The 15th International *Aspergillus* Meeting

Asperfest 15

February 25, 2018

Biology Auditorium, Main Campus, Technion,

Haifa, Israel.

Artwork Credit: Edyta Szewcyzk

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).

2017 AGRPC

Gerhard Braus, 2017-2019, Georg-August-University Goettingen, Germany; gbraus@gwdg.de
Elaine Bignell, 2017-2019, University of Manchester, UK; elaine.bignell@manchester.ac.uk
Vera Meyer, 2017-2019, Berlin University of Technology, Germany; vera.meyer@tu-berlin.de
David Cánovas, 2017-2019, University of Seville, Spain; davidc@us.es
Chris, Koon Ho Wong, 2017-2019, University of Macau, Macau SAR, China; koonhowong@umac.mo
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
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
Kevin McCluskey (Ex officio), Curator, Fungal Genetics Stock Center; mccluskeyk@ksu.edu

Asperfest15 Local Organizer: Nir Osherov, Tel Aviv University, Israel; nosherov@post.tau.ac.il

THANKS TO OUR MEETING SPONSORS!

novozymes® Rethink Tomorrow

FUNGAL GENETICS STOCK CENTER

zymergen®

Time	SA2 –PROGRAM - February 25th -Sunday	
	Faculty of Biology - Auditorium (Ground Floor)	
	The Fifteenth International Aspergillus Meeting Asperfest 15	**Local organizer: Nir Osherov**
08:30-09:00	**Registration and poster hang up**	
09:00-09:15	Welcome, introductions and announcements	Michelle Momany, University of Georgia, USA.
09:15-10:15	Session I	David Canovas, University of Seville, Spain.
	Mining of secondary metabolites from *Aspergillus.* Clay Wang, USC.	
	Comparative genomics analysis of 6 new species of *Aspergillus* section Sparsi, Ochraceorosei, Tanneri and Robusti. Tammi Vesth, DTU.	
	nsdD-mediated sexual development and ascospore-specific gene expression in *Aspergillus nidulans.* Kap-Hoon Han, Woosuk University.	
10:15-10:45	**Coffee break**	
10:45-12:00	Session II: Genomic Tools	Mikael Andersen, DTU, Denmark.
	New CRISPR tech for *Aspergillus* engineering. Uffe Mortensen, DTU.	
	FungiDB update. Evelina Basenko and David Roos, FungiDB.	
	Updates on the *A. fumigatus* K/O project. Mike Bromley, University of Manchester.	
	Using new *Aspergillus* genomes for linking compounds to clusters. Inge Kjaerboelling, DTU.	
12:00-12:30	Community directions discussion; Elections	Michelle Momany
12:30-13:30	**Lunch**	
13:30-15:00	Novozymes Poster Session	Richard Todd, Chair, Kansas State University, USA.
15:00-16:15	Session III: Talks from Abstracts	Ling Lu, Nanjing Normal University, China.
	Substrate specificity of the FurE transporter is determined by cytoplasmic terminal domain interaction. George Diallinas, National and Kapodistrian University of Athens.	
	Asp30 and Asp73 of *Aspergillus oryzae* cutinase CutL1 are involved in the ionic interaction with fungal hydrophobin RolA. Yuki Terauchi, Tohoku University.	
	The *Aspergillus nidulans* pyruvate dehydrogenase kinases are essential to integrate carbon source metabolism. Laure Nicolas Annick Ries, Universidade de São Paulo.	
	Biosynthesis of histidine, arginine, riboflavin and pantothenic acid, but not siroheme, is crucial for virulence of *Aspergillus fumigatus*. Anna-Maria Dietl , Medical University of Innsbruck.	
16:15-17:00	Pontecorvo Lecture (sponsored by Zymergen)	Ken Bruno & Edyta Szewczyk, Zymergen, Inc.
	The Hunger Games: What starvation response genes reveal about how fungi cope with nutrient stress. Margaret Katz, University of New England.	
17:00	Election results; Novozymes student poster prizes; other discussion items	
17:30	**Dismiss and poster take down**	
18:00	ECFG Reception (10 minute walk to reception site)	

List of Posters

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

1. High-throughput format for the phenotyping of fungi on solid substrates
David Cánovas, Lena Studt, Ana T. Marcos, and Joseph Strauss

2. Novel regulator induces biosynthesis of cryptic natural products in the fungus *Aspergillus sydowii
Maria C. Stroe, Tina Netzker, Vito Valiante, Kirstin Scherlach, Volker Schroeckh, Christian Hertweck, Axel A. Brakhage

***3. Hyperbranching in the filamentous fungus *Aspergillus niger* following deletion of the GTPase RacA leads to altered glucoamylase secretion upon overexpression of the enzyme**
Markus RM Fiedler, ***Lars Barthel,** Christin Kubisch, **Corrado Nai,** Vera Meyer

***4. Substrate specificity of the FurE transporter is determined by cytoplasmic terminal domain interaction**
Georgia F. Papadaki, Sotiris Amillis, and George Diallinas

5. CRISPR-mediated expression platform for multi-species Aspergilli
Zofia Dorota Jarczynska, Ferdinand Hans Kirchner, Christina Spuur Nødvig, Uffe Hasbro Mortensen

6. Organic acid production in *Aspergillus niger*: Rewiring endogenous metabolic pathways by introducing and modifying the itaconic acid pathway from *Aspergillus terreus*
Abeer Hossain, Roy van Gerven, Peter S Lubeck, **Peter Punt**

7. Fungal strain development for screening and production of enzymes: development of a suite of tailored *Aspergillus* host strains with improved characteristics regarding proteolytic degradation, enzyme screening and fermentation characteristics
Wouter de Bonte, Sylvia Segers, Vivi Joosten, **Peter Punt**

8. Regulation of arabinose-induced gene expression in *Aspergillus niger
Jos Reijngoud, Malte Deseke, Ebru Alazi, Arthur FJ Ram

9. Biosynthesis of acurin A and B, two novel isomeric fusarin C-like compounds from *Aspergillus aculeatus
Peter Persson Wolff, Maria Lund Nielsen, Lene Maj Petersen, Lasse Norup Andersen, Thomas Isbrandt, Dorte Koefoed Holm, Uffe H. Mortensen, Christina Spuur Nødvig, Thomas Ostenfeld Larsen, and Jakob Blæsbjerg Hoof

***10. Elucidating the Biosynthetic Pathway of Felinone A in *Aspergillus nidulans* Through serial Promoter Replacement**
Yi-En Liao, Tzu-Shyang Lin, Clay C. C. Wang

***11. Characterization of *Aspergillus niger* isolated from the International Space Station**
Jillian Romsdahl, Adriana Blachowicz, Abby Chiang, Yi-Ming Chiang, Jason E. Stajich, Markus Kalkum, Kasthuri Venkateswaran, and Clay C.C. Wang

12. Pulses of Ca2+ coordinate actin assembly and exocytosis for stepwise cell extension.
Norio Takeshita

13. Genome-wide chromatin mapping of *Aspergillus nidulans* reveals BasR, a novel regulator of bacteria-triggered fungal natural product biosynthesis
Tina Netzker, Juliane Fischer, Sebastian Y. Müller, Agnieszka Gacek-Matthews, Nils Jäger, Kirstin Scherlach, Maria C. Stroe, María García-Altares, Francesco Pezzini, Mario K. C. Krespach, Ekaterina Shelest, Volker Schroeckh, Vito Valiante, Thorsten Heinzel, Christian Hertweck, Joseph Strauss, Axel A. Brakhage

14. Comparative analysis for transcription start sites of enolase genes in *Aspergillus oryzae* and *Aspergillus nidulans
Taishi Inoue, Mizuki Tanaka, Takahiro Shintani, Katsuya Gomi

15. The small GTPase ArfA controls secretion, morphology, and growth in *Aspergillus niger* via actin ring positioning
Cairns, T., Feidler, M., Koch, O., Kubisch C. & Meyer, V.

***16. Asp30 and Asp73 of *Aspergillus oryzae* cutinase CutL1 are involved in the ionic interaction with fungal hydrophobin RolA**
Yuki Terauchi, Yoon-Kyung Kim, Takumi Tanaka, Kei Nanatani, Akira Yoshimi, Toru Takahashi, and Keietsu Abe

***17. HapX iron sensing in *Aspergillus fumigatus* involves the interaction with the monothiol glutaredoxin GrxD**
Mareike Scheven, Matthias Misslinger, Peter Hortschansky, Thomas Krüger, Hubertus Haas & Axel A. Brakhage

18. The *Aspergillus nidulans* pyruvate dehydrogenase kinases are essential to integrate carbon source metabolism
Laure Nicolas Annick Ries, Leandro José de Assis, Fernando José Santos Rodrigues, Camila Caldana, Marina Campos Rocha, Iran Malavazi, Özgür Bayram, Gustavo H. Goldman

19. Paxillin protein PaxB and actinin-like protein AcnA are required for cytokinesis via regulating actin ring assembly in *Aspergillus nidulans
Xiaogang Zhou, Jing Ye, Weiran Qiao, Steven D. Harris, Ling Lu

***20. Involvement of an *Aspergillus fumigatus* putative sphingolipid-synthesis related protein OrmA in antifungal azole stress responses**
Pengfei Zhai, Jinxing Song,Ling Lu

21. Iron sensing is governed by mitochondrial, but not by cytosolic iron-sulfur cluster biogenesis in *Aspergillus fumigatus
Matthias Misslinger, Hubertus Haas

***22. Friends and foes - comparative genomics of 23 *Aspergillus Flavi* species**
Inge Kjærbølling, Tammi C. Vesth, Jane L. Nybo, Sebastian Theobald, Jens C. Frisvad, Martin E. Kogle, Ellen K. Lyhne, Alan Kuo, Asaf Salamov, Robert Riley, Thomas O. Larsen, Uffe H. Mortensen, Igor V. Grigoriev, Scott E. Baker and Mikael R. Andersen.

***23. Activation of a silent gene cluster in *Aspergillus nidulans* through the development of a hybrid transcription factor**
Michelle F. Grau, Ruth Enwistle, Tomohiro Akashi, Richard B. Todd, Berl R. Oakley, Clay C. C. Wang

24. Spore heterogeneity of food spoilage fungi; *Aspergillus niger
Sjoerd J. Seekles, Tom van den Brule, Maarten Punt, Jan Dijksterhuis, Jos Houbraken, Arthur F. J. Ram, Han A. B. Wösten

25. Biosynthesis of histidine, arginine, riboflavin and pantothenic acid, but not siroheme, is crucial for virulence of *Aspergillus fumigatus
Anna-Maria Dietl, Nir Osherov, Hubertus Haas

26. The Aspmine - comparative genomics analysis of 6 new species of Aspergillus section Sparsi, Ochraceorosei, Tanneri and Rubusti
Tammi Vesth, Jane Lind Nybo, Sebastian Theobald, Jens Frisvad, Ronald de Vries, Igor V. Grigoriev, Scott E. Baker, Ellen K. Lyhne, Martin E. Kogle, Asaf Salamov, Alan Kuo, Robert Riley, Matthieu Hainaut, Mikael R. Andersen

27. A lariat branch point motif-interrupted spliceosomal twin intron in *Aspergillus nidulans*
Napsugár Kavalecz, Michel Flipphi, Norbert Ág, Levente Karaffa, Claudio Scazzocchio, **Erzsébet Fekete**

28. Itaconic acid production from D-xylose by *Aspergillus terreus*
István S. Kolláth, Ákos P. Molnár, Erzsébet Fekete, and **Levente Karaffa**

Presenting Authors

(Alphabetical; Student presenters in bold type with asterisk)

Presenter	Poster number
Barthel, Lars	**3***
Cairns, Timothy	15
Cánovas, David	1
Diallinas, George	4
Dietl, Anna-Maria	**25***
Fekete, Erzsébet	27
Grau, Michelle F.	**23***
Inoue, Taishi	**14***
Jarczynska, Zofia Dorota	5
Levente Karaffa	28
Kjærbølling, Inge	**22***
Liao, Yi-En	**10**
Misslinger, Matthias	**21*.**
Netzker, Tina	13
Nai, Corrado	3
Papadaki, Georgia F.	**4***
Punt, Peter	6,7
Ries, Laure Nicolas Annick	18
Reijngoud, Jos	**8*.**
Romsdahl, **Jillian**	**11***
Scheven, Mareike	**17***
Seekles, Sjoerd J.	**24***
Stroe , Maria C.	**2***
Takeshita, Norio	12
Terauchi, Yuki	**16**
Vesth, Tammi	26
Wolff, Peter Persson	**9*.**
Zhai, Pengfei	**20***
Zhou, Xiaogang	**19***

Student Presenting Authors

(Alphabetical)

Presenter	Poster number
Barthel, Lars	**3***
Dietl, Anna-Maria	**25***
Grau, Michelle F.	**23***
Inoue, Taishi	**14***
Kjærbølling, Inge	**22***
Liao, Yi-En	**10**
Misslinger, Matthias	**21*.**
Papadaki, Georgia F.	**4***
Reijngoud, Jos	**8*.**
Romsdahl, **Jillian**	**11***
Scheven, Mareike	**17***
Seekles, Sjoerd J.	**24***
Stroe , Maria C.	**2***
Terauchi, Yuki	**16**
Wolff, Peter Persson	**9*.**
Zhai, Pengfei	**20***
Zhou, Xiaogang	**19***

Abstracts

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

1. High-throughput format for the phenotyping of fungi on solid substrates

David Cánovas[1,2], Lena Studt[1,3], Ana T. Marcos[2], and Joseph Strauss[1,3]
[1] Division of Microbial Genetics and Pathogen Interaction, Department of Applied Genetics and Cell Biology, BOKU University of Natural Resources and Life Science, Campus Tulln, Tulln/Donau, Austria
[2] Department of Genetics, Faculty of Biology, University of Seville, Spain
[3] Research Platform Bioactive Microbial Metabolites, BOKU University and University of Veterinary Medicine Vienna, Campus Tulln, Austria

Filamentous fungi naturally grow on solid surfaces, yet most genetic and biochemical analyses are still performed in liquid cultures. Here, we report a multiplexing platform using high-throughput photometric continuous reading that allows parallel quantification of hyphal growth and reporter gene expression directly on solid medium, thereby mimicking natural environmental conditions. Using this system, we have quantified fungal growth and expression of secondary metabolite GFP-based reporter genes in saprophytic *Aspergillus* and phytopathogenic *Fusarium* species in response to different nutrients, stress conditions and epigenetic modifiers. With this method, we provide not only novel insights into the characteristic of fungal growth but also into the metabolic and time-dependent regulation of secondary metabolite gene expression.

2. Novel regulator induces biosynthesis of cryptic natural products in the fungus *Aspergillus sydowii

Maria C. Stroe[a,b], Tina Netzker[a], Vito Valiante[c], Kirstin Scherlach[d], Volker Schroeckh[a], Christian Hertweck[b,d], Axel A. Brakhage[a,b]
[a]Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany, [b]Friedrich Schiller University Jena, [c]Research Group Biobricks of Microbial Natural Product Synthetases, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany, [d]Department of Biomolecular Chemistry, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany, maria.stroe@leibniz-hki.de

Natural products are low-molecular mass compounds with diverse chemical structures and important pharmacological activities, ranging from antibiotics to cholesterol-lowering agents (1). Filamentous fungi are well-known producers of such molecules, and recent advances in sequencing and genome mining have revealed that the biosynthetic potential of fungi is far greater than the number of currently identified compounds (2). This finding prompted the development of new methods to activate the biosynthesis of cryptic natural products. One successful approach is to simulate natural environmental conditions through co-cultivation of microorganisms (3). We previously showed that in a mixed fermentation, the bacterium *Streptomyces rapamycinicus* leads to the activation of the silent orsellinic acid (*ors*) gene cluster of the fungus *Aspergillus nidulans* (4). The metabolite production was further shown to be dependent on a novel regulator termed BasR (5), which is induced during the bacterial-fungal interaction. Here, we show that this transcription factor is responsible for the activation of the *ors* cluster in the related fungus *Aspergillus sydowii*, where its induced expression is able to activate the fungal secondary metabolism and triggers the biosynthesis of cryptic compounds.
(1) Brakhage (2013) Nature, (2) Macheleidt et al., (2016) Annu Rev Genet, (3) Netzker et al., (2015) Front Microbiol, (4) Schroeckh et al., (2009) PNAS, (6) Fischer et al., in preparation

***3. Hyperbranching in the filamentous fungus *Aspergillus niger* following deletion of the GTPase RacA leads to altered glucoamylase secretion upon overexpression of the enzyme**

Markus RM Fiedler[1], ***Lars Barthel[1],** Christin Kubisch[1], **Corrado Nai[1],** Vera Meyer[1]
[1]Department Applied and Molecular Microbiology, Institute of Biotechnology, Technische Universität Berlin, Gustav-Meyer-Allee 25, 13355 Berlin (DE)

Filamentous fungi secrete hydrolytic enzymes to degrade polymeric substances into smaller molecules which are then taken up as to sustain growth and metabolism. The accepted paradigm in fungal biology is that the tips of fungal hyphae are the highly active regions of a fungal colony, where polarised growth and secretion are coupled processes. However, it is currently debated if the amount of growing hyphal tips in filamentous fungi correlates with an increase in secretion, with previous studies showing either a positive or no correlation. In this study, we investigated the previously described hyperbranching strain of the industrial cell factory *Aspergillus niger*, which is deleted in the GTPase RacA and builds more hyphal tips but shows otherwise identical growth rate and total protein secretion as the wildtype.
Here, we use a v-SNARE reporter strain (SncA-GFP) to show that the hyperbranching strain exhibits an increased level in secretory vesicles at the hyphal tip upon overexpression of glucoamylase driven by the

Tet-on system. Thus, we establish for the first time a link between level of transcript/secretory cargo load with the gradient of secretory vesicles at hyphal tip. We show that Δ*racA* secretes altered amounts of glucoamylase upon Tet-on driven overexpression of the enzyme in comparison to the parental strain despite unaltered biomass yields, total secretory vesicles, or total protein secretion. Our results contribute to the understanding of fungal protein secretion at the hyphal tip, and have profound implications for biotechnology and applied mycology.

***4. Substrate specificity of the FurE transporter is determined by cytoplasmic terminal domain interaction**

Georgia F. Papadaki, Sotiris Amillis, and George Diallinas
Department of Biology, National and Kapodistrian University of Athens, Panepistimioupolis, Athens 15784, Greece**,** diallina@biol.uoa.gr

FurE, a member of the NCS1 transporter family in *Aspergillus nidulans*, is specific for allantoin, uric acid, uracil and related analogues. Herein, we show that C- or N-terminally truncated FurE transporters (FurE-ΔC or FurE-ΔN) present increased protein stability, but also inability for uric acid transport. To better understand the role of cytoplasmic terminal regions, we characterized genetic suppressors that restore FurE-ΔC-mediated uric acid transport. Suppressors map in the periphery of the substrate-binding site (Thr133 in TMS3 and Val343 in TMS8), an outward-facing gate (Ser296 in TMS7, Ile371 in TMS9, Tyr392 and Leu394 in TMS10) or in flexible loops (Asp26 in L_N, Gly222 in L5, Asn308 in L7). Selected suppressors were shown to also restore the wild-type specificity of FurE-ΔN, suggesting that both C- and/or N-terminal domains are involved in intramolecular dynamics critical for substrate selection. A direct, substrate-sensitive, interaction of C- and/or N-terminal domains was supported by bimolecular fluorescence complementation assays. To our knowledge, this is the first case where not only the function, but also, the specificity of a eukaryotic transporter is regulated by its terminal cytoplasmic regions.

5. CRISPR-mediated expression platform for multi-species Aspergilli

Zofia Dorota Jarczynska[1], Ferdinand Hans Kirchner[1], Christina Spuur Nødvig[1], Uffe Hasbro Mortensen[1]
[1] Eukaryotic Molecular Cell Biology group, Department of Biotechnology and Biomedicine, Søltofts Plads, Building 223, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark
Corresponding author: um@bio.dtu.dk

The recent sequencing survey on many fungal species revealed a large repertoire of industrially or medically relevant enzymes and secondary metabolites. However, the industrial potential of these species is often hindered by the difficulties with the cultivation at laboratory conditions or in bioreactors. It is therefore beneficial to create heterologous expression platforms that facilitate the screening process of fungal genes for production of relevant enzymatic activities or secondary metabolites. Unfortunately, in many cases it is not possible to predict whether a given host possesses a physiology and metabolism compatible for formation of these products, resulting in an inefficient heterologous production. To increase the chance of successful heterologous production, we have created a flexible expression platform in various *Aspergillus* species. The system is based on the insertion of a reporter gene into defined locus in each of the different expression hosts. The reporter allows to assess the strength of expression from the various defined loci, as well as it can be replaced by a gene-expression cassette containing your favourite gene or pathway via marker-free homologous recombination mediated by CRISPR-Cas9 technology. Importantly, our setup allows different species to be transformed by the same gene-expression cassette to reduce DNA construction work. As a proof-of-concept, we chose three representatives of *Aspergillus*: the model fungus *A. nidulans*, and the fungal industrial workhorses *A. niger* and *A. oryzae*. We have used red fluorescent protein (RFP) as a reporter gene and inserted it into several defined integration sites in all three species. RFP production was confirmed through fluorescence microscopy and the three different strains constitute our versatile *Aspergillus* expression platform. We have tested the platform and replaced RFP in the different species with genes allowing for production of relevant enzymes and secondary metabolites.

6. **Organic acid production in *Aspergillus niger*: Rewiring endogenous metabolic pathways by introducing and modifying the itaconic acid pathway from *Aspergillus terreus***

Abeer Hossain, Roy van Gerven, Peter S Lubeck, **Peter Punt**
Dutch DNA Biotech, Utrecht Netherlands

Rising carbon emissions due to increased industrialization and its effect on the global climate are raising awareness to move from a fossil fuel-based economy to a bio-based economy. Organic acids have huge potential as alternative for petrochemicals and concomitantly its derivatives as commodities [1]. Filamentous fungi are widely known as efficient organic acid producers, in particular members of the genus Aspergillus.

Itaconic acid (IA), a C5-dicarboxylic acid, has been identified as one of the top twelve building block chemicals that can be produced by biotechnological means. The potential applications of IA in green chemistry are numerous and IA is already naturally produced by *Aspergillus terreus*. However, for several reasons heterologous production in the related species *Aspergillus niger* has been proposed. Previously we have shown that rewiring of a non-canonicla citrate synthase gene (*citB*) derived from an *A. niger* secondary metabolism cluster has led to an increased yield, titer and productivity of IA, reaching to the highest levels reported for heterologous IA production.

In our research we have now performed a RNA-Seq analysis of high, medium and low IA producing strains to further improve our optimized IA pathway and understand the effect of heterologous IA production on *A. niger* metabolism. It was found that apart from *citB*, another non-canonical citrate synthase displayed a similar role in itaconic acid production upon overexpression. Further rewiring of metabolic pathways was seen by specific gene deletion of pathways involved in byproduct formation and overexpression of canonical primary metabolic pathways genes. Several of these strain modifications were found to improve production of itaconic acid. Finally, our research also showed a hitherto unknown involvement of N-metabolism on prolonged itaconic acid production to achieve higher titers and yields.

7. Fungal strain development for screening and production of enzymes: development of a suite of tailored *Aspergillus* host strains with improved characteristics regarding proteolytic degradation, enzyme screening and fermentation characteristics

Wouter de Bonte, Sylvia Segers, Vivi Joosten, **Peter Punt**
Dutch DNA Biotech, Utrecht Netherlands

Fungal host strains such as *Aspergillus niger*, *Aspergillus sojae* and *Trichoderma reesei* are used for the production of a wide variety of industrially relevant enzymes. About 80% of all industrial enzymes are derived from filamentous fungi, making these organisms also the hosts of choice for the production of new enzymes and proteins.

To develop suitable strain platforms to exploit the unique characteristics of fungi several strain improvement topics are being addressed in our research. These include development of protease deficient host strains using different classical genetic and molecular genetic approaches, followed up by systems biology approaches to further explore the details of the regulation of protease production in different filamentous host strains. From our research it has become clear that various different pathways may operate in different fungi. Besides protease production also strain improvement aimed at improved fermentation characteristics is highly relevant for protein production. In particular aspects of fungal morphology have been addressed in our research in line with fungal fermentation process engineering. This research has resulted in improved fermentation design and performance.

In many cases prior to producing specific proteins of interest also selection of genes and gene-designs for optimal protein secretion is an important step in fungal strain development. For this purpose we have developed various fungal host strains suitable for this screening phase. An example of this is a line of host strains unable to use specific polymeric carbon sources and their use to develop selective biological screens for specific protein activities.

8. Regulation of arabinose-induced gene expression in *Aspergillus niger*

***Jos Reijngoud,** Malte Deseke, Ebru Alazi, Arthur FJ Ram
Leiden University, Institute of Biology Leiden, Molecular Microbiology and Biotechnology, Sylviusweg 72, 2333 BE Leiden, The Netherlands

The AraR transcription factor of *Aspergillus niger* encodes a Zn(II)2Cys6 transcription factor required for the induction of several arabinolytic genes. One of the target genes of AraR is AbfA encoding an arabinofuranosidase that is specifically induced on arabinan and arabinose in an AraR-dependent way. Expression of AbfA as well as other arabinolytic genes in *A. niger* requires the presence of L-arabinose as an inducer to activate AraR.
With the goal to isolate mutants that constitutively express arabinolytic genes independent on the presence of L-arabinose as an inducer, we designed a positive selection method using the arabinose-responsive promoter (*PabfA*) fused to the acetamidase (*amdS*) reporter gene. Expression of the *amdS* gene enables the fungus to grow on acetamide as the sole nitrogen source. Hence, mutants constitutively expressing the *amdS* gene can be selected on agar-plates with acetamide as a N-source. Growth analysis of the *PabfA-amdS* reporter strain indicated that *abfA* is specifically induced by arabinose, arabitol and arabinan. The *in vivo* reporter strain was also used to monitor carbon catabolite repression control. The *PabfA-amdS* reporter was repressed by glucose, fructose and sorbitol in a concentration dependant manner. CreA is important in mediating carbon catabolite repression and deletion of the *creA* gene in the *PabfA-amds* reporter strain

abolished repression by glucose, fructose and sorbitol. Interestingly, the *PabfA-amdS* reporter construct in the Δ*creA* background was induced not only by arabinose but also by xylose indicating a regulatory overlap between AraR and XlnR transcription factors.

9. Biosynthesis of acurin A and B, two novel isomeric fusarin C-like compounds from *Aspergillus aculeatus*

***Peter Persson Wolff**, Maria Lund Nielsen, Lene Maj Petersen, Lasse Norup Andersen, Thomas Isbrandt, Dorte Koefoed Holm, Uffe H. Mortensen, Christina Spuur Nødvig, Thomas Ostenfeld Larsen, and Jakob Blæsbjerg Hoof
Department of Biotechnology and Biomedicine, Technical University of Denmark, Søltofts Plads, Kongens Lyngby, Denmark

This study presents the identification and proposed biosynthetic pathway for two stereoisomeric compounds of mixed polyketide-nonribosomal peptide origin that we named acurin A and acurin B. The compounds were discovered in extracts from *Aspergillus aculeatus*, a filamentous fungus known for the commercial utilization in the production of several enzymes. The structures of acurin A and B highly resemble the mycotoxin fusarin C produced by several *Fusarium* species. In our work, we used CRISPR-Cas9 to construct a non-homologous end-joining deficient strain of *A. aculeatus*, which enabled efficient gene deletions in the acurin gene cluster. Using gene-expression analysis in combination with metabolite profiling of gene-deletion strains, the gene cluster responsible for acurin production was delineated, which allowed us to propose a biosynthetic pathway for formation of acurin. Our results show that acurin is biosynthesized by an individual polyketide synthase and non-ribosomal synthetase. Moreover, at least six other enzymatic activities are required to complete the biosynthesis of acurin. This study shows how we exploit the CRISPR-Cas9 system in filamentous fungi for the rapid construction of fungal host strains that can be readily engineered to elucidate biosynthetic pathways.

***10. Elucidating the Biosynthetic Pathway of Felinone A in *Aspergillus nidulans* Through serial Promoter Replacement**

Yi-En Liao, Tzu-Shyang Lin, Clay C. C. Wang

Fungal secondary metabolites (SMs) are an important source for drug discovery. Thousands of biosynthetic pathways of SMs are revealed by several approaches and classified based on their characteristics. In this study, we focus on one of SMs: felinone A. In our previous study, we replaced the promoter of nonreducing polyketides synthase (NR-PKS) AN7901 and isolated 2,4-dihydroxy-3-methyl-6-(2-oxopropyl)-benzaldehyde (compound **1**)[1]. However, since we did not activate the surrounding genes that may modify compound **1**, the final product of this gene cluster remains unknown. By homolog comparison, we propose that AN7901 is involved in the biosynthetic pathway of Felinone A as a transcription factor, and AN7902 encodes the enzyme that oxidize compound 1 to final product, felinone A. We conducted BLAST analysis to determine the putative biosynthesis genes involved, and will replace their promoters serially. The data will allow us to confirm our proposed biosynthetic pathway and determine the boundary of Felinone A cluster.

***11. Characterization of *Aspergillus niger* isolated from the International Space Station**

Jillian Romsdahl[1], Adriana Blachowicz[1,2], Abby Chiang[3], Yi-Ming Chiang[1], Jason E. Stajich[4], Markus Kalkum[3], Kasthuri Venkateswaran[2], and Clay C.C. Wang[1,5]

[1] Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, California, USA; [2] Biotechnology and Planetary Protection Group, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California, USA; [3] Department of Molecular Immunology, Beckman Research Institute of City of Hope, Duarte, California, USA; [4] Department of Microbiology & Plant Pathology and Institute of Integrative Genome Biology, University of California-Riverside, Riverside, California, USA; [5] Department of Chemistry, College of Letters, Arts, and Sciences, University of Southern California, Los Angeles, California, USA

As strives are made toward human interplanetary exploration, a thorough understanding of how fungi respond and adapt to the various stimuli encountered during spaceflight is imperative for the health of crew. In the current study, we used a combination of genomics, proteomics, and metabolomics to characterize the molecular phenotype of a strain of *Aspergillus niger* isolated from the International Space Station (ISS). As a predominant ISS isolate that is frequently detected in other built environments, current and future studies of *A. niger* strains that have inhabited spacecraft environments will become increasingly important as the duration of manned space missions increase. The ISS isolate exhibited an increased rate of growth compared to a terrestrial strain. Whole-genome sequencing revealed increased genetic variance when compared to several other genome sequenced strains *A. niger*. Additionally, a distinct molecular phenotype of the ISS isolate was observed that suggests increased resistance to irradiation and oxidative stress, and an

enhanced ability to acquire nutrients. Increased abundance was also observed for numerous secondary metabolites, including naphtho-gamma-pyrones, which are involved in melanin production, and pyranonigrin A, an antioxidant. These findings provide insight into the adaptive evolutionary mechanism of melanized fungal species and demonstrate the need for more studies on the biological alterations of microbes adapted to extreme spaceflight environments.

12. Pulses of Ca2+ coordinate actin assembly and exocytosis for stepwise cell extension.
Norio Takeshita
University of Tsukuba, Faculty of Life and Environmental Science

Many eukaryotic cells grow by extending their cell periphery in pulses. The molecular mechanisms underlying this process are not yet fully understood. Here we present a comprehensive model of stepwise cell extension by using the unique tip growth system of filamentous fungi. Live-cell imaging analysis, including superresolution microscopy, revealed that the fungus *Aspergillus nidulans* extends the hyphal tip in an oscillatory manner. The amount of F-actin and secretory vesicles (SV) accumulating at the hyphal tip oscillated with a positive temporal correlation, whereas vesicle amounts were negatively correlated to the growth rate. The intracellular Ca2+ level also pulsed with a positive temporal correlation to the amount of F-actin and SV at the hyphal tip. Two Ca2+ channels, MidA and CchA, were needed for proper tip growth and the oscillations of actin polymerization, exocytosis, and the growth rate. The data indicate a model in which transient Ca2+ pluses cause depolymerization of F-actin at the cortex and promote SV fusion with the plasma membrane, thereby extending the cell tip. Over time, Ca2+ diffuses away and F-actin and SV accumulate again at the hyphal tip. Our data provide evidence that temporally controlled actin polymerization and exocytosis are coordinated by pulsed Ca2+ influx, resulting in stepwise cell extension.
Takeshita et al, PNAS, 114(22):5710-06, 2017.

13. Genome-wide chromatin mapping of *Aspergillus nidulans* reveals BasR, a novel regulator of bacteria-triggered fungal natural product biosynthesis
Tina Netzker[a], Juliane Fischer[a], Sebastian Y. Müller[b], Agnieszka Gacek-Matthews[c], Nils Jäger[d], Kirstin Scherlach[e], Maria C. Stroe[a,h], María García-Altares[e], Francesco Pezzini[f,h], Mario K. C. Krespach[a,h], Ekaterina Shelest[f], Volker Schroeckh[a], Vito Valiante[g], Thorsten Heinzel[d], Christian Hertweck[e,h], Joseph Strauss[c], Axel A. Brakhage[a,h]
[a]Departments of Molecular and Applied Microbiology, [e]Biomolecular Chemistry and [f]Systems Biology and Bioinformatics, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany, [b]Department of Plant Sciences, University of Cambridge, Cambridge, UK, [c]Department for Applied Genetics and Cell Biology, BOKU-University of Natural Resources and Life Sciences, Campus Tulln, Tulln/Donau, Austria, [d]Department of Biochemistry, Friedrich Schiller University Jena, Jena, Germany, [g]Leibniz Research Group – Biobricks of Microbial Natural Product Syntheses, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany, [h]Friedrich Schiller University Jena, Jena, Germany, tina.netzker@leibniz-hki.de

Microorganisms can produce a plethora of secondary metabolites (SMs), which often have pharmacological potential (1). In nature, microorganisms live in multispecies communities, in which the produced SMs are often used as signal molecules. Mimicking the natural habitat in the laboratory by mixed fermentation experiments has been developed into a useful strategy to identify new SMs (2). We have been intensively studying the interaction between the model organism *Aspergillus nidulans* and the soil bacterium *Streptomyces rapamycinicus*, which leads to the activation of the silent fungal orsellinic acid (*ors*) gene cluster (3). Essential for the *ors* gene cluster activation is the activity of the lysine-acetyltransferase GcnE, which specifically acetylates lysine 9 and 14 of histone H3 during the co-cultivation (4). Furthermore we could show that the exchange of several amino acids of histone H3 in *A. nidulans* resulted in major changes in the penicillin, sterigmatocystin and orsellinic acid biosynthesis (5). This specific microbial interaction provides an excellent model system to study molecular and regulatory mechanisms underlying interspecies crosstalk. A genome-wide chromatin immunoprecipitation (ChIP) analysis was performed to analyse the distribution of the acetylation events during the interaction. Our data reveal major changes in the fungal chromatin landscape induced by the bacterium and led to the identification of the transcription factor BasR, required for the bacteria-induced activation of secondary metabolism.
(1) Macheleidt et al. (2016) Annu Rev Genet, (2) Netzker et al. (2015) Front Microbiol, (3) Schroeckh et al. (2009) PNAS, (4) Nützmann et al. (2011) PNAS, (5) Nützmann, Fischer et al. (2013) AEM

14. Comparative analysis for transcription start sites of enolase genes in *Aspergillus oryzae* and *Aspergillus nidulans

Taishi Inoue[1], Mizuki Tanaka[2], Takahiro Shintani[1], Katsuya Gomi[1]
[1]Department of Bioindustial Informatics and Genomics, Graduate School of Agricultural Science, Tohoku University, Japan
[2]Depatment of Food and Nutritional Sciences, Graduate School of Integrated Pharmaceutical and Nutritional Sciences, the University of Shizuoka, Japan

Aspergillus oryzae is a domesticated filamentous fungus used for Japanese fermentation industries and has been well known for its high ability of starch assimilation. In *A. oryzae*, transcription of glycolytic genes is induced at high level in the presence of fermentable carbon sources such as glucose. This feature would be important for the growth in industrial culture conditions of *A. oryzae*. However, it has not been evaluated whether or not the transcriptional induction of glycolytic genes is a specific feature to *A. oryzae* compared with the related species existing in natural environments such as *Aspergillus nidulans*.

Our previous studies in *A. oryzae* uncovered that the enolase gene (*AoenoA*), one of the most strongly expressing glycolytic genes, has two alternative transcription start sites (TSSs) dependent on the difference of two types of carbon source; the TSS located at -510 nt upstream of the start codon (+1) is strictly used under condition with nonfermentable carbon sources and another TSS located at -36 nt is used under condition with fermentable carbon sources.

To elucidate the conservation of alternative TSSs in *enoA* among the genus *Aspergillus*, we investigated the TSS usage of *A. nidulans enoA* (*AnenoA*) under glucose or acetate culture condition. Although 5′ RACE analysis revealed that *AnenoA* had also two TSSs located at around -440 nt and -19 ~ -67 nt, transcription from the downstream TSS was not preferentially observed under glucose condition unlike in *AoenoA*. In addition, Northern blot and qRT-PCR analyses showed that the induction levels of the transcript from the downstream TSS in the presence of glucose were approx. 18-fold in *AnenoA* and approx. 100-fold in *AoenoA* compared those in the presence of acetate. These results indicated that transcription from the downstream TSS under glucose condition is markedly upregulated in *AoenoA* compared to that in *AnenoA*.

15. The small GTPase ArfA controls secretion, morphology, and growth in *Aspergillus niger* via actin ring positioning

Cairns, T.[1]**,** Feidler, M.[1], Koch, O.[1], Kubisch C.[1] & Meyer, V.
[1]Department Applied and Molecular Microbiology, Institute of Biotechnology, Technische Universität Berlin, Gustav-Meyer-Allee 25, 13355 Berlin (DE)

In filamentous fungi, growth and protein secretion occurs predominantly at the hyphal tip. This requires coordinated regulation of multiple processes, including vesicle trafficking, exocytosis, and endocytosis, which are facilitated by a complex cytoskeletal apparatus. In this study, functional analyses of the small GTPase ArfA from *Aspergillus niger* demonstrate that this protein functionally complements the *Saccharomyces cerevisiae* ARF1/2, and that this protein is essential for *A. niger*, where it regulates hyphal growth rates, protein secretion, and hyphal morphology. ArfA co-localizes to Golgi equivalents and post-Golgi carriers, but not the endoplasmic reticulum in hyphae. Moreover, localization of the endocytic machinery, visualized via fluorescent tagging of the actin ring, was found to be abnormal in ArfA under- and overexpressed conditions, indicating that ArfA mechanistically regulates secretion by affecting actin ring positioning. Finally, we provide evidence that secretion in *A. niger* occurs subapically, which may be a mechanism to compensate for defective and/or excess secretion at the hyphal apex due to ArfA misregulation. Taken together, our results demonstrate that ArfA fulfils multiple functions in the secretory pathway of A. niger. We propose that ArfA is a critical regulator that controls the endocytotic machinery at the hyphal apex.

***16. Asp30 and Asp73 of *Aspergillus oryzae* cutinase CutL1 are involved in the ionic interaction with fungal hydrophobin RolA**

Yuki Terauchi [1], Yoon-Kyung Kim [1], Takumi Tanaka [1], Kei Nanatani [2], Akira Yoshimi [3], Toru Takahashi [3], and Keietsu Abe [1,2,3,]
[1]Department of Microbial Biotechnology, Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan, [2]Department of Microbial Resources, Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan, [3] Microbial Genomics Laboratory, New Industry Creation Hatchery Center, Tohoku University, Sendai, Miyagi, Japan

When the industrial fungus *Aspergillus oryzae* is grown with polyesters as a sole carbon source, the fungus co-produces a hydrophobin RolA and an esterase CutL1. RolA attached to polyesters specifically recruits CutL1 and consequently promotes hydrolysis of polyesters by CutL1. The mechanism of its recruitment is attributed to the ionic interaction between positively charged residues (H32, K34) of RolA and negatively charged residues (E31, D142, D171) of CutL1. The K_D for the interaction of RolA with the CutL1-E31S/D142S/D171S was considerably higher than that for its interaction with wild-type CutL1. In the

presence of 250 mM NaCl, both K_D values were similar, suggesting that some additional charged residues in CutL1 are involved in the CutL1—RolA interaction besides E31, D142 and D171. In this study, we investigated whether D30 and D73 of CutL1 are also involved in the CutL1—RolA interaction. First, we compared amino acid sequences of CutL1 and CutL1 orthologs, and analyzed CutL1 3D-model, leading to prediction of D30 and D73 as the candidate residues involved in RolA-CutL1 interaction. Next, we purified CutL1-D30S, CutL1-D73S, CutL1-D30S/E31S/D142S/D171S and CutL1-E31S/D73S/D142S/D171S, and measured Circular Dichroism (CD) spectra of them to confirm their structures. To analyze the kinetics of binding of the CutL1 mutants to RolA, we used a Quartz Crystal Microbalance (QCM) and calculated K_D. The QCM approach revealed that the K_D values of the CutL1 single mutants to RolA were higher than that of wild-type CutL1 to RolA, and the K_D values of CutL1 quadruple mutants to RolA were higher than that of CutL1 E31S/D142S/D171S. We conclude that D30 and D73 are important for CutL1–RolA interaction. These results also imply that CutL1 3D-model is useful for prediction of amino acid residues that are involved in the interaction with RolA despite little conservation of the residues among CutL1 orthologs.

***17. HapX iron sensing in *Aspergillus fumigatus* involves the interaction with the monothiol glutaredoxin GrxD**

Mareike Scheven[1,2], Matthias Misslinger[3], Peter Hortschansky[1], Thomas Krüger[1], Hubertus Haas[3] & Axel A. Brakhage[1,2]
(1) Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute (HKI), Jena, Germany, (2) Friedrich Schiller University, Jena, Germany, (3) Division of Molecular Biology, Biocenter, Innsbruck Medical University, Innsbruck, Austria. Mareike.Scheven@leibniz-hki.de

Aspergillus fumigatus is a ubiquitous saprophytic mold, which causes life-threatening diseases in immunocompromised patients. During infection, sufficient iron supply is crucial for fungal growth. Iron is a vital nutrient, but can be harmful in excess by triggering the formation of cell damaging reactive oxygen species. As a result, *A. fumigatus* has evolved fine-tuned mechanisms to maintain iron equilibrium. Adaptation to iron limitation and iron excess is mediated by the bZIP transcription factor HapX, which functions *via* physical interaction with the heterotrimeric CCAAT-binding complex. During iron starvation, iron consuming pathways are repressed and iron uptake is activated. During iron overload, the cell is detoxified from iron by activation of vacuolar iron storage [1].
Currently, the molecular mechanisms of iron sensing by HapX are unknown and remain to be elucidated. As shown for iron regulators in other ascomycetes, *A. fumigatus* HapX senses the cellular iron status most likely by interaction with other regulators, like monothiol glutaredoxin (GrxD). We applied a co-immunoprecipitation approach for identification of possible GrxD as well as HapX interaction partners. VENUS-tagged GrxD and MYC-tagged HapX proteins were enriched from crude cell extracts by GFP-Trap and MYC-Trap, respectively. Immunoprecipitated proteins were identified by nano LC-MS/MS measurement. HapX co-precipitated during $GrxD^{VENUS}$ enrichment under iron starvation, sufficiency and excess. *Vice versa,* GrxD was co-enriched during ^{MYC}HapX immunoprecipitation. The interaction of GrxD and HapX was subsequently confirmed *in vivo* by bimolecular fluorescence complementation analysis. In line with the *in vivo* results, recombinant *A. fumigatus* GrxD and HapX proteins were also co-purified with an unknown Fe-S ligand *in vitro* from *Escherichia coli*. In summary, these data provide first evidence that HapX iron sensing in *A. fumigatus* involves the interaction with GrxD.
Reference: [1] Gsaller, F.; Hortschansky, P. *et al. EMBO J* **2014,** 33, 2261-2276.

18. The *Aspergillus nidulans* pyruvate dehydrogenase kinases are essential to integrate carbon source metabolism

Laure Nicolas Annick Ries[1], Leandro José de Assis[1], Fernando José Santos Rodrigues[2], Camila Caldana[3], Marina Campos Rocha[4], Iran Malavazi[4], Özgür Bayram[5], Gustavo H. Goldman[1]
[1]Faculdade de Ciências Farmacêuticas de Ribeirão Preto (FCFRP), Universidade de São Paulo, CEP 14040-903, Ribeirão Preto, São Paulo, Brazil
[2]Instituto de Investigação em Ciências da Vida e Saúde, Campus de Gualtar, Universidade do Minho, 4710-057, Braga, Portugal
[3]Laboratório Nacional de Ciência e Tecnologia do Bioetanol (CTBE), Centro Nacional de Pesquisa em Energia e Materiais (CNPEM), Caixa Postal 6192, CEP 13083-970, Campinas, São Paulo, Brazil
[4]Departamento de Genética e Evolução, Centro de Ciências Biológicas e da Saúde, Universidade Federal de São Carlos, São Carlos, São Paulo, Brazil
[5]Maynooth University, Biology Department, Maynooth, Co. Kildare, Ireland

The pyruvate dehydrogenase complex (PDH), that converts acety-coA to pyruvate, is regulated by a consortium of pyruvate dehydrogenase kinases (PDHK) and phosphatases (PDHP) that have been shown to be important for morphology, pathogenicity and carbon source utilisation in different fungal species. The aim of this study was to investigate the role played by the three PDHKs PkpA, PkpB and PkpC in glucose,

cellulose and acetate utilisation in the reference filamentous fungus *Aspergillus nidulans*, in order to unravel regulatory mechanisms which could prove useful for fungal biotechnological and biomedical applications. All three PDHKs were shown to be mitochondrial with PkpA positively regulating PDH activity. In the presence of glucose, PkpA and PkpC function in the same pathway and deletion of the respective genes resulted in reduced glucose utilisation, which affected carbon catabolite repression (CCR) and hydrolytic enzyme secretion, due to de-regulated glycolysis and TCA cycle enzyme activities. Furthermore, PkpC was shown to be required for the correct metabolic utilisation of cellulose and acetate. PkpC negatively regulated the activity of the glyoxylate cycle enzyme isocitrate lyase (ICL), required for acetate metabolism. In summary, this study identified PDHKs important for the regulation of central carbon metabolism in the presence of different carbon sources, with effects on the secretion of biotechnologically important enzymes and carbon source-related growth. This work demonstrates how central carbon metabolism can affect a variety of fungal traits and lays a basis for further investigation into these characteristics with potential interest for different applications.
Financial support: FAPESP and CNPq

19. Paxillin protein PaxB and actinin-like protein AcnA are required for cytokinesis via regulating actin ring assembly in *Aspergillus nidulans

Xiaogang Zhou[1], Jing Ye[1], Weiran Qiao[1], Steven D. Harris[2], Ling Lu[1]*
[1]Jiangsu Key Laboratory for Microbes and Functional Genomics, Jiangsu Engineering and Technology Research Center for Microbiology; College of Life Sciences, Nanjing Normal University, Nanjing, 210023, China.
[2]Center for Plant Science Innovation and Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska 68588-0660, United States of America

Cytokinesis, as the final step of cell division, plays an important role in fungal growth and proliferation. In filamentous fungus *Aspergillus nidulans*, abnormal multinuclear or non–nucleated cells induced by defected cytokinesis will cause defected hyphal growth and sporulation. Previous studies have demonstrated that proper cytokinesis is accompanied with actin ring formation and contraction, which are regulated by (SIN) septation–initiation network that is consist of several conserved components. In our previous study, we found that actinin-like protein AcnA is essential for cytokinesis but underlined relationship between AcnA and SIN or between AcnA and actin cytoskeleton are not known yet.
In the present study, we have identified a cytoskeletal protein paxillin PaxB has a similar phenotype to that of AcnA, suggesting it is also essential for cytokinesis. In the absence of AcnA or PaxB, a key component of SIN pathway -MobA, was unable to contract at the predict septation site. Comparably, loss of function of SIN pathway could affect localization of AcnA and PaxB at the septation site. These results suggest that two cytoskeletal proteins AcnA and PaxB and the SIN pathway are reciprocal required to drive the proper cytokinesis. Moreover, deletion of *acnA* or *paxB* caused actin rings disappeared, which implies that AcnA and PaxB are crucial for actin ring formation. In addition, deletion of *acnA* leads to undetectable PaxB at the septation site, in comparison, deletion of *paxB* did not affect the location of AcnA but block its contraction during cytokinesis, which demonstrate that AcnA and PaxB are required for the function of each other. In *paxB* deletion mutant strains, septation and sporulation defects can be rescued by overexpressed *acnA*. These data suggest that AcnA and PaxB probably have an overlapping function for the proper function of SIN pathway and the formation of actin ring in *A. nidulans*.
*Correspondence to: Ling Lu, Email: linglu@njnu.edu.cn

***20. Involvement of an *Aspergillus fumigatus* putative sphingolipid-synthesis related protein OrmA in antifungal azole stress responses**

Pengfei Zhai, Jinxing Song,*Ling Lu
Jiangsu Key Laboratory for Microbes and Functional Genomics, Jiangsu Engineering and Technology Rese arch Center for Microbiology; College of Life Sciences, Nanjing Normal University, Nanjing, 210023, China.

Previous studies have identified that the sphingolipid, which is a major structural component of eukaryotic cytoplasmic membrane, is involved in many important biological processes, including cell metabolism and stress tolerance response, etc. It has been reported that there are two sphingolipid-synthesis related protein Orm1p and Orm2p in *Saccharomyces cerevisiae*s, which negatively regulate activities of the serine palmitoyl transferase that is the first speed-limit enzyme of sphingolipid synthesis.
In this study, through genome-wide homolog search analysis, we found that the *A. fumigatus* genome only contains one Orm homolog, referred as OrmA. Deletion of *ormA* causes hypersensitivity to azole while overexpression of OrmA shows the azole resistance. Moreover, Western blotting results indicate that OrmA protein expression could be induced by azole antifungals in a dose dependent way. These data suggest that OrmA in *A. fumigatus* is required for responding to the azole stress. In addition, rescued phenotypes for susceptibility to azole drugs by adding an sphingolipid synthesis inhibitor-myriocin in *ormA*-defective strains

combined with data for deletion of *ormA* leads to significant increased main components of sphingolipid ceramide, suggesting that OrmA may work as a negative regulator for the sphingolipid synthesis.

Further mechanism analysis verified that OrmA is involved in drug susceptibility through affecting endoplasmic reticulum stress responses in an unfolded protein response pathway (UPR) HacA-dependent way. Our data suggest that endoplasmic reticulum stress caused by azole drugs could stimulate HacA activation accompanied with inducing the increased expression of OrmA so that affecting the sensitivity of azole drugs by regulating the synthesis of sphingolipid. Most importantly, virulence tests demonstrated that OrmA deletion caused a significantly decreased virulence in immunosuppressive mice model. Our findings suggest that the unexplored sphingolipid metabolism pathway in *A. fumigatus* plays important roles for fungal virulence and azole susceptibilities and it may be used as new antifungal drug targets.

*Correspondence to: Ling Lu, Email: linglu@njnu.edu.cn

21. Iron sensing is governed by mitochondrial, but not by cytosolic iron-sulfur cluster biogenesis in *Aspergillus fumigatus

Matthias Misslinger, Hubertus Haas
Division of Molecular Biology, Medical University of Innsbruck, Biocenter, Innsbruck, Austria

For optimal growth, microorganisms have to adapt their iron metabolism to the requirements of their ecological niche to avoid iron shortage as well as iron toxicity. Therefore, mechanisms have been evolved to tightly regulate iron uptake, consumption and detoxification, respectively, which depend on sensing the cellular iron status. In the facultative anaerobic yeast *Saccharomyces cerevisiae,* iron sensing has been shown to depend on mitochondrial (MIA) but not cytosolic iron-sulfur cluster assembly (CIA), while in mammals the cellular iron state is sensed via cytosolically synthesized iron-sulfur clusters. To address the question how the obligatory aerobic mold *Aspergillus fumigatus* senses the cellular iron state, mutant strains allowing down-regulation of MIA and CIA were generated. These studies revealed that: (i) Af-Nfs1 (Afu3g14240) and Af-Nbp35 (Afu2g15960), which are required for MIA and CIA, respectively, are essential for growth; (ii) inactivation of the Frataxin homolog Af-FxnA (Afu4g10510), which is involved in MIA, is not lethal, but results in a severe growth defect; (iii) a decrease in MIA (Af-Nfs1 depletion, Af-FxnA-deficiency) but not CIA (Af-Nbp35 depletion) results in an iron starvation response accompanied by increased iron toxicity; and, likewise, (iv) a decrease in mitochondrial iron import results in an iron starvation response. Taken together, these data underline that iron sensing in *A. fumigatus* depends on the mitochondrial, but not the cytosolic iron-sulfur cluster machinery. Moreover, depletion of the glutathione pool caused an iron starvation response underlining a crucial role of glutathione in iron sensing in *A. fumigatus*.

***22. Friends and foes - comparative genomics of 23 *Aspergillus Flavi* species**

Inge Kjærbølling[1], Tammi C. Vesth[1], Jane L. Nybo[1], Sebastian Theobald[1], Jens C. Frisvad[1], Martin E. Kogle[1], Ellen K. Lyhne[1], Alan Kuo[2], Asaf Salamov[2], Robert Riley[2], Thomas O. Larsen[1], Uffe H. Mortensen,[1] Igor V. Grigoriev[2], Scott E. Baker[3] and Mikael R. Andersen[1].
(1) Department of Bioengineering, Technical University of Denmark, Kgs. Lyngby, Denmark
(2) DOE Joint Genome Institute, Walnut Creek, CA, USA
(3) Pacific Northwest National Laboratory, Richland, WA, USA

A. oryzae is widely used in food fermentation for the production of soy sauce, sake and miso in addition to enzyme production and it has GRAS status. A close relative *A. flavus* on the other hand produces some highly toxic compounds such as aflatoxin and is an opportunistic pathogen. Both species belong to section *Flavi* consisting of at least 29 species[1].

In this study, we have whole genome-sequenced 19 novel *Flavi* species to examine the core of this section and the differences based on comparative genomics. The genomes reveal a highly diverse section with the number of predicted genes ranging from 9,078 to 14,216 in *A. coremiiformis* and *A. transmontanensi* respectively. We have identified 1,119 *Flavi* specific core protein families corresponding to approximately 9% of the proteome while the number of species specific protein families ranges from 395 for *A. nomius* NRRL 13137 to 2,219 for *A. leporis*.

Of particular interest is enzymes for degradation of carbohydrates, due to their essentiality both for food fermentation, plant pathogenicity, and biotechnology. Thus, the Carbohydrate-Active enZYmes (CAZY) potential was investigated ranging from 353 to 617 identified proteins belonging to a CAZy family for *A. coremiiformis* and *A. novoparasiticus* respectively.

In addition, we have investigated the secondary metabolite (SM) potential of this section since it is vital for food safety but also represents potential useful bioactive compounds. The total number of predicted SM clusters in the *Flavi* section is 1,527 constituting 283 cluster families with an average of 73 clusters per species. No SM gene cluster family is shared between the all the *Flavi* species however 106 unique cluster families are only found in one species. Overall this investigation paints a picture of a highly diverse section encompassing friends and foes.

[1] Varga *et al.* 2011 Studies in Mycology 69:57-80

***23. Activation of a silent gene cluster in *Aspergillus nidulans* through the development of a hybrid transcription factor**

Michelle F. Grau[a], Ruth Enwistle[b], Tomohiro Akashi[c], Richard B. Todd[d], Berl R. Oakley[b], Clay C. C. Wang[a,e]
[a] *Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, School of Pharmacy, Los Angeles, California, USA.*
[b] *Department of Molecular Biosciences, University of Kansas, Lawrence, Kansas, USA*
[c] *Division of OMICS analysis, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan*
[d] *Department of Plant Pathology, Kansas State University, Manhattan, Kansas, USA*
[e] *Department of Chemistry, University of Southern California, College of Letters, Arts, and Sciences, Los Angeles, California, USA.*

Many efforts have focused on activating silent gene clusters in fungal species since they are a rich source of structurally diverse compounds with medicinal and agricultural value. Fungal genome analyses have revealed that many species contain a surprisingly large number of secondary metabolite (SM) genes, the products of which are mostly unknown. In this investigation, we use a genetically engineered host strain of *Aspergillus nidulans,* developed in earlier work, which allows genes to be deleted or have their promoters replaced with relative ease. This genetic system has allowed us to express cryptic SM pathways in *A. nidulans* through promoter replacement. Then through deletion analysis, define the genes responsible for the synthesis of each SM. While most studies have focused on nonreducing polyketide synthase (NR-PKS) and non-ribosomal peptide synthetase pathways, this investigation highlights the discovery of a novel metabolite produced by one of the less-studied, highly reducing (HR)-PKS pathways. Preliminary work focused on generating an overexpression strain of the HR-PKS AN11191, and subsequent experiments determined the product released by AN11191 to be octatrienoic acid (OTA). Efforts to overexpress the entire AN11191 pathway through overexpression of the its cluster-specific transcription factor (TF), AN9221, proved to be unsuccessful, and prompted the design of a highly activated hybrid TF. This chimeric TF is characterized by the native DNA binding domain of AN9221 fused to the TF activation domain from the highly expressed pathway for asperfuranone. Heterologous expression of this hybrid TF in place of AN9221 resulted in the successful production of the SM derived from the AN11191 cluster. Gene deletion studies have allowed us to elucidate the biosynthetic pathway for this compound.

24. Spore heterogeneity of food spoilage fungi; *Aspergillus niger

Sjoerd J. Seekles[1], Tom van den Brule[2], Maarten Punt[3], Jan Dijksterhuis[2], Jos Houbraken[2], Arthur F. J. Ram[1], Han A. B. Wösten[3]
[1]Molecular Microbiology and Biotechnolody, Institute of Biology Leiden, Leiden, Zuid-Holland, Netherlands
[2]Applied and Industrial Mycology, Westerdijk Fungal Biodiversity Institute, Utrecht, Utrecht, Netherlands
[3]Microbiology, Department of Biology, Utrecht University, Utrecht, Utrecht, Netherlands
Presenting author is a PhD student.

Food production needs to increase by 70% to feed the world population in 2050. Reducing post-harvest food spoilage could significantly contribute to this challenge. At the moment, 25% of the food is lost due to spoilage, and a significant part due to fungal contamination. Industry aims prevent food spoilage by applying preservation techniques such as heat inactivation or salt inhibition. However, current methods are still incapable of fully controlling fungal food spoilage during minimal food processing. Therefore, more research is needed to investigate resistances of food spoilage fungi such as *Aspergillus niger*.
The filamentous fungus *Aspergillus niger* is a known food spoiler of coffee beans, fresh fruits and vegetables. Food spoilage by *Aspergillus niger* starts with contamination of food products with conidia. These dormant asexual reproduction structures are massively produced and naturally resilient to stressors and abundant in the environment, making contamination inevitable. A commonly used preservation method against spores is heat inactivation. However, experimental data strongly indicates the existence of subpopulations of conidia with different levels of resistance to heat stress. This heat resistant subpopulation makes targeted minimal food processing challenging.
In this work, we have studied impact of melanin on the heat resistance properties of *Aspergillus niger* conidia. As melanin deficient mutants do not show an altered heat sensitivity, we conclude that melanin is not involved in determining heat resistance. Additionally, we have studied the effect of spore maturation and show that young spores are very sensitive to heat stress. The heat sensitivity of young spores correlated with low concentrations of mannitol and trehalose in the spores, indicating that intracellular levels of trehalose and mannitol are important factors in determining heat resistance in conidia of *Aspergillus niger*.

25. Biosynthesis of histidine, arginine, riboflavin and pantothenic acid, but not siroheme, is crucial for virulence of *Aspergillus fumigatus

Anna-Maria Dietl[1], Nir Osherov[2], Hubertus Haas[1]
[1]Division of Molecular Biology/Biocenter, Medical University of Innsbruck, Austria; [2]Department of Clinical Microbiology and Immunology, Sackler School of Medicine, Tel Aviv, Israel

Aspergillus fumigatus is the most prevalent airborne fungal pathogen causing invasive fungal infections in immunosuppressed individuals. Limitations in antifungal therapy arise from non-specific symptoms of infection, poor diagnostics and comparatively few options for treatment. The aim of this study is to explore the metabolism of *A. fumigatus* on a comprehensive scale as essential virulence determinant to generate a collection of *A. fumigatus* strains with a focus on primary metabolism to target fungal pathways that are absent in mammals. Based on the annotated genome of *A. fumigatus*, metabolic network reconstruction served to identify fungal-specific pathways and key reactions. Predictions for unique enzymes resulted in a candidate list of genes, the inactivation of which is likely to result in an auxotrophic phenotype. The virulence potential of the generated auxotrophic mutant strains was then analyzed in various host niches. We identified five *A. fumigatus* pathways that are essential for growth in minimal medium: biosynthesis of the amino acids histidine and arginine, the vitamins riboflavin and pantothenic acid, and the heme-like prosthetic group siroheme, which is essential for sulfate and nitrate assimilation as well as nitric oxide detoxification. Inactivation of biosynthesis of histidine, arginine, riboflavin and pantothenic acid, but not siroheme, resulted in attenuated virulence of *A. fumigatus* in murine models for invasive aspergillosis with intranasal and systemic infection. The results characterize the availability of nutrients in the host niche and reveal targets for development of novel antifungal therapeutic approaches.

26. The Aspmine - comparative genomics analysis of 6 new species of Aspergillus section Sparsi, Ochraceorosei, Tanneri and Rubusti

Tammi Vesth [1], Jane Lind Nybo [1], Sebastian Theobald [1], Jens Frisvad [1], Ronald de Vries [4], Igor V. Grigoriev [3], Scott E. Baker [2], Ellen K. Lyhne [1], Martin E. Kogle [1], Asaf Salamov [3], Alan Kuo [3], Robert Riley [3], Matthieu Hainaut [5], Mikael R. Andersen [1]
1) Department of Bioengineering, Technical University of Denmark, Lyngby, Denmark;
2) Joint Bioenergy Institute, Berkeley, CA, USA
3) Joint Genome Institute, Walnut Creek, CA, USA
4) Fungal Physiology, CBS -KNAW Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands
5) Architecture et Fonction des Macromolécules Biologiques (AFMB), Marseille, France

In this work, we present the new whole genome sequences, functional annotation and comparative analysis of 6 filamentous fungi of the Aspergillus genus. The new species belong to the sections of Sparsi (3), Ochraceorosei (1), Tanneri (1) and Rubustii (1).
Species of section Sparsi, *A.* funiculosus, *A.* implicatus and *A.* biplanus are found in warm soil climates and produce antimicrobial compounds and toxins such as kojic acid, auraglaucin, gregatins, funicin and sidins. The section Ochraceorosei (suggested 2009) consists of *A.* ochraceoroseus and A. rambelii. *A.* ochraceoroseus, these species produce the mycotoxin aflatoxin B1.
The analysis presented here include genome sequence quality, secondary metabolism potential, carbohydrate degradation potential, shared proteomes and species as well as section specific genes. Comparisons were made to other filamentous fungi, Penicillium (3), Neurospora (1) and Aspergillus (49 species, 32 from section Nigri)
The species of Ochraceorosei have a much smaller number of predicted genes than the other species in the set (7.800-8.200). This is in comparison to some of the Nigri species with up to 18.000 genes. Section Sparsi species have a very wide range of predicted genes (9.000-15.000) while *A.* tanneri falls in the midrange (13.000). The large range of predicted genes illustrates the large diversity within these species.
Analyzing the CaZyme distribution of the 6 species revealed a diversity comparable to that of section Nigri. In the analysis of secondary metabolism, we find shared and conserved clusters within some sections while other sections have not associated clusters. Unique gene clusters are found in all the newly sequenced genomes, to the same extent as found in the Aspergilli in general.
The six new species provide additional information to the comparative genomics studies of Aspergillus and illustrate the large diversity and application of species in this genus.

27. A lariat branch point motif-interrupted spliceosomal twin intron in *Aspergillus nidulans*

Napsugár Kavalecz[1], Michel Flipphi[1], Norbert Ág[1], Levente Karaffa[1], Claudio Scazzocchio[2], **Erzsébet Fekete[1]**

1: Department of Biochemical Engineering, University of Debrecen, Debrecen, Hungary

2: Department of Microbiology, Imperial College, London SW7 2AZ, United Kingdom

In the primary transcript of nuclear genes, coding sequences – exons –alternate with non-coding sequences – introns. The latter are removed and former are joined to create the mRNA ORF that translates into the functional peptide product. Ubiquitous intron splicing provides a means of post-transcriptional regulation of expression by coupling alternative splicing with nonsense-mediated mRNA decay, hardily addressed in fungi. We use spliceosomal twin introns ("stwintrons") as model systems to study spliceosomal introns and their excision. Stwintrons are unconventional intervening sequences where a standard "internal" intron interrupts one of the three canonical splicing motifs of an "external" intron, and that consequently, can only be removed by consecutive splicing reactions. Previously, we have characterised stwintrons where the internal intron interrupts either the donor- or the acceptor sequence of the external intron (**). We have demonstrated that stwintrons can emerge by the appearance of a new intron within a pre-extant intron, consistent with mechanisms of intron gain from an endogenous origin. Here we present a new type of stwintron in which the internal intron is nested in the conserved sequence element around the lariat branch point adenosine of the external intron. This particular lariat branch point motif-interrupted stwintron is a recently evolved feature in *Aspergillus nidulans* and we show that it emerged by an alternative mechanism which involves intronisation of exonic sequences on either side of a pre-extant standard intron.

This work was supported by the EU and co-financed by the European Regional Development Fund [GINOP-2.3.2-15-2016-00008], and the Hungarian Scientific Research Fund [OTKA NN116519 to LK]

(**) Flipphi et al. (2013) Fungal Genet. Biol. 57:48; Ág et al. (2015) Fungal Genet. Biol. 85:7; Fekete et al. (2017) Nucleic Acids Res. 45:9085; Flipphi et al. (2017) Fungal Biol. Biotechnol. 4:7

28. Itaconic acid production from D-xylose by *Aspergillus terreus*

István S. Kolláth, Ákos P. Molnár, Erzsébet Fekete, and **Levente Karaffa**

Department of Biochemical Engineering, University of Debrecen, Debrecen, Hungary

Itaconic acid (2-methylenesuccinic acid; IA) is a five-carbon dicarboxylic acid, frequently used as a building block chemical for the synthesis of plastics, coatings and resins. IA is commercially produced by large-scale submerged fermentations employing the filamentous Ascomycete fungus *Aspergillus terreus* and using molasses or hydrolized corn starch as primary carbon sources. The objective of this study was to test whether IA can be produced on D-xylose in concentrations and specific yields ($Y_{p/s}$) similar to D-glucose by *A. terreus*.

Production of IA is the result of the metabolic overflow of primary metabolism. High ($Y_{p/s}$>0.8) molar yields on D-glucose require high (>10%, w/v) concentrations of carbon, strong aeration and carefully set cultivation parameters, of which Mn(II) ion limitation is the most prominent. When D-glucose was replaced with D-xylose under identical fermentation conditions, the plot depicting specific IA yield vs. initial carbon concentration was notably different. Maximum IA yield was significantly reduced ($Y_{p/s}$=0.55), but it was achieved at a relatively low (5%, w/v) initial D-xylose concentration. Any further increase above this level did not affect yield, which was, however, subject to severe Mn(II)-related regulation. Mn(II) ion concentrations as low as 5 ppb decreased IA yield on D-xylose by 15%. In contrast to the situation on D-glucose, IA yield did not drop below 0.3 on D-xylose even in the presence of 1000 ppb of Mn(II) ions. In conclusion, while it is possible to produce IA from D-xylose by *A. terreus*, the technology has to be improved considerably to be competitive with traditional glucose-based fermentations.

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 and by the Hungarian Scientific Research Fund (NN116519) to LK.

List of Registrants

Last name	First name	Affiliation	Email
Andersen	Mikael Rordam	Technical University of Denmark	mr@bio.dtu.dk
Arnau Martinez	Jose	Novozymes	joau@novozymes.com
Attias	Shani	Tel Aviv University	shaniattias@mail.tau.ac.il
Baerends	Richard	Novozymes A/S	rjb@novozymes.com
Barthel	Lars	Technische Universitt Berlinn	lars.barthel@tu-berlin.de
Binder	Ulrike	Medical University Innsbruck	ulrike.binder@i-med.ac.at
Blachowicz	Adriana	University of Southern California	blachowi@usc.edu
Braus	Gerhard	Georg-August-Universitaet	gbraus@gwdg.de
Bromley	Michael	University of Manchester	mike.bromley@manchester.ac.uk
Bruno	Kenneth	Zymergen	bruno@zymergen.com
Cairns	Timothy	Technical University Berlin	t.cairns@tu-berlin.de
Canovas	David	University of Sevilla	davidc@us.es
Diallinas	George	National and Kapodistrian University of Athens	diallina@biol.uoa.gr
Dietl	Anna Maria	Medical University of Innsbruck	anna-maria.dietl@i-med.ac.at
Dzikowska	Agnieszka	University of Warsaw	adzik@igib.uw.edu.pl
Ellena	Valeria	ACIB GmbH	valeria.ellena@boku.ac.at
Fekete	Erzsebet	University of Debrecen	kicsizsoka@yahoo.com
Frandsen	Rasmus	Technical University of Denmark	rasf@bio.dtu.dk
Grau	Michelle	University of Southern California	mgrau@usc.edu
Haarmann	Thomas	AB Enzymes	thomas.haarmann@abenzymes.com
Han	Kap-Hoon	Woosuk University	khhan@woosuk.ac.kr
Inoue	Taishi	Tohoku University	t.inoue.a812@gmail.com
Jaeger	Nils	Friedrich-Schiller University Jena, Center for Molecular Medicine	nils.jaeger@uni-jena.de
Jarczynska	Zofia Dorota	Technical University of Denmark	zofja@bio.dtu.dk
Jun	Sang-Cheol	Woosuk University	pekkman@hotmail.com
Karaffa	Levente	University of Debrecen	dr.kicsizsoka@gmail.com
Katz	Margaret	University of New England	margaret.katz@bigpond.com
Kim	Jong-Hwa	Woosuk University	jhkim@woosuk.ac.kr
Kjaerboelling	Inge	Technical University of Denmark	ingek@bio.dtu.dk
Kroes	Wouter	DSM	wouter.kroes@dsm.com
Lehmbeck	Jan	Novozymes	jal@novozymes.com
Liao	Yien	University of Southern California	yienliao@usc.edu
Lu	Ling	Nanjing Normal University	linglu@njnu.edu.cn
Maiyuran	Suchindra	Novozymes Inc	smai@novozymes.com
Masuo	Shunsuke	University of Tsukuba	ma_shun7775@hotmail.com
Meir	Zohar	Tel Aviv University	lightenzm@gmail.com
Misslinger	Matthias	Medical University of Innsbruck	matthias.misslinger@i-med.ac.at
Momany	Michelle	University of Georgia	mmomany@uga.edu
Mortensen	Uffe	Technical University of Denmark	um@bio.dtu.dk
Muzzi	Gloria	Novozymes	GMER@novozymes.com

Last name	First name	Affiliation	Email
Nai	Corrado	Technische Universitt Berlinn	corrado.nai@tu-berlin.de
Netzker	Tina	Hans Knoell Institute (HKI)	Tina.Netzker@leibniz-hki.de
Norio	Takeshita	University of Tsukuba	takeshita.norio.gf@u.tsukuba.ac.jp
Nybo	Jane Lind	Technical University of Denmark	jlnr@bio.dtu.dk
Osherov	Nir	Tel Aviv University	nosherov@post.tau.ac.il
Partosh	Tamir	Tel Aviv University	tamirpartosh@gmail.com
Punt	Peter	Dutch DNA Biotech	peter.punt@ddna-biotech.com
Ram	Arthur	Leiden University Institute of Biology	a.f.j.ram@biology.leidenuniv.nl
Reijngoud	Jos	Institute of Biology Leiden	j.reijngoud@biology.leidenuniv.nl
Ries	Laure Nicolas Annick	University of Sao Paulo	rieslaure13@gmail.com
Robeck	Logan	Concordia University	loganrobeck@gmail.com
Romsdahl	Jillian	University of Southern California	romsdahl@usc.edu
Sandovsky	Hana	Tel Aviv University	hana.sandovsky5@gmail.com
Scheven	Mareike	Hans Knoell Institute	mareike.scheven@leibniz-hki.de
Seekles	Sjoerd	Institute of Biology Leiden	s.j.seekles@biology.leidenuniv.nl
Segers	Frank	Westerdijk Fungal Biodiversity Institute	f.segers@westerdijkinstitute.nl
Segers	Sylvia	Dutch DNA Biotech	peter.punt@ddna-biotech.com
Shadkhan	Yona	Tel Aviv University	yanka@post.tau.ac.il
Song	Letian	Concordia University	letian.song@concordia.ca
Stroe	Maria Cristina	Leibniz Institute for Natural Product Research - HKI	maria.stroe10@outlook.com
Szewczyk	Edyta	Zymergen	eszewczyk@zymergen.com
Terauchi	Yuki	Tohoku University	yuki.terauchi.s8@dc.tohoku.ac.jp
Todd	Richard	Kansas State University	rbtodd@ksu.edu
Udagawa	Hiroaki	Novozymes Japan	huda@novozymes.com
Vesth	Tammi	Technical University of Denmark	tcve@bio.dtu.dk
Vind	Jesper	Novozymes	jvi@novozymes.com
Vollebregt	Aad	DSM	aad.vollebregt@dsm.com
Wanka	Franziska	TU Wien c/o ACIB	franziska.wanka@acib.at
Wolff	Peter	DTU Bioengineering	pewol@dtu.dk
Zhai	Pengfei	Nanjing Normal University	302547367@qq.com
Zhou	Xiaogang	Nanjing Normal University	929002698@qq.com
Zhu	Bo-Han	Woosuk University	allen9055@163.com