. O. Atiq, Elisabet Olsen, Lance Myers, Terry W. Hill

34. An *in-silico* reconstructed gene regulation network for *Aspergillus niger* for the prediction of protein functions
Sascha Jung, Norman Paege, Paul Schäpe and Vera Meyer

35*. Hidden in Plain Sight: Heterogeneity in Asexual Spores of *Aspergillus fumigatus*
S.E. Kang and M. Momany

36. Growth-phase sterigmatocystin formation on lactose is mediated via low specific growth rates in *Aspergillus nidulans*.
L. Karaffa, Z. Németh, Á.P. Molnár, B. Fejes, L. Novák, N.P. Keller and E. Fekete

37*. Culture independent detection of *Aspergillus fumigatus* Cyp51A in human lung
Aiah Khateb, Michael Bromley and Paul Bowyer

38*. L-rhamnose induction and metabolism in *Aspergillus niger*
C. Khosravi, Maria Victoria Aguilar-Pontes, Eline Majoor and Ronald De Vries

39. Genomic and transcriptomic analysis of *Aspergillus niger* producing thermophilic bacterial cellulases
J. Kim, S. A. Campen, B. A. Simmons, S. E. Baker, J. M. Gladden and J. K. Magnuson

40*. Identifying more than 300 biosynthetic gene clusters with potential resistance genes in over 75 *Aspergillus* species using resistance gene-guided genome mining
I. Kjaerboelling, T.C. Vesth, J.C. Frisvad, J.L. Nybo, S. Theobald, I.V. Grigoriev, A. Salamov, T.O. Larsen, U.H. Mortensen and M.R. Andersen

41*. COP9 signalosome and Cand mediated Cullin-RING ligase control and *Aspergillus nidulans* development
A. M. Koehler and G. H. Braus

42*. The transcriptional activators RhaR, AraR and GaaR co-regulate pectin degradation in *Aspergillus niger*.
J. E. Kowalczyk, R. J. M. Lubbers, E. Battaglia and R. P. de Vries

43. Genomics of the first 100 Aspergilli
A. Kuo, R. Riley, S. Mondo, S. Haridas, A. Salamov, F. Korzeniewski, B. Simmons, S. Baker, M. Andersen and I. Grigoriev

44*. Genome-wide analysis of the GATA transcription factor AreA in *Aspergillus nidulans*
A. Li, Z. Miao, D.J. Downes, G.Y. Busot, R.B. Todd and K. Wong

45*. Genetic drivers of diversity in secondary metabolic gene clusters in *Aspergillus fumigatus* populations
Abigail Lind, Jennifer Wisecaver, Fernando Rodrigues, Gustavo Goldman and Antonis Rokas

46. Overexpression of a C_4-dicarboxylate transporter is the key for converting citric acid production to C_4-dicarboxylic acid production in *Aspergillus carbonarius*
Lei Yang, Eleni Christakou, Jesper Vang, Mette Luebeck and **Peter Luebeck**

47. Genomic and bioinformatics analyses of biosynthesis and production enhancement of a novel antifungal antibiotics, FR901469, from a filamentous fungus
Masayuki Machida, Makoto Matsui, Tatsuya Yokoyama, Hiroya Itoh, Akira Ohyama and Takashi Shibata

48. Alteration of polyamine metabolism negatively affects *Aspergillus flavus* development and pathogenesis during its interaction with corn kernels
R. Majumdar, J. Cary, S. Minocha, M. Lebar, B. Mack, C. Sickler, C. Carter-Wientjes, K. Rajasekaran and D. Bhatnagar

49. The *A. fumigatus* Fumiquinazoline C is potentially cytotoxic to macrophages and soil amoeba *Dictyostelium discoideum*
Marina Campos Rocha, Taicia Pacheco Fill, Juliana Issa Hori, Lilian Pereira Silva, João Henrique Tadini Marilhano Fabri, Anderson Ferrira da Cunha, Gustavo Henrique Goldman and **Iran Malavazi**

50*. Internuclear diffusion of histone H1 within cellular compartments in *Aspergillus nidulans*
Alexander Mela and Michelle Momany

51*. Heterokaryon incompatibility and phenotypic characterisation of *Aspergillus flavus* isolates in low and high risk zones in Kenya
Alfred Mitema, Sheila Okoth, Revel Lyer, Amelia Hilgart and Suhail Rafudeen

52*. Comparative analysis of the function of α-1,3-glucan syntheses, AgsA and AgsB, in *Aspergillus nidulans*.
K. Miyazawa, A. Yoshimi, S. Yano, S. Kasahara, F. Hasegawa and K. Abe

53*. Population structure of *Aspergillus flavus* in soil and corn kernels from four different states
M.S. Molo, R. Singh, J.B. White, T. Isakeit, K.A. Wise, C.P. Woloshuk, B.H. Bluhm, B.W. Horn, R. Heiniger and I. Carbonw

54. A CRISPR/Cas9 based Toolbox for Efficient Genome Editing of Filamentous Fungi
U.H. Mortensen, C. S. Nødvig, Z.D. Jarczynska, M.L. Nielsen, F. H. Kirchner and J.B. Hoof

55*. An oxylipin signal mediates hyphal branching in pathogenic Aspergilli
M. Niu, G. Fischer and N. Keller

56*. What drives speciation? Examination into the evolutionary events of more than 100 Aspergillus species.
J.L. Nybo, T.C. Vesth, S. Theobald, I. Kjaerboelling, J.C. Frisvad, T.O. Larsen, R. Riley, A. Salamov, I.V. Grigoriev, S.E. Baker and M.R. Andersen

57. Development of genome editing method by direct introduction of Cas9-protein in *Asepergillus oryzae*.
K. Shimamoto, R. Saitou, Y. Wada, O. Mizutani, **K. Oda**, M. Okuda and K. Iwashita

58*. Comparative genomics of *Aspergillus flavus* S and L morphotypes provides insights into niche adaptation
Mana Ohkura, Peter Cotty and Marc Orbach

59. Fast and highly efficient gene replacement in *Aspergillus niger* using CRISPR/Cas9
Jean Paul Ouedraogo, Yun Zheng, Tricia John, Letian Song and Adrian Tsang

60. Improvements to the *Aspergillus nidulans* molecular genetic system: Creation of new selectable markers, cloning of genes that complement them and construction of *A. nidulans* strains with up to seven selectable markers
James Dohn, Alex Grubbs, C. Elizabeth Oakley and **Berl R. Oakley**

61. The cullin-1 gene in *Aspergillus nidulans* interacts genetically with γ-tubulin and plays critical roles in regulating the G1 to S transition and in suppressing septation near the hyphal tip.
Tori Paolillo, James Dohn, **Berl Oakley**

62*. Utilizing next generation sequencing to revitalize a forward genetic screen for mutants deficient in the production of sterigmatocystin in *Aspergillus nidulans*
B. T. Pfannenstiel, K. J. Affeldt, J. Bok, R. A. E. Butchko, T. Choera, G. J. Fischer, B. P. Knox, F. Y. Lim[b], X. Luo[d], A. A. Soukup, J. E. Spraker, K. Throckmorton, P. Wiemann, N. P. Keller, and J. M. Palmer

63. CgXbp1 modulates temporal transcriptional dynamics in human fungal pathogen *Candida glabrata* during interaction with host macrophages.
M. N. Rai, C. Parsania, R. Rai and K. H. Wong

64*. Universal expression system for eukaryotic microorganisms
A. Rantasalo, C. Landowski, J. Kuivanen, J. Jäntti and D. Mojzita

65. Analysis of aspirochlorine (ACL) productivity and ACL cluster sequence in *Aspergillus oryzae* strain.
S. Ryota, O. Tami, U. Miyuki, O. Ken and I. Kazuhiro

66. Comparative genomics of the *Aspergillus* section *Flavi.*
A. Sato, K. Matsushima, K. Ito and T. Mituyama

67*. Microtubules are reversibly depolymerized in response to changing gaseous microenvironments within Aspergillus biofilms.
N. Shukla, A. Osmani and S. Osmani

68. The role of lifecycle in azole-resistance development in *Aspergillus fumigatus*.
E. Snelders, J. Zhang, A.J.M. Debets, S.E. Schoustra, W.J.G. Melchers, B.J. Zwaan and P.E. Verweij

69*. Are G protein-coupled receptor proteins involved in thigmoregulation of aflatoxin inhibition by *Aspergillus flavus*?
R.R. Sweany and K.E. Damann, Jr.

70. *Aspergillus oryzae* requires unfolded protein response for growth under condition inducing amylases production.
Mizuki Tanaka

71. Crosstalk of LaeA and CreA in controlling virulence and secondary metabolism in *Penicillium expansum*
J. Tannous, X. Luo, D. Kumar, S. Barad, Y. Chen, A. Dubey, N.G. Matana, S. Tian, B. Li, D. Prusky and N. Keller

72*. Genus level analysis of secondary metabolism reveals the origin of hybrid NRPS-PKS gene clusters
S. Theobald, T.C. Vesth, J.L. Nybo, I. Kjærbølling, J.C. Frisvad, K.F. Nielsen, T.O. Larsen, I.V. Grigoriev, A. Salamov, U.H. Mortensen, S.E. Baker and M.R. Andersen

73*. An intrinsically disordered domain of the VelB velvet domain provides specificity for heterodimer formation in *Aspergillus nidulans*
S. Thieme, J. Gerke, C. Sasse, O. Valerius, K. G. Thieme and G. H. Braus

74. Genetic diversity of 100+ Aspergillus species - the aspMine analysis resource
T. C. Vesth, J. L. Nybo, S. THEOBALD, R. P. DE VRIES, I. V. GRIGORIEV, S. E. BAKER and M. R. ANDERSEN

75. Resistance Gene-Guided Genome Mining: Serial Promoter Exchanges in *Aspergillus nidulans* Reveal the Biosynthetic Pathway for Fellutamide B, a Proteasome Inhibitor
Hsu-Hua Yeh, Manmeet Ahuja, Yi-Ming Chiang, C. Elizabeth Oakley, Shauna Moore, Olivia Yoon, Heather Hajovsky, Jin-Woo Bok, Nancy P. Keller, **Clay C. C. Wang** and Berl R. Oakley

76*. Are dormant spores really dormant? A perspective from the transcription level.
Fang Wang, Liguo Dong and Chris Koon Ho Wong

77*. Functional reconstitution of the Trypacidin Gene Cluster in *Aspergillus fumigatus* by Advanced Gene Editing
J. Weber, V. Valiante, C.S. Nødvig, D.J. Mattern, R.A. Slotkowski, U.H. Mortensen and A.A. Brakhage

78. A global co-expression network approach for connecting genes to specialized metabolic pathways
Jennifer Wisecaver, Abigail Lind and Antonis Rokas

79. Global transcriptional regulation and chromatin dynamics of primary metabolism in *Aspergillus nidulans.*
Koon Ho Wong, Ang Li, Yingying Chen, Liguo Dong, Md Ashiqul Alam, Fang Wang, Kaeling Tan, Michael J. Hynes, Joan M. Kelly[c] and Richard B. Todd

80*. WetA is a DNA-binding protein governing cellular and chemical development in Aspergillus
Ming-Yueh Wu*, Julia Martien, Matthew Mead, Daniel Amador-Noguez, Antonis Rokas, and Jae-Hyuk Yu

81. Palmitoylated protein ArkA and its putative targeted proteins in *Aspergillus*
Yuanwei Zhang, Congcong Sun and Ling Lu

82. Discovery of a novel azole-resistance mutation in *Aspergillus fumigatus* and the possible role of sexual reproduction in its evolution
J. Zhang, Bas Zwaan, E Snelders, S Schoustra, K Dijk, J Meis, F Hagen, E Kuijper, G Kampinga, J Zoll, W Melchers, P Verweij and A Debets

83*. Genetic parts screening and artificial N-glycosylation motif engineering for heterologous protein production in *Aspergillus niger*
Jinxiang Zhang, Saori Amaike-Campen, Sam Deutsch, Ljiljana Pasa-Tolic, Erika Zink, Jon Jacobs, Blake Simmons, John Gladden and Jon Magnuson

Presenting Authors

(Alphabetical; Student presenters in bold type with asterisk)

Presenter	Poster number
Abe, K.	1
Aguilar-Pontes, M. V.	**2***
Al Abdallah, Q.	3
Alazi, E.	**4***
ALFURAIJI, N.	**5***
Andersen, Mikael Rørdam	6
Anglin, Sarah Lea	7
Basenko, Evelina	8
Bromley, Mike	9
Bruno, K.S.	10
Canovas, D.	11, 12
Cary, J.	13
CHEN, Yingying	14
de Reus, P.E.	**15***
Dorter, I.	**16***
Fekete, E.	17
Frisvad, J.	18
Geib, E.	**19***
Gell, R.M.	**20***
Geranios, P.	**21***
Gerke, J.	22
Gomi, K.	23
Grijseels, Sietske	**24***
Gsaller, F.	25, 26
Han, Kap-Hoon	27, 28
Hoof, J.B.	29
Hunter, C.C.	**30***
Ichinose, S.	**31***
Inoue, Taishi	**32***
Jackson-Hayes, Loretta	33
Jung, Sascha	34
Kang, S.E.	**35***
Karaffa, L.	36
Khateb, Aiah	**37***
Khosravi, C.	**38***
Kim, J.	39
Kjaerboelling, I.	**40***
Koehler, A. M.	**41***
Kowalczyk, J. E.	**42***
Kuo, A.	43

Presenter	Poster number
Li, A.	**44***
Lind, Abigail	**45***
Luebeck, Peter	46.
Machida, Masayuki	47
Majumdar, R.	48
Malavazi, Iran	49
Mela, Alexander	**50***
Mitema, Alfred	**51***
Miyazawa, K.	**52***
Molo, M.S.	**53***
Mortensen, U.H.	54
Niu, M.	**55***
Nybo, J.L.	**56***
Oakley, Berl R.	60, 61
Oda, K.	57
Ohkura, Mana	**58***
Ouedraogo, Jean Paul	59
Pfannenstiel, B.T.	**62***
Rai, M. N.	63
Rantasalo, A.	**64***
Ryota, S.	65
Sato, A.	66
Shukla, N.	**67***
Snelders, E.	68
Sweany, R.R.	**69***
Tanaka, Mizuki	70
Tannous, J.	71
Theobald, S.	**72***
Thieme, S.	**73***
Vesth, T. C.	74
Wang, Clay C. C.	75
Wang, Fang	**76***
Weber, J.	77*
Wisecaver, Jennifer	78
Wong, Koon Ho	79
Wu, Ming-Yueh	**80***
Zhang, Yuanwei	81
Zhang, J.	82
Zhang, Jinxiang	**83***

Student Presenting Authors

(Alphabetical)

Presenter	Poster number
Aguilar-Pontes, Maria Victoria	2*
Alazi, Ebru	4*
ALFURAIJI, NARJES	5*
de Reus, P.Elise	15*
Dorter, Ilkay	16*
Geib, Elena	19*
Gell, Richard M.	20*
Geranios, Pavlos	21*
Grijseels, Sietske	24*
Hunter, Cameron C.	30*
Ichinose, Sakurako	31*
Inoue, Taishi	32*
Kang, S. Earl	35*
Khateb, Aiah	37*
Khosravi, Claire	38*
Kjaerboelling, Inge	40*
Koehler, Anna Maria	41*
Kowalczyk, Joanna E.	42*
Li, Ang	44*
Lind, Abigail	45*
Mela, Alexander	50*
Mitema, Alfred	51*
Miyazawa, Ken	52*
Molo, Megan S.	53*
Niu, Mengyao	55*
Nybo, Jane L.	56*
Ohkura, Mana	58*
Pfannenstiel, Brandon T.	62*
Rantasalo, A.	64*
Shukla, Nandini	67*
Sweany, Rebecca R.	69*
Theobald, Sebastian	72*
Thieme, Sabine	73*
Wang, Fang	76*
Wu, Ming-Yueh	80*
Zhang, Jinxiang	83*

Abstracts

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

1. Adsorption kinetics and self assembled structures of *Aspergillus oryzae* hydrophobin RolA on chemically modified solid surfaces.

Y. Terauchi[a], M. Nagayama[a], T. Tanaka[a], H. Tanabe[a], T. Arita[b], H. Higuchi[b], F. Hasegawa[c] and **K. Abe[a,c]**
[a]Dept. Microbial biotechnology, Grad. Sch. Agricult. Sci., Tohoku Univ., Sendai, Miyagi, JP [b]IMRAM, Tohoku University [c] NICHe, Tohoku University

Hydrophobins are amphipathic proteins that are ubiquitous among filamentous fungi, and required for the formation of fungal aerial structures (1). Hydrophobins also play an important role in the infection of pathogenic fungi because hydrophobins are not recognized by pattern recognition receptors of animals and plants (2, 3). The industrial fungus *Aspergillus oryzae* produces a hydrophobin RolA (4). RolA attaches and self-assembles on solid surfaces such as polyesters, and recruits an esterase CutL1, which results in stimulation of the hydrolysis of polyester by CutL1 (4). RolA also evades from recognition by mouse immune system (unpublished results). Because of these properties, RolA is expected to be applicable to industrial and medical fields. Therefore, kinetic properties and self-assembled properties of RolA-adsorption on solid surfaces are important. To investigate kinetic properties and self-assembled structures of RolA on solid surfaces, we constructed self-assembled monolayers (SAM) of 1-undcanethiol (hydrophobic), 11-amino-1-undecanethiol (cationic), and 10-carboxy-1-undecanethiol (anionic) on the electrodes of a Quartz Crystal Microbalance (QCM). We analyzed kinetics of RolA-adsorption on SAM-electrodes at various pH, and then observed self assembled structures of RolA by an AFM. Affinities between RolA and all SAM-electrodes were highest at pH 4 and lowest at pH 10. The forms of self-assembled structure of RolA depended on the pH values, amounts of adsorbed RolA, and functional groups of SAMs. Rod-like structures of self-assembled RolA were observed on all SAM-electrodes at pH 4. In addition, surface tensions of SAM-electrodes were modified by RolA adsorption. Overall, these results suggest that hydrophobic and electrostatic interactions between RolA molecules adsorbed on SAM-electrodes and soluble RolA molecules in water-phase depend on pH values and mainly contributed to the kinetics of RolA adsorption and self-assembly of RolA molecules on solid surfaces.

1) Wösten H. A., Annu Rev Microbiol. 55: 625 (2001)
2) Aimanianda V. et al., Nature. **460**:1117 (2009)
3) Martellini F. et al., Mol Biotechnol. 55: 27 (2013)
4) Takahashi T. et al., Mol Microbiol. 96: 14 (2015)

2*. Expression data integration in an *Aspergillus niger* genome-scale metabolic model

M. V. Aguilar-Pontes[a], E. McDonnell[b], K. Strasser[b], D. Fulton[b], A. Tsang[b] and R. P. de Vries[a]
[a]Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre & Utrecht University, Utrecht, NL [b]CSFG-Concordia University, Montreal, Canada

Filamentous fungi include important species used in industrial applications. One of the main representatives is *Aspergillus niger*, an industrial workhorse used for enzyme and metabolite production. In order for *A. niger* to achieve its full potential as a cell factory, deeper knowledge of its metabolism is needed. We propose a new metabolic network based on a manually curated annotation of the *Aspergillus niger* genome, including literature references, as well as information from curated and automated protein, metabolite and reaction databases and RNA-seq data. Reconstruction of the model and manual curation of the network based on different expression data sets was performed using Pathway Tools. In order to assess and improve our model, a comparison with previously publish genome scale models was performed (Andersen *et al.*, 2008; Lu *et al.,* 2016). Experimental results will be used to validate predictions of essential genes involved in growth as well as mutant behavior.
Our aim is to create a model that will give us new insights into the carbon metabolic pathways of *A. niger* and obtain leads to improve industrial processes. This model will also enable us and other researchers to study carbon utilization by fungi in more detail.

3. Components of the *Aspergillus fumigatus* Ras Post-translational modification pathway regulate conidial viability, hyphal growth, and virulence

Q. Al Abdallah[a], TA Norton[a] and JR Fortwendel [a]
[a]Department of Clinical Pharmacy, UTHSC, Memphis, TN

The Ras superfamily is comprised of highly conserved, membrane-associated GTPase proteins and activate several downstream signaling cascades. Localization of Ras proteins to the plasma membrane (PM) is an essential step for fungal growth and pathogenesis. In order to be properly localized, Ras proteins must undergo multiple steps of post-translational modifications (PTMs) at the conserved C-terminal CAAX box. In yeast and mammalian cells, these steps include farnesylation, followed by protease-mediated cleavage of the -AAX amino acids, carboxymethylation, and palmitoylation. Our *in silico* analysis suggests that this pathway is conserved in *Aspergillus fumigatus*. Additionally, our previous work shows that palmitoylation of *A. fumigatus* RasA is required for PM localization and for full virulence. The purpose of this study is to investigate the roles of the sole farnesyltransferase complex, as well as the CAAX protease and the methyltransferase in the growth of *A. fumigatus*. To achieve this, we deleted the genes that encode for RasA PTM homologues in *A. fumigatus* and characterized the deletion mutants for their growth rates, RasA localization and activation, and fungal virulence. Conidial germination, overall growth rate, and virulence were all reduced in the farnesylation-deficient mutant, although hyphal morphology appeared normal. Conidial viability was also decreased in the farnesylation mutant and was associated with formation of anuclear conidia. As expected, loss of farnesylation led to decreased RasA PM association. Surprisingly, fungal growth and development was not affected by either single or double deletion of the enzymes contributing to CAAX proteolysis. In addition, RasA localization to the PM and steady-state activation of RasA in the double mutant was unperturbed. However, loss of one of the CAAX proteolysis homologs, *rce1*, is associated with increased sensitivity to cell wall disruption. Together, our results suggest that CAAX proteolysis is not an essential step for fungal growth or regulation of RasA in *A. fumigatus*, whereas farnesylation contributes heavily to these processes.

4*. Evidence for inducer function of 2-keto-3-deoxy-L-galactonate in D-galacturonic acid induced gene expression in *Aspergillus niger*

E. Alazi[a], C. Khosravi[b], T.G. Homan[a], M. Arentshorst[a], M. Di Falco[c], M. Peng[b], M.V. Aguilar Pontes[b], A. Tsang[c], J. Visser[a], R.P. de Vries[b] and A.F.J. Ram[a]
[a]Molecular Microbiology and Biotechnology, Institute of Biology Leiden, Leiden University, The Netherlands [b]Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre & Fungal Molecular Physiology, Utrecht University, The Netherlands [c]Centre for Structural and Functional Genomics, Concordia University, Canada

Pectin is an important carbon source for the saprotrophic fungus *Aspergillus niger*, which is an efficient producer of pectin-degrading enzymes. D-galacturonic acid (GA) is the main product of pectin degradation. In *A. niger*, GA is transported into the cell by the sugar transporter GatA and subsequently catabolized into pyruvate and glycerol through a pathway consisting of four enzymes: GaaA, D-galacturonate reductase; GaaB, L-galactonate dehydratase; GaaC, 2-keto-3-deoxy-L-galactonate aldolase; and GaaD, L-glyceraldehyde reductase. It has been shown that GA or a derivative of GA is required for the induction of the genes needed for pectin degradation, GA transport, and GA catabolism. In order to identify the GA derivative that acts as an inducer, we constructed GA catabolic pathway deletion mutants (Δ*gaaA*, Δ*gaaB*, Δ*gaaC* and Δ*gaaD*) anticipating that the mutants would accumulate the substrate of the deleted enzyme when grown on GA. The growth of both Δ*gaaB* and Δ*gaaC* was abolished on GA pointing out that there are no redundant enzymes replacing GaaB and GaaC. Δ*gaaB* and Δ*gaaC*, pregrown in fructose medium and then transferred to GA medium, accumulated pathway intermediates L-galactonate and 2-keto-3-deoxy-L-galactonate, respectively. Northern analysis showed that the expression of the GA induced genes is drastically reduced in Δ*gaaB* and highly increased in Δ*gaaC* compared to the wild type strain. Genome wide gene expression analysis via RNA-seq indicated that the accumulation of 2-keto-3-deoxy-L-galactonate in Δ*gaaC* results in the induction of the GA induced genes. Identification of the inducer of the pectinase genes, together with the recent identification of the transcriptional activator-repressor module controlling pectinase expression would facilitate the industrial use of *A. niger* in pectinase production.

5*. Parallel fitness using barcode sequencing in *A. fumigatus*: Assessment of the role of Aspergillus Kinases in azole resistance and as Targets for Antifungal Drug Discovery

N. ALFURAIJI[a], J. Mabey [a], M. Bromley [a] and P. Bowyer[a]
[a]Manchester Fungal Infection Group,Division of Immunity, Infection and Respiratory Medicine,Faculty of Biology, Medicine and Health Sciences, University of Manchester

Fungi cause a wide range of infections including invasive and life threatening disease, superficial infections of the skin and mucosal membranes, as well as allergic disorders. The mortality rate due to fungal diseases remains unacceptably high, and is thought to exceed one million patients annually. Currently, only four groups of antifungals are available to treat systemic fungal infections: the polyenes, flucytosine, triazoles and echinocandins. The emergence of resistance to the available antifungal drugs and toxicity associated with some classes necessitates the exploration of novel pharmacologically effective antifungal drugs. Protein phosphorylation by protein kinases (PK) impacts all areas of cellular activity. Their critical roles in a wide range of cellular functions, along with the relative ease in which drug inhibitor assays can be developed have highlighted this class of enzymes as potential drug targets.
A bioinformatic analysis of the genomes of 9 Aspergillus species has revealed, in contrast to a previous study, that the number of protein kinases in each species is relatively consistent ranging from a minimum of 140 in *A. nidulans* up to 175 in *A. niger*. The kinases are distributed over 11 sub-groups including genes which apparently encode tyrosine like kinases and tyrosine kinases. We present comparative analysis which suggests that there are a sub-group of kinases that are specific to filamentous fungi.
To assess the role of these kinases in growth, virulence and drug tolerance, we have generated a library of 90 genetically barcoded knockout mutants. We have validated a barcode-sequencing approach to assess in pooled cultures, the fitness defect in each null mutant when in standard culture conditions and in the presence of the antifungal drug itraconazole. We show that 2 genes, the mitogen-activated protein kinase *mpkB* (AFUB_078810) and a serine/threonine protein kinase *ssn3* (AFUB_035220) are critical for azole tolerance. In addition, we have identified 25 genes that encode kinases that are essential for viability. Our assessment of virulence of these strains is currently in progress.

6. Expansions and reductions in fungal primary metabolism studied across 100 fungal species

Julian Brandl[a], Jane Nybo[a], Tammi C. Vesth[a] and **Mikael Rørdam Andersen**[a]
[a]Department of Biotechnology and Biomedicine, Technical University of Denmark, Kgs Lyngby, DK

The primary metabolism of fungi is the power house that drives nearly all cellular functions. Primary metabolism is involved in converting the surroundings of the fungus to a food source as well as delivering metabolite precursors for everything from cellular growth and maintenance over biological responses to external stimuli to producing secreted secondary metabolites and protein effectors. Furthermore, it is known that fungal metabolism is highly versatile. Saphrophytic fungi can grow on a very wide range of carbon and nitrogen sources and utilize this for production of even more diverse range of secondary metabolites and secreted proteins.

Within this project, we have been interested in two main things: 1) What is the "roadmap" of fungal metabolism within a single species? What is the total sum of genes involved in primary metabolism? For studying this, we have been focusing on the industrial workhorse and model fungus *Aspergillus niger*. 2) What is the diversity of metabolism across fungi? Which gene functions are expanded, reduced, added and lost across species? For this we have used the metabolic "roadmap" of *Aspergillus niger*, and used it to query the genomes of approximately 100 different species, primarily of the genus Aspergillus.

For the initial roadmap of fungal metabolism, we have reconstructed the metabolism of *A. niger* ATCC 1015 at genome-scale. The metabolic reconstruction covers 1801 metabolic conversions, 997 genes, and 1411 metabolites across six compartments. Phenotype arrays have been applied to evaluate the ability to germinate on 180 carbon sources and 92 nitrogen sources. Examining the metabolism shows a high degree of isoenzymes across both central and outer metabolism.

Employing this metabolic network to our database of fungal genomes allows us to examine the diversity of metabolic strategies through the identification of orthologs across species and map this to the species. Our analysis shows that some specialized fungi have a more reduced genome and survives with 1-2

isoenzymes for most of central metabolism, while others apply a diversification strategy and often have 2-5 isoenzymes, even for the highly conserved functions in central metabolism.

7. Mutations in *set2*, the Histone H3-K36 methyltransferase, rescue transcriptional down-regulation in *snxA1* and *snxA2* mutants of *Aspergillus nidulans*.
SW James[a], JM. Palmer[b], A Soukup[b], NP Keller[b], L Ciraku[a], M Dunworth[a], S Francisco[a], and **SL Anglin**[c].
[a]Gettysburg College, Gettysburg, PA; [b]University of Wisconsin-Madison, Madison, Wisconsin; [c]Millsaps College, Jackson, MS

The *Aspergillus nidulans snxA* (suppressor of *nimX-cdc2*) gene encodes an ortholog of the budding yeast SR/RRM family protein Hrb1/Gbp2, which participates in mRNA export and prevents the export of defective or abnormally spliced mRNAs. The A. nidulans *snxA1* and *snxA2* mutations specifically suppress defects in regulators of the CDK1 mitotic induction pathway (James et al., 2014. GENETICS 198: 617-633). Recessive *snxA1* and *snxA2* mutations exhibit strongly repressed mRNA and protein expression at the permissive temperature, indicating that snxA acts normally by antagonizing the CDK1 mitotic induction pathway to restrain the G2/M transition. In order to identify genetic changes in the mutants, we performed whole-genome sequencing, followed by de novo genome assembly. This approach revealed a reciprocal translocation in which a portion of the *snxA* locus on right arm of Chromosome II was joined to a large uncharacterized gene on the left arm of Chromosome I. The Chromosome II breakpoint occurs in the first snxA intron, separating the promoter region and first exon from most of the coding region and thereby explaining the dramatic reduction of expression in *snxA1* and *snxA2* mutants. The Chromosome I breakpoint occurs within an exon in a novel GYF-domain gene, AN6228. To better understand *snxA* transcriptional control, we undertook a mutational screen for extragenic suppressors of *snxA2* cold-sensitivity. Whole-genome sequencing of suppressors identified *set2*, a histone H3-K36 methyltransferase, loss of which strongly rescued *snxA2* and weakly suppressed *snxA1*. These phenotypes were copied in the cognate Histone H3-K36L mutant, indicating that methylation of H3-K36 acts to repress transcription in *snxA* mutants. Restoration of transcriptional proficiency in *snxA1* and *snxA2* mutants by the *set2* mutation and the H3-K36L mutation suggest the activation of a cryptic promoter proximal to the translocation break point on chromosome II.

8. FungiDB: An integrated functional genomics database for fungi and oomycetes.
Evelina Basenko[a], Omar Harb[b], Achchuthan Shanmugasundram[a] and David Roos[b]
[a]Centre for Genomic Research, Functional and Comparative Genomics, University of Liverpool, Liverpool, UK [b]Penn Center for Bioinformatics and Department of Biology, University of Pennsylvania, Philadelphia, PA

FungiDB (http://FungiDB.org) is a free online resource for data-mining and functional genomics analysis. As part of the Eukaryotic Pathogen Bioinformatics Resource Center (http://EuPathDB.org), FungiDB provides an easy to use and interactive interface to explore genomes, annotation, functional data (transcriptomics or proteomics), metabolic pathways and results from numerous genome wide analyses (ie. InterPro scan, signal peptide and transmembrane domain predictions, orthology, etc..). FungiDB contains an expanding number of genomes from species spanning the Ascomycota*,* Basidiomycota*,* zygomycete, and chytrid fungi; including pathogenic species from the *Cryptococcus, Histoplasma*, and *Coccidiodes* genera.

The graphical user interface in FungiDB allows users to conduct *in silico* experiments that leverage the available data and analyses. For example, a search in FungiDB can identify all genes in *Candida albicans* that do not have orthologs in mammals, have a predicted signal peptide, are annotated as a kinase and are expressed under conditions of high oxygen stress. Results from any search can be further analyzed visually using a companion genome browser or through analysis tools such as genome ontology and metabolic pathway enrichment. All results and searches can be saved in a user's profile or downloaded in multiple formats.

Users of FungiDB can also privately analyze their own data (ie. RNAseq or genomic sequence) via the EuPathDB galaxy implementation. All genomes in EuPathDB are preloaded into this galaxy instance

allowing more streamlined data analysis. Results from galaxy workflows can be downloaded or analyzed within FungiDB, such as through visualization of RNAseq read coverage plots in the genome browser.

Input from the community (images, files, PubMed records, *etc*) can be easily added to FungiDB records (ie. gene pages) via user comments; these comments are attributed to the submitted and become immediately visible and searchable.

FungiDB is supported in part by NIH HHSN272201400030C and the Welcome Trust grant WT108443MAAuthors are individuals present at this meeting and representing the EuPathDB team.

9. The Negative Cofactor 2 complex mediates azole resistance through transcriptional regulation of an efflux transporter and ergosterol biosynthesis in *Aspergillus fumigatus.*

Takanori Furukawa[a], Fabio Gsaller[a], Josie Parker[b], Steve Kelly[b], Scott Moye-Rowley[c], Paul Bowyer[b] and **Mike Bromley[a]**
[a]Manchester Fungal Infection Group, University of Manchester, Manchester, UK [b]Institute of Life Science, Swansea University Medical School , UK [c]Department of Molecular Physiology and Biophysics, University of Iowa

A. fumigatus is the most important airborne mould pathogen and allergen worldwide. Estimates suggest that over 3 million people have invasive or chronic infections that lead to in excess of 600,000 deaths every year. Very few drugs are available to treat the various forms of aspergillosis and we rely predominantly on the azole class of agents (Itraconazole, Voriconazole, Posoconazole and the recently licensed Isavuconazole). Resistance to the azoles is emerging. For individuals that are infected with a resistant isolate the mortality rate exceeds 88%. Therapy failure is in part attributed to delays in administering alternative therapies so methods to rapidly detect resistance is critical. While resistance in around 50% of clinical isolates has been linked to modification of the gene encoding the target of the azoles, *cyp51A*, our understanding of what leads to resistance in the remaining strains is lacking. To examine the mechanistic basis of non-target driven azole resistance we have carried out a systematic evaluation of the role of transcription factors in drug tolerance.
We have screened a library of 401 transcription factor *A. fumigatus* knockout strains and identified a cohort of factors governing azole resistance and sensitivity. Here we report on two CBF/NF-y family transcription regulators, AFUB_029870 (NctA) and AFUB_045980 (NctB) where loss of function leads to azole resistance. The orthologues of these regulators in yeast, known respectively as BUR6 and NCB2, are subunits of a heterotrimeric transcriptional regulator called Negative Cofactor 2 (NC2). In keeping with the hypothesis that NctA and NctB are part of the same transcriptional regulatory complex, we show the transcriptional regulons governed by these two factors are essentially identical. Furthermore, reciprocal co-immunoprecipitation studies using S-tagged versions of the proteins indicate that they interact directly with each other.
With a view to understanding which genes are directly regulated by NctA, we performed genome-wide protein-DNA interaction analysis using ChIP-seq. Over 1500 NctA interacting loci were identified, including 13 genes which encode members of the ergosterol biosynthetic pathway, and the efflux transporter cdr1B. Eight of these sterol biosynthetic genes along with cdr1B are transcriptionally upregulated in our NctA null leading to elevated levels of ergosterol and Cdr1B protein. Evaluation of the clinical significance of these findings is under investigation.

10. Adapting filamentous fungi to an automated environment for the application of a Design-Build-Test-Analyze-Learn approach to strain optimization

K.S. Bruno[a], E. Szewczyk[a], K Rothschild-Mancinelli[a] and P.J. Westfall[a]
[a]Zymergen, Inc, Emeryville, CA

Identifying, understanding and ultimately engineering the complex set of interacting genes responsible for any cellular phenotype is extremely difficult and traditionally requires years of study. For example, optimizing metabolic flux to a native or non-native small molecule, or increasing the diversity of carbon sources that a microbe can use are well studied topics and yet very few novel compounds have made it through the high risk path to commercialization. To address this problem, Zymergen has built a platform for automated and high-throughput microbial strain improvement that is focused on improving the

economics of large-scale fermentation processes and accelerating the industrialization of novel bio-products. The platform has been designed to be flexible, highly reliable, and host-agnostic.

Filamentous fungi are sought after as production hosts for their ability to tolerate a variety of feedstocks as well as their capacity for high production of enzymes and other small compounds. However, adapting fungi to modern automation platforms for high throughput manipulation poses some unique challenges. Large scale, routine preparation of protoplasts, automated liquid handling during transformation, reduction of heterokaryon formation and minimizing cross-contamination with volatile spores are just some of the engineering challenges that need to be overcome to ensure successful strain improvement outcomes. Here we describe the adaptation of Aspergillus niger to an automated liquid handling and data capture environment that allows large scale implementation of a Design-Build-Test-Analyze-Learn cycle for strain improvement and pathway engineering.

11. Genome wide consequences of the deletion of the Aspergilli non-homologous end joining (NHEJ) DNA repair mechanism

I. Álvarez-Escribano[a], C. Sasse[b], J. Woo Bok[c], A. Lipzen[d], W. Schackwitz[d], J. Marin[d], K. Barry[d], I. Grigoriev[d], A.T. Marcos[a], N.P. Keller[c], G.H. Braus[b] and **D. Canovas[a]**
[a]Department of Genetics, Faculty of Biology, University of Seville, Spain [b]Department of Molecular Microbiology and Genetics and Göttingen Center for Molecular Biosciences (GZMB), Georg-August-University, Göttingen, Germany [c]Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, Wisconsin, USA; Department of Bacteriology, University of Wisconsin-Madison, Madison, Wisconsin, USA [d]DOE Joint Genome Institute, Walnut Creek, California, USA

The relevance of *Aspergillus* for human daily life is immeasurable: not only because of its industrial applications but also because of its clinical implications. Most *Aspergillus* laboratories world-wide use mutants in the non-homologous end joining (NHEJ) pathway (*KU70* or *KU80*) to do genetic modifications in the strains under study due to a higher frequency of homologous integrations after transformation of DNA constructs. *KU70* is involved in a DNA repair mechanism highly conserved in eukaryotes, and genetic manipulations are much faster in NHEJ mutant (Δ*KU70*) strains in many fungal species.
Three *Aspergillus* species were selected for this study to allow for comparative genetics and genomics: *A. flavus, A. fumigatus* and *A. nidulans*. Wild-type and NHEJ mutants were grown on solid media for 60 growth passages using asexual spores (ca. 3,000 mitosis). In the case of *A. nidulans* ten passages with sexual cleistothecia were also independently performed. Genome sequences were obtained and analyzed. In both *A. flavus* and *A. fumigatus*, the *KU70* mutants accumulated on average more mutations than the wild type strains (8.37 +/- 1.80 vs 6.12 +/- 1.76 in *A. flavus*, and 5.75 +/- 2.33 vs 4.12 +/- 1.76 in *A. fumigatus*). The higher number of non-synonymous mutations in *A. fumigatus* and of mutations in the intergenic regions in *A. flavus* in the *KU70* mutants was statistically significant. None of the sexual passages of the *A. nidulans KU70* strain could be completed due to the lack of cleistothecia formation, while the majority of the wild type passages reached ten passages. Collectively here we provide an assessment of the effects of the NHEJ pathway in the genomic stability in Aspergilli.

12. Role of the urea cycle in the synthesis of nitric oxide

D. Canovas[a,b], A.T. Marcos[a], J.F. Marcos[c], T. Schinko[b] and J. Strauss[b]
[a]Department of Genetics, University of Sevilla, Sevilla, Spain [b]Division of Microbial Genetics and Pathogen Interactions, Department of Applied Genetics and Cell Biology, BOKU-University of Natural Resources and Life Sciences Vienna, Austria [c]Department of Food Science, Institute of Agrochemistry and Food Technology (IATA), Valencia, Spain

Nitric oxide (NO), and its role in signalling, has been extensively studied in mammals and to some extent in plants. However, little is known about the role of NO in fungi and how it is synthesized in these organisms. Recently, we reported that NO production in *A. nidulans* is coupled to conidiation and requires a functional nitrate reductase (NR) gene (*niaD*) that is upregulated under these conditions even in the presence of the repressing nitrogen source ammonium. NO levels influence the balance between conidiation and sexual reproduction.
Here we report that NO levels are also modulated by light, a general environmental cue and a regulator of

fungal development. The light-dependent modulation of nitric oxide levels involves NO catabolism by the mitochondrial flavohemoglobin *fhbB*, and *agaA*, an arginase that controls the intracellular concentration of the NO precursor arginine. Addition of arginine to the cultures provokes a transient increase of the production of NO. However, analogues of arginine did not affect the production of NO. Mutants in the urea cycle genes show differences in NO levels compared to the wild type strain. Taken together our findings indicate that light-dependent developmental processes in *A. nidulans* interfere with nitric oxide metabolism which – in addition to nitrate reduction - is modulated by enzymes of the urea cycle.
Reference: Marcos AT, Ramos MS, Marcos JF, Carmona L, Strauss J, Cánovas D. Nitric oxide synthesis by nitrate reductase is regulated during development in *Aspergillus*. Mol Microbiol (2016) 99:15-33.

13. Identification and characterization of the *Aspergillus flavus* aspergillic acid gene cluster
J. Cary[a], M. Lebar[a], C. Carter-Wientjes[a], B. Mack[a], R. Majumdar[a], J. Diana Di Mavungu[b] and S. De Saeger[b]
[a]USDA-ARS-SRRC, New Orleans, LA [b]Laboratory of Food Analysis, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium

Aspergillus flavus produces toxic and carcinogenic aflatoxins. *In silico* analysis of the *A. flavus* genome revealed 56 gene clusters encoding for secondary metabolites (SMs). Although about 20 SMs have been identified from *A. flavus* cultures, studies by our group and others have experimentally assigned metabolites to just ten *A. flavus* gene clusters. We are interested in how these SMs affect fungal development, survival, and virulence. We are particularly interested in *A. flavus* metabolites that are produced during infection of corn seed. RNA-Seq analysis of all predicted *A. flavus* secondary metabolic gene cluster 'backbone' genes during corn kernel infection showed that in addition to the aflatoxin cluster polyketide synthase (PKS) gene, *aflC*, one of the earliest genes expressed was the uncharacterized Cluster 11 nonribosomal peptide synthetase (NRPS) gene, *asaB* (AFLA_023020). SMURF analysis indicated that Cluster 11 may be composed of as many as 12 genes, however we focused on five genes encoding the putative NRPS, desaturase, P450 oxidoreductase, MFS transporter and C6 transcription factor. LC-MS analysis of extracts from knockout mutants of these genes showed that they were responsible for the synthesis of the previously characterized pyrazinone, deoxyaspergillic acid (deoxy-AA), and the antimicrobial hydroxamic acid-containing mycotoxins aspergillic acid (AA) and hydroxy-AA (OH-AA). From the LC-MS data we have proposed a biosynthetic pathway for the production of these metabolites. In addition, we found that AA can form a trimer with one atom of iron to generate ferriaspergillin, perhaps indicating that this cluster is involved in regulation of iron homeostasis in *A. flavus*.

14. Global Analysis of CreA Regulatory Network in *Aspergillus nidulans*
Yingying CHEN[a], Liguo DONG[a], Md Ashiqul Alam[b], Fang Wang[a], Joan Kelly[b] and Koon Ho Wong[a]
[a]Faculty of Health Science, University of Macau, Macau, CN [b]Department of Genetics and Evolution, School of Biological Science, The University of Adelaide

Carbon metabolism is central to all living organisms governing many physiological processes.
In *Aspergillus nidulans*, carbon metabolism is tightly controlled at the transcription level by a process known as carbon catabolite repression (CCR). CCR is mediated by a global transcriptional repressor called CreA. Despite decades of research on CreA, the full spectrum of CreA targets and how it globally regulates and coordinates expression of genes remain unclear. In this research, we applied two powerful approaches RNA-seq and ChIP-seq for a genome-wide study of CreA under repressing and derepressing conditions. We discovered that CreA binds to several thousands promoters in the genome. The bindings are found mainly at the nucleosome-depleted promoter regions and are enriched with GC-rich motifs along with many other motifs. The set of CreA direct targets include genes involved in biosynthetic process, transmembrane transport, response to stimulus, regulation of transcription, ion homeostasis and sexual reproduction. Some of these roles have been confirmed by functional tests. Interestingly, our analysis reveals that the CreA binding intensity, the number of CreA binding sites and additional motifs are associated with different sets of CreA target genes of different physiological functions. Moreover, our genome-wide ChIP-seq data also revealed that CreA constitutively binds to the target promoters at similar levels under both repression and de-repression conditions, even for genes whose expression is markedly increased (i.e. de-repressed) under the de-repression condition. This observation strongly indicates that

CreA binding alone is not sufficient for CreA regulation, and in turn implies that post-translational modification(s) play(s) crucial role in CreA regulation. Transcription profiling analysis by RNA-seq of wildtype and *creA*∆ mutant strains reveals that CreA not only acts as a transcription repressor, but also has a positive role for many target genes. Surprisingly, we found that expression of more than half of CreA bound genes are not significantly affected in the *creA*∆ strain. This suggests that either CreA pauses at many promoters pending modification for function or the activators responsible for activating those genes are not available for function under our experimental conditions. Taken together, our results reveal a comprehensive global CreA regulatory network at a whole-genome level and illuminate novel CreA regulating patterns and functions.

15*. An experimental approach to studying the role of Horizontal Gene Transfer in shaping fungal secondary metabolism

P.E. de Reus[a], K.F.N. Nielsen[a] and R.J.N. Frandsen[a]
[a]Biotechnology and Biomedicine, Technical University Denmark, Copenhagen, DK

The *in-silico* discovery rate of putative secondary metabolite gene clusters in filamentous fungi is on a surge. With it come increasingly many reports of Horizontal Gene Transfer (HGT) of these gene clusters. Such events are inferred from discrepancies in the synteny, nucleotide composition or taxonomical distribution of the gene clusters in question. Clustered genes have been reported to undergo HGT more often than unclustered genes, for which various hypotheses can be offered. This 'genetic sharing economy' is very interesting from an evolutionary perspective, and yet *in-silico* studies leave fundamental questions about the functional implications of HGT unanswered.

Our study takes an experimental approach to studying the role of HGT in shaping fungal secondary metabolism, focusing on the immediate impact of a whole-cluster HGT event on the recipient. Four naphto-y-pyrone gene clusters - two confirmed and two putative - were selected from four different native fungal hosts (*Fusarium graminearum*, *Fusarium fujikuroi*, *Trichophyton rubrum*, and *Aspergillus eucalypticola*). Each cluster was transferred to the same locus in host organism *Aspergillus nidulans* using a one-step PCR-based *in-vivo* recombination method. Alongside the clusters, a second library was created in which the cluster pathway-specific transcription factors were overexpressed in a second locus. In a third library, the transcription factors were overexpressed in strains lacking the cluster.

Transcriptional activity of the clusters was investigated using RT-qPCR to address splicing and trans-regulatory interactions as two of the potential barriers to the functional expression of HGT gene clusters in the new host. Metabolic profiling of the libraries was done by HPLC-UV/Vis-High Resolution MS to screen for production of pathway products and intermediates, as well as other novel metabolites arising from cross-chemistry or cross-regulation.

This case study, on a small scale, demonstrates the added value of *in-vivo* experimental work to complement *in-silico* findings and hypotheses, offering additional insights into the role of HGT in fungal secondary metabolism, as well as providing an additional tool for cluster validation.

16*. Understanding the role of septin AspD in the early vegetative growth of *Aspergillus nidulans*

I. Dorter[a] and M. Momany[a]
[a]Plant Biology, UGA, Athens, GA

Basic research on septin dynamics in the model organism *Aspergillus nidulans* helps us better understand the cellular and molecular mechanisms of these cytoskeletal elements and can help develop new therapeutic approaches against diseases. Under the right conditions *Aspergillus nidulans* spores emerge a germ tube to explore new growth medium. Thereby fungal hyphal tips grow into the food substrate forming a hyphal network with evenly spaced lateral branches. This basic process requires a fine and precise coordination of biochemical and cellular processes. We performed single deletions of the genes encoding for the core septins in *A. nidulans* (*AspA*-*D*). In the absence of AspA, AspB and AspC we could observe an abnormal increase of lateral branches, which is also known as hyperbranching. The knock-out of the fourth core septin gene *AspD* did not result in obvious phenotypic changes whereas

fluorescence microscopy revealed abnormal nuclear structures that worsened with each mitotic division. In contrast to WT the average distance between two neighboring nuclei was greatly reduced in the Δ*aspD* mutant background, whereas the nuclei themselves were slightly elongated. Time-lapse analyses with a strain expressing AspD-GFP fusion protein showed septin bars contacting both nucleus and cell cortex. Shortly after nuclear division the septin bar located between the two newly divided nuclei suggesting that the septin AspD plays a role after mitosis. In addition, the disruption of the septins revealed an increased number of cells harboring an odd number of nuclei. This may point to an asynchronous nuclear division cycle of the multinucleated fungus in the absence of core septins. We asked the question whether septins could be part of a checkpoint that regulates nuclear division by monitoring cell cycle progression. We treated WT and septin deletion mutants with benomyl, which depolymerizes microtubules and inhibits spindle formation thereby activating the spindle assembly checkpoint. In contrast to the other cells all *ΔaspD* mutants showed changes in conidiospore density and color, a sectoring phenotype, which is the result of chromosome missegregation. This supports the idea that the SAC checkpoint did not function properly in the absence of AspD. We were able to construct a strain, which expresses both AspB-CFP and AspD-GFP and could confirm that at least two distinct septin heteropolymer populations co-exist. Future time-lapse analyses will show if these two populations play different roles during nuclear division in *A. nidulans*.

17. Pivotal role for the alternatively spliced, spliceosomal twin intron in one-bp intron drift

E. Fekete[a], M. Flipphi[a], N. Ág[a], N. Kavalecz[a], G.C. Cerqueira[b], C. Scazzocchio[c] and L. Karaffa[a]
[a]Department of Biochemical Engineering, University of Debrecen, Debrecen, HU [b]Broad Institute of MIT & Harvard, Cambridge MA, USA [c]Dept. of Microbiology, Imperial College London, UK

In the primary transcript of nuclear genes, coding sequences – exons – usually alternate with non-coding sequences – introns. The latter are removed and former are joined by means of splicing to create the mRNA ORF that translates into the functional peptide product. In the study of the control of fungal gene expression, the ubiquitous splicing process is largely ignored. In the evolution of genic intron-exon structure, introns are gained at new positions or lost from extant positions or they move to a new position. The latter "intron drift" or "intron slide" leads to discordant introns in ortholog genes.
Previously we described stwintrons (spliceosomal twin introns) in filamentous ascomycota (*). These are complex intervening sequences in which a canonical "internal" intron interrupts one of the three conserved domains of a canonical "external" intron and consequently, they can only be removed with two subsequent splicing reactions. A stwintron in which the donor of the external intron is interrupted between the first and second bp – [D1,2] – can also be removed alternatively if the acceptor of the other external intron is interrupted between the penultimate and ultimate bp – [A2,3] – where the resultant mRNA is identical for both splicing routes.

Here we present a new stwintron, the first uncovered in *Aspergillus nidulans*. Ortholog genes occur in 4 Pezizomycotina classes. Most encompass either a [D1,2] or an alternatively spliced [D1,2] /[A2,3] stwintron at the same position. However, 9 species harbor there a discordant, canonical intron that conforms a one-bp intron drift: 7 have a phase-2 intron and appear to miss the [D1,2] internal intron while two carry a phase-0 intron and they appear to lack the [A2,3] internal intron instead. This is one of the few instances of intron drift where its mechanism could be elucidated.

(*) Ág et al. (2015) Fungal Genet Biol 85:7 & Flipphi et al. (2013) Fungal Genet Biol 57:48.

This research was supported by the EU and co-financed by the European Regional Development Fund under the project GINOP-2.3.2-15-2016-00008.

18. Using intra-species fungal diversity to get different epigenetic imprints on fungal exometabolite production

J. Frisvad[a]
[a]CMB, Dept Systems Biol - DTU, Kongens Lyngby, DK

Fungi that are primarily Competition-selected (C-selected), such as species of *Aspergillus*, *Penicillium Paecilomyces* and *Talaromyces*, have been reported to produce many different secondary metabolites (exometabolites). Some of these exometabolites are produced on commonly used agar or broth media, while others need to be epigenetically induced by exometabolites from other species. Alternatively histone de-methylation or deacetylation inhibitors may be added to a medium in order to increase the number and kinds of exometabolites produced by the fungus. Often the use of an additional medium will increase the number of members of one biosynthetic family of exometabolites. On the commonly used media Czapek yeast autolysate (CYA) agar and yeast extract sucrose (YES) agar *Aspergillus taichungensis* did not produce any prenylated indol alkaloids, while on rice the same isolate produced 21 different taichunamides. However exometabolites coded by apparently silent gene clusters can also be discovered by examining isolates of the same species from different geographic regions. *Penicillium antarcticum* is a marine-derived fungal species producing a series of bioactive exometabolites. 47 strains from all over the world were analyzed using HPLC-DAD after growth on CYA and YES agar. Of these 45 produced asperentins, 40 patulin, 31 antarones, 28 fischerin, 25 atlantinone A, 23 chrysogines, 22 phthalides, 12 penitrems, 12 deacetoxyfructigenine, 5 a terrestric acid-like metabolite, 4 atrovenetins, 4 austalides, 3 cytochalasins, 1 an orthosporin-like metabolite and 1 patulodins. Furthermore potentially new extrolites (chromophore families not observed in any other *Penicillium* species) were produced by 26, 11, 5, 4, 3, 2, and 6 singleton isolates respectively. Examining several isolates within a species from different niches and geographic regions is an alternative way of discovering potentially bioactive exometabolites that could otherwise only be discovered by genome sequencing and using exometabolite gene cluster search algorithms. These observations indicate that Aspergilli and Penicillia can be epigenetically imprinted to express their exometabolites in certain instances.

19*. *Aspergillus niger versus Aspergillus oryzae*: Expression platforms for heterologous secondary metabolite production

E. Geib[a] and M. Brock[a]
[a]Fungal Genetics and Biology, University of Nottingham, Nottingham, GB

Filamentous fungi are treasure chests for novel secondary metabolites and genome mining has uncovered a multitude of yet unexplored secondary metabolite biosynthesis gene clusters. Their identification and characterisation is crucial for the development of drugs that combat various kinds of diseases. Unfortunately, most of these gene clusters appear silent under laboratory cultivation conditions, which requires heterologous gene expression in well-characterised expression systems. Previously, we developed such an expression system in *Aspergillus niger*, which bases on regulatory elements from the *Aspergillus terreus* terrein biosynthetic gene cluster. We used this platform to produce polyketides (e.g. lecanoric acid), non-ribosomal peptide synthetase-like products (e.g. aspulvinone E) and reconstituted the Asp-melanin biosynthesis pathway from *A. terreus*.
The latter studies led to an interest in understanding the chemistry of NRPS-like enzymes that produce metabolites with antifungal, cytotoxic, antitumorigenic and antiviral activity. Enzymes of this class may accept the same substrate, but form different products depending on their thioesterase domain. To study these domains, we compared the aspulvinone E synthetase MelA from *A. terreus* with the atromentin synthetase InvA5 from *Paxillus involutus*. While recombinant expression of *melA* in *A. niger* resulted in aspulvinone E production, expression of *invA5* led to a range of yet unknown products, but failed to produce atromentin. In contrast, recombinant and purified InvA5 produced atromentin *in vitro*. We therefore speculated that the physiology of *A. niger* might lead to a modification of the InvA5-derived metabolite. Consequently, the expression system was transferred to the alternative host *Aspergillus oryzae*. Indeed, *A. oryzae* produced aspulvinone E from MelA and, even more, atromentin from InvA5. In conclusion, our recombinant expression system is perfectly suited for heterologous production of secondary metabolites. However, the metabolic physiology of *A. niger* and *A. oryzae* differs and at least

two different expression platforms should be selected when aiming in the characterisation of novel secondary metabolite biosynthesis genes.

20*. Sexual fertility in *Aspergillus flavus*: understanding genetic exchange and phenotypic inheritance through analysis of F1 progeny

R.M. Gell[a] and I. Carbone[a]
[a]Center for Integrated Fungal Research, Program of Genetics, Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, USA

The carcinogenic mycotoxin aflatoxin is a constant threat and economic burden to corn and oil seed crops grown within the United States and globally. Aflatoxin is produced by species in *Aspergillus* section *Flavi*, primarily *Aspergillus flavus*. Within the US and Africa, aflatoxin contamination is controlled through the high-density application of non-aflatoxin producing *A. flavus* strains. Though previously thought of as only asexual, *A. flavus* has recently been found to undergo sexual reproduction both in laboratory crosses and in the field. During the mating process, the sclerotium, a survival structure, of one strain acts as the female parent providing both the mitochondria and a matrix for the ascocarps and progeny to grow, while a spore or propagule from a second compatible strain fertilizes as the male. The fertility of mating pairs is highly variable and strongly influenced by the directionality of the cross but little is known about the genetic basis of fertility and how it is regulated. We are examining crosses that exhibit high fertility in one direction, but low fertility when male and female parents are reversed. Genome wide data using double digest Restriction Associated DNA sequencing was obtained for the progeny of these biased crosses. These data are being used to explore genome-wide recombination and as markers for mapping genomic regions that influence phenotypes, such as bias in fertility. By understanding this variation, we create opportunities to utilize strain fertility in the selection of biological control agents and increase our understanding of genetic exchange within populations of *A. flavus*.

21*. Understanding cell tropisms in *Aspergillus fumigatus* hyphae

P. Geranios[a], N. Al-Furaji[a], K. Lord[b], M. Bromley[a] and N. Read[a]
[a]Manchester Fungal Infection Group, University of Manchester, Manchester, GB [b]University of Edinburgh, Edinburgh, GB

Fungal cell tropisms involve directional changes of growing fungal cells or hyphae in response to an external stimulus. Negative cell tropisms are ubiquitous in filamentous fungi. Two clear examples are: (1) avoidance of adjacent germ tubes during colony initiation; and (2) avoidance of adjacent vegetative hyphae and branches at the colony periphery. Both tropisms have been proposed to be important in reducing the competition of neighbouring germ tubes/hyphae for nutrients. Despite the widespread occurrence of negative tropisms in fungi, little is known about the signalling processes governing the phenomenon. The aim of our work is to understand the mechanistic basis of negative cell tropism (self-avoidance) during spore germination and hyphal growth in the human pathogen Aspergillus fumigatus. To this end, confocal live-cell imaging and quantitative image analysis was employed to image and measure the angles formed between germlings when visualized in both 2D and 3D as the conidial germlings invade the agar substrata of different hardnesses. The angles formed between germlings were found to be dependent on the number of germlings in a given group. Our time-lapse imaging data indicated that re-arrangement of the growth axis occurs almost immediately when hyphae approach each other. Furthermore, the avoiding hyphae also exhibited a propensity to invade their agar substratum and this was influenced by its hardness. Mutants blocked in secondary metabolite synthesis or in the presence of a NO scavenger or CO_2indicated that the avoidance signal was probably not a secondary metabolite or NO.
A kinase knockout library of 96 mutant strains of A. fumigatus has been screened with the aim of identifying strains defective in hyphal self-avoidance. The initial screening has involved classifying the mutants into three categories based on their growth phenotype: i) no obvious effect on polarized growth, ii) possibly defective in self-avoidance, and iii) major growth defects. During the kinase mutant screen we have found out that the best way to visualise negative tropisms in a screen like this is to image leading hyphae at the periphery of approaching colonies.
The chemical signal responsible for these negative tropisms has not yet been identified but is suspected

to be likely a volatile because it occurs on cellophane overlying growth medium. Currently, the volatiles released by the developing colony are being identified and analysed.

22. ZtfA is a novel regulator of asexual development, secondary metabolism and oxidative stress defense in *Aspergillus nidulans*

K.G. Thieme[a], **J. Gerke[a]**, C. Sasse[a], S. Thieme[a], O. Valerius[a] and G.H. Braus[a]
[a]Institute of Microbiology and Genetics, Georg-August University, Göttingen, DE

In the filamentous fungus *Aspergillus nidulans*, asexual and sexual development are tightly interconnected with secondary metabolism, orchestrated by the velvet protein transcriptional network. Here, we analyze the new $Zn(II)_2$-Cys_6 transcription factor ZtfA, which regulates asexual development, secondary metabolite production and oxidative stress response downstream of the velvet factor VosA. A *ztfA* deletion strain produces drastically diminished numbers of conidiospores, whereas a *ztfA* overexpression strain forms conidiophores even in vegetative growth, where normally only vegetative mycelia is formed. Moreover, conidiospores show a rapid loss in viability in Δ*ztfA*. Genetic analyses indicate that ZtfA activates the conidiation pathway via the major regulator encoding *brlA*. In the absence of *ztfA* the fungus is not able to produce the secondary metabolites austinol and dehydroaustinol, whereas the biosynthesis of sterigmatocystin in Δ*ztfA* as well as in the overexpression of *ztfA* is increased. In addition, ZtfA is involved in the oxidative stress response system. In the absence of *ztfA*, the fungus shows an increased tolerance towards H_2O_2 compared to the wild type.

23. Functional and subcellular localization analyses of transcription factors, AtrR and SrbA, involved in azole drug resistance in *Aspergillus oryzae*

K. Sugiyama[a], A. Ohba-Tanaka[a], D. Hagiwara[b], S. Kawamoto[b], M. Tanaka[a], T. Shintani[a] and **K. Gomi[a]**
[a]Graduate School of Agricultural Science, Tohoku University, Sendai, JP [b]Medical Mycology Research Center, Chiba University, Chiba, JP

We previously demonstrated that a novel $Zn(II)_2Cys_6$ transcriptional factor, AoAtrR, regulates gene expression of the ABC transporters that would function as drug efflux pumps and contributes to the azole drug resistance in *Aspergillus oryzae, and* a disruption mutant of the *AoatrR* ortholog (*AfatrR*) in *Aspergillus fumigatus* was similarly hypersensitive to azole drugs. In addition, RNA-seq analysis in *A. fumigatus* showed that AtrR regulated several ergosterol biosynthetic genes including *erg11*. Surprisingly, most of these ergosterol biosynthetic genes regulated by AtrR were nearly identical to those regulated by the basic helix-loop-helix transcription factor, SrbA (1). Therefore, we are interested in and investigate the difference in function between AtrR and SrbA in *Aspergillus oryzae*.
The expression of ergosterol biosynthetic genes such as *erg11*, *erg24*, and *erg25*, as well as ABC transporter genes, was significantly down-regulated in the *AoatrR* disruptant. Similarly, *AosrbA* disruption resulted in remarkable down-regulation of ergosterol biosythetic genes same as in *A. fumigatus*, but not ABC transporter genes. In contrast, *AoatrR* overexpression did not resulted in up-regulation of ergosterol biosythetic genes. These results suggested that AtrR and SrbA coordinately regulate ergosterol biosynthetic genes in aspergilli. However, *AoatrR* or *AosrbA* disruption had apparently no effect on another gene expression level, suggesting their expression would be regulated independently of each other. On the other hand, the *AoatrR* disruptant was more hypersensitive to azole drugs compared to the *AosrbA* disruptant and sensitive comparable to the *AoatrR*/*AosrbA* double disruptant, suggesting that hypersensitivity of the *atrR* disruptant to azole drugs is attributed not only to lowered ergosterol levels owing to down-regulation of ergosterol biosynthetic genes, but also to reduced efflux transport of the drugs owing to down-regulation of ABC transporter genes. Subcellular localization analysis of AoAtrR and AoSrbA showed that AoAtrR was constitutively localized in the nucleus and AoSrbA was likely localized in the nuclear envelope and/or endoplasmic reticulum.
(1) Hagiwara et al., *PLoS Pathogens*, in press.

24*. Elucidating the biosynthetic pathway of the anticancer secondary metabolite calbistrin in *Penicillium decumbens*

Sietske Grijseels[a], Carsten Pohl[b], Zahida Wasil[a], Jens Christian Nielsen[c], Yvonne Nygård[b], Jens Nielsen[c], Jens C. Frisvad[a], Kristian Fog Nielsen[a], Mhairi Workman[a], Thomas Ostenfeld Larsen[a], Arnold Driessen[b] and Rasmus John Normand Frandsen[a]

[a]Department of Biotechnology and Biomedicine, Technical University of Denmark, DK 2800 Kgs. Lyngby, Denmark [b]Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands [c]Department of Biology and Biological Engineering, Chalmers University of Technology, SE412 96 Gothenburg, Sweden

Filamentous fungi are important producers of secondary metabolites, low molecular weight molecules that often have bioactive properties. One interesting secondary metabolite is calbistrin, a compound recently found to have bioactivity against leukemia cells. This compound consists of two polyketides linked by an ester bond; a decalin containing polyketide similar to lovastatin, and a linear 12 carbon dioic acid structure. Calbistrin is known to be produced by several uniseriate black Aspergilli, *Aspergillus versicolor*-related species, and several Penicillia. Among the Penicillia, the recently genome sequenced *P. decumbens* is interesting as it produces several putative intermediates of the calbistrin pathway, such as decumbenone A and B and versiol. In this study, the molecular and enzymatic mechanisms underlying the biosynthesis of calbistrin are elucidated using a combinatorial approach of bioinformatics, molecular biology and analytical chemistry. Comparative studies of the polyketide synthase (PKS) sequences from the three genome sequenced species *A. versicolor*, *A. aculeatus* and *P. decumbens* resulted in the identification of a putative gene cluster for production of the decalin part of calbistrin. Implementation of CRISPR/Cas9 technologies in *P. decumbens* facilitated the deletion of the putative PKS in this species. Subsequent UHPLC-MS analysis of extract metabolites revealed that calbistrin and putative intermediate compounds were absent, proving the involvement of the PKS in calbistrin production. Further characterization of the predicted gene cluster is achieved by targeted deletion of the individual biosynthetic genes in the cluster.

25. The opposing roles of SrbA and the HapX/CCAAT binding complex in the regulation of sterol biosynthesis and azole tolerance in *Aspergillus fumigatus*

F. Gsaller[a], P. Hortschansky[b], T. Furukawa[a], P.D. Carr[a], B. Rash[a], J. Capilla[c], C. Müller[d], F. Bracher[d], H. Haas[e], A.A Brakhage[b,f] and M.J. Bromley[a]

[a]Division of Infection, Immunity & Respiratory Medicine, University of Manchester, Manchester, GB [b]Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany [c]Microbiology Unit, Medical School, Universitat Rovira i Virgili, Spain [d]Department of Pharmacy, Center for Drug Research, Ludwig-Maximilians University of Munich, Germany [e]Division of Molecular Biology, Biocentre, Medical University of Innsbruck, Austria [f]Institute for Microbiology, Friedrich Schiller University Jena, Jena, Germany

Azole drugs selectively target fungal sterol biosynthesis and are critical to our antifungal therapeutic arsenal. Resistance to this class of agents in the major human mould pathogen *Aspergillus fumigatus* is reaching levels that suggest that they could be lost for clinical use. One family of pan-azole resistant isolates, characterized by the presence of a tandem repeat of at least 34 bases (TR34) within the promoter of sterol C14-demethylase encoding gene *cyp51A* dominates and is found globally. We demonstrate the transcriptional mechanisms underpinning TR34 mediated resistance showing the repeat is bound by both the sterol regulatory element binding protein SrbA, and the CCAAT binding complex (CBC). We show that the CBC acts complementary to SrbA as a negative regulator of ergosterol biosynthesis and TR34 driven overexpression of *cyp51A* results from effective duplication of SrbA but not CBC binding sites leading us to suggest possible routes to tackle the problem of TR34 mediated resistance.

26. Unveiling a novel regulatory role of the pH regulator PacC and the CCAAT-Binding-Complex in *Aspergillus fumigatus* 5-Flucytosine resistance

F. Gsaller[a], T. Furukawa[a], P.D. Carr[a] and M.J. Bromley[a]
[a]Manchester Fungal Infection Group, Division of Infection, Immunity & Respiratory Medicine, University of Manchester, Manchester, GB

Annually >1.5 million people die from fungal disease, a big proportion results from invasive and chronic mould infections, predominantly caused by *Aspergillus fumigatus*. Only four classes of agents, the azoles, polyenes, candins and 5-Flucytosine (5FC) are available to treat clinical infection. 5FC, a derivative of the nucleobase cytosine is rarely used as a monotherapeutic agent to treat *Aspergillus* infection as resistance develops rapidly. In addition at neutral pH (pH 7.0) the *in vitro* activity of 5FC against *A. fumigatus* is insignificant, however, 5FC is highly active at low pH (pH 5.0).
In this work we provide an explanation for the low efficacy of 5FC at pH 7.0 and uncover two transcription factors that mediate resistance to 5FC - The CCAAT binding complex (CBC) and the pH regulator PacC. Genetic inactivation of the CBC and PacC results in derepression of the purine-cytosine permease encoding gene *fcyB*, which results in 5-FC hypersusceptibility phenotypes. We show that *fcyB* expression is repressed at pH 7.0 and transcriptionally activated at pH 5.0. Disruption of *fcyB* results in hyperresistance to 5FC in wt and, moreover, significantly increases 5FC resistance in both the CBC and PacC mutant backgrounds independent of the pH. In contrast, CBC/PacC independent overexpression of *fcyB* using a conditional expression system results in 5FC hypersusceptibility. ChIP-Seq based genome-wide binding analysis using a strain expressing *gfp*-tagged *hapC* (*hapC*^{GFP}) uncovers the CBC as direct regulator of *fcyB.*
Collectively, within this study we characterised the cytosine permease encoding gene *fcyB* the expression of which is under combinatorial transcriptional control of the CBC and PacC. Furthermore, we show that both regulators act as repressors of *fcyB* during pH 7.0, which results in 5FC resistance.

27. Developmental regulation by constitutive activation and inactivation of MpkB MAPK pathway in *Aspergillus nidulans*

Sang-Cheol Jun[a], Kwang-Yeop Jahng[b], Jong-Hwa Kim[c] and **Kap-Hoon Han**[c]
[a]Institute for Genetic Engineering, Chonbuk National University, Jeonju, South Korea [b]Department of Life Sciences, Chonbuk National University, Jeonju, South Korea [c]Department of Pharmaceutical Engineering, Woosuk Univ, Wanju, Jeonbuk, South Korea

The *mpkB* gene is a member of the multiple MAPK pathways in *Aspergillus nidulans*. The MpkB has been known to play a key role in the asexual and sexual development and secondary metabolite production. The MAPK kinase MkkB can be phosphorylated at Ser218 and Thr222 residues within the activation loop site by SteC MAPKK kinase and consequently, activates MpkB by phosphorylating at threonine and tyrosine residues of MpkB in *A. nidulans*. By introducing negatively charged (aspartic acid) or non-polar (proline and lysine) residues that may mimic the effect of phosphorylation at positions 218 and 222 in MkkB, we constructed mutants which constitutively phosphorylate MpkB MAPK and constitutively non-phosphorylate MpkB MAPK. Expression of the constitutively activated MkkB in *A. nidulans* led to the hyper-activation of MpkB MAPK and resulted in a great elevation of sexual development, while asexual sporulation was remarkably reduced. In contrast, constitutively inactivated MkkB mutant could not produce sexual organs, while asexual sporulation and sterigmatocystin production were normally processed. Differential expression pattern of proteins among these mutants were analyzed by using the 2D proteomic analysis, which revealed that expression level of 375 spots are significantly different.

28. Ascospore-specific gene expression analysis in *Aspergillus nidulans*

Mi-Kyoung Lee[a], Jong-Hwa Kim[b], Jae-Hyuk Yu[a] and **Kap-Hoon Han**[b]
[a]Department of Bacteriology, University of Wisconsin-Madison, WI, USA [b]Department of Pharmaceutical Engineering, Woosuk University, Wanju, Jeonbuk, South Korea

Developmental process and spore formation in a model filamentous fungus *Aspergillus nidulans* is environmentally and genetically regulated. Asexual spores or conidia differentiation is controlled by various orchestrated developmental pathways, including the *brlA* gene-mediated conidiophore and conidia morphogenesis. However, a few genes have been elucidated for playing an important role in

sexual development and ascospore formation. The *nsdD*, *nsdC* and *veA* genes are well-known key regulators of sexual and asexual developmental processes. However, unlike conidia, physiological and genetic studies of ascospores are remained to be characterized. To know more about the ascospores biology, we performed RNA-seq analysis from from *A. nidulans* conidia and ascospores RNA samples. Comparative analysis of transcription profiles of conidia and ascospores revealed many genes that are expressed differentially in both spores. Detailed investigation of the differentially expressed genes is in progress.

29. Biosynthesis of acurin A and B in *Aspergillus aculeatus*

M.L. Nielsen[a], P.P. Wolff[a], L.M. Petersen[a], L.N. Andersen[a], T.I. Petersen[a], D.K. Holm[a], U.H. Mortensen[a], C.S. Nødvig[a], T.O. Larsen[a] and **J.B. Hoof[a]**
[a]Department of Biotechnology and Biomedicine, Technical University of Denmark, Kongens Lyngby, Denmark

Aspergillus aculeatus is known for the commercial utilization in production of several enzymes. We have identified two stereoisomeric compounds of mixed polyketide-nonribosomal peptide origin in the extracts of *A. aculeatus* that we named acurin A and acurin B. The structures of the compounds strongly resemble the structure of the mycotoxin fusarin C produced by several *Fusarium* species. CRISPR-Cas9 was used to construct a non-homologous end-joining deficient strain of *A. aculeatus*, which enabled efficient gene deletions in the acurin gene cluster. Using RT-qPCR in combination with metabolite profiling of gene deletion strains, the acurin producing gene cluster was delineated, which allowed us to propose a biosynthetic pathway for formation of acurin. Our results show that acurin, in contrast to fusarin C, is biosynthesized by an individual polyketide synthase and non-ribosomal synthetase. At least six other enzymatic activities are required for the biosynthesis of acurin. This study shows how we exploit the CRISPR-Cas9 system for the rapid construction of fungal host strains that can be readily engineered to generate valuable knowledge.

30*. Nuclear accumulation of the *Aspergillus nidulans* GATA transcription factor AreA is independent of DNA binding.

C.C. Hunter[a], M.J. Hynes[b], J.A. Fraser[b], D.F. Clarke[b], M.A. Davis[b] and R.B. Todd[a]
[a]Department of Plant Pathology, Kansas State University, Manhattan, KS [b]Department of Genetics, The University of Melbourne, Melbourne, Australia.

The primary regulator of nitrogen metabolic genes in fungi is the GATA transcription factor AreA. Subcellular localization of AreA in *A. nidulans* is dependent on nutrient availability. When glucose and nitrogen sources are available, AreA is found throughout the cytoplasm and the nucleus. When the cell becomes nitrogen starved, AreA accumulates in the nucleus due to blocked AreA nuclear export. We have recently found redundancy in the nuclear localization signals (NLSs) of AreA. Five conserved canonical NLSs and one conserved noncanonical arginine-based bipartite NLS within the zinc-finger DNA binding domain all function together to mediate nuclear import. Only mutation of the noncanonical bipartite NLS within the DNA binding domain had any significant effect on AreA function, completely abolishing transcriptional activity. In order to determine whether AreA DNA binding affects the intracellular localization of AreA we have HA-epitope-tagged and analyzed the two classical DNA binding mutants; an altered DNA binding specificity mutant, AreA102, and a non-binding mutant, AreA217. The $AreA102^{HA}$ mutant protein showed a similar pattern of subcellular localization to wildtype AreA except when transferred to uric acid (a nitrogen source the *areA102* mutant cannot utilize). On uric acid $AreA102^{HA}$ accumulated in the nucleus as observed during nitrogen starvation. The $AreA217^{HA}$ non-binding mutant protein accumulated in the nuclei of nitrogen-starved cells, demonstrating that DNA binding is not required for AreA nuclear accumulation. $AreA217^{HA}$ does not show nuclear accumulation when ammonium is present. In contrast to wildtype, $AreA217^{HA}$ accumulated in the nucleus when alternative nitrogen sources were available. These findings suggest that while nuclear accumulation of AreA is independent of DNA binding, nuclear export is dependent on AreA DNA binding function. We show using nitrogen metabolism mutants that this is likely due to the requirement of AreA for metabolic signaling of nitrogen availability.

31*. Subcellular localization and stability of deubiquitinase CreB involved in carbon catabolite repression in *Aspergillus oryzae*

S. Ichinose[a], M. Tanaka[a], T. Shintani[a] and K. Gomi[a]
[a]Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan

Aspergillus oryzae produces large amounts of amylolytic enzymes in the presence of maltooligosaccharides, In the presence of glucose, however, their production is repressed by carbon catabolite repression (CCR) system. In filamentous fungi, it has been proposed that CCR is regulated by Cys_2His_2 type transcriptional factor CreA, and *creA* gene deletion results in high production level of α-amylase in *A. oryzae* (1). In addition, it is known that the ubiquitin processing protease CreB is also involved in CCR regulation. We generated a *creB* deletion mutant in *A. oryzae*, and revealed that CCR was relieved by deleting the *creB* gene. However, it is not clear how CreB is involved in CCR regulation. In this study, to elucidate the mechanism of CCR regulation by CreB, we generated GFP- or 3FLAG-fused CreB and observed the subcellular localization and stability of CreB. CreB-GFP was localized in the cytoplasm in both glucose and maltose media. In contrast, the amount of CreB-3FLAG in maltose medium was larger than that in glucose medium. Concomitantly, the *creB* gene expression level in maltose medium was much higher than that in glucose medium. These results suggested that CreB is regulated at transcriptional level and the *creB* gene is induced under CCR relieved condition.
In *Aspergillus nidulans*, it was reported that CreB interacts with WD40 repeat protein CreC. Thus, we generated a *creC* deletion mutant in *A. oryzae*, and observed that CCR was also relieved by deleting the *creC* gene in *A. oryzae*. Interestingly, *creC* gene deletion resulted in a significant decrease in the amount of CreB-3FLAG and increase in the *creB* gene expression level compared with the wild-type, while the subcellular localization of CreB was not substantially altered. These results suggested that stability and transcriptional level of CreB was regulated by CreC

(1) Ichinose et al., *Appl. Microbiol. Biotechnol.,* **98**, 335–343 (2014)

32*. Intron within 5′ untranslated region enhances transcription of the enolase-encoding gene (*enoA*) in *Aspergillus oryzae*

Taishi Inoue[a], Hiroki Toji[a], Mitsuru Takama[a], Mizuki Tanaka[a], Takahiro Shintani[a] and Katsuya Gomi[a]
[a]Tohoku University, Sendai, Miyagi, Japan

Background: The glycolytic pathway is the primary metabolic process which is essential to metabolize a wide range of carbon sources in fungi. Hence, it is important for fungal survival to optimize the level of glycolytic genes expression in response to various environments. Interestingly, in *Aspergillus oryzae*, several genes which are involved in both glycolysis and gluconeogenesis, have alternative transcription start sites (TSSs). In addition, we demonstrated that selection of TSSs was dependent on two types of carbon sources; one is metabolized via glycolysis such as glucose and fructose, and another via gluconeogenesis such as acetate and ethanol. Particularly, stringent selection of alternative TSS was observed in the enolase-encoding gene (*enoA*). The similar transcriptional control of enolase gene was suggested in *Aspergillus nidulans* (Hynes et al., 2007). These findings seem to be important because it provides novel insight into environmental adaptation in the stage of transcriptional regulation of glycolytic genes in *Aspergillus* spp. However, the molecular details of alternative TSS selection remain to be elucidated. Remarkably, in enoA, there is an intron containing a downstream TSS (dTSS) within 5′ untranslated region (5′ UTR) when it is transcribed from an upstream TSS (uTSS). The length of the intron is 440 bp and quite long in fungi. In this study, we investigated the importance of this 5′ UTR intron in *enoA* expression.
Results: To this end, we analyzed the enoA promoter plus 5′ UTR whose intron was deleted (P*enoA*-Δi) or mutated at a splice site (P*enoA*-issm) using GUS reporter system. Under culture condition with acetate where transcription from uTSS is induced while transcription from dTSS is suppressed, both the GUS activity and the mRNA level were significantly decreased in P*enoA*-Δi. On the other hand, in P*enoA*-issm, the mRNA level was unaffected but the GUS activity was almost lost, presumably caused by emergence of uORF within intron unspliced. These results indicated that the deletion of dTSS within intron does not contribute to the reduction of gene expression in P*enoA*-Δi. Additionally, when the *enoA* gene was expressed by P*enoA*-Δi under condition that a resident *enoA* expression is suppressed, a resulting strain showed a significant reduction of the mRNA transcribed from uTSS and a poor growth under acetate

culture condition. These results suggested that 5′ UTR intron enhances the *enoA* transcription from uTSS in *A. oryzae*.

33. *Aspergillus nidulans* Protein kinase C and SepA physically interact at sites of polarized growth.

Loretta Jackson-Hayes[a], Z. O. Atiq[a], Elisabet Olsen[a], Lance Myers[a], Terry W. Hill[a]
[a]Departments of Chemistry and Biology, Rhodes College, Memphis, TN 38112

Our work focusses on proteins that localize to hyphal tips and septation sites. In Aspergillus nidulans, Protein kinase C (PkcA) is one such protein. Our previous work identified a mutation in the *pkcA* gene (*calC2*) which results in reduced cell wall integrity. We also showed that wild type PkcA localizes to growing hyphal apices and septation sites, and we identified amino acid sequences within PkcA that are required for PkcA to localize to these sites of cell wall synthesis. Our work, as well as work done in other labs, has also brought to light many other proteins that play roles in polarized growth and that also localize to septation sites and growing tips. Among these are the formin SepA which has been observed at septation sites and at the hyphal tip in both a spot and crescent pattern. SepA is associated with contractile actomyosin ring (CAR) formation, and a mutation in the *sepA* gene (*sepA1*) renders *A. nidulans* aseptate at elevated temperature. Using time lapse fluorescence microscopy we have observed overlap of PkcA::RFP and SepA::GFP signals in some tips in the crescent pattern, while other tips displayed SepA spot and PkcA crescent localization within the same tip or separately. At septation sites PkcA::RFP and SepA::GFP consistently appear concurrently; both initially appear at the outer edges of the septation site and contract in a lockstep fashion until both signals dissipate. A functional interaction between the two proteins is shown by the ability of the *sepA1* mutation to block PkcA::GFP localization to septation sites, but not to hyphal tips. Additionally, we have demonstrated reciprocal complementation of the septation defect in the *sepA1* mutant by *pkcA* overexpression and of hypersensitivity to cell wall perturbing agents calcofluor white, Congo red, and sodium dodecyl sulfate by *sepA* overexpression in the *calC2* mutant. Using a bimolecular fluorescence complementation strategy we have found evidence that SepA and PkcA physically interact at both hyphal tips and septation sites. Interaction of these two proteins was confirmed using a GAL4-based yeast two-hybrid assay.

34. An *in-silico* reconstructed gene regulation network for *Aspergillus niger* for the prediction of protein functions

Sascha Jung[a], Norman Paege[a], Paul Schäpe[a] and Vera Meyer[a]
[a]Institute of Biotechnology, Department Applied and Molecular Microbiology, Berlin University of Technology, Gustav-Meyer-Allee 25, 13355 Berlin, Germany

The genome of *A. niger* belongs to the best annotated genomes among *Aspergillus* species; however, only 2% of its ~14,000 genes are functionally verified and 50% of the predicted open reading frames encode for hypothetical proteins. Hence, the genetic basis for almost all cellular processes in *A. niger* and its physiological peculiarities is unknown. On the other hand, hundreds of post-genomic data including transcriptomic and proteomic data are available for *A. niger* for more than 150 different growth conditions. This holistic dataset can be scrutinized and used to predict gene functions and gene interactions. For a proof-of-concept, we have focused on the *anafp* gene encoding the antifungal protein AnAFP known to selectively inhibit the growth of filamentous fungi. As more than 50 AnAFP orthologs have been identified in many different genera of the *Ascomycota* tree of life, we wished to understand which regulatory systems control expression of the *anafp* gene.
Our analysis uncovered that *anafp* displays a highly coordinated temporal and spatial transcriptional profile which is concomitant with key nutritional and developmental processes. Its expression profile coincides with early starvation response and parallels with genes involved in nutrient mobilization and autophagy. Using fluorescence- and luciferase reporter strains we could demonstrate that the *anafp* promoter is indeed under control of CreA and FlbA as predicted by the *in silico* data. A co-expression network analysis further predicted that *anafp* expression is embedded in several cellular processes including allorecognition, osmotic and oxidative stress survival, development, secondary metabolism and autophagy, and predicted StuA and VelC as additional regulators (1). We currently prove these predictions by respective wet-lab experiments. First results match the expected knock out phenotype in *A. niger*.

(1) Paege N, Jung S, et al. A Transcriptome Meta-Analysis Proposes Novel Biological Roles for the Antifungal Protein AnAFP in *Aspergillus niger*. PlosOne 2016; DOI:10.1371/journal.pone.0165755.s009

35*. Hidden in Plain Sight: Heterogeneity in Asexual Spores of *Aspergillus fumigatus*

S.E. Kang[a] and M. Momany[a]
[a]Department of Plant Biology, University of Georgia, Athens, GA

Aspergillus fumigatus spores are found ubiquitously in the environment. The asexual clonal spores (conidia) produced in the environment are inhaled where they can germinate and cause high mortality rates in immunocompromised patients. Germination of conidia is the most crucial step in the development of aspergillosis. Given that conidia are produced in a variety of environments, we tested whether sporulation conditions impact the ability of conidia to break dormancy and grow. Spores from a single colony were bulked on complete media and cultured with environmentally- and medically-relevant stress conditions. Spores from each condition were collected and transferred to all other conditions. Germination and growth kinetics were analyzed for 25,000 conidia from each condition swap experiment using flow cytometry. We found that clonal populations of conidia are heterogeneous for germination and growth phenotypes and the stress increases this phenotypic heterogeneity.

36. Growth-phase sterigmatocystin formation on lactose is mediated via low specific growth rates in *Aspergillus nidulans*.

L. Karaffa[a], Z. Németh[a], Á.P. Molnár[a], B. Fejes[a], L. Novák[b], N.P. Keller[c,d] and E. Fekete[a]
[a]Department of Biochemical Engineering, University of Debrecen, Debrecen, HU [b]Department of Physical Chemistry, Faculty of Science and Technology, University of Debrecen, HU [c]Department of Medical Microbiology and Immunology, University of Wisconsin, Madison, USA [d]Department of Bacteriology, University of Wisconsin, Madison, USA

Seed contamination with polyketide mycotoxins such as aflatoxin (AF) and sterigmatocystin (ST) produced by *Aspergillus spp*., is an agricultural, economic, and medical issue worldwide. ST is the penultimate intermediate in the biosynthesis of AF, and in several fungi including the model fungus *A. nidulans*, it is the end product of the AF pathway. This biosynthetic pathway is well-characterized in *A. nidulans*, but many of the regulatory aspects related to the carbon source available for the fungus are still enigmatic. This is particularly true for the heterodisaccharide lactose (milk sugar; 1,4-*O*-beta-D-galactopyranosyl-D-glucose), inasmuch as some ST production mutant strains still synthesize ST on lactose but not on other carbon substrates including the customary D-glucose. Here, kinetic data from well-controlled single-carbon substrate submerged fermentations revealed that on D-glucose, ST forms only after the sugar is depleted from the medium, while on lactose, ST appears when the majority of the carbon source is still available. Maximal biomass-specified ST production in lactose medium was significantly higher than on D-glucose. These data suggested that ST formation may either be mediated by a carbon catabolite regulatory mechanism prominent on D-glucose, or induced by low specific growth rates attainable on lactose. These hypotheses were tested by constant-mass chemostat-type continuous fermentations on D-glucose as a sole carbon source at two different dilution rates (D = 0.090 h^{-1} and D = 0.020 h^{-1}), representing a state of carbon catabolite repression and derepression, respectively. ST production under such conditions negatively correlated with the dilution rate, i.e., no ST formed at high growth rate, while low growth rate led to the formation of 0.4 mg L^{-1} ST. Essentially identical results were obtained with a CreA mutant strain, indicating that CreA does not regulate the formation of ST during growth on D-glucose. We concluded that low specific growth rates may be the primary cause of sustained, mid-growth ST formation on the slowly assimilating lactose in *A. nidulans*, and that carbon utilization rates likely play a general regulatory role during biosynthesis.

This research was supported by the EU and co-financed by the European Regional Development Fund under the project GINOP-2.3.2-15-2016-00008.

37*. Culture independent detection of *Aspergillus fumigatus* Cyp51A in human lung
Aiah Khateb[a,b], Michael Bromley[b] and Paul Bowyer[b]
[a]Faculaty of Applied Sciences, Medical Laboratory Technology, University of Taibah, Medina, Kingdom of Saudi Arabia [b]Fungal Infection Group, Centre for Respiratory Medicine and Allergy, Faculty of Biology, Medicine and Health,Institute of Inflammation and Repair, Manchester Academic Health Science Centre, The University of Manchester and University Hospital of South Manchester

Background *Aspergillus fumigatus* is the main causative agent of aspergillosis. Traditional culture methods recover a small fraction of all the lung resident species and therefore resistant isolates are usually missed. Long-term triazole oral therapy, particularly itraconazole and voriconazole has been used in treatment of chronic pulmonary Aspergillosis or allergic aspergillosis. Azole resistance is associated with poorer disease outcome in allergic and chronic disease and has increased in recent years. This study aims to detect resistant mutations in *cyp51A* in the context of uncultured *Aspergillus* strains present in clinical samples.
Methods DNA was extracted directly from 38 BAL samples (8 healthy controls, 9 ABPA, 8 SAFS, 7 SA and 3 MA). The *cyp51A* gene was directly amplified from extracted DNA and amplicons sequenced using illumina Miseq. Sequence analysis was done using the following software pipline; trimmomatic 0.36>bowtie2>samtools1.2>bcftools htslib 1.3.1.>VarScan.
Results 61 SNPS were identified, 29 resulted in amino acid change. Three SNPs matched cyp51A mutations that have previously been shown to confer azole resistance; M220I/M220T and F46L. 9 further mutations occurred only in azole treated patients; N218T, F219Y, N218Y, M220T, M220I, I217S, A62C, I135T, T112I and 17 were common to sequences obtained from patients with no azole treatment history.
Conclusions This study has identified new *cyp51A* mutations in the healthy, chronically colonized patients. It also broadened our understanding of *cyp51A* resistance mechanisms in the context of uncultured *Aspergillus* strains present in clinical samples.

38*. L-rhamnose induction and metabolism in *Aspergillus niger*
C. Khosravi[a], Maria Victoria Aguilar-Pontes[a], Eline Majoor[a] and Ronald De Vries[a]
[a]CBS Fungal Biodiversity Centre, Utrecht, The Netherlands

Aspergillus species are potent producers of enzymes involved in plant polysaccharide degradation. In nature, *Aspergillus* degrades the polysaccharides to monomeric sugars that can serve as a carbon source. Therefore, *Aspergillus* uses a variety of catabolic pathways to efficiently convert all the monomeric components of plant biomass.
L-rhamnose catabolism in fungi has been described for the yeasts *Pichia sptipitis* and *Debaryomyces hansenii* (Watanabe *et al.*, 2008). In *Aspergillus niger* this pathway is poorly studied, and none of the putative L-rhamnose pathway genes or enzymes have been characterized. This project aims to confirm the function of the putative L-rhamnose pathway genes and to identify which metabolite in the L-rhamnose pathway is the inducer of the L-rhamnose responsive regulator (RhaR) (Gruben et al., 2014). After identification of the candidates, deletion mutants for these genes were obtained. Growth profile result showed no or only minor growth for all of the metabolic mutants. To study the effect of deletion of these genes in more detail, transcriptomics analysis of the reference strain and the KO strains Δ*lraA*, Δ*lraB*, Δ*lraC* and Δ*rhaR* have been done. The results indicate that L-rhamnose, L-rhamnono-Y-lactone and L-rhamnonate are not the inducers of RhaR. Further experiments will be required in order to determine if the inducer is located at the end of the metabolic pathway.

39. Genomic and transcriptomic analysis of *Aspergillus niger* producing thermophilic bacterial cellulases
J. Kim[a,b], S. A. Campen[a,b,e], B. A. Simmons[a,c], S. E. Baker[a,b], J. M. Gladden[a,d] and J. K. Magnuson[a,b]
[a]Joint BioEnergy Institute, Emeryville, CA [b]Pacific Northwest National Laboratory, Richland, WA [c]Lawrence Berkeley National Laboratory, Berkeley, CA[d]Sandia National Laboratories, Livermore, CA [e]J. Craig Venter Institute, La Jolla, CA (current affiliation)

Enzymatic hydrolysis of pretreated lignocellulosic biomass is important in efficient conversion of lignocellulosic biomass to biofuel. Thermophilic bacterial cellulase enzymes have advantages in industrially relevant conditions due to their stability in high temperature and tolerance to inhibitory

compounds generated during biomass pretreatment. *Aspergillus niger* is a filamentous fungus known for its ability to produce high levels of enzymes, and it has been used for the industrial production of many useful enzymes. In this work, we employ genomics and transcriptomics approaches to understand and improve the production of thermophilic bacterial cellulases in *A. niger*.

Comparative transcriptomic analysis of the *A. niger* ATCC 11414 parent strain and an engineered strain expressing a thermophilic bacterial cellulase using RNA-Seq shows that more than two-thirds of the genes are differentially expressed. We also investigated differential RNA polymerase binding and histone modification using ChIP-Seq, in order to examine these potential causes of differential expression. Using whole-genome resequencing, we identified the genetic variation in the engineered *A. niger* strain as well as the copy number and integration locus of the introduced bacterial cellulase gene. These genetic changes could be responsible for the epigenetic changes and subsequent differential gene expression in heterologous enzyme producing *A. niger* strain.

40*. Identifying more than 300 biosynthetic gene clusters with potential resistance genes in over 75 *Aspergillus* species using resistance gene-guided genome mining

I. Kjaerboelling[a], T.C. Vesth[a], J.C. Frisvad[a], J.L. Nybo[a], S. Theobald[a], I.V. Grigoriev[b], A. Salamov[b], T.O. Larsen[a], U.H. Mortensen[a] and M.R. Andersen[a]
[a]Department of Biotechnology and Biomedicine, Technical University of Denmark, Kongens Lyngby, DK [b]Joint Genome Institute, Walnut Creek, CA, USA

With the increasing number of genome sequences, an increasing number of orphan /cryptic secondary metabolite biosynthetic gene clusters (BCGs) has followed. These clusters potentially encode pathways for bioactives, however linking genes to compound and elucidating bioactivity requires extensive work. Thus, the question remains how to select the most interesting clusters in a rational manner and find their bioactivity. By using Resistance Gene-Guided Genome mining, it is possible to identify novel bioactive compounds and their clusters.

The hypothesis for this approach is that some BGCs include a gene which is a resistant form of the enzyme targeted by the compound produced by the cluster. In filamentous fungi, this mechanism has been seen for mycophenolic acid as well as Fellutamide B. By using this knowledge and a large number of fungal genomes, we have set up a pipeline, which extracts predicted clusters with potential resistance genes.

A total of over 300 clusters were found to exhibit this specific pattern by running our algorithm on more than 75 *Aspergillus* species and 5000 (SMURF) predicted gene clusters. With further filtering and analysis, we ended up with a manageable number of potential resistance genes and clusters. The filters used in this algorithm will return clusters where one gene in the cluster has a homolog conserved in 98% of the examined species. Although we use a conservative approach it ensures that the identified clusters are most likely of interest. Selected clusters and resistance genes are now being validated experimentally both to confirm the resistance mechanism and to identify the compounds produced.

41*. COP9 signalosome and Cand mediated Cullin-RING ligase control and *Aspergillus nidulans* development

A. M. Koehler[a] and G. H. Braus[a]
[a]Molekulare Mikrobiologie und Genetik, Georg-August-Universität Göttingen, D-37077 Göttingen, Germany

Cullin RING ligases (CRLs) are the specificity factors which mark target substrates for degradation by the ubiquitin 26S proteasome system. CRLs are activated by linkage of the ubiquitin-like modifier NeddH/Nedd8 to a conserved lysine residue within the cullin core module of this enzyme complex. *Aspergillus nidulans* posseses approximately 70 F-box proteins as receptors for different substrates, which are linked through the adaptor SkpA to cullins. Fungal development requires the controlled degradation of various substrates and therefore exchanges of F-box proteins within CRLs. This exchange depends on the inactivation of CRLs by removing NeddH and subsequent reassembly. At the molecular level this process is catalysed by the two deneddylases COP9 signalosome and DenA [1, 2],

and the Cand complex [3]. In fungi a seven-subunit pre-COP9 signalosome is first assembled and activated by the integration of the eighth CsnE NeddH-specific isopeptidase subunit [4]. Cand binds to deneddylated cullins at the SkpA binding and the neddylation site and its function is a prerequisite for CRL reassembly. Defects in COP9 or Cand subunits result in impaired fungal development and a dysregulated secondary metabolism. Cand represents a single polypeptide in numerous filamentous fungi and mammals, but is split in *A. nidulans* into at least two polypeptides (34kDa CandAN blocks neddylation site; 114 kDa CandAC blocks SkpA binding site), which form a cullin binding heterodimeric complex and are presumably the result of a genomic rearrangement event [3]. The gene locus of the two *A. nidulans cand* genes was analysed in more detail and revealed that the fungal Cand complex consists apparently of even three proteins, which all are required for fungal development and coordinated secondary metabolism. The interplay between Cand subunits and the two deneddylases as well as their molecular function and consequences for fungal differentiation are presented.

[1] Christmann M *et al.*, 2013. **PLoS Genet**. 9, e1003275.
[2] Schinke J *et al.,* 2016.**PLoS Genetics** 12, e1005949.
[3] Helmstaedt K *et al.,* 2011. **Mol Biol Cell**. 22, 153-164.
[4] Beckmann EA *et al.,* 2015. **Mol Microbiol** 97: 110-124

42*. The transcriptional activators RhaR, AraR and GaaR co-regulate pectin degradation in *Aspergillus niger*.

J. E. Kowalczyk[a], R. J. M. Lubbers[a], E. Battaglia[a] and R. P. de Vires[a]
[a]Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre & Fungal Molecular Physiology, Utrecht University, the Netherlands

Aspergillus niger produces an arsenal of extracellular enzymes that allow step-by-step degradation of organic matter found in its environment. Pectin is a heteropolymer abundantly present in the primary cell wall of plants. The complex structure of pectin requires multiple enzymes to act sequentially. Production of pectinolytic enzymes in *A. niger* is highly regulated, which allow flexible and efficient capture of nutrients. So far, three transcriptional activators have been linked to regulation of pectin degradation in *A. niger.* The L-rhamnose-responsive regulator RhaR controls the production of enzymes that degrade rhamnogalacturonan-I (RG-I) [1]. The L-arabinose-responsive regulator AraR controls the production of enzymes that decompose the arabinan and arabinogalactan side chains of RG-I [2]. The D-galacturonic acid-responsive regulator GaaR controls the production of enzymes that act on the polygalacturonic acid backbone of pectin [3]. This project aims to understand how RhaR, AraR and GaaR co-regulate pectin degradation. For that reason, we constructed single, double and triple disruptant strains of those regulators and analyzed their growth phenotype and pectinolytic gene expression in *A. niger* grown on different pectins.

1. Gruben, B.S., Zhou, M., Wiebenga, A., Ballering, J., Overkamp, K.M., Punt, P.J., and de Vries, R.P. (2014) *Aspergillus niger* RhaR, a regulator involved in L-rhamnose release and catabolism. *Applied Microbiol Biotechnol* 98(12): 5531-5540.
2. Battaglia, E., Visser, L., Nijssen, A., van Veluw, J., Wösten, H.A.B., and de Vries, R.P. (2011) Analysis of regulation of pentose utilisation in *Aspergillus niger* reveals evolutionary adaptations in the Eurotiales. *Stud Mycol* 69: 31-38.
3. Alazi, E., Niu, J., Kowalczyk, J.E., Peng, M., Aguilar Pontes, M.V., Van Kan, J.A.L. et al. (2016) The transcriptional activator GaaR of *Aspergillus niger* is required for release and utilization of D-galacturonic acid from pectin. *FEBS Letters* 590(12): 1804-1815.

43. Genomics of the first 100 Aspergilli

A. Kuo[a], R. Riley[a], S. Mondo[a], S. Haridas[a], A. Salamov[a], F. Korzeniewski[a], B. Simmons[b], S. Baker[b], M. Andersen[c] and I. Grigoriev[a]
[a]DOE Joint Genome Institute, Walnut Creek, CA [b]DOE Joint BioEnergy Institute, Emeryville, CA [c]Technical University of Denmark, Kongens Lyngby, Denmark

Aspergillus is a ubiquitous and phenotypically diverse genus of filamentous Ascomycota, many of which play key roles as fermenters in food production, platforms for biotechnology and industrial production of enzymes and chemicals, plant and opportunistic animal pathogens, and agents of agricultural toxigenesis and biomass conversion for bioenergy. As part of a Joint BioEnergy Institute initiative to characterize the

entire genus, the Joint Genome Institute will sequence, assemble, and annotate the genomes of each of the ~300 species of the genus *Aspergillus*. To accomplish this massive task in a timely manner without sacrificing quality, we streamlined and optimized our processes for *Aspergillus* genomes. Over the past year we have released on MycoCosm the genomes of 100 *Aspergillus* sp. which represent a broad spectrum of phylogenetic diversity and gene content, including significant variability of transporters, carbohydrate-active enzymes, proteases, and secondary metabolism clusters. The high resolution of genomic differences between closely related species is being mapped to their distinctive phenotypes to improve gene annotation in the entire genus. The next 100 species are expected soon.

44*. Genome-wide analysis of the GATA transcription factor AreA in *Aspergillus nidulans*

A. Li[a], Z. Miao[a], D.J. Downes[b], G.Y. Busot[b], R.B. Todd[b] and K. Wong[a]
[a]Faculty of Health Sciences, University of Macau, Macau, CN [b]Department of Plant Pathology, Kansas State University, KS

In *Aspergillus nidulans,* nitrogen utilization is precisely regulated at the gene expression level. Expression of genes encoding enzymes to utilize alternative nitrogen sources or to scavenge nitrogen compounds during nitrogen starvation is dependent on the GATA transcription factor AreA. In general for typical nitrogen metabolic genes, AreA-dependent activation is minimal during nitrogen sufficiency, increased when nitrogen is limiting, and strongest during nitrogen starvation. Although many AreA target genes are known, the genome-wide targets of AreA and AreA DNA binding properties remain unknown. To address this, we profiled AreA DNA binding under nitrogen sufficiency, nitrogen limitation and nitrogen starvation via Chromatin Immuno-precipitation followed by Sequencing (ChIP-seq) and identified more than 1,600 promoters bound by AreA. Interestingly, AreA binds to more than 400 promoters under nitrogen sufficiency, when its protein level in the nucleus is low. Expression analysis shows that less than 5% of these targets are affected by *areA* deletion. In contrast, under nitrogen starvation expression of more than 40% of AreA bound genes is significantly altered by *areA* deletion. These results indicate that AreA binds to many target promoters poised to activate upon appropriate signaling. Comparisons of targets among the different nitrogen conditions revealed many (~200) common binding sites as well as nitrogen source-specific AreA binding events, indicating that AreA targets different promoters according to nitrogen sources and availability. De novo motif discovery analysis showed that besides “GATA”, several other motifs are also enriched among AreA bound regions, consistent with cooperation of AreA with pathway-specific transcription factors. In addition to nitrogen metabolism, Gene Ontology analysis of AreA target genes suggests roles in development and stress response. Moreover, our data also provides compelling evidence for positive feedback control of AreA on the co-repressor NmrA that negatively regulates its function. Therefore, this work not only reveals the genome-wide functions of AreA, but also uncovers a new regulatory loop for nitrogen metabolism repression in *A. nidulans*.

45*. Genetic drivers of diversity in secondary metabolic gene clusters in *Aspergillus fumigatus* populations

Abigail Lind[a], Jennifer Wisecaver[b], Fernando Rodrigues[c], Gustavo Goldman[d] and Antonis Rokas[b]
[a]Biomedical Informatics, Vanderbilt University, Nashville, TN [b]Biological Sciences, Vanderbilt University, Nashville, TN [c]Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal [d]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, São Paulo, Brazil

Filamentous fungi produce a diverse array of secondary metabolites that play ecological roles in defense, virulence, and inter- and intra-species communication. The biosynthetic genes required for the production of individual secondary metabolites are arranged in contiguous genomic clusters. These pathways are narrowly taxonomically distributed and highly diverse between species, and are among the most fast-evolving protein-coding elements in filamentous fungal genomes. To gain insight into the diversity of secondary metabolic gene clusters in fungal populations and its drivers, we examined the conservation and divergence of secondary metabolic gene clusters across the genomes of 40 representative isolates of the cosmopolitan opportunistic pathogen *Aspergillus fumigatus*. We found that a core set of secondary metabolic gene clusters were present in all isolates, but that other gene clusters were only present in subsets of isolates. We additionally identified multiple different types of gene cluster polymorphisms, including fusion of different gene clusters, alternative genomic locations for gene clusters, and clusters

with multiple alternative idiomorphs (i.e., non-homologous alleles). In particular, two gene clusters flanked by transposable elements were consistently found in different genomic locations; one of these clusters showed a phylogenetic pattern consistent with horizontal gene transfer between fungi. Our results suggest that multiple genetic factors, including recombination, gene loss, and horizontal gene transfer, drive the diversification of secondary metabolism pathways.

46. Overexpression of a C_4-dicarboxylate transporter is the key for converting citric acid production to C_4-dicarboxylic acid production in *Aspergillus carbonarius*

Lei Yang[a], Eleni Christakou[a], Jesper Vang[a], Mette Luebeck[a] and **Peter Luebeck[a]**
[a]Chemistry and Bioscience, Aalborg University, Aalborg, DK

C_4-dicarboxylic acids, including malic acid, fumaric acid and succinic acid, are valuable organic acids that can be produced and secreted by a number of microorganisms. *Aspergillus carbonarius* is capable of producing high amounts of citric acid, however, all attempts to change the citric acid production into C_4-dicarboxylic production by pathway engineering have been with very limited success.
In this study, a glucose oxidase deficient strain of *A. carbonarius* was used as the parental strain to overexpress a native C_4-dicarboxylate transporter and the *frd* gene encoding fumarate reductase from *Trypanosoma brucei* individually and in combination to investigate their impacts on organic acid production. Overexpression of the C_4-dicarboxylate transporter alone and in combination with the *frd* gene significantly increased the production of C_4-dicarboxylic acids and reduced the accumulation of citric acid, whereas expression of the *frd* gene alone did not result in any significant change of the organic acid production profile.
This study demonstrates that the key to change the citric acid production into production of C_4-dicarboxylic acids in *A. carbonarius* is the C_4-dicarboxylate transporter. Furthermore it shows that the C_4-dicarboxylic acid production in *A. carbonarius* can be further increased via metabolic engineering.

47. Genomic and bioinformatics analyses of biosynthesis and production enhancement of a novel antifungal antibiotics, FR901469, from a filamentous fungus

Masayuki Machida[a,d], Makoto Matsui[b,d], Tatsuya Yokoyama[b,d], Hiroya Itoh[b,d], Akira Ohyama[c,d] and Takashi Shibata[b,d]
[a]Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Sapporo, Japan [b]Biotechnology labs, Astellas Pharma Inc., Japan [c]in silico biology, inc., Yokohama, Japan [d]Technology Research Association of Highly Efficient Gene Design (TRAHED), Tokyo, Japan

A novel antifungal antibiotics, FR901469, is a non-ribosomal peptide (NRP) compound produced by a filamentous fungus, No. 11243. We have sequenced an entire genome of this strain, revealing genome size of 21.7 Mb with 9,920 predicted genes. Of the 4 putative non-ribosomal peptide synthetase (NRPS) genes existing on the genome, we have identified a 45 kbp NRPS gene for the core biosynthesis of FR901469 and associated nine genes as a gene cluster of 79 kbp in length. To date, fourteen mutants producing higher amount of FR901469 than the parental strain (up to 10 times) have been obtained by UV irradiation. Genome-wide mutation analysis revealed 14-183 mutations for each strain, among which those for genes categorized as “Replication, recombination and repair”, “Signal transduction mechanisms” and “Transcription” are highly enriched. Interestingly, the region consisting of approximately 1.6 Mbp in size that showed 50% higher depth of mapping of the short reads from NGS possessed the FR901469 biosynthesis gene cluster above. Transcription expression analysis by RNA-sequencing revealed strong relationship between the expression of NRPS and the FR901469 productivity. In accordance of this result, artificial overexpression of a C6 zinc cluster transcription factor found in the gene cluster of the parental strain showed 3- to 4-times higher production of FR901469. Besides the enhancement of transcription expression of the cluster member genes, we have found genes relating to amino acid biosynthesis were overexpressed commonly in the mutant strains as well.
During the progress above, we have developed a software tool to further analyze secondary metabolism (SM) gene clusters, In silico Molecular Cloning Genome Design Suite (IMCDS). Based on the unique feature of this tool, by which multiple clusters are aligned according to the sequence similarity of each cluster member gene, and in combination with MIDDAS-M and MIPS-CG, which we previously developed

to predict SM gene cluster in a sequence motif-independent manner, further characterization of the FR901469 biosynthesis gene cluster will be discussed

48. Alteration of polyamine metabolism negatively affects *Aspergillus flavus* development and pathogenesis during its interaction with corn kernels

R. Majumdar[a], J. Cary[a], S. Minocha[b], M. Lebar[a], B. Mack[a], C. Sickler[a], C. Carter-Wientjes[a], K. Rajasekaran[a] and D. Bhatnagar[a]
[a]Food and Feed Safety Research Unit, USDA-ARS, New Orleans, LA [b]Dept. of Biological Sciences, University of New Hampshire, Durham, NH

Polyamines (PAs) are low molecular weight polycations that regulate DNA replication, transcription and translation in living cells. They have been shown to positively influence growth, development, and pathogenicity in both plant and human pathogens. Among the three predominant polyamines (putrescine, Put; spermidine, Spd; spermine, Spm), Spd is absolutely required for cell division. Spermidine (triamine) is synthesized from Put (diamine) by spermidine synthase. Spermidine serves as the donor of a 4-aminobutyl group to the Lys50 residue of eukaryotic elongation factor 5A (eIF5A) to produce the rare amino acid, hypusine. Hypusination-mediated activation of eIF5A is critical for initiation of translation. *A. flavus* is an opportunistic fungus that infects corn and other oilseed crops and produces the toxic and carcinogenic mycotoxins, aflatoxins, upon infection. To explore the role of spermidine synthase (s*pds)* in *A. flavus* pathogenesis, we disrupted the single copy of the *spds* gene and studied the ability of *spds* mutants to grow on artificial media as well as infected corn kernels. Inactivation of s*pds* significantly reduced mycelial growth and sporulation on artificial media and an exogenous supply of Spd (auxotroph) was required to support fungal growth and development. Infection of corn kernels using *spds* mutant, shows significantly less fungal growth and sporulation (vs. controls). These results suggest that impairment of Spd biosynthesis negatively affects *A. flavus* pathogenesis and pre-treatment (prior to seed inoculation) of a *spds* mutant with Spd and possible Spd acquisition from the host, are not sufficient to restore wild-type levels of pathogenesis during seed infection. On the other hand, dual disruption of the ornithine decarboxylase (*odc)* gene (involved in Put biosynthesis) and a putative PA uptake transporter had little effect on fungal growth and sporulation. The significance of these observations will be discussed in context to the expression of *A. flavus* genes associated with polyamine metabolism (biosynthesis, uptake, and catabolism) and aflatoxin biosynthesis, and aflatoxin contents in the *spds* mutant vs. wild-type *A. flavus* infected corn kernels.

49. The *A. fumigatus* Fumiquinazoline C is potentially cytotoxic to macrophages and soil amoeba *Dictyostelium discoideum*

Marina Campos Rocha[a], Taicia Pacheco Fill[b], Juliana Issa Hori[c], Lilian Pereira Silva[d], João Henrique Tadini Marilhano Fabri [a], Anderson Ferrira da Cunha[a], Gustavo Henrique Goldman[d] and **Iran Malavazi**[a]
[a]Departamento de Genética e Evolução, Centro de Ciências Biológicas e da Saúde, Universidade Federal de São Carlos, São Carlos, Brazil. [b]Instituto de Química, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil. [c]Departamento de Farmacologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil. [d]Departamento de Ciências Farmacêuticas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil.

Fungi remarkably produce a variety of secondary metabolites as a consequence of different environmental stimuli. These compounds can ultimately provide fitness attributes to the producing organism. Recently, we characterized two components of the *A. fumigatus* cell wall integrity pathway (CWI), *pkcA* and *rlmA* and observed that in addition to the cell wall related-phenotypes, the perturbation of the signaling circuit coordinated by the PkcA-MpkA-RlmA module impacts on the production of fumiquinazolines (Fq). FqC is the major Fq produced by *A. fumigatus* which accumulation was associated with conidia formation. Here we show that *pkcA*^{G579R} and Δ*rlmA* mutant strains produce lower FqC (24.7% and 27.9%, respectively) and that FqC concentrations were 10.5- fold lower in the Δ*mpkA* strain. This decrease is accompanied by global down-regulation in mRNA expression of the Fq cluster genes during the asexual development. Aiming to understand if other cell stresses could influence the production of FqC, we performed a screening using different null mutants and found that the deletion of the transcription factor SebA, (primarily involved in heat shock and oxidative stress) overproduced FqC

(about 4.5-fold increase) indicating that *sebA* is a negative regulator of FqC production. *A. fumigatus* is sensitive to FqC and this tolerance is decreased in the CWI pathway mutants and increased in the Δ*sebA* strain. In addition, FqC can induce pore formation on the membrane of macrophages and highly stimulates the secretion the pro-inflammatory cytokine TNF-α by this cell type. We also used the soil amoeba Dictyostelium *discoideum* to study the phagocytic interaction of this organism with conidia from the Δ*sebA* strain. Interestingly, conidia of the Δs*ebA* were significantly less phagocytized by *D. discoideum* and the opposite occurred when conidia from the CWI pathway mutants were tested. Our results suggest that Fq production is regulated at different levels in *A. fumigatus* and that FqC can serve as a defense compound against other microorganisms or soil predators. Although we could not detect FqC in the lung of infected mice, this molecule is potentially cytotoxic to fungi and mammalian cells.

50*. Internuclear diffusion of histone H1 within cellular compartments in *Aspergillus nidulans*
Alexander Mela[a] and Michelle Momany[a]
[a]Plant Biology, University of Georgia, Athens, GA

Histone H1 is an evolutionarily conserved 'linker' histone protein which serves the important dynamic function of arranging and stabilizing chromatin structure. The mechanism by which histone H1 carries out its function remains unclear. One thing that is certain is the common use of fluorophore-tagged histone H1 protein in microscopy to track nuclei within cells. In time-lapse analyses, we observed stochastic exchange of photo-activated Dendra2-Histone-H1 protein between nuclei. We also observed heterogeneous mixing of histone proteins between nuclei in hyphae and conidiophores derived from heterokaryon fusions of histone H1-RFP and H1-GFP strains. Subsequent analysis of the resulting conidia that contained both RFP- and GFP-labeled histone H1 proteins, showed only parental genotypes. These data together suggest the stochastic exchange of histone H1 protein between nuclei is likely a result of diffusion rather than genetic recombination during karyogamy.

51*. Heterokaryon incompatibility and phenotypic characterisation of *Aspergillus flavus* isolates in low and high risk zones in Kenya
Alfred Mitema[a,b], Sheila Okoth[b], Revel Lyer[b], Amelia Hilgart[b] and Suhail Rafudeen[b]
[a]Molecular and Cell Biology, University of Cape Town, Cape Town, Western Cape, ZA [b]University of Nairobi, Kenya

Species *Aspergillus flavus* (*A. flavus*) has three morphotypes: S-, L- and S_{BG}-type strains, with the first repeatedly being associated with acute aflatoxicosis. Aflatoxicosis in Kenya is a serious health problem with outbreak 317 cases and 215 deaths. In 2013, we choose to address this crisis by characterising the diversity of *A. flavus* isolates through comparison of vegetative compatibility groups (VCGs), phenotypic characteristics and mycotoxin profiles across various agricultural regions in Kenya where aflitoxicosis has occurred previously. Using diagonal transect random household sampling, Maize kernels were collected from Makueni, Homa Bay, Nandi, and Kisumu counties between November and December 2013. Out of 37 isolates, the nitrate non-utilising auxotrophs (*nit* mutants) complementation test revealed 20 VCGs. KVCG14 and KVCG15 had highest distribution frequency (n = 13; 10.8%) while KVCG10 and KVCG20 (n = 1; 0.8%) the least. Analyses of VCG diversity using the Shannon Index (H) showed that Nandi (H'= 0.108) and Kisumu (H'= 0.324) counties recorded the lowest and highest VCG diversities respectively. Results also showed that, with a few exceptions, within-county isolates were self-compatible but they were incompatible across any two adjacent counties and across all the sampled counties. Heterokaryon incompatibility in Nandi (n = 6; 67%) and Makueni (n =3; 33%) were the greatest and least respectively. Mycotoxin detection by coconut cream agar under UV light (365 nm) revealed blue fluorescence {(57%, n =21) aflatoxin B} and green {(43%, n =16) aflatoxin G}. The study further revealed L-, S- type strains (57%; n =21 and 7%; n =2) respectively. The findings of the study could provide reliable information in determining biocontrol strategies to mitigate aflatoxin contamination of maize by *A. flavus* in the studied counties.

52*. Comparative analysis of the function of α-1,3-glucan syntheses, AgsA and AgsB, in *Aspergillus nidulans*.

K. Miyazawa[a], A. Yoshimi[b], S. Yano[c], S. Kasahara[d], F. Hasegawa[b] and K. Abe[a,b]
[a]Grad. Sch. Agric. Sci., Tohoku Univ., Sendai, JP [b]NICHe, Tohoku Univ., Sendai, JP [c]Yamagata Univ., Yonezawa, JP [d]Miyagi Univ., Sendai, JP

Although α-1,3-glucan (AG) is one of major polysaccharides in the cell wall of *Aspergillus* species, the biological function of AG remains unclear, except for the role as a virulence factor in some pathogenic fungi. Previously, we carried out functional analysis of two α-1,3-glucan synthase (AGS) genes (i.e. *agsA* and *agsB*) in the model filamentous fungus *Aspergillus nidulans*. The *agsB*Δ strain lost most cell wall AG, suggesting that a main AGS in this fungus is AgsB. Interestingly, the hyphae of the *agsB*Δ strain was dispersed under liquid culture conditions, whereas the wild-type strain formed hyphal pellets under same culture conditions. These results suggest that AG has the role as an adhesive factor for hyphal cells. On the other hand, because the expression of *agsA* gene was scarcely detected under normal growth conditions and the *agsA*Δ strain did not show phenotypic defects, the role of *agsA* remains unclear. In this study, in order to investigate the roles of AgsA and AgsB in cell wall AG synthesis, we comparatively analyzed cell wall polysaccharides synthesized by AgsA and AgsB. First, we constructed *agsA* or *agsB* gene overexpression (O/E) strain by replacing the promoter region of *agsA* or *agsB* with *tef1* promoter under the genetic background of the other *AGS* gene disruption, and confirmed the high expression of either of the *AGS* genes in the O/E strains. The O/E of *agsA* restored the growth characteristics of the *agsB*Δ strain under liquid culture conditions: the O/E *agsA* strain formed the hyphal pellets. This suggests that the *agsA* gene encodes a functional AGS. To elucidate the differences of cell wall structure between these two strains, we performed the alkaline-fractionation of cell wall and analyzed the sugar composition of the fractions. The carbohydrate analyses revealed that the sugar compositions of the AS2 fraction were similar in these two strains, but the texture of the AS2 fraction derived from these two strains was markedly different from each other, suggesting that the detailed chemical structure of AG obtained from the O/E *agsA* differs from that of AG derived from the O/E *agsB* strains.

53*. Population structure of *Aspergillus flavus* in soil and corn kernels from four different states

M.S. Molo[a], R. Singh[a], J.B. White[a], T. Isakeit[b], K.A. Wise[c], C.P. Woloshuk[c], B.H. Bluhm[d], B.W. Horn[e], R. Heiniger[a] and I. Carbonw[a]
[a]North Carolina State University, Raleigh, NC [b]Texas A&M University, College Station, TX [c]Purdue University, West Lafayette, IN [d]University of Arkansas, Fayetteville, AR [e]United States Department of Agriculture, Agriculture Research Service, Dawson, GA

Aspergillus flavus is a cosmopolitan soil-borne fungus that contaminates oil seed crops by producing carcinogenic aflatoxins (AFs). Ingesting AF-contaminated grains has been linked to hundreds of deaths in Africa. Due to the health risks AFs pose, the FDA has strict regulations on grain contaminated with AFs. Therefore, AFs have a huge economic impact on corn growers through management practices, yield loss, and adverse health effects. Previous reports indicate that certain *A. flavus* genotypes exist in the soil and others are specialized to colonize corn kernels, but this is based on sequence data from a relatively small number of genetic markers. Understanding the genetic differences that separate corn and soil isolates could lead to insights into the development of the next generation of biological control agents. We sampled *A. flavus* from soil early in the growing season and from kernels at harvest in Texas, North Carolina, Arkansas, and Indiana in 2013. This allowed us to examine genetic structure between populations from different states as well as between soil and kernel substrates. We sequenced a total of 246 isolates using double digest restriction-site associated DNA sequencing, which represents the genome in a manageable size by providing markers across each chromosome and the mitochondria. A preliminary analysis revealed that each state had native strains of *A. flavus* as well as shared evolutionary lineages. Further work will examine the distribution of genetic variation between soil and kernel populations.

54. A CRISPR/Cas9 based Toolbox for Efficient Genome Editing of Filamentous Fungi
U.H. Mortensen[a], C. S. Nødvig[a], Z.D. Jarczynska[a], M.L. Nielsen[a], F. H. Kirchner[a] and J.B. Hoof[a]
[a]Biotechnology and Biomedicine, Technical University of Denmark, Kgs. Lyngby, DK

Large numbers of fungi are currently being fully sequenced and will contribute dramatically to our understanding of fungal biology. However, the fact that gene targeting is inefficient in most fungal species hampers exploitation of the genome sequences. This problem has been significantly reduced after efficient CRISPR/Cas9 gene editing has been introduced in several different fungal species. A challenge of the CRISPR/Cas9 system is to deliver gRNAs to form the Cas9 ribonuclease. Several approaches have been presented in the literature including methods where gRNAs are co-transformed into the cells along with the gene editing DNA substrates, methods where the gRNA is produced by RNA polymerase III, and methods where the gRNA is liberated by ribozymes from a larger transcript produced by RNA polymerase II. Since the different methods have different advantages/disadvantages, we envision that they may work with different efficiencies in different fungal species. We have therefore developed a flexible CRISPR/Cas9 toolbox adapted for filamentous fungi to facilitate genome editing. Our toolbox includes bio-bricks containing e.g. different genetic markers and polymerase promoter types allowing for a rapid and efficient vector assembly and bricks that allow for quick insertion of new genes into strong expression sites for heterologous expression. Moreover, it includes bricks to facilitate trouble shooting including a *cas9-RFP* reporter gene to evaluate Cas9 levels in new hosts and a system allowing the efficiency of individual gRNA species to be tested in vivo. Using our toolbox, we have successfully edited the genomes of more than 10 species and used it to make a different range of genetic alterations including site specific mutations by using oligonucleotides as repair templates and deletions. In this way we have linked secondary metabolites to genes in species that have not previously been genetically engineered. For strains where we plan to do extensive gene targeting, we typically use CRISPR to mutate the *pyrG* gene, hence, producing a marker than can be selected/counter selected. Next, we mutate a gene in the NHEJ pathway to produce a strain where gene targeting is very efficient. We will show how this strategy can be used investigate the biosynthetic pathway of gene clusters. Lastly, we will show how markers and mutations in NHEJ genes can be easily reverted to wild-type if a wild-type background is desirable in the subsequent analyses.

55*. An oxylipin signal mediates hyphal branching in pathogenic Aspergilli
M. Niu[a], G. Fischer [a] and N. Keller[a,b]
[a]Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI, USA [b]Department of Bacteriology, University of Wisconsin-Madison, Madison, WI, USA

Oxylipins are a group of diverse oxygenated polyunsaturated fatty acids found in all eukaryotes that modulate growth, development, and cellular communication. Three oxylipin generating oxygenases, PpoA (Psi producing oxygenase A), PpoB, and PpoC, mediate development and stress responses in pathogenic *Aspergillus* species. However, the cellular targets of these oxylipin metabolites, their cellular functions, and signal transduction pathway(s) transmitting the signal are yet to be investigated. Our laboratory has recently identified that exogenous treatment of 5(S),8(R)-dihydroxide octadecadienoic acid (5,8-diHODE), the final oxylipin product of PpoA, resulted in stunted apical growth, increased lateral growth or hyper-branching, and decreased septal distance in the human fungal pathogen *A. fumigatus* and plant pathogen *A. flavus*. Our results suggested that the observed hyperbranching phenotype is specific to C18 diol-oxylipin acids with specific structural features yet to be identified. In addition, the branching phenotype by 5,8-diHODE treatment was remediated by high amount of Ca^{2+} to the wildtype level, suggesting that Ca^{2+} is involved in oxylipin signal transduction in the pathogenic Aspergilli species.

56*. What drives speciation? Examination into the evolutionary events of more than 100 Aspergillus species.

J.L. Nybo[a], T.C. Vesth[a], S. Theobald[a], I. Kjaerboelling[a], J.C. Frisvad[a], T.O. Larsen[a], R. Riley [b], A. Salamov[b], I.V. Grigoriev[b], S.E. Baker[c] and M.R. Andersen[a]
[a]Biotechnology and Biomedicine, Technical University of Denmark, Kongens Lyngby, DK [b]Joint Genome Institute, Walnut Creek, CA, USA [c]Joint Bioenergy Institute, Berkeley, CA, USA

The study of speciation - how new species arise, diverge and remain separate, has a central role in evolutionary biology. Partly because it embraces so many disciplines, including population genetics, behavioral sciences, comparative genomics, evolutionary biology, biodiversity, biogeography and ecology. It also remains one of the most fascinating questions in evolution.

Speciation is nearly impossible to study and in most cases, we know very little about the genetic basis of species formation. But in this project we aim to identify evolutionary events that can drive speciation, such as gene duplications, creations and losses, and horizontal gene transfers between closely or distantly related species within the genus of the filamentous fungi Aspergillus. This diverse genus holds species relevant to both plant and human pathology, food biotechnology, enzyme and bulk chemical production, model organisms, and it even contains some extremophiles.

To identify these events, we have developed a homologous protein prediction software that has been used to generate a high-resolution pan-genomic map. From where, we have identified genes specific to species, clades and core that allows for guilt-by-association-based mapping of genotype-to-phenotype.

Our results illustrate a highly diverse genus where 500-2000 genes are unique to each species. These genes are predominantly within regulation or compound biosynthesis, supporting the notion of natural selection. A conservative estimate of the number of protein families shared by all Aspergillus species is surprisingly low, only about 2600 core families, suggesting high environmental adaptation within this genus.

57. Development of genome editing method by direct introduction of Cas9-protein in *Asepergillus oryzae*.

K. Shimamoto[a,b], R. Saitou[a], Y. Wada[c], O. Mizutani[a], **K. Oda[a]**, M. Okuda[a,b] and K. Iwashita[a,b]
[a]Brewing mycology, National Research Institute of brewing, Higashi-hiroshima, Hirhoshima, JP [b]Hiroshima Univ. [c]FASMAC Co.Ltd.

Aspergillus oryzae is the one of attractive industrial strains used for traditional fermentation industries and recent enzyme production and pharmaceutical industries. Molecular breeding of *A. oryzae* is still difficult and troublesome, because of its genetical difficulty such as multi-nuclear and deficient of cross. Recently, genome editing with CRISPR/Cas9 is focused on for editing target genes. This system was generally introducing *cas9* gene with plasmid vector and expressed in mycelium, and as a result the transformants becomes gene modified organisms. Considering the utilization in industry, we developed the genome editing method by directly Cas9 introducing into the experimental strain of RIB40 and applied to various industrial strains.

We chose 5-FOA selectable *pyrG* in *A. oryzae* for genome editing target. Cas9 and *pyrG* single guide RNA (sgRNA) are mixed and formed ribonucleoproteins (RNPs), then introduced into *A. oryzae* by conventional protoplast-PEG method. 289 candidates were selected on 5-FOA containing plates. We confirmed the sequence of *pyrG* locus in 24 candidates and found that about half of the strains occurred 1bp deletion at target locus, indicating that genome editing was succeeded by this method. We further examined the amount of Cas9 use, and found that more than 10 mg is enough for *A. oryzae* genome editing by this method.

To confirm the practicality of the method, we carried out *pyrG* genome editing against some *A. oryzae* industrial strains, such as OIS01 (current Japanese Sake brewing), RIB128 (ancient Sake brewing), RIB163 (Japanese Sake brewing) and RIB915 (Soy sauce making) strains. We acquired genome editing candidates in the all industrial strains, indicating that this Cas9 direct introducing genome editing method is useful for molecular breeding of *A. oryzae* industrial strains.

58*. Comparative genomics of *Aspergillus flavus* S and L morphotypes provides insights into niche adaptation

Mana Ohkura[a], Peter Cotty[a] and Marc Orbach[a]
[a]School of Plant Sciences, University of Arizona, Tucson, AZ

Aspergillus flavus is a widely distributed saprotrophic fungus and the most common causal agent of aflatoxin contamination on food crops. Despite the notoriety of *A. flavus* to produce aflatoxin, strains vary greatly in their levels of toxin production and atoxigenic strains are not uncommon in nature. Within *Aspergillus flavus,* there are two distinct morphologies, namely morphotypes: the S morphotype produces numerous small sclerotia and limited conidiospores and the L morphotype produces relatively fewer large sclerotia and abundant conidiospores. S strains are consistently toxigenic, whereas L strains vary greatly in toxin production ranging from atoxigenic to highly toxigenic. Morphological differences between the two morphotypes suggest the production of abundant sclerotia in S strains is advantageous for long-term survival in the soil, whereas the production of abundant conidia in the L strains is advantageous for aerial dispersal to the phyllosphere. The selection pressure to maintain aflatoxin production may be higher in the soil compared to the phyllosphere, leading to the retention of consistently toxigenic S strains in the soil. In contrast, the selection pressure may be lower in the phyllosphere, allowing the survival of atoxigenic or low toxin-producing L strains in that niche. To develop hypotheses on differential niche adaption, we compared the genomes of three S strains and three L strains to identify structural differences and genes unique to each morphotype. A 530 kb inversion was identified between the morphotypes that affects a secondary metabolite gene cluster and a cutinase gene on the margins. The L strain genomes contained 10 deletions, many of which involve secondary metabolite genes. Each morphotype had unique genes that play a role in carbon/nitrogen metabolism, secondary metabolism, and antimicrobial defense. These findings indicate that the genomes of the two morphotypes differ beyond developmental genes, and that they may have diverged as they adapted to their respective niches.

59. Fast and highly efficient gene replacement in *Aspergillus niger* using CRISPR/Cas9

Jean Paul Ouedraogo[a], Yun Zheng[a], Tricia John[a], Letian Song[a] and Adrian Tsang[a]
[a]Concordia University, CSFG, 7141 Sherbrooke St.W.Montreal, QC, H4B1R6

The CRISPR (clustered regularly interspaced short palindromic repeat)–Cas9 (CRISPR-associated nuclease 9) system is transforming biology by providing a simple and efficient method to precisely edit the genome of any organism. As an important bio industrial workhorse, *Aspergillus niger* has attracted widespread interest for exploring broaden application in the sectors of industrial enzymes and organic acids. The availability of its genome sequence enables the redesign and engineering novel stains of *A. niger* based on CRISPR/Cas9 tools. In this study, we have extended CRISPR/Cas9 system for gene editing in *A. niger*. The endogenous tRNA gene as RNA polymerase III promoter was employed to lead the expression of guide-RNA (gRNA) cassette to delete and replace gene by homologous recombination in *Aspergillus niger.* The combination of CRISPR/Cas9 system and non-homologous end joining deficient strain allows fast and highly efficiency gene replacement in an organic acids producer *A. niger* strain with 100 % of transformants showing correct gene replacement.

Keywords CRISPR/Cas9, genome editing, *Aspergillus niger*

60. Improvements to the *Aspergillus nidulans* molecular genetic system: Creation of new selectable markers, cloning of genes that complement them and construction of *A. nidulans* strains with up to seven selectable markers

James Dohn[a], Alex Grubbs[a], C. Elizabeth Oakley[a] and **Berl R. Oakley**[a]
[a] Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045 USA

Progress in transformation methods and in production of transforming DNA fragments has greatly facilitated molecular genetic manipulations of *Aspergillus nidulans*. Complex molecular genetic manipulations may require several, serial transformations, however, and there is a need for additional selectable markers for transformation. In particular, it would be useful to have multiple selectable markers in a single strain. To this end, we have carried out a series of gene replacements and marker rescues in strain LO4389, creating new strains with multiple selectable markers. All strains carry *pyrG89*, *riboB2* and

pyroA4, as well as a deletion of the *nkuA* gene (reducing transformation by heterologous integration) and a deletion of the sterigmatocystin gene cluster. In addition, the strains carry deletions of one or more of the following genes: *biA*, *pabaA*, *lysB* and *choA*. LO9771 carries all of these mutations and deletions and thus has seven selectable markers available for transformation. This strain is healthy and transforms well. In addition, we have cloned the *biA*, *pabaA*, *lysB* and *choA* homologs from *Aspergillus terreus*, transformed them into strains carrying deletions of these genes and shown that they complement the deletions well. These strains and complementing markers will be made available at the Fungal Genetics Stock Center. Supported by the Irving S. Johnson fund of the University of Kansas Endowment.

61. The cullin-1 gene in *Aspergillus nidulans* interacts genetically with γ-tubulin and plays critical roles in regulating the G1 to S transition and in suppressing septation near the hyphal tip.
Tori Paolillo [a], James Dohn [a], **Berl Oakley**[a]
[a]Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045 USA

In the coenocytic fungus *Aspergillus nidulans*, a cold-sensitive γ-tubulin allele, *mipAD159*, causes a nuclear autonomous failure of the Cdh1 homolog, CdhA, to dissociate from spindle-pole bodies at the G1/S transition resulting in continuous activation of the anaphase promoting complex/cyclosome in affected nuclei (Edgerton-Morgan and Oakley, 2012, J. Cell Biol. 198, 785-791). This, in turn, results in continuous destruction of cyclin B and failure of affected nuclei to progress through the cell cycle (Nayak et al., 2010, J. Cell Biol. 190, 317-330). These data reveal a role for γ-tubulin in regulation of CdhA, but the underlying molecular mechanisms are unknown. In many organisms, one of the regulators of Cdh1 is the Skp1-Cullin1-F-box (SCF) complex, an E3 ubiquitin ligase that has been shown to regulate Cdh1 ubiquitination and subsequent degradation. We were consequently interested in investigating the function of this complex in A. nidulans. We have deleted *culA*, the *A. nidulans* cullin-1 gene and found that it is essential for viability. Utilizing the heterokaryon rescue technique, we have found that *culAΔ* germlings undergo several rounds of nuclear division before growth arrest and, while some nuclei in tip cells accumulate cyclin B, other nuclei in the same cell fail to accumulate it. This probabilistic, nuclear autonomous failure to accumulate cyclin B recapitulates a *mipAD159* phenotype. These data suggest that deletion of culA likely prevents CdhA degradation, resulting in nuclei with constitutively active APC/CCdhA that fail to progress through the cell cycle. We also determined the in vivo localization of CulA and found CulA-GFP is nuclear during interphase and exits nuclei during mitosis. Although CulA-GFP strains grew like wild-type controls at all temperatures, there was a noticeable decrease in conidiation at lower temperatures, suggesting that the GFP tag may cause a slight loss of function. Interestingly, CulA-GFP, mipAD159 strains barely grew. The strong, synthetic genetic interaction between *mipAD159* and culA-GFP indicates that γ-tubulin and CulA are involved in a common function that is required for growth or viability. Our heterokaryon rescue experiments also revealed an unexpected role for CulA, and by inference the SCF complex, in the regulation of septation. Septation is normally suppressed near growing hyphal tips in A. nidulans resulting in a multinucleate tip cell that is hypothesized to facilitate rapid tip growth. Live-cell imaging revealed that in culA deletion germlings, the timing of septation was normal, occurring shortly after the completion of mitosis as in wild-type cells. Suppression of septation near the tip was obviated, however, resulting in a dramatic increase of septa near the tip that sometimes resulted in a single nucleus in the tip cell. Preliminary data indicate that deletion of another SCF complex gene, *skpA* (A.K.A. *sconC*), confers a similar, but perhaps more dramatic, hyperseptation phenotype. These data indicate that the SCF complex plays an important role in suppressing septation near growing hyphal tips in *A. nidulans*. Supported by the Irving S. Johnson Fund of the KU Endowment.

62*. Utilizing next generation sequencing to revitalize a forward genetic screen for mutants deficient in the production of sterigmatocystin in *Aspergillus nidulans*

B. T. Pfannenstiel[a], K. J. Affeldt[b], J. Bok[b], R. A. E. Butchko[c], T. Choera[b], G. J. Fischer[a], B. P. Knox[b], F. Y. Lim[b], X. Luo[d], A. A. Soukup[a], J. E. Spraker[d], K. Throckmorton[a], P. Wiemann[b], N. P. Keller[b,e], and J. M. Palmer[f]

[a]Department of Genetics, University of Wisconsin-Madison, Madison, WI, 53706, USA. [b]Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI, 53706, USA. [c]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, USA. [d]Department of Plant Pathology, University of Wisconsin-Madison, Wisconsin 53706. [e]Department of Bacteriology, University of Wisconsin-Madison, Madison, WI 53706, USA. [f]Center for Forest Mycology Research, Northern Research Station, U.S. Forest Service, Madison, WI 53706, USA.

The study of aflatoxin regulation in *Aspergillus* spp. has warranted the attention of many researchers due to its carcinogenic properties and frequency as a food and feed contaminant. Significant progress on the regulation of the AF cluster has been conducted in the model organism *Aspergillus nidulans* by assessing regulation of the sterigmatocystin (ST) gene cluster as ST is the penultimate precursor of AF. A screen using a chemical mutagen identified 23 loci involved in regulating ST production. Only six of these loci were characterized from this screen using classical mapping (*mcsA)* and complementation with a cosmid library (*laeA*). Recently the remaining mutants were backcrossed and sequenced using an Ion Torrent PGM™. Each mutant contained one or more SNPs in predicted genes. Deletion of these genes resulted in identification of mutant alleles responsible for the loss of ST production in 12 out of the 17 remaining mutants. Three of the causative mutations were in uncharacterized genes unknown to be involved with ST production, of which two appear to regulate the ST cluster via transcriptional regulation of its cluster specific transcription factor, *aflR*. Based on protein domains and homologs, the remaining uncharacterized gene has a predicted role in the plasma membrane fusion. Nine mutations were in genes already known to affect ST synthesis (*laeA*, *mcsA*, *fluG*, *stcA*).

63. CgXbp1 modulates temporal transcriptional dynamics in human fungal pathogen *Candida glabrata* during interaction with host macrophages.

M. N. Rai[a], C. Parsania[a], R. Rai[a] and K. H. Wong[a]

[a]Faculty of Health Sciences, University of Macau, Macau, MO

Candida glabrata, an opportunistic human fungal pathogen in immunocompromised patients, remodels its carbon metabolism to survive in nutrient-deprived intracellular macrophage microenvironment. Here, we have identified a transcription factor, CgXbp1, required for remodeling carbon metabolic pathways in *C. glabrata* upon exposure to host macrophages. Employing the next generation sequencing approaches, we showed that *Cgxbp1Δ* mutant exhibited constitutively active fatty acid catabolic and proteolytic pathways even in the presence of sufficient carbohydrate in the growth medium, suggesting that carbon metabolic processes are deregulated upon *CgXBP1* deletion. Next, we demonstrated that CgXbp1 is essential for arresting the cells in G1 phase of cell cycle during carbon starvation. Global gene expression analysis on *Cgxbp1Δ* mutant revealed that genes associated with GO terms – mitochondrion organization, mitochondrial transport and mitochondrial translation were highly enriched among down-regulated genes, indicating that CgXbp1 may have pivotal roles in cellular energy homeostasis maintenance. Comparison of the transcriptional profiles of macrophage-engulfed wild-type and *Cgxbp1Δ* mutant cells revealed that unlike wild-type cells, *Cgxbp1Δ* mutant was unable to repress genes associated with GO terms - Translation, Ribosome biogenesis and RNA metabolic processes during early stage of macrophage infection. Overall, our gene expression data indicated that CgXbp1 not only is central in carbon metabolic remodeling, but also affects global transcriptional response of *C. glabrata* cells in host macrophages.

64*. Universal expression system for eukaryotic microorganisms

A. Rantasalo[a], C. Landowski[a], J. Kuivanen[a], J. Jäntti[a] and D. Mojzita[a]
[a]VTT Technical Research Centre of Finland Ltd

We have developed a novel orthogonal expression system that functions in a wide spectrum of eukaryotic microorganisms. The expression system is based upon a synthetic transcription factor (sTF) that regulates expression of the target gene via a sTF-dependent promoter. The sTF expression is driven by an engineered, universal core promoter that provides a low, but sufficient expression level of the sTF. The sTF expression is constitutive and thus present in the cells in all growth conditions and in all developmental and growth stages. The sTF-dependent promoter regulating the expression of the target gene also contains a similar type of universal core promoter functional in diverse species. This core promoter can be further modified by inserting synthetic repressor (sR) binding sites, through which an additional expression control is gained upon expression of the sR. The combination of multiple sTF-binding sites and the core promoters (including the repressible versions) enables specific adjustment over a wide range of target gene expression levels, from very low to very high. This expression system provides robust, stable, and tuneable expression levels of the target genes in a broad spectrum of host organisms. Further it simplifies the genetic tools needed for the construction of new protein expression hosts, including those with undeveloped know-how. The method for selecting the universal core promoters, constructions of the expression systems, and demonstrations of their performance in diverse hosts, including yeasts and filamentous fungi, will be presented.

65. Analysis of aspirochlorine (ACL) productivity and ACL cluster sequence in *Aspergillus oryzae* strain.

S. Ryota[a], O. Tami[a], U. Miyuki[a], O. Ken[a] and I. Kazuhiro[a,b]
[a]Nstional Research Institution of Brewing, Hiroshima, Hiroshima, Japan [b]Graduate School of Advanced Science of Matter, Hiroshima, Hiroshima, Japan

Aspergillus oryzae is GRAS (generally recognized as safe) and various type of strains are used in the industry. The *A*. *oryzae* genome sequence analysis conducted in 2005, revealed the presence of many conserved secondary metabolism clusters in *A*. *oryzae*. In these studies, a cluster similar to the gliotoxin synthesis cluster was identified in the *A. oryzae* genome. Recently, the product of this cluster was identified as aspirochlorine (ACL), and this cluster was designated as the ACL cluster. ACL is antibiotics A30641, which inhibits fungal protein synthesis and represses the growth of *Candida albicans.* Although, some *A*. *oryzae* strains are known to produce ACL, the ACL productivity in several *A*. *oryzae* strains remain unclear. In this study, we investigated the physiological and molecular biological features of ACL productivity among different *A*. *oryzae* strains. First, we investigated ACL productivity in 3 different media (WATM, CYA, YES) in agar plate condition and rice koji condition by using *A*. *oryzae* RIB40. We observed that the amount of ACL was different in each plate culture medium. However, in rice koji conditions, RIB40 did not produce ACL even after 8 days of cultivation. We then examined the ACL production of 13 *A*. *oryzae* strains, one from each of the 13 phylogenetic clusters of *A. oryzae*. We examined the ACL productivity of these strains in YES agar medium, as RIB40 showed the maximum ACL production; ACL productivities among these strains were considerably different. In particular, RIB301, RIB430 and RIB1172 did not produce ACL. Furthermore, we examined the ACL production by these strains in rice koji conditions and found that none of the strains produced ACL. This difference in ACL productivity likely be based on the difference between the ACL cluster genes sequences. Hence, we examined the genome sequence of ACL clusters in these 13 strains. The ACL cluster was found to be conserved in all the 13 strains and many mutations were found in each strain. Some strain-specific mutations were also observed; in particular, in the strains that did not produce ACL.

In this study, it is clear that ACL productivity is affected by nutrient conditions. Interestingly, in the rice koji condition, no strains produced ACL, and it supposed that Japanese sake does not contain ACL. Hence, we investigated the ACL concentration in Japanese sake. We collected 11 types of sake from 20 prefectures, and did not find ACL.

66. Comparative genomics of the *Aspergillus* section *Flavi.*
A. Sato[a], K. Matsushima[a], K. Ito[a] and T. Mituyama[b]
[a]R&D, Kikkoman Corporation, Noda, JP [b]Artificial Intelligence Research Centre, National Institute of Advanced Industrial Science and Technology (AIST), Tokyo, Japan

Aspergillus section *Flavi* includes industrial fungi; *A. sojae* (*As*), *A. oryzae* (*Ao*), as well as aflatoxigenic fungi; *A. parasiticus* (*Ap*) and *A. flavus* (*Af*). It is difficult to clarify the evolutionary relationships among these four species because these fungi exhibit considerable morphological and physiological variations which hinder ordinary phylogenetics analysis. In order to clarify the relationship of these fungi at single base resolution, we performed re-sequencing and re-assembling of *As* NBRC 4239 upon our previous sequencing and obtained high quality 39.4-Mb chromosomal sequences with a 29-kb mitochondrial genome. We conducted computational genome comparison for these fungi and found that *As* is more homologous to *Ap* than to *Ao* and *Af*. In order to identify major factors to explain differences of genomic sizes (As and Ap 39Mb, Ao 37.2Mb, Af 36.9Mb) among these species, we conducted multiple compuation analyses. We found that genomic duplication can not be one of the factors because we did not find blocks to infer that *As* and *Ap* gained extra DNA by genome duplication. We conducted computational gene prediction by using Scipio (http://www.webscipio.org/) for these genomes. Then we computed gene-wise similarities among the species. The averaged alignment score over all predicted genes between *As* and *Ap* is 0.93 while that between *As* and *Ao* is 0.85. These results suggest that these fungi can be classified into two groups. We compared *As* and *Ap* genomes in terms of genomic regions of putative gene clusters for secondary metabolites. We found that *As* is deficient in some backbone genes which are essential for the secondary metabolite synthesis. This result suggests that *As* has lost abilities to generate some mycotoxins in the course of evolution. We developed a database of *As* in order to share our genome analysis results with the research community. Our database provides *As* genome browsing feature using Zenbu browser software (http://fantom.gsc.riken.jp/zenbu/). We integrated our genome annotation results into a GenBank formatted file which is supplied to the Model SEED server (http://modelseed.org/) to generate a SBML formatted genome-scale model. They can be downloaded from our database server at https://genome.cbrc.jp/sojae/.

67*. Microtubules are reversibly depolymerized in response to changing gaseous microenvironments within Aspergillus biofilms.
N. Shukla[a,b], A. Osmani[b] and S. Osmani[a,b]
[a]The Ohio State Biochemistry Program, The Ohio State University, Columbus, Ohio 43210 [b]Department of Molecular Genetics, The Ohio State University, Columbus Ohio 43210

Fungal persistence and drug resistance during infections occurs mainly due to their ability to form biofilms. Concentration gradients of oxygen and nutrients caused by cell crowding and potentially secreted signaling factors cause cells in different areas of the biofilm to experience different microenvironments. The goal of our study was to understand how microenvironments within different areas of a fungal biofilm impact cell physiology.

Using confocal microscopy and genetic analyses we have uncovered a new physiological response that *Aspergillus nidulans* cells in a biofilm undergo in response to their microenvironment. We found that after adhesion to a surface, cells at the base of a forming biofilm first halt their growth and later on depolymerize their microtubules (MTs) in a cell-autonomous manner. During such MT-disassembly, MT plus-end binding proteins like EB1, ClipA and Dynein transiently locate to unique bar-like structures. To our surprise, we also found that biofilm mediated MT-disassembly involves soluble gases since cells instantly repolymerize their MTs upon simple air exchange above the static biofilm media. Further experiments revealed that biofilm-mediated MT disassembly occurs as a regulated response to hypoxia. Upon further maturation, and possibly due to the formation of the extracellular matrix (ECM), cells no longer respond to simple gaseous exchange above the biofilm media. At this time, physical removal of a part of the biofilm is required to promote MT reassembly that then occurs in cells at the new biofilm edge. In experiments aimed at further understanding the volatile agent involved in the regulation of MT dynamics within biofilms we tested the effects of the gaseous signaling agent, hydrogen sulfide (H_2S). We find that all aspects of MT depolymerization and repolymerization, including EB1, ClipA and Dynein bar formation, can be mimicked in growing cells under normoxia by H_2S addition and removal. Collectively,

our study has implications in the areas of MT regulation, responses to hypoxia, gaseous signaling, and potentially fungal pathogenesis.

68. The role of lifecycle in azole-resistance development in *Aspergillus fumigatus*.
E. Snelders[a], J. Zhang[a], A.J.M. Debets[a], S.E. Schoustra[a], W.J.G. Melchers[b], B.J. Zwaan[a] and P.E. Verweij[b]
[a]Laboratory of Genetics, Wageningen University, Wageningen, NL [b]Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, NL

Aspergillus fumigatus is a ubiquitous fungus that plays an important role in carbon and nitrogen recycling in nature by degrading organic biomass. It is also a fungus that can cause several diseases in humans ranging from allergic conditions to acute invasive aspergillosis. To survive and thrive, *A. fumigatus* needs to rapidly adapt to these environments that often entail various challenges. Genetic adaptations by mutation and recombination can be defined as the acquisition of heritable modifications in an organism through natural selection, which enable it to survive and reproduce in the prevailing or new environment. One example of this adaptive process is the development of azole resistance. Triazoles have become the cornerstone of medical treatment of aspergillus-related diseases. Although triazole resistance can develop during patient therapy, another route of resistance selection in *A. fumigatus* can be through exposure to azole fungicides in the environment. Using the case of triazole resistance development, we show that the process of reproduction, sexual, parasexual, or asexual, is crucial for the adaptive potential of *A. fumigatus*. In this study we describe the different life cycles in *A. fumigatus* and their characteristics and probability of occurring in the human host or in the environment.

69*. Are G protein-coupled receptor proteins involved in thigmoregulation of aflatoxin inhibition by *Aspergillus flavus*?
R.R. Sweany[a] and K.E. Damann, Jr.[a]
[a]Plant Pathology and Crop Phys, Louisiana State University, Baton Rouge, LA

Aspergillus flavus can contaminate corn, groundnuts and other oil seed crops with acutely toxic and carcinogenic aflatoxin. Atoxigenic biocontrol strains of *A. flavus* reduce aflatoxin of toxigenic strains in a thigmoregulated manner. G protein-coupled receptor (GPCR) mutants of *A. flavus* were obtained from Nancy Keller at the University of Wisconsin. Since GPCRs are membrane bound proteins involved in signaling, we determined whether loss of signal receptor proteins affected intraspecific aflatoxin inhibition. Aflatoxin production was quantified using HPLC of extracts from four-day old, glucose-salts medium cultures grown in 24-well plates. Several atoxigenic isolates, both commercially developed biocontrol strains and isolates from Louisiana, were screened against the wild-type CA14 and the CA14N1 *Nit* mutant (the genetic background for gene knock outs) for intraspecific aflatoxin inhibition. Only two strains from Louisiana completely inhibited aflatoxin production of CA14 and CA14N1. One inhibitory biocontrol strain was grown with the GPCR mutants to look for loss of biocontrol function. The biocontrol strain inhibited aflatoxin production in all mutants. Toxigenic strains can also have biocontrol ability and inhibit aflatoxin production, therefore the mutants were grown with four toxigenic strains representing small or large sclerotial strains of both mating types. Only two GPCR mutants did not inhibit aflatoxin production of the toxigenic strains. When non-inhibitory GPCR mutants were germinated 12 hours prior to inoculation (due to slow growth) with competing toxigenic strains, the ability to inhibit toxin production was restored. Giving the slow-growing GPCR mutants a head start presumably reduced aflatoxin production because competing germinated hyphae now touched within the 1st 12 hours. This reiterates earlier observations that touch must occur early during the germination of the toxigenic isolate, otherwise aflatoxin production will be unaltered by a competing strain. Single G protein-coupled receptor protein knockouts did not change aflatoxin inhibition, but further studies are needed during the 12-18 hour critical window to understand what signaling leads to successful intraspecific aflatoxin inhibition.

70. *Aspergillus oryzae* requires unfolded protein response for growth under condition inducing amylases production.

Mizuki Tanaka [a]
[a]Laboratory of Bioindustrial Genomics

Unfolded protein response (UPR) is an intracellular signaling pathway for adaptation to endoplasmic reticulum (ER) stress. In yeast UPR, Ire1 cleaves the unconventional intron of HAC1 pre-mRNA, and the functional Hac1 protein translated from the spliced HAC1 mRNA induces the expression of ER chaperone genes and ER-associated degradation genes for the refolding or degradation of unfolded proteins. To examine the role of UPR under conditions inducing secretory protein production in Aspergillus oryzae, we attempted to construct a disruption mutant of IRE1 ortholog (ireA). However, no homokaryotic ireA disruption mutant could be obtained. Hence, we constructed an ireA conditionally expressing strain, and examined the contribution of UPR to ER stress adaptation under physiological conditions. Repression of ireA completely blocked A. oryzae growth under conditions inducing the production of amylases. This growth defect was restored by the introduction of unconventional intronless hacA. Furthermore, UPR was observed to be induced by amylolytic gene expression, and the disruption of the transcriptional activator (AmyR) for amylolytic genes resulted in partial growth restoration of the ireA-repressing strain. In addition, a homokaryotic ireA disruption mutant was successfully generated using the strain harboring unconventional intronless hacA as a parental host. These results indicated that UPR is required for A. oryzae growth to alleviate ER stress induced by excessive production of hydrolytic enzymes.

71. Crosstalk of LaeA and CreA in controlling virulence and secondary metabolism in *Penicillium expansum*

J. Tannous[a], X. Luo[b], D. Kumar[c], S. Barad[c,d], Y. Chen[e], A. Dubey[c], N.G. Matana[c,d], S. Tian [e], B. Li [e], D. Prusky[c] and N. Keller[a,b,f]
[a]Department of Medical Microbiology and Immunology, University of Wisconsin – Madison, Madison, WI 53706-1598, USA [b]Department of Plant Pathology, University of Wisconsin - Madison, Madison, WI 53706-1598, USA [c]Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel [d]Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel [e]Key Laboratory of Plant Resources, Institute of Botany, Chinese Academy of Sciences, Beijing, 100093, China [f]Department of Bacteriology, University of Wisconsin - Madison, Madison, WI 53706-1598, USA

The plant pathogenic fungus *Penicillium expansum* is a major concern of the global food industry due to its wide occurrence and ability to produce various mycotoxins, of which the most significant is patulin. Although the patulin gene cluster has been recently identified and characterized, little is known on how it is regulated. Here we demonstrate that LaeA, a global regulator of mycotoxins in other fungi, is also required for the production of not only patulin but also other putative toxic secondary metabolites (SMs) produced by *P. expansum* as well as virulence on apple. We find that an increase of the sucrose molarity in the culture medium from 15 to 175 mM positively impacts *creA* expression, but conversely, decreases the expression of *laeA* and the accumulation of patulin by 5 and 175 fold, respectively. The increase in sucrose also negatively influences the expression of most of the SM synthase genes in *P. expansum* Pe-D1 strain. Independently, LaeA was deemed as a positive regulator of at least 6 SM gene clusters. Here we address the hypothesis that CreA regulation of *laeA* mediates many of the ΔlaeA phenotypes including regulation of SM clusters and virulence on apple.

72*. Genus level analysis of secondary metabolism reveals the origin of hybrid NRPS-PKS gene clusters

S. Theobald[a], T.C. Vesth[a], J.L. Nybo[a], I. Kjærbølling[a], J.C. Frisvad[a], K.F. Nielsen[a], T.O. Larsen[a], I.V. Grigoriev[c], A. Salamov[c], U.H. Mortensen[a], S.E. Baker[b] and M.R. Andersen[a]
[a]Department of Biotechnology and Biomedicine, Technical University of Denmark, Lingby, DK [b]Joint Bioenergy Institute, Berkeley, CA, USA [c]Joint Genome Institute, Walnut Creek, CA, USA

The World Health Organization is reporting a rising number of multiple drug resistant pathogens every year, increasing the need for new drug development. However, current methods for natural product discovery rely on time consuming experimental work, making them unable to keep up with this demand.

In the Aspergillus genus sequencing project, we are sequencing and analyzing over 300 species of Aspergilli, a group of filamentous fungi rich in natural compounds. Natural products are encoded by genes located in close proximity, called secondary metabolic gene clusters (SMGC), which makes them interesting targets for genomic analysis. Important classes of SMGC include non ribosomal peptide synthetases (NRPS), polyketide synthases (PKS) and hybrids thereof.

We use a modified version of the Secondary Metabolite Unique Regions Finder (SMURF) algorithm, combined with InterPro annotations, network analysis and approximate maximum likelihood (ML) trees of conserved domains, giving insights into the secondary metabolism gene diversity and evolution. In this study we describe the diversity of hybrid SMGC among Aspergilli, horizontal gene transfers within and outside Aspergilli and identify possible ancestors of hybrids.

ML analysis indicated PKS-NRPS hybrids being of bacterial origin and having an ancestor cluster in the Streptomyces and Achromobacter genus. NRPS-PKS hybrids showed a wide diversity with orthologs being found in different Ascomycete classes like Dothideomycetes, Eurotiomycetes, Sordariomycetes and others. Here, our genus level study enables us to determine which hybrids might have been horizontally transferred to the precursor of a whole section, driving speciation of this section by a selective advantage. Examining these events helps us to identify the origin of secondary metabolites in Aspergilli and also provides us with new variants of compounds for industrial applications.

73*. An intrinsically disordered domain of the VelB velvet domain provides specificity for heterodimer formation in *Aspergillus nidulans*

S. Thieme[a], J. Gerke[a], C. Sasse[a], O. Valerius[a], K. G. Thieme[a] and G. H. Braus[a]
[a]Molekulare Mikrobiologie und Genetik, Georg-August-Universität Göttingen, D-37077 Göttingen, Germany

Genetic networks are often controlled by DNA binding proteins, which act as homo- or heterodimers. The velvet domain represents a conserved fungal DNA binding and dimerization domain of approximately 150 amino acids, which is reminiscent to the *rel* domain of NF-kB in the mammalian immune and infection response [1]. Aspergilli possess four members of velvet family proteins (VeA, VelB, VelC and VosA), which can form homo- or heterodimers and are required to control development coordinated with the appropriate interconnected secondary metabolism and spore viability [2]. VeA interacts with several epigenetic methyltransferases and shuttles VelB as a VeA-VelB heterodimer into the nucleus [3, 4]. VelB is also part of the VelB-VosA complex supporting reduced and delayed asexual spore formation and promoting spore viability. Only the velvet domain of VelB is interrupted by an insertion of additional amino acids in ascomycetes. The *A. nidulans* insert consists of 99 amino acids and is conserved among Aspergilli. We compared a VelB with or without insert and found that the insert is required for an accurate light control which normally promotes asexual development and reduces sexual development as well as secondary metabolite production [5]. This is reflected on the molecular level by the finding that the VelB interaction partners with insert differ to the VelB interaction partners without insert. This suggests that the insertion changes the potential of velvet domains and provides a surface for altered protein-protein interactions.

[1] Ahmed *et al*. (2013) Fungal *velvet* regulators contain a DNA binding domain reminiscent of NF-kB. **PLoS Biol.** 11, e1001750.
[2] Bayram and Braus (2012) Coordination of secondary metabolism and development in fungi: the velvet

family of regulatory proteins. **FEMS Microbiol. Rev**. 36, 1-24.
[3] Sarikaya-Bayram *et al.* (2015) One Juliet and four Romeos: VeA and its methyltransferases. **Front. Microbiol.** 6, 1. 1-7.
[4] Bayram *et al.* (2008) VelB/VeA/LaeA complex coordinates light signal with fungal development and secondary metabolism. **Science**. 320, 1504-1506.
[5] Bayram *et al.* (2016) Changes of global expression and secondary metabolite accumulation during light-dependent *Aspergillus nidulans* development. **Fung. Genet. Biol.** 87, 30-53.

74. Genetic diversity of 100+ Aspergillus species - the aspMine analysis resource

T. C. Vesth[a], J. L. Nybo[a], S. THEOBALD[a], R. P. DE VRIES [d], I. V. GRIGORIEV[c], S. E. BAKER[b] and M. R. ANDERSEN[a]
[a]Department of Bioingineering, Technical University of Denmark, Lyngby, Denmark [b]Joint Bioenergy Institute, Berkeley, CA, USA, Berkeley, CA, USA [c]Joint Genome Institute, Walnut Creek, CA, USA, Walnut Creek, CA, USA [d]Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands, Utrecht, The Netherlands

The filamentous fungal species of the Aspergillus genus are of broad interest to the scientific community including applied, medical and basic research. These fungi are prolific producers of native and heterologous proteins, organic acids, and secondary metabolites (including bioactives and toxins such as ochratoxin A). Because of these abilities, they represent a substantial economic interests in pharmaceutical, biotechnology, and bioenergy applications. In a project collaboration with the US Joint Genome Institute and JBEI we are de novo sequencing 300 different species of Aspergillus and establishing an online analysis platform for the scientific community, aspMine. The goal of this project is to develop a targeted tool to expand and improve our knowledge and expertise about this versatile group of fungi. At time of writing, 200 genomes are in various stages of sequencing and a bioinformatic pipeline has been established to analyze and store the data. This project covers a wide range of biologically interesting ideas surrounding the concept of speciation, such as genetic diversity, primary and secondary metabolism and proteome diversity. Complementary to the tools offered by FungiDB and JGI, the aspMine analysis resource offers tools for tracking genes and functions across species, allowing for investigation of shared genes and clusters across the genus as well as species- and clade-specific genes. The online platform also offers comparative analysis of secondary metabolism gene clusters with focus on synteny and functional conservation across species. The aspMine is implemented as a number of web applications created in R shiny, a graphical interface for analysis. The different tools are collected on a webpage which also includes method descriptions and relevant literature. The webpage is available from the beginning of 2016 and will be continually expanded. It is our goal to provide a comprehensive analysis platform for the community for comparative analysis of Aspergillus species.

75. Resistance Gene-Guided Genome Mining: Serial Promoter Exchanges in *Aspergillus nidulans* Reveal the Biosynthetic Pathway for Fellutamide B, a Proteasome Inhibitor

Hsu-Hua Yeh[a], Manmeet Ahuja[b], Yi-Ming Chiang[b,c], C. Elizabeth Oakley[b], Shauna Moore[b], Olivia Yoon[b], Heather Hajovsky[b], Jin-Woo Bok[d], Nancy P. Keller[d], **Clay C. C. Wang**[a,e] and Berl R. Oakley[b]
[a]Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, California 90089, United States [b]Department of Molecular Biosciences, University of Kansas, Lawrence, Kansas 66045, United States [c]Department of Pharmacy, Chia Nan University of Pharmacy and Science, Tainan City 71710, Taiwan [d]Department of Bacteriology and Department of Medical Microbiology and Immunology, University of Wisconsin, Madison, Wisconsin 53706, United States [e]Department of Chemistry, University of Southern California, Dornsife College of Letters, Arts, and Sciences, Los Angeles, California 90089, United States

Fungal genome projects are revealing thousands of cryptic secondary metabolism (SM) biosynthetic gene clusters that encode pathways that potentially produce valuable compounds. Heterologous expression systems should allow these clusters to be expressed and their products obtained, but approaches are needed to identify the most valuable target clusters. The *inp* cluster of *Aspergillus nidulans* contains a gene, *inpE*, that encodes a proteasome subunit, leading us to hypothesize that the inp cluster produces a proteasome inhibitor and *inpE* confers resistance to this compound. Previous efforts to express this

cluster have failed, but by sequentially replacing the promoters of the genes of the cluster with a regulatable promotor, we have expressed them successfully. Expression reveals that the product of the *inp* cluster is the proteasome inhibitor fellutamide B, and our data allow us to propose a biosynthetic pathway for the compound. By deleting *inpE* and activating expression of the *inp* cluster, we demonstrate that *inpE* is required for resistance to internally produced fellutamide B. These data provide experimental validation for the hypothesis that some fungal SM clusters contain genes that encode resistant forms of the enzymes targeted by the compound produced by the cluster.

76*. Are dormant spores really dormant? A perspective from the transcription level.
Fang Wang[a], Liguo Dong[a] and Chris Koon Ho Wong[a]
[a]Faculty of Health Sciences, University of Macau, Macau SAR, China

Fungal spores are specialized reproductive cells of filamentous fungi with properties for dispersal as well as for survival under harsh conditions and over a long period of time. It has been shown in *Aspergillus niger* that the resting spores have little metabolic activities and consume minimal energy. Previous studies have detected mRNA transcripts in dormant spores of some filamentous fungi, but it is not clear whether the transcripts were carried over from the process of conidiation or were actively synthesized in the resting spores. Moreover, a previous study in *A. niger* has been shown that genes involved in protein biosynthesis, RNA degradation, respiration and nitrogen metabolism are induced within an hour upon shifting to favorable growth conditions, suggesting that spores are constantly sensing and readily responding to the environment for growth. In this work, we aim to find out 1) whether resting spores of *Aspergillus nidulans* have any active transcription, 2) whether dormant spores can respond to stresses during dormancy and 3) whether resting spores of different ages and spores kept under different stresses behave differently during the break of dormancy. To address these, we have used Chromatin Immuno-precipitation followed by Next Generation Sequencing (ChIPseq) against RNA polymerase II (Pol II) to map active transcription events in spores kept under different conditions (e.g. fresh spores at 37˚C, spores aged at RT or 4˚C with or without oxygen) and during the germination process. Our preliminary results show that Pol II is engaged on many genes genome-wide in resting spores. Gene Ontology analysis shows that these genes are enriched with stress response, ATP generation and the conidiation process. Unexpectedly, we found a significant level of Pol II accumulation near the 5' end of many genes in resting spores but absent in germinated spores and mycelia. The accumulation pattern is similar to the promoter-proximal pausing phenomenon in higher eukaryotes. We are in the process of confirming and understanding the functional significance of this phenomenon. We speculate that Pol II may be preloaded to specific promoters during the spore maturation process such to reduce the steps and energy needed by activation of genes for growth and survival in energetically dormant spores. Therefore, this Pol II "pausing" phenomenon might have important implications in the physiology and durability of spores.

77*. Functional reconstitution of the Trypacidin Gene Cluster in *Aspergillus fumigatus* by Advanced Gene Editing
J. Weber[a,b], V. Valiante[c], C.S. Nødvig[d], D.J. Mattern[a,b], R.A. Slotkowski[a,b], U.H. Mortensen[d] and A.A. Brakhage[a,b]
[a]Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI) –, Jena, Germany [b]Institute of Microbiology, Friedrich Schiller University Jena, Germany [c]Leibniz Research Group – Biobricks of Microbial Natural Product Syntheses, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany [d]Eukaryotic Molecular Cell Biology, Section for Eukaryotic Biotechnology, Department of Systems Biology, Technical University of Denmark, Søltofts Plads, Kongens Lyngby, Denmark

The human pathogenic fungus *Aspergillus fumigatus* is known to produce various spore-borne natural products. One of these is the polyketide trypacidin which has been shown to be involved in the interactions with alveolar macrophages as well as with the amoeba *Dictyostelium discoideum*. Even though recent studies could elucidate the corresponding gene cluster in *A. fumigatus*, it still remained elusive why several isolates do not produce trypacidin. We addressed this question employing a CRISPR/Cas9-based gene editing strategy. Thus, we could link a single nucleotide insertion in the polyketide synthase of the trypacidin biosynthetic pathway and also reconstitute its production in a nonproducing strain. In addition, we developed a split-marker approach for the selection of edited strains,

since the selectable marker could not be directly linked to the target site. The here established tool could be useful in next generation fungal genetics *e.g.* for the investigation of single nucleotide polymorphism, or amino acid substitutions.

78. A global co-expression network approach for connecting genes to specialized metabolic pathways

Jennifer Wisecaver[a], Abigail Lind[a] and Antonis Rokas[a]
[a]Vanderbilt University, Nashville, TN

Both fungi and plants produce a tremendous diversity of specialized metabolites (SMs) to interact with and manage their environment. A major challenge hindering efforts to tap this seemingly boundless source of pharmacopeia is the identification of SM pathways and their constituent genes. To address this challenge, much attention has been paid to fungal SM gene clusters, but the number of SM pathways in fungi that are non-clustered or only partially clustered is completely unknown. The genome of *Aspergillus fumigatus,* an opportunistic human pathogen and one of the leading causes of fungal-related human deaths, provides a stark example; 21 of its 37 SM gene clusters are missing either transporters, transcription factors or both, suggesting these genes may be located elsewhere in the genome. Given the well-established observation that the genes comprising a SM pathway are co-regulated in response to specific environmental conditions, we hypothesized that genes from a given SM pathway would form tight associations (modules) with each other in gene co-expression networks, facilitating their identification even without knowledge of the genome sequence. To evaluate this hypothesis, we first used 10 global co-expression datasets—each a meta-analysis of hundreds to thousands of expression experiments—across eight plant model organisms to identify hundreds of modules of co-expressed genes for each species. In support of our hypothesis, 15.3-52.6% of modules contained two or more known SM biosynthetic genes (e.g., cytochrome P450s, terpene synthases, and polyketide synthases), and module genes were enriched in SM functions. Moreover, modules recovered many experimentally validated SM pathways in these species, including all those known to form SM gene clusters. For each clustered SM pathway, the module analysis expanded the known genetic repertoire of the pathway by identifying additional unclustered enzymatic genes as well as unclustered genes involved in pathway regulation and product transport. We are currently expanding our network analysis to fungal taxa including *Aspergillus* and submit that global gene co-expression is a rich, but largely untapped, data source for discovering the genetic basis and architecture of natural products.

79. Global transcriptional regulation and chromatin dynamics of primary metabolism in *Aspergillus nidulans.*

Koon Ho Wong[a], Ang Li[a], Yingying Chen[a], Liguo Dong[a], Md Ashiqul Alam[c], Fang Wang[a], Kaeling Tan[a], Michael J. Hynes[d], Joan M. Kelly[c] and Richard B. Todd[b]
[a]Faculty of Health Sciences, University of Macau, Macau [b]Department of Plant Pathology, Kansas State University, KS [c]Department of Genetics and Evolution, School of Biological Science, The University of Adelaide, Australia [d]School of BioSciences, University of Melbourne, Australia

Fungi adapt their metabolism to nutrient availability primarily via transcriptional control of nutrient uptake and metabolic genes. In *Aspergillus nidulans,* genes involved in carbon and nitrogen metabolism are tightly controlled by carbon catabolite repression (CCR) and nitrogen metabolite repression (NMR), respectively. Two key global regulators are involved in CCR and NMR; the repressor CreA controls expression of carbon metabolic genes, while the activator AreA modulates nitrogen metabolic gene expression. An extensive set of genes is expected to be under the control of the two regulators, which often act in concert with different pathway-specific transcription factors to turn on different subsets of metabolic genes according to nutritional conditions. In an attempt to identify the genome-wide targets of the global and pathway-specific regulators and to learn how they collaborate with each other to bring about the regulation, we have mapped whole-genome binding sites of the two global regulators and some pathway specific factors under different nutrient conditions and integrated the information with genome-wide transcription profiles of wildtype, *creA*Δ and *areA*Δ strains. In addition, we have also mapped global chromatin dynamics and chromatin modifications under different nutrient conditions in order to understand the mechanism of regulation at the chromatin level. A summary of CreA and AreA global regulation and chromatin dynamics will be presented.

80*. WetA is a DNA-binding protein governing cellular and chemical development in Aspergillus
Ming-Yueh Wu*[a], Julia Martien[b], Matthew Mead[c], Daniel Amador-Noguez[b], Antonis Rokas[c], and Jae-Hyuk Yu[a,b]
[a]Program of Genetics, University of Wisconsin, Madison, Wisconsin, USA [b]Department of Bacteriology, University of Wisconsin, Madison, Wisconsin, USA [c]Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee, USA

The main reproductive mode of aspergilli is the formation of conidia. BrlA, AbaA, and WetA are the central regulators of conidiation in aspergilli. BrlA and AbaA are transcription factors, yet the WetA-mediated regulatory mechanism is still unknown. Here, we characterized WetA's functions and further investigated the underlying molecular regulatory mechanism along with genetics, transcriptomics, and metabolomics approaches. The deletion of wetA causes conidia autolysis, disrupted conidia wall integrity, and reduced stress tolerance. Moreover, in A. flavus, WetA is involved in light-stimulated hyphae development, vegetative growth, and aflatoxin production. Northern analysis shows that WetA is a negative regulator of its upstream regulators and exerts feed-back control for development in both species. We further carried out RNA-seq of WT and wetA-null mutant conidia from A. nidulans, A. flavus, and A. fumigatus. The genome-wide expression studies showed that WetA has a broad effect on development and metabolism in conidia. For in vivo WetA-DNA interaction studies, ChIP-seq was performed on A. nidulans WT conidia. The result showed that WetA directly interacts with several transcription factors in conidia, suggesting that WetA may be a transcription factor. Overall, WetA is an evolutionarily conserved central developmental regulator in Ascomycetes. This is the first study demonstrating that WetA is a DNA-binding protein, which plays multiple regulatory roles of development and metabolism in Aspergillus.

81. Palmitoylated protein ArkA and its putative targeted proteins in *Aspergillus*
Yuanwei Zhang[a,b], Congcong Sun[a] and Ling Lu[a]
[a]Nanjing Normal University, Nanjing, China [b]University of Dundee, Dundee, GB

Finely tuned changes in cytosolic free calcium ($[Ca^{2+}]_c$) mediate numerous intracellular functions resulting in the activation or inactivation of a series of target proteins. Palmitoylation involves the reversible posttranslational modification that addition of palmitate to cysteines and promotes protein binding and subcellular localization, yet the relationship between palmitoylation and calcium signaling is poorly understood. Here, we present proof that the yeast palmitoyl transferase ScAkr1p homolog, AkrA in *Aspergillus nidulans*, regulates $[Ca^{2+}]_c$ homeostasis and plays an important role in hyphal growth and conidiation under low calcium conditions. We utilized calcium reporter aequorin expression system and showed that the $[Ca^{2+}]_c$ responses in *akrA* mutants to high extracellular calcium or ER/plasma membrane stress were defective. Furthermore, we demonstrated that all of these effects on the $[Ca^{2+}]_c$ responses mediated by AkrA were tightly related to the cysteine of the DHHC motif which is required for AkrA auto-palmitoylation. When auto-palmitoylation of AkrA was inhibited, by either mutation of cysteine or truncated the DHHC motif, the susceptibility of the mutants to azole drugs was increased. By acyl-biotin exchange assay, we identified potential substrates palmitoylated by AkrA including calcium-related proteins and ergosterol biosynthetic proteins which involved in azole drug resistance in *A. nidulans*. In addition, deletion of AkrA homolog in *A. fumigatus* displays a similar phenotype of the colony growth defect to that in *A. nidulans*, indicating function of AkrA in *Aspergillus* or in fungi might be conserved.
Thus, this study provides new insights into the relationship among palmitoylation, calcium homeostasis and azole resistance, which has implications for the development of novel antifungal drugs and therapeutic methods. Further details about the cellular function for putative proteins targeted by AkrA in *A. fumigatus* are ongoing.

82. Discovery of a novel azole-resistance mutation in *Aspergillus fumigatus* and the possible role of sexual reproduction in its evolution

J. Zhang[a], Bas Zwaan[a], E Snelders[a], S Schoustra[a], K Dijk[b], J Meis[c,d], F Hagen[c,d], E Kuijper[e], G Kampinga[f], J Zoll[d,g], W Melchers[d,g], P Verweij[d,g] and A Debets[a]
[a]Laboratory of Genetics, Wageningen University, Wageningen, NL [b]Department of Medical Microbiology, Vu University Medical Centre, Amsterdam, The Netherlands [c]Department of Medical Microbiology and Infectious Diseases, CWZ Hospital, Nijmegen, The Netherlands [d]Expert Centre in Mycology Radboudumc/CWZ (EMRC) [e]Department of Medical Microbiology, Leiden University Medical Centre, Leiden, The Netherlands [f]Department of Medical Microbiology, University Medical Centre Groningen, Groningen, The Netherlands [g]Department of Medical Microbiology, Radboud University Medical Centre, Nijmegen, The Netherlands

We investigated two compost-heaps, one containing azoles and one without azoles, for the presence of azole-resistant and azole-sensitive *A. fumigatus* isolates. The azole-free compost yielded 98% (49/50) sensitive and 2% (1/50) azole resistant isolates, whereas the azole-containing compost yielded 8% (4/45) sensitive and 92% (41/45) resistant isolates. From the latter, 84% (37/45) were of the highly resistant TR_{46} Y121F/ T289A genotype and 8% (4/45) had a novel pan–triazole-resistance harbouring a triple tandem repeat: TR_{46}^{3}/Y121F/ M172I/T289A/G448S. The stark contrast between the two composts indicates that azole–containing compost is a hot spot for the development and maintenance of azole resistance. Subsequent screening of the clinical *A. fumigatus* collection from the Dutch national surveillance programme indicated that this resistance mechanism was already present in 2012, and is now found in all participating medical centres. We were able to recover this novel TR_{46}^{3} mutation among the sexual progeny in the lab, from a cross between two TR_{46} isolates of opposite mating type that were from the same compost. This strongly indicates a role of sex in the emergence of this novel azole–resistance mechanism in *A. fumigatus*. Furthermore, we provide further evidence indicating the potential occurrence of sexual reproduction in compost, but this still needs further confirmation. Our findings alarm the fast development of the azole resistance issue in *A. fumigatus* and further indicate the role of sexual reproduction of *A. fumigatus* in the development of azole resistance.
Key words: *Aspergillus fumigatus*; novel new mutation; compost; azole resistance; hot spot; sexual reproduction; ascospores; conidiospores

83*. Genetic parts screening and artificial N-glycosylation motif engineering for heterologous protein production in *Aspergillus niger*

Jinxiang Zhang[a,c], Saori Amaike-Campen[a,b], Sam Deutsch[d], Ljiljana Pasa-Tolic[e], Erika Zink[e], Jon Jacobs[e], Blake Simmons[a,f], John Gladden[a,c] and Jon Magnuson[a,b]
[a]Joint BioEnergy Institute, Emeryville, CA [b]Pacific Northwest National Laboratory, Richland, WA [c]Sandia National Laboratories, Livermore, CA [d]Joint Genome Institute, Walnut Creek, CA [e]Environmental Molecular Sciences Laboratory, PNNL, Richland, WA [f]Lawrence Berkeley National Laboratory, Berkeley, CA

Aspergillus niger is a genetically tractable model organism for scientific discovery and a platform organism used in industry for the production of enzymes. Expression of secreted native enzymes at tens of grams per liter have been discussed by those in industry, but high level production of heterologous enzymes remains elusive. Strategies to increase production include the use of strong promoters, protease-deficient strains, fusion proteins, multiple gene copies, etc. However, yields of heterologous proteins are still lower than desired.

We generated proteomics data from secretome samples of A. niger grown on a variety of minimal and rich media, with the goal of identifying useful genetic elements for increasing heterologous protein production. Twenty promoters, six signal sequences and four introns from the most highly secreted proteins were identified as candidate genetic elements to enhance heterologous gene expression. These candidate elements were tested for their ability to drive expression of a prokaryotic glycoside hydrolase. A vector was designed to target integration of the modified expression cassette to the native glucoamylase (glaA) gene locus by homologous recombination. Considerable diversity was seen in heterologous protein production driven by these various elements. Interestingly, a signal peptide from

GPI-anchored cell wall protein showed promising results.

In addition, based on the 3D structure of heterologous protein, we generated seven individual artificial N-glycosylation motifs on the surface of the heterologous protein. Analysis of these sites for glycosylation via top-down proteomics is just beginning. Correlation of glycosylation with any changes in kinetic and thermodynamic properties of the altered enzymes is the goal of this aspect of the research.

List of Registrants

Last Name	First Name	Institute	Email
Agarwal-Jans	Sheba	Elsevier BV	s.agarwal-jans@elsevier.com
Aguilar Pontes	Maria Victoria	CBS-KNAW Fungal Biodiversity Ctr	v.aguilar@cbs.knaw.nl
Al Abdallah	Qusai	UTHSC-Memphis	galabdal@uthsc.edu
Alazi	Ebru	Leiden University	ebrualazi@gmail.com
Andersen	Mikael	Technical University of Denmark	mr@bio.dtu.dk
Anglin	Sarah	Millsaps College	mcguisl@millsaps.edu
Bakhuis	Janny	DSM Biotechnology Center	Janny.Bakhuis@DSM.COM
Basenko	Evelina	University of Liverpool	evelina.basenko@liverpool.ac.uk
Bennett	Joan	Rutgers Univ	profmycogirl@yahoo.com
Benoit	Isabelle	Concordia University	isabelle.benoit@concordia.ca
Braus	Gerhard	Georg-August Univ	gbraus@gwdg.de
Brock	Matthias	University of Nottingham	Matthias.brock@nottingham.ac.uk
Bruno	Kenneth	Zymergen	bruno@zymergen.com
Cánovas López	David	University of Sevilla	davidc@us.es
Cary	Jeff	USDA-ARS-SRRC	Jeff.cary@ars.usda.gov
CHEN	YINGYING	University of Macau	yychen@umac.mo
Cohrs	Kim	WWU Münster	kim.cohrs@uni-muenster.de
Cramer	Robert	Geisel School of Medicine at Dartmouth	robert.a.cramer.jr@dartmouth.edu
de Reus	Elise	Technical University Denmark	elisedr@dtu.dk
de Vries	Ronald	CBS-KNAW Fungal Biodiversity Centre	r.devries@cbs.knaw.nl
Dorter	Ilkay	UGA	s2iydoer@uga.edu
Doty	Tammy	Novozymes, Inc.	tmmd@novozymes.com
Dzikowska	Agnieszka	University of Warsaw	adzik@igib.uw.edu.pl
Fekete	Erzsébet	University of Debrecen	kicsizsoka@yahoo.com
Fischer	Amanda	Novozymes Inc	jwat@novozymes.com
Fortwendel	Jarrod	UTHSC-Memphis	jfortwen@uthsc.edu
Frandsen	Rasmus	Technical University of Denmark	rasf@bio.dtu.dk
Frisvad	Jens	Technical University of denmark	jcf@bio.dtu.dk
Geib	Elena	University of Nottingham	elena.geib@nottingham.ac.uk
Gell	Richard	North Carolina State University	rmgell@ncsu.edu
Geranios	Pavlos	University of Manchester	pavlos.geranios@manchester.ac.uk

Gerke	Jennifer	Georg-August-University	jgerke@gwdg.de
Gielesen	Bianca	DSM Food Specialties	bianca.gielesen@dsm.com
Gomi	Katsuya	Tohoku Univ	gomi@biochem.tohoku.ac.jp
Grijseels	Sietske	Technical University of Denmark	sigri@bio.dtu.dk
Gsaller	Fabio	University of Manchester	fabio.gsaller@manchester.ac.uk
Gundersen	Amy	Novozymes, Inc.	aygm@novozymes.com
Guo	Shuhui	University of Macau	gshcsu@gmail.com
Haarmann	Thomas	AB Enzymes GmbH	thomas.haarmann@abenzymes.com
Harting	Rebekka	Georg August University	rhartin@gwdg.de
Hill	Terry	Rhodes College	hill@rhodes.edu
Hoof	Jakob	DTU Bioengineering	jbn@bio.dtu.dk
Hunter	Cameron	Kansas State University	cameron.hunter15@gmail.com
Ichinose	Sakurako	Tohoku university	cherry-co@live.jp
Inoue	Taishi	Tohoku University	t.inoue.a812@gmail.com
Jackson-Hayes	Loretta	Rhodes Col	jacksonhayesL@rhodes.edu
John	Tricia	Concordia University	tricia.john@concordia.ca
Jung	Sascha	TU Berlin	s.jung@tu-berlin.de
Kang	Earl	University of Georgia	sekang2@uga.edu
Karaffa	Levente	University of Debrecen	karaffa.levente@science.unideb.hu
Keller	Nancy	Univ Wisconsin, Madison	npkeller@wisc.edu
Khosravi	Claire	CBS KNAW Fungal Biodiversity Centre	c.khosravi@cbs.knaw.nl
Kim	Joonhoon	Joint BioEnergy Institute	joonhoonkim@lbl.gov
Kjaerboelling	Inge	Technical University of Denmark	ingek@bio.dtu.dk
Koehler	Anna Maria	Georg August University	akoehle3@gwdg.de
Kowalczyk	Joanna	CBS-KNAW	j.kowalczyk@cbs.knaw.nl
Kuo	Alan	DOE Joint Genome Institute	akuo@lbl.gov
Li	Ang	University of Macau	beautylee14@gmail.com
Lind	Abigail	Vanderbilt University	abigail.l.lind@Vanderbilt.edu
Love	Dona	NIDDK, National Institutes of Health	donalove@mail.nih.gov
Lu	Ling	Nanjing Normal Univeristy	linglu6465@hotmail.com
Machida	Masayuki	AIST	m.machida@aist.go.jp
Majumdar	Raj	USDA-ARS-SRRC	raj.majumdar@ars.usda.gov
Malavazi	Iran	Universid. Federal de São Carlos	imalavazi@ufscar.br
Marschall	Robert	Institute of Plant Biology	robert.marschall@uni-muenster.de

McFarland	Sarah	Novozymes Inc	samf@novozymes.com
Mela	Alexander	University of Georgia	apm8190@gmail.com
Menke	Jon	Cargill	Jon_Menke@cargill.com
Meyer	Vera	TU Berlin	vera.meyer@tu-berlin.de
MIYAZAWA	Ken	Tohoku University	ken.m.tubbt@gmail.com
Molo	Megan	North Carolina State University	mlsexto3@ncsu.edu
Momany	Michelle	Univ Georgia	mmomany@uga.edu
Mortensen	Uffe	Technical University of Denmark	um@bio.dtu.dk
Muraguchi	Hajime	Akita Prefectural Univ	muraguchi@akita-pu.ac.jp
Muzzi-Erichsen	Gloria	Novozymes Inc	GMEr@novozymes.com
Müller	Ulrike	EW Nutrition GmbH	ulrike.mueller@ew-nutrition.com
Nielsen	Michael	Novozymes	mlxn@novozymes.com
NIU	Mengyao	University of Wisconsin-Madison	mniu5@wisc.edu
Nybo	Jane	Technical University of Denmark	jlnr@bio.dtu.dk
Oakley	Berl	Univ Kansas	boakley@ku.edu
Oda	Ken	Nat. Res. Inst. of Brewing	k.oda@nrib.go.jp
Osmani	Stephen	Ohio State Univ	osmani.2@osu.edu
Ouedraogo	Jean-Paul	Centre for Structural and Functional Genomics	jean-paul.ouedraogo@concordia.ca
Pfannenstiel	Brandon	University of Wisconsin-Madison	btpfannenstiel@gmail.com
Pham	Thi Thanh My	Concordia University	my.pham@concordia.ca
RAI	Maruti Nandan	University of Macau	nandanrai@gmail.com
Ram	Arthur	Leiden Univ	a.f.j.ram@biology.leidenuniv.nl
Rassinger	Alice	Vienna University of Technology	alice.rassinger@tuwien.ac.at
Riley	Robert	Lawrence Berkeley National Lab	rwriley@lbl.gov
Roos	David	University of Pennsylvania	droos@sas.upenn.edu
Ryota	Saito	NRIB	mamoteny0621@yahoo.co.jp
Samson	Robert	Royal Academy of the Netherlands	r.samson@cbs.knaw.nl
Sato	Atsushi	Kikkoman Corporation	a.sato@mail.kikkoman.co.jp
Schmoll	Monika	Austrian Institute of Technology AIT	monika.schmoll@ait.ac.at
Schumacher	Julia	WWU Muenster	julia.schumacher@wwu.de
Shanmugasundram	Achchuthan	University of Liverpool	achchu@liverpool.ac.uk
Shukla	Nandini	The Ohio State University	shukla.25@buckeyemail.osu.edu

Skaar	Ida	Norwegian Veterinary Institute	ida.skaar@vetinst.no
Snelders	Eveline	Wageningen University	eveline.snelders@wur.nl
Sweany	Rebecca	Louisiana State University	rsweany@agcenter.lsu.edu
Szewczyk	Edyta	Zymergen, Inc.	eszewczyk@zymergen.com
Tanaka	Mizuki	Tohoku University	mizuki.tanaka.a7@tohoku.ac.jp
Tannous	Joanna	University of Wisconsin Madison	jtannous@wisc.edu
Theobald	Sebastian	Technical University of Denmark	setd@bio.dtu.dk
Thieme	Sabine	Georg August University	sreen@gwdg.de
Todd	Richard	Kansas State University	rbtodd@ksu.edu
van Peij	Noel	DSM Biotechnology Center	noel.peij-van@dsm.com
Vesth	Tammi	Technical University of Denmark	tcve@bio.dtu.dk
Wang	Fang	university of macau	yb57660@umac.mo
Weber	Jakob	Hans Knoell Institute, Jena	jakob.weber@leibniz-hki.de
Westfall	Patrick	Zymergen, Inc	pjwestfall@zymergen.com
Wisecaver	Jennifer	Vanderbilt University	jen.wisecaver@vanderbilt.edu
Wong	Koon Ho	University of Macau	koonhowong@umac.mo
Wu	Ming-Yueh	The University of Wisconsin-Madison	mwu33@wisc.edu
Zhang	Jianhua	Wageningen University	jianhua.zhang@wur.nl
Zhang	Jinxiang	Joint BioEnergy Institute	jinxiang.zhang@gmail.com
Zheng	Yun	Concordia University	zhyun627@gmail.com