

The 7th International *Aspergillus* Meeting

“Asperfest 7”

**NH Conference Centre
Leeuwenhorst, The Netherlands**

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

2009 Aspergillus Genomes Research Policy Committee

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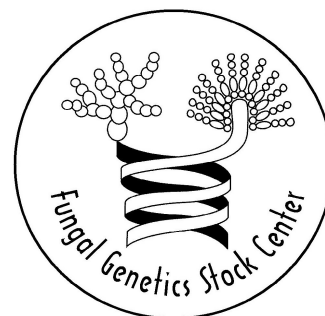
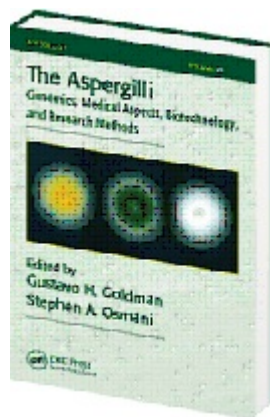
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THANKS TO OUR MEETING SPONSORS!!!



**Gustavo H. Goldman
and
Stephen A. Osmani**

The 7th International *Aspergillus* Meeting
“Asperfest 7”
NH Conference Centre
Leeuwenhorst, The Netherlands

March 28, 2010 (Sunday)

19:00 -21:00 **Posters and Welcome Reception Sponsored by Novozymes**

March 29 (Monday)

9:00 - Welcome, introductions and announcements Michelle Momany

Session I: New Labs Masa Machida

9:20 - David Canovas, University of Seville, Spain
Tracing the activation path of conidiation by light

9:40 - Ronald P. deVries, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands
Fungal physiology and biodiversity in relation to the utilisation of natural carbon sources

10:00 - Samara Reck-Peterson, Harvard Medical School, USA
Towards a Global Analysis of Microtubule-Based Transport in *Aspergillus nidulans*

10:20 - *Coffee Break*

Session II: Genomics updates Jennifer Wortman

10:40 - Colin DeSouza, Ohio State University, USA
A. nidulans deletion cassette project

10:55 - Mark Caddick, University of Liverpool, UK
High throughput sequencing of the *A. nidulans* transcriptome

11:10 - Kenneth Bruno, Pacific Northwest National Laboratory, USA
Tools and resources for *A. niger* at PNNL

11:25 - Jane Mabey Gilsenan, University of Manchester, UK
CADRE and Ensembl Fungi update

11:40 – Martha Arnaud, Stanford University, USA
AspGD: tools for exploration of *Aspergillus* gene, protein, and sequence information

11:55 - Jennifer Wortman on behalf of Bill Nierman and Natalie Fedorova, JCVI, USA
NIAID Genome Sequencing Center project for *A. fumigatus*

12:20 - *Lunch*

14:00 - Community directions discussion; Election Michelle Momany

Session III: Talks from abstracts

Arthur Ram

14:20 - Hideaki Koike, National Institute of Advanced Industrial Science and Technology, Japan
Engineering intracellular metabolism by altering gene expression of *Aspergillus oryzae*

14:35 - Vera Meyer, Leiden University, The Netherlands
Fine-tuning gene expression in filamentous fungi: An inducible and tunable promoter system for *Aspergillus niger*

14:50 - Özgür Bayram, Georg-August-University, Germany
Dynamic and functional interactions between the components of the trimeric velvet complex in the filamentous fungus *Aspergillus nidulans*

15:05 *Coffee Break*

15:25 -16:10 - **Pontecorvo Lecture Sponsored by AlerGenetica** Gerhard Braus

Geoff Turner, University of Sheffield, UK

Dreaming up excuses for *Aspergillus*: protoplasts, penicillin, polarity and pathogens

16:10-16:45 Election results; poster prizes; other discussion items

16:45 End of Asperfest 7

18:00 ECFG10 Keynote and opening reception

Poster Abstracts

1) A Glutathione S-Transferase, GliG, May Mediate Thiol Incorporation in Gliotoxin Biosynthesis And Is Not Involved in Auto-Protection Against Gliotoxin

Carol Davis^{1*}, Stephen Carberry¹, Markus Schrettl¹, Dermot Brougham³, Kevin Kavanagh¹, John Stephens² and Sean Doyle¹.
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Biosynthesis of gliotoxin is directed by the multi-gene (*gli*) cluster in the opportunistic fungal pathogen, *Aspergillus fumigatus*. Minimal functional cluster annotation is available. The gene *gliG*, located in the *gli* cluster, is classified as a glutathione *s*-transferase by *in silico* analysis and recombinant GliG exhibits GST and glutathione reductase activity. Two overlapping constructs, each containing part of a marker gene (*ptrA*) and with homology to *gliG* flanking regions, were used to disrupt *gliG* in *A. fumigatus* (Δ *aku80* and *Af293* strains). The generation of a *gliG* mutant was confirmed using Southern Blot analysis using a digoxigenin-labelled probe specific for an *XbaI* digested fragment size of 2124 bp in the wild-type and 1668 bp in the *gliG* mutant. Absence of *gliG* expression in the mutant was confirmed by Northern analysis. RP-HPLC-DAD and LC-MS analysis of extracts from *A. fumigatus* wild-type and Δ *gliG* revealed that gliotoxin (Rt= 14.4 min) was absent from the mutant strains, strongly indicating that *gliG* is involved in gliotoxin biosynthesis. Interestingly, an additional metabolite (Rt = 12.3 min) was present in mutant culture supernatants which may represent a precursor of gliotoxin (GT_p). LC-ToF analysis determined that the metabolic intermediate had a mass of 263 Da and targeted alkylation demonstrated the lack both free thiol residues and an intact disulphide bridge. Reconstitution of *gliG* into *A. fumigatus* Δ *gliG* restored gliotoxin biosynthesis. Unlike another component of the *gli* cluster, *gliA*, it appears that *gliG* is not involved in the auto-protection of *A. fumigatus* against exogenous gliotoxin. In conclusion, we confirm a key role for the glutathione *s*-transferase, GliG, in the biosynthesis of, and not auto-protection against, gliotoxin- which, to our knowledge, is the first time this enzyme has been shown to play a pivotal function in ETP biosynthesis.

2) Engineering intracellular metabolism by altering gene expression of *Aspergillus oryzae*

Junichiro Marui¹, Sumiko Ohashi¹, Marie Nishimura², Hideaki Koike¹ and Masayuki Machida¹ 1 Natl. Inst. Adv. Indus. Sci. Tech. (AIST), 2 Natl. Inst. Agrobiol. Sci.

Aspergillus oryzae is one of the most important organisms in Japanese fermented food industry. Although it hardly produces secondary metabolites, related organisms are producers of diverse metabolites. A major objective of our project is to develop a system using *A. oryzae* to generate diverse metabolites. The factors affecting expression level of metabolic genes are being studied. To develop the system, novel vectors and host strains of *A. oryzae* have been constructed. We have replaced promoter regions of some metabolic genes and successfully altered the level of some metabolite productions. Although it is well known that *A. oryzae* does not produce toxic metabolites, less attention has been paid to its non-toxic secondary metabolites. Genomic analysis revealed that *A. oryzae* possessed the orthologous gene cluster for penicillin production. The penicillin production was positively regulated by a global gene regulator required for transcriptional expression of the penicillin biosynthetic genes. Overexpression of the biosynthetic genes by a strong promoter yielded a greater than 100-fold increase in penicillin production. Transcriptional repression of a wide range of secondary metabolism genes in *A. oryzae* is a valuable characteristic for the production of a particular secondary metabolite with higher purity and safety. It appears that genetically engineered *A. oryzae* should be extremely useful as a cell factory for industrial production of beneficial secondary metabolites.

3) Non-Ribosomal Peptides play an Important Role in the Virulence of the Opportunistic Pathogen *Aspergillus fumigatus*
K. O'Hanlon¹, D. Stack¹, M. Schrettel², T. Larsen³, K. Kavanagh¹ and S. Doyle¹ National Institute for Cellular Biotechnology, Department of Biology, National University of Ireland, Maynooth, Co. Kildare, Ireland² Division of Molecular Biology, Innsbruck Medical University, A-6020 Innsbruck, Austria³ Center for Microbial Biotechnology, Department of Systems Biology, Technical University of Denmark, Søtofts Plads 221, 2800 Kgs. Lyngby, Denmark karen.a.ohanlon@nuim.ie

Aspergillus fumigatus is a ubiquitous filamentous fungus, and a serious opportunistic human pathogen. Availability of the complete genome sequence for *A. fumigatus* has revealed that there are at least eighteen genes coding for non-ribosomal peptide synthetases (NRPS). NRPS's are usually large, multi-modular enzymes, comprised of discrete domains, which synthesise bioactive peptides via a thio-template mechanism. To date, a wide range of virulence factors have been reported for *A. fumigatus*, including adhesions, conidial pigments and proteases. Some of the best documented virulence factors for *A. fumigatus* include Gliotoxin and the iron-chelating Siderophores, which are of NRPS origin. Despite these important findings, there have been few studies relating the majority of *A. fumigatus* NRPS encoding genes to specific peptide products. This work aims to elucidate the peptide product encoded by a mono-modular NRPS, *pesL* (Afu6g12050/NRPS11), and to determine a possible role in virulence. A *pesL* deletion strain was generated, termed $\Delta pesL$. $\Delta pesL$ displays severely reduced virulence in the *Galleria mellonella* model ($p < 0.0001$). Phenotypic analysis has confirmed increased sensitivity of $\Delta pesL$ to H_2O_2 (> 1 mM) compared to the wild-type ($p = 0.05$), and severely increased susceptibility towards the antifungal voriconazole (> 0.25 μ g/ml) compared to wild-type ($p < 0.01$). These results indicate a role for *pesL* in protection against oxidative and antifungal stress within *A. fumigatus*. Comparative RP-HPLC analysis identified conidial specific material ($R_t = 15.9$ min; λ_{max} at 220 nm) synthesised by *A. fumigatus* wild-type. This metabolite was absent from $\Delta pesL$ conidia. Increased production of this metabolite was observed in conidial extracts cultured in 2 mM H_2O_2 , indicating up-regulation in response to oxidative stress. This material is currently undergoing further analysis. Furthermore, a recombinant PesL enzyme has been purified for use in an assay to determine the specific PesL amino acid substrate. This will contribute to the currently limited information on fungal NRPS substrate selectivity. Interestingly, another NRPS mutant generated previously, termed $\Delta pes3$ ($\Delta Afu5g12030/\Delta NRPS8$) displays increased virulence in the *Galleria mellonella* model ($p < 0.0001$). Furthermore, $\Delta pes3$ exhibited severely increased susceptibility towards the antifungal voriconazole (> 0.5 μ g/ml) compared to wild-type ($p < 0.001$). RP-HPLC has not yet revealed a candidate *pes3* peptide. However, the search is on-going. This data further highlights the importance that NRPS plays in this serious human pathogen, and may reveal novel drug targets in the future.

4) The master regulator of the Unfolded Protein Response revisited

N.D.S.P. Carvalho, T.R. Jørgensen, M. Arentshorst, C.A.M.J.J van den Hondel and A.F.J. Ram Institute of Biology Leiden, Leiden University, Molecular Microbiology and Biotechnology, Kluyver Centre for Genomics of Industrial Fermentation, Sylviusweg 72, 2333 BE Leiden, The Netherlands. n.carvalho@biology.leidenuniv.nl

In *Aspergillus niger*, the beta-zip transcription factor HacA plays a central role in the activation of genes involved in the UPR pathway. Activation of HacA is mediated via an unconventional splicing event of 20 nucleotides in its mRNA. In the present study we engineered an *A. niger* strain that expresses only the activated form of HacA and we show that the removal of the 20 nucleotides results in a constitutive activation of the well established UPR target genes. The wild type strain (HacAWT) and the strain expressing the constitutive active form of HacA (HacACA) were cultured in glucose-limited batch cultures using bioreactors. RNA from the batch cultures was isolated for transcriptomic analysis of the effect of expressing a constitutive activated HacA. Statistical analysis defined 1119 genes as differentially expressed (significance $p < 0.005$) relative to the wt. GO enrichment analysis revealed that the expression of several secretion and protein modification related genes was up-regulated in the HacACA mutant. Biological processes overrepresented in the down-regulated genes include several metabolic pathways, and terms related to transcription and translation. A comprehensive overview of the transcriptional response in the HacACA mutant will be presented.

5) Mutations in two Golgi apparatus COG proteins affect growth and glycosylation in *Aspergillus nidulans*

Sara Gremillion¹, Darlene Loprete², and Terry Hill². ¹Department of Biology, Armstrong Atlantic State University, 11935 Abercorn Street, Savannah, GA 31419, USA, ²Departments of Biology and Chemistry, Rhodes College, 2000 N. Parkway, Memphis, TN 38112, USA. Sara.Gremillion@armstrong.edu

The *swoP1* (swollen cell) and *podB1* (polarity defective) mutations in *Aspergillus nidulans* interfere with establishment and maintenance of polarity. At restrictive temperatures, conidia of both mutants swell to approximately 1.5 times the normal diameter. Conidia of *swoP* also produce abnormally wide hyphae and establish multiple points of polarity, which grow isotropically before arrest. Genes complementing the mutations of *swoP1* and *podB1* have strong sequence homology to COG4 (ANID7462.1) and COG2 (ANID8226.1), respectively. In mammals and yeast, COG2 and COG4 are part of a multi-protein structure called the COG (conserved oligomeric Golgi) complex associated with retrograde transport within the Golgi apparatus. A GFP-tagged COG2 displayed a punctuate distribution within fungal hyphae, a pattern consistent with other Golgi protein localization. COG4 was not successfully GFP tagged. Protein overexpression studies provided evidence of intra-complex interactions between COG2 and COG4 as well as between COG2 and COG3. To study the role of these proteins in growth of filamentous fungi, an *AlcA* promoter replacement strategy was performed. When grown on *AlcA*-suppressive media, the COG4 *AlcA*-replaced promoter strain displayed over-swelling and a lack of polarity, while the COG2 *AlcA*-replaced promoter strain failed to grow. These results link both COG2 and COG4 to normal filamentous growth. A lectin blot using concanavalin A revealed significant differences in protein glycosylation patterns between the *swoP1* and *podB1* mutants when compared to wild type when grown at restrictive temperatures. The glycosylation patterns of the two mutants were indistinguishable under these conditions.

6) Signaling through the *Aspergillus nidulans* orthologue of Pkc mediates septum formation

Loretta Jackson-Hayes, Terry W. Hill, Darlene M. Loprete, Britany Chavez, Chassidy Groover, Erinn Ogburn, and Michael Pluta

We have shown that the *Aspergillus nidulans* orthologue of protein kinase C (PkcA) participates in regulating cell wall integrity (CWI) and localizes to sites of cell wall synthesis, including growing hyphal tips and septa. To better understand the mechanisms by which PkcA localizes to tips and septa, we have observed the formation of cortical rings at sites of septation by fluorescently tagged PkcA in hyphae defective in expression of other proteins necessary for septum formation, using either temperature-sensitive mutants or regulation under the *AlcA* promoter. We have also co-imaged PkcA and other septation proteins bearing complementary tags. Here we report that localization of PkcA to septa lies “downstream” of the functions performed by MobA (Mob1p orthologue), TpmA (tropomyosin), SepA (formin), SepD, SepG, and proteins encoded by two other not-yet-cloned *Sep* loci. In the absence of function of these proteins, PkcA cortical rings were not observed. PkcA localization lies “upstream” of MyoB (myosin II orthologue), the *A. nidulans* orthologue of Bud4p (in yeast, a bud site selection marker), and a protein encoded by a third uncloned *Sep* locus. PkcA cortical rings still form in the absence of function of these proteins, though septa do not develop. SepA, TpmA, MyoB, and MobA all appear to colocalize with PkcA during normal septum formation. While PkcA localizes to the very apex of hyphal tips and to the leading edge of growing septa, the protein phosphatase BimG localizes to sites lateral to the most active sites of growth. Studies with other septation-related proteins are ongoing.

7) Withdrawn

8) ColA, a white spore color mutant in *Aspergillus niger*, identifies the phosphopantetheinyl transferase (PptA) protein which is required for melanin biosynthesis

Joohae Park, Thomas R. Jørgensen, Mark Arentshorst, Patricia A. vanKuyk, Robbert A. Damveld, C.A.M.J.J. van den Hondel, Arthur F.J. Ram *Institute of Biology Leiden, Leiden University, Molecular Microbiology and Biotechnology, Kluyver Centre for Genomics of Industrial Fermentation, Sylviusweg 72, 2333 BE, Leiden, The Netherlands*

A characteristic hallmark of *Aspergillus niger* is the formation of black conidiospores. In this study, we report the characterization of a color mutant, *colA*, which was isolated because of a complete loss of pigmentation resulting in white conidia. Pigmentation of the *colA* mutant was restored by a gene that encodes the *A. niger* ortholog of the 4'-phosphopantetheinyl transferase protein (PptA). 4'-phosphopantetheinyl transferase activity is required for the activation of Polyketide synthases (PKSs) and Non-Ribosomal Peptide Synthases (NRPSs). Complementation analysis showed that the *colA* mutant is allelic to a previously isolated color mutant, *gryA*. Sequencing of the *colA* and *gryA* loci and the targeted deletion of the *pptA* gene further confirmed that the *colA* /*gryA* mutants are mutated in the *pptA* gene. Spores from the *pptA* deletion are paler in color than spores of an *A. niger* strain disrupted in the *pksA* gene. PksA encodes the polyketide synthase required for melanin biosynthesis and spores from the *pksA* disruption strain become fawn colored. Spores from both the *pksA* and the *pptA* were hypersensitive to UV-radiation indicating that melanin is required for resistance against UV-radiation. The *pksA* strain was equally sensitive to hydrogen peroxide as the parental strain, but spores from the *pptA* strain showed increased sensitivity. The results suggest the involvement of PKS or NRPS-derived metabolites that confer resistance towards

9) Identification of Cell Wall Factor(s) Adsorbing Taka-amylase in Submerged Culture of *Aspergillus oryzae*

Hiroki Sato, Takahiro Shintani, Katsuya Gomi Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan

We have observed that Taka-amylase (TAA) activity disappeared in submerged culture of *Aspergillus oryzae* at the later-stage of cultivation. This disappearance was revealed to be caused by adsorption of TAA on fungal mycelia. We have also showed that cell wall of *A. oryzae* prepared from mycelia at the later-stage of cultivation has an adsorption ability for TAA. This suggested that a certain cell wall factor(s) can adsorb TAA, resulting in the disappearance of TAA in liquid medium during cultivation. To identify the adsorption factor(s) in fungal cell wall, we carried out stepwise fractionation of cell wall prepared from mycelia at the later cultivation stage by alkali extraction and cell wall lytic enzymes. The alkali-insoluble fraction of cell wall, CW4, showed high adsorption ability for TAA, but digestion of CW4 with chitinase resulted in a significant decrease in the adsorption ability. These results indicated that the adsorption factor for TAA is chitin, which is one of major polysaccharides in fungal cell wall. However, the cell wall prepared from mycelia at the earlier cultivation stage barely adsorbed TAA, although it contained equivalent amount of chitin to that of later-stage mycelia. Taken together, it is suggested that there exists unidentified factor(s) that could prevent from adsorption of TAA onto the cell wall at the earlier-stage of cultivation and the factor(s) would be removed from or decreased in the cell wall with longer cultivation periods.

10) Essentiality of RNA exosome subunit encoding genes in *Aspergillus oryzae*

Mizuki Tanaka, Takahiro Shintani, and Katsuya Gomi Grad. Sch. Agric. Sci., Tohoku Univ., Japan

The exosome is a multi-subunit 3'-5' exonucleolytic complex that is conserved in eukaryotes. The ring-shaped core structure of the exosome is constituted of nine subunits. In yeast, all of nine exosome subunits are essential for viability. On the other hand, in plant, Csl4 is dispensable for growth and development, whereas Rrp41 and Rrp4 are essential for the development of female gametophytes and embryogenesis. Since there has been no report on the exosome itself in filamentous fungi, we attempted to construct the disruptants of genes encoding exosome subunits in *Aspergillus oryzae*. We have chosen two orthologous genes for *csl4* and *rrp4* as targets for disruption, and successfully obtained a *csl4* disruptant but not an *rrp4* disruptant. The disruption of *csl4* gene had no apparent defect on growth in *A. oryzae*. Since the *rrp4* disruptant could not be obtained, this gene would be essential for cell viability. Thus, we generated the conditional *rrp4* expression strain by using the promoter of *nmtA*, expression level of which is regulated by riboswitch existed within its 5'-untranslated region and is repressed considerably in the presence of thiamine. The resultant conditional *rrp4* expression strain displayed a remarkable growth defect when thiamine was added to the medium. These results suggested that Rrp4 is essential but Csl4 is not for cell growth in *A. oryzae* and that function of individual exosome components in *A. oryzae* is similar to that in plant.

11) Chitinases of *Aspergillus niger* upregulated during autolysis

Jolanda van Munster¹, Benjamin Nitsche², Rachel van der Kaaij³, Arthur Ram², Lubbert Dijkhuizen¹, Marc van der Maarel¹.
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The filamentous fungus *Aspergillus niger* is well known for its capacity to secrete high amounts of proteins and metabolites, and is therefore used in industry for the production of enzymes and chemicals. The mycelium of this fungus is highly differentiated. After stationary growth phase, part of the mycelium is degraded in a process called autolysis. Autolysis is characterized by hyphal fragmentation, loss of biomass, ammonia release and the production of enzymes such as proteases and glycoside hydrolases. These glycoside hydrolases could function in degradation of cell wall polymers such as chitin. However, knowledge about the exact mechanism of autolysis is currently limited. During industrial fermentation processes, autolysis can cause the productive biomass to decrease, causing reduced product yield. A better understanding of autolysis can contribute to the formation of strategies to increase efficiency of fermentations. In order to increase understanding of the dynamics of the fungal mycelium, a consortium of academic and industrial partners investigates autolysis and differentiation in *Aspergillus niger*. One goal of this project is the identification and characterization of glycoside hydrolases that are involved in autolysis. By using microarrays to monitor transcription levels during growth, we have identified genes that are upregulated during the autolytic phase compared to exponential growth phase. Four of these genes belong to glycoside hydrolyse family 18, which consists mainly of (putative) chitinases. In order to investigate the properties of these enzymes we performed heterologous gene expression in *E. coli* with subsequent purification using affinity tags. The activity of purified proteins is investigated.

12) Tools for Exploration of *Aspergillus* Gene, Protein, and Sequence Information at the *Aspergillus* Genome Database (AspGD)

Martha B. Arnaud¹, Jonathan Binkley¹, Marcus C. Chibucos², Maria C. Costanzo¹, Jonathan Crabtree², Diane O. Inglis¹, Joshua Orvis², Prachi Shah¹, Marek S. Skrzypek¹, Gail Binkley¹, Stuart R. Miyasato¹, Jennifer R. Wortman² and Gavin Sherlock¹ Department of Genetics, Stanford University School of Medicine, Stanford, CA ²Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD

The *Aspergillus* Genome Database (AspGD) is an online genomic resource for *Aspergillus* genomics and molecular biology, with information curated from the literature and web-based research tools for exploration and analysis of these data. The Sybil Comparative Genomics tool displays alignments of the genomic regions encoding clusters of homologous proteins and syntenic regions from *A. nidulans*, *A. fumigatus*, *A. flavus*, *A. oryzae*, *A. niger*, *A. clavatus*, *A. terreus*, and *Neosartorya fischeri*. The GBrowse Genome Browser supports navigation and searching of genes and chromosomal regions of the *A. nidulans* FGSC A4 and *A. fumigatus* Af293 genomes, and will be extended to other *Aspergilli* in the future. Additional tools are available for search, retrieval, analysis, and download of *A. nidulans* sequence and curated gene and protein information. While AspGD curation has initially focused on *A. nidulans*, we will begin curation of the scientific literature on *A. fumigatus* and other *Aspergillus* species in 2010 and will provide the full suite of AspGD tools for each of these species in the future. We also provide tools for community interaction, including a colleague registry and a list of *Aspergillus* research laboratories. Our mission is to be responsive to the needs of the research community, and we welcome your feedback and suggestions, at aspergillus-curator@genome.stanford.edu. All of the data in AspGD are freely available to the public from <http://www.aspgd.org/>. AspGD is supported by grant RO1 AI077599 from the NIAID at the NIH.

13) High throughput sequencing of the *A. nidulans* transcriptome

Mark Caddick, Christopher Sibthorp, Huihai Wu, Neil Hall, Prudence Wong. School of Biological Sciences, The University of Liverpool, Crown Street, Liverpool L69 7ZB, UK.

Next generation sequencing is transforming the way in which we can analyse the transcriptome. It potentially provides quantitative information which is superior to microarray data, whilst also providing information about differential use of promoters, splicing and transcript 3' ends. We are applying this approach to *A. nidulans*. In the first instance we have specifically investigated transcript start site location, by sequencing mRNA 5' ends. This has provided genome wide data with over ~3500 transcript start sites being localised to within two nucleotide and over 7000 localised to within four nucleotides. From this we have been able to investigate DNA motifs and features associated with transcription start sites. We will present these data and current work focused on characterising the full transcriptome. Our aim is to provide a community resource that will significantly improve genome annotation and greatly increase our understanding of its flexibility and regulation.

14) Comparison of the polysaccharide degrading ability of 8 *Aspergilli*

Isabelle Benoit(1), Pedro Coutinho(2), Hala Al-Bushari(1), Guillermo Aguilar(1), Evy Battaglia(1) Birgit Gruben(1), Blanca Trejo-Aguilar(1), Ad Wiebenga(3), Bernard Henrissat(2), Ronald de Vries(1,3). (1)Microbiology, Utrecht University; (2)AFMB, Univ. Aix-Marseille I & II ; (3)CBS-KNAW Fungal Biodiversity Centre. r.devries@cbs.knaw.nl.

Plant polysaccharides are among the most prominent carbon sources for fungi, which degrade these substrates through the production of diverse sets of extracellular enzymes. This topic has been best studied in the *Aspergilli*, in particular in *A. niger* and *A. oryzae* due to the many industrial application of plant polysaccharide degrading enzymes and the good fermentative properties of these fungi. The availability of genome sequences for several *Aspergilli* has allowed detailed comparisons between these species and enables us to identify differences in the strategies that they employ to release their carbon source from crude plant biomass. CAZy-annotation of the genomes revealed significant differences in the sets of hydrolytic enzymes and growth profiling of these fungi demonstrated strong correlations between genome content and ability to degrade specific polysaccharides. In addition, analysis of the secreted enzymes demonstrated further differences that are likely caused by differences in transcriptional or post-transcriptional regulation. Highlights from these results will be presented. These data will help with the further development of improved enzyme cocktails as they enable a link between the composition of the enzyme set and the efficiency with which different polysaccharides are degraded.

15) Two GATA factors AREA and AREB negatively regulate arginine catabolism genes of *Aspergillus nidulans* in response to nitrogen and carbon source

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In *A. nidulans* arginine is utilised as a nitrogen and carbon source and it is a good system for investigation the connection between the two global carbon and nitrogen repression regulatory systems. Utilization of arginine depends on the presence and inducibility of two arginine catabolic enzymes arginase and ornithine aminotransferase (OAT) encoded by *agaA* and *otaA* genes, respectively. Analysis of different single and double *areA* and *areB* mutants have shown that two GATA factors AREA and AREB negatively regulate the expression of arginine catabolism genes under nitrogen repression conditions. AREA and AREB activities depend on carbon source. AREA regulator is necessary for the ammonium repression of arginine catabolism genes under carbon repressing conditions while AREB - under carbon limited, non-repressing conditions. AREA activity was shown to be modulated by a direct protein – protein interaction with NMRA protein which is proposed to bind to the C terminus of AREA and repress its activity in the presence of glutamine (Platt et al., 1996; Andrianopoulos et al., 1998; Lamb 2003 et al., Lamb et al., 2004; Wong et al., 2007). We have shown that these interactions are also important in nitrogen metabolite repression of arginine catabolism genes.

16) Curation of *Aspergillus* gene and protein information at the *Aspergillus* Genome Database (AspGD)

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The *Aspergillus* Genome Database (www.aspgd.org) is a web-based genomics resource for researchers studying the genetics and molecular biology of an important group of fungal microorganisms, the aspergilli. AspGD provides high-quality manual curation of the experimental scientific literature, including gene names, general descriptions, phenotypes, and Gene Ontology (GO) annotations, as well as tools for exploring these data. The curated information for each gene appears on its Locus Summary page with links to details pages that provide additional information, including phenotype and GO details, sequence and annotation history and a comprehensive list of references. Initially, we have focused on the manual curation of genomic information for *Aspergillus nidulans*, the best-characterized species of the group. In the future, we will expand our efforts to include curation of *A. fumigatus*, *A. flavus*, *A. oryzae*, *A. niger*, *A. clavatus*, *A. terreus*, and *Neosartorya fischeri* genomes. AspGD is supported by grant RO1 AI077599 from the NIAID at the NIH.

17) Signaling through the *Aspergillus nidulans* orthologue of Pkc mediates septum formation

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We have shown that the *Aspergillus nidulans* orthologue of protein kinase C (PkcA) participates in regulating cell wall integrity (CWI) and localizes to sites of cell wall synthesis. To better understand such localization, we have observed the formation of cortical rings at sites of septation by fluorescently tagged PkcA in hyphae defective in expression of other proteins necessary for septum formation, using either temperature-sensitive mutants or regulation under the *AlcA* promoter. We have also co-imaged PkcA and other septation proteins bearing complementary tags. Here we report that localization of PkcA to septa lies "downstream" of the functions performed by MobA (Mob1p orthologue), TpmA (tropomyosin), SepA (formin), SepD, SepG, and proteins encoded by two other not-yet-cloned *Sep* loci. In the absence of function of these proteins, PkcA cortical rings were not observed. PkcA localization lies "upstream" of MyoB (myosin II orthologue), the *A. nidulans* orthologue of Bud4p (in yeast, a bud site selection marker), and a protein encoded by a third uncloned *Sep* locus. PkcA cortical rings still form in the absence of function of these proteins, though septa do not develop. SepA, TpmA, MyoB, and MobA all appear to colocalize with PkcA during normal septum formation. While PkcA localizes to the very apex of hyphal tips and to the leading edge of growing septa, the protein phosphatase BimG localizes to sites lateral to the most active sites of growth. Studies with other septation-related proteins are ongoing.

18) RRMA, an RNA binding protein involved in regulated mRNA degradation

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RRMA is the RNA binding protein involved in posttranscriptional regulation of gene expression in *Aspergillus nidulans*. *rrmA* gene was identified as a suppressor of mutations in arginine/proline catabolic pathway. Independently RRMA protein was shown to bind to the 3'UTR of *areA* transcript (nitrogen positive regulator). Δ *rrmA* mutation results in slow growth phenotype and higher sensitivity to oxidative stress. Analysis of main antioxidant enzymes revealed different activity pattern during early development stages in Δ *rrmA* strain comparing to the control strain. Transcriptional analysis has shown that Δ *rrmA* mutation results in higher stability of specific transcripts under conditions of oxidative stress and nitrogen starvation. Our results indicate that RRMA plays important role in metabolism of *A. nidulans* and can be involved in the mechanism of regulated degradation of specific mRNAs in response to specific signals.

19) Comparative analysis of koji mold's genomes

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Koji mold is the traditional name of *Aspergillus* species that are used for Japanese fermentation industries. *Aspergillus oryzae* has been widely used in Japanese fermentation industries, Japanese alcohol beverage, soy sauce and so on for longer than a thousand of years. Comparison of *A. oryzae* genome with those of other *Aspergillus* species of smaller genome size revealed existence of non-syntenic blocks (NSBs) specific to the *A. oryzae* genome. *Aspergillus awamori* is another industrial filamentous fungus, widely used for brewing Japanese traditional spirits, Awamori, in Okinawa prefecture. We have sequenced *A. awamori* NBRC 4314 (RIB 2604). The total length of non-redundant sequences reached 34.7 Mb consisting of contigs fallen into 44 major linkage groups. High potential of secretory production of proteins has led *A. oryzae* and *A. awamori* to extensive use also in modern biotechnology. *A. awamori* is genetically very close to *Aspergillus niger* and close to *A. oryzae*. Like *A. niger*, *A. awamori* vigorously produces citric acid, lowering pH of the product. *A. awamori* is genetically very close to *Aspergillus niger*. However, mapping of short reads from *A. awamori* by SOLiD revealed that the species have remarkable difference. Comparison between the genomes of *A. awamori* and *A. oryzae* showed higher diversity of genes located on the non-syntenic blocks of the *A. oryzae* genome. We are currently sequencing various strains of *A. awamori* by SOLiD. Analysis of the relationship between genetical and phenotypical differences among the strains should provide important information for gene function.

20) Targeted functional proteomics: A putative translation elongation factor with glutathione s-transferase activity protects *Aspergillus fumigatus* against oxidative stress

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Sequencing of the *Aspergillus fumigatus* genome has led to an increased understanding of the organism; however the functions of many genes remain unknown. A putative translation elongation factor 1Bgamma (EF1Bgamma, termed *elfA*; 750 bp) is expressed, and exhibits glutathione s-transferase activity, in *A. fumigatus* [1]. Normally, EF1Bgamma plays a key role in the elongation step of protein synthesis. Our hypothesis is that *elfA* may also play a role in regulating the cellular redox state adjacent to the ribosome during protein synthesis. Consequently, *elfA* was disrupted in *A. fumigatus* ATCC46645 (wild- type) using a bipartite construct containing overlapping fragments of a pyrithiamine resistance gene (*ptrA*). The *elfA* mutant (*deltaelfA*) was complemented using a hygromycin resistance marker (*hph*). Southern Blot analysis was used to confirm the generation of *deltaelfA* and the complemented strain. RT-PCR confirmed the expression of *elfA* in wild-type and complemented strains, and absence of expression in *deltaelfA*. The availability of the mutant has facilitated phenotypic analysis of *elfA* functionality. *A. fumigatus* wild-type and *deltaelfA* were grown on AMM plates with the oxidant H₂O₂ (1 - 5 mM), voriconazole (0.25 - 1 microg/ml), and the thiol-reactive reagent, 4,4'-dithiodipyridine (3 - 7.5 microM). At 37°C, the *elfA* mutant was significantly more sensitive (p=0.0003) to H₂O₂ and (p=0.0056) to 4,4'-dithiodipyridine than wild-type. However, *deltaelfA* was significantly less sensitive (p=0.0251) to voriconazole than wild-type. These results implicate *elfA* in the oxidative stress response in *A. fumigatus* and also strongly indicate that *elfA* may play a role in the sensitisation of *A. fumigatus* to voriconazole. Global proteomic studies are currently underway using 2D-PAGE and MALDI-MS to explore alterations in the proteome consequential to *elfA* disruption with a view to gaining further insight into the function of *elfA* in *A. fumigatus*. 1. Carberry, S, et al. (2006), *Biochem Biophys Res Commun*, 341, 1096-1104.

21) Oxylipins Exchange During the Interaction Between *A. ochraceus* and *Triticum durum* Seeds

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The oxylipin metabolism controls mycotoxin biosynthesis, conidiogenesis, sclerotia formation and the interaction with the host in *Aspergillus nidulans*, *A. flavus* and *A. parasiticus*. The subsequent finding of a lox-like gene sequence (*AoloxA*; DQ087531) in the genome of *A. ochraceus* indicate that also in this fungus oxylipin metabolism can play a pivotal role in controlling different pathways. The *AoloxA* deleted mutant displays a different colony morphology with a remarkable delay in conidia formation and an induction of sclerotia development. *AoloxA* (-) shows oxylipins biosynthetic pathways switching from 13-HPODE to a prevalent formation of 7,8 and 8,13-DiHODE. A large number of sclerotia, which are considered to be vestigial of the sexual cleistotecia are formed in vitro by *AoloxA* (-), possibly due to an increment of diols formation. Further, the reduced oxylipin formation induce a strong reduction of OTA biosynthesis in comparison with the wild type. The seeds of *T. durum* cv Ciccio contaminated with *AoloxA* (-) did not accumulate 9- hydroperoxyoctadecadienoic acid and did not express PR1 mRNA whereas WT stimulated both these events. The generalized down-regulation of oxylipins synthesis in *AoloxA* (-)grown on wheat seeds also confirms the existence of a cross-talk between wheat seeds and *A. ochraceus* mediated by oxylipins. In conclusion, we suggest that similarly to *A. nidulans*, *A. flavus* and *A. parasiticus*, some of the mechanisms that drive toxin biosynthesis and morphogenesis, which are modulated by fatty acids oxidation by-products, are active in *A. ochraceus*.

22) Efficient gene targeting in *Aspergillus niger* using a transiently disrupted *ku70* gene

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Homologous recombination frequencies in filamentous fungi, including *Aspergillus niger*, are low. Several reports over the last years have shown that mutants defective in the Non-Homologous-End Joining (NHEJ) pathway display increased homologous integration efficiencies up to 80 to 100%. Recently, we described that deletion of the *A. niger kusA* gene, encoding the orthologue of the Ku70 protein in other eukaryotes, dramatically improved homologous integration efficiency (1). However, deletion of *kusA* also causes increased sensitivity of *A. niger* towards UV and X-ray and the consequences of loss of *kusA* in relation to DNA repair and genome stability are currently unknown. To avoid any potential side effects of a *kusA* loss-of-function mutation on growth and viability of *A. niger*, we transiently disrupted *kusA*. We made use of the counter selectable *amdS* marker, flanked by 300 bp direct repeats of the *kusA* gene. Disruption of the *kusA* gene resulted in similar homologous recombination frequencies compared to the *deltakusA* strain. After completion of the gene targeting approach, we re-established an intact *kusA* copy using counter selection on fluoroacetamide, proven by sequencing of the *kusA* locus. In *A. niger*, which lacks an sexual reproduction cycle, the transient disruption system is especially important as the NHEJ pathway cannot be restored by performing a sexual cross. References: 1. Meyer, V., Arentshorst, M., El-Ghezal, A., Drews, A.C., Kooistra, R., van den Hondel, C.A.M.J.J., Ram, A.F.J. *Journal of Biotechnology*, 128 (4), 770-5. 2007.

23) Significant differences in pentose regulation between two *Aspergilli*

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D-xylose and L-arabinose are highly abundant components of plant biomass and therefore major carbon sources for many fungi. Fungi produce extracellular enzymes to release these sugars, which are subsequently taken up into the cell and converted through the pentose catabolic pathway. In *Aspergillus niger* and *Aspergillus nidulans*, D-xylose release and part of the pentose catabolic pathway are regulated by the transcriptional activator XlnR. In *Aspergillus niger*, we recently described the transcriptional activator AraR, a homologue of XlnR that controls L-arabinose release and the pentose catabolic pathway. Analysis of *A. nidulans* AraR showed that it is also involved in pentose (L-arabinose) catabolism in this fungus. However, the growth phenotype of the *delta araR* from these two species showed significant differences. *A. niger delta araR* is not able to grow on L-arabinose, whereas for *A. nidulans delta araR* growth is not affected. The reverse was observed for L-arabitol. We will present data on the molecular difference between the regulation of AraR and the interaction with XlnR in these 2 *Aspergilli* by analysis of expression of genes of the pentose catabolic pathway. We will demonstrate significant evolutionary differences in regulation of the pentose metabolism, and will hypothesize about the implications hereof for other sugar catabolic pathways.

24) Implication of FarA transcriptional factor in the expression of lipolytic genes in *Aspergillus oryzae*

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FarA is a Zn₂Cys₆ transcription factor which upregulates genes required for growth on fatty acids in filamentous fungi like *Aspergillus nidulans*. Homology of all the amino acid sequences and the homology of Zn₂Cys₆ motifs of FarA between the *A. oryzae* and the *A. nidulans* are 83% and 97.5%, respectively. This study determines whether FarA transcriptional factor also works in the regulation of genes responsible for the production of lipolytic enzymes including cutinase particularly in *A. oryzae*. FarA disruptant was constructed (*delta farA::sC*) and confirmed by Southern blotting analysis. The wild-type (WT) and the disruptant strains were grown and induced in minimal liquid medium with 2% of flaxseed oil at different time intervals. Supernatants were analyzed for Western blot and the WT showed the presence of cutinase (CutL1) protein while the disruptant did not. In addition, qRT-PCR and RT-PCR revealed that the expression of *cutL1* gene was significantly reduced in the disruptant compared to the WT. Difference of monoacylglycerol lipase gene (*mdlB*) expression was also found between the WT and the disruptant. These results indicated that the FarA transcriptional factor may be implicated in the gene expression of lipolytic enzymes including cutinase. *Acknowledgement*: I would like to extend my gratitude to the Federation of European Microbiological Societies and to the ECFG10 Organizing Committee for partly providing me the financial assistance.

25) RacA is required for actin distribution and affects the localization of the exocytosis machinery in the filamentous fungus *Aspergillus niger*

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Tip growth in filamentous fungus requires coordination of basic cellular processes in the cell such as exocytosis, polarity maintenance, endocytosis and cell wall biosynthesis. The small GTPase RacA, the fungal orthologue of the human Rac1 protein is important for fungal growth. In the absence of RacA, apical dominance of tip growth is lost resulting in apical branching and a hyperbranching phenotype. Actin patches in the *racA* deletion mutant were found to be hyperpolarized at the extreme apex, while in the wild type strain a smoother gradient of actin patches towards the tip was observed. GFP-RacA localizes to the plasma membrane at the extreme apex of growing hyphae probably, marking the site of exocytosis. To understand the function of RacA in relation to exocytosis, secretory vesicles were visualized by tagging the V-SNARE (SynA) with GFP (GFP-SynA). In wild type cells, GFP-SynA is present on intracellular structures representing secretory and/or endocytotic vesicles. High levels of GFP-SynA are also present in the Spitzenkörper. Finally, GFP-SynA is localized in the plasma membrane. Fluorescence of GFP-SynA at the plasma membrane is highest at the extreme apex (the sites of exocytosis) and decreases in subapical regions. In the *racA* deletion mutant, SynA localization was similar to the wild type, but the intensity of the signal was less. Whereas the tips of wild type hyphae display a ~10-15 µm gradient of GFP-SynA protein, tips in the $\Delta racA$ show a much shorter (< 5 µm) gradient of GFP-SynA. We conclude that the *A. niger* RacA protein is necessary for precise actin localisation and distribution in hyphal tip cells and that the gradient of GFP-SynA towards the tip is actin dependent.

26) Reduced Expression of *SccA* Increases Sensitivity to Wall Stress in the fungus *Aspergillus nidulans*

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Decreased expression of the extragenic suppressor, designated *SccA*, affects cell wall integrity in the filamentous fungus *Aspergillus nidulans*. Overexpression of *SccA* suppresses the phenotype of the *calC2* mutation in the *A. nidulans* orthologue of protein kinase C (PkcA), which results in hypersensitivity to the chitin-binding agent Calcofluor White (CFW). In addition, we have shown that *SccA* complements 6 wall-sensitive strains. In filamentous fungi, as in yeasts, hypersensitivity to CFW correlates with defects in cell wall integrity. *SccA* is predicted to have a single transmembrane domain, an extracellular domain rich in serine and threonine residues, and a C-terminal domain of 59 amino acids. Homologues exist in the genomes of other filamentous fungi, but not in yeasts or other organisms. A *SccA*-GFP hybrid localizes to the plasma membrane and septa of vegetative hyphae. When *SccA* is placed under the control of the regulatable *AlcA* promoter and grown under low expression conditions (glucose), we observed a sensitivity to CFW, indicating it plays an important role in cell wall integrity. Taking into consideration the protein's cell surface location and its influence on the function of PkcA, we hypothesize that *SccA* plays a role in signal transduction as part of a cell wall integrity pathway.

27) The RmsA protein – a hub in the protein interaction networks of *Aspergillus niger*?

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Many cells and organisms go through polarized growth phases during their life. Cell polarization is achieved by local accumulation of signaling molecules which guide the cytoskeleton and vesicular trafficking to specific parts of the cell and thus ensure polarity establishment and maintenance. Polarization of signaling molecules is also fundamental for the lifestyle of filamentous fungi such as *Aspergillus niger* and essential for their morphogenesis, development and survival under environmental stress conditions. Considerable advances in our understanding on the protagonists and processes mediating polarized growth in filamentous fungi has been made over the past years. However, how the interplay of different signaling pathways is coordinated has yet to be determined. We found recently that the *A. niger* RmsA protein is central for the polarization of actin at the hyphal tip (1). However, we show here that RmsA is also of vital importance for the metabolism, viability and stress resistance of *A. niger*. This suggests that RmsA could occupy an important position in the global network of pathways that balance growth, morphogenesis and survival of *A. niger*. 1. Meyer V, Arentshorst M, Flitter SJ, Nitsche BM, Kwon MJ, Reynaga-Pena CG, Bartnicki-Garcia S, van den Hondel CA, Ram AF (2009) Reconstruction of signalling networks regulating fungal morphogenesis by transcriptomics. *Eukaryot Cell* 8: 1677-1691

28) Transposon mutagenesis using a resident DNA transposon *Crawler* in *Aspergillus oryzae*

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An active DNA transposon *Crawler* isolated from the genome of industrially important fungus *Aspergillus oryzae* transposes under extreme stress conditions. The DNA sequencing surveys revealed that the *Crawler* element is widely distributed among *A. oryzae* and *A. sojae* strains, which are commonly used in Japanese traditional fermentation manufacturing. In the present study, we analyzed the relationship between various stress stimuli and inhibition of cryptic splicing of the *Crawler* mRNA by qRT-PCR to enhance the frequency of *Crawler*-mediated mutagenesis in an *A. oryzae* industrial strain, AOK139. Under the optimized stress conditions, in which conidiospores were treated in 20mM CuSO₄ or 52 °C for 6hr, various phenotypic mutants different from the parent strain were isolated. Those exhibited white color in conidiospores, less number of spores formed, shortened aerial hyphae, thin colony mat and so on. DNA sequencing analyses of a white conidia mutant revealed that *Crawler* was newly inserted within a coding region of *wA* gene encoding polyketide synthetase, which resulted in *wA* deficiency. The insertion occurred also at a TA site with duplication according to the manner of *Crawler* transposition. These results suggested that transposon mutagenesis using active *Crawler* is potentially valuable to improve characteristics of *A. oryzae* industrial strains.

29) The transcription activator AtrR regulates gene expression of ABC transporters and contributes to azole drug resistance in *Aspergillus fumigatus*

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Previously, we demonstrated that a novel transcriptional factor 'AtrR' regulated gene expression of these ABC transporters that would function as drug efflux pumps and contributes to the azole resistance in *A. oryzae*. Orthologous genes of the *A. oryzae atrR* have been found widely in genomes of *Aspergilli*, including *A. fumigatus*, *A. nidulans*, and *A. niger*. In the present study, we constructed a disruption mutant of the *atrR* ortholog in *A. fumigatus* and investigated the role of the gene in drug resistance. The mutant was similarly hypersensitive to azole drugs, especially susceptible to fluconazole that is not effective to *Aspergillus* fungi. The mutant also showed reduced expression level of several ABC transporter genes that would function as drug efflux pumps. These results indicate that the transcription factor AtrR also regulates gene expression of ABC transporters and contributes to azole resistance in *A. fumigatus*. In addition, this suggests that fluconazole resistance of *Aspergillus* fungi is attributed to the expression of drug efflux ABC transporters regulated by AtrR. This transcriptional factor AtrR provides novel therapeutic targets for the treatment of azole drug resistant fungal infections.

30) Blue- and red-light photoreceptors regulate the activation by light of conidiation genes in *Aspergillus nidulans*

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The ascomycete *A. nidulans* is a model organism to study fungal development. Conidiation is controlled by the product of the *brlA* gene. Many gene products act upstream of *brlA*, probably allowing the synthesis of chemicals or allowing the transduction of environmental signals to trigger *brlA* transcription. The *A. nidulans* genome contains genes for a phytochrome (*fphA*), two homologs of *N. crassa* WC-1 and WC-2 (*lreA* and *lreB*) and a *veA* gene. Red and blue light stimulate conidiation in *A. nidulans*, but mutations in the *veA* gene allow conidiation in the dark. Recently, it has been shown that the phytochrome *FphA* interacts with *VeA* and *LreA* and *LreB*. However, the mechanism that the photoreceptors employ to activate conidiation remains unknown. We have found that the expression of several conidiation genes, including *brlA*, *fluG*, *flbA*, *flbB* and *flbC*, was regulated by light. The photoactivation of these genes showed a quick response with mRNA accumulation increasing after 5 minutes of illumination. *brlA* mRNA accumulation after illumination increased with time showing maximum values between 30 to 60 minutes and revealed a two-component activation. Deletion of the photoreceptor genes *fphA*, *lreA* and *lreB* reduced the activation by light of the studied genes. None of these genes are essential for gene photoactivation since we observed light-dependent mRNA accumulation in strains with single deletion of photoreceptor genes. On-going experiments provides a model for the light-dependent activation of conidiation.

31) Dynamic and functional interactions between the components of the trimeric *velvet* complex in the filamentous fungus *Aspergillus nidulans*

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We have recently discovered the trimeric *velvet* complex which is comprised of the light-dependent regulator *VeA*, Velvet-like protein *VelB*, and master regulator of secondary metabolism *LaeA*. The *velvet* complex coordinates development and secondary metabolism upon light signal in *Aspergillus nidulans*. *VeA* protein serves as a light-dependent bridging function between *VelB* and *LaeA* proteins. We are currently analysing the functional as well as physical relationships between the components of the *velvet* complex. First data suggest that there might be some subcomplexes regulating development. We have new insights into the function of the complex

32) Fine-tuning gene expression in filamentous fungi: An inducible and tunable promoter system for *Aspergillus niger*

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The function of genes is usually inferred from mutants in which the desired gene has been deleted or strongly overexpressed. However, studies at these extreme discrete points give only limited information about the gene functions. Moreover, many overexpression studies make use of metabolism-dependent promoters which often cause pleiotropic effects and thus impose further limitations on their use and significance. Here we report a promoter system for *Aspergillus niger* that can be fine-tuned to user-specified expression levels, that is independent from carbon and nitrogen metabolism, that can be induced within minutes and that shows remarkable reproducibility. The system is based on the tetracycline-dependent promoter and the bacterial rtTA transcriptional activator protein and has been validated under various cultivation conditions. From the data obtained we conclude that the tetracycline-dependent promoter provides rapid and tunable gene control in *A. niger*. The system should be applicable to other filamentous fungi with only minor modifications.

33) Secrelection: A Novel Fungal Expression System For Selection Of Secreted Enzymes

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Modern biotechnology has generated an impressive set of molecular tools: for instance the ability to generate large sets of error prone mutant libraries or cDNA libraries. When these libraries are expressed in a host (e.g. *Aspergillus niger*) not all strains produce a secreted protein. This is mainly dependent on the quality of the library. Here we describe a novel expression system that was developed by using genome expression profiling under different conditions. We were able to identify promoters that fit the required expression profile. These promoters were both up regulated during protein secretion and were not expressed during overexpression of intracellular proteins. By making use of transcriptomics for useful promoter identification, we were able to generate reporter construct(s) that allow us to easily select clones that secrete proteins. This technology can speed up novel protein discovery significantly. Additionally we have shown this approach is not limited to fungi but can also be applied to other production organisms.

34) Improving *A. niger* asparaginase via directed evolution

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The filamentous fungus *Aspergillus niger* has extensively been used for recombinant protein production. The sequencing of the *A. niger* CBS513-88 genome, an ancestor of our current enzyme production strains, yielded easy access to numerous protein encoding genes. Some of these genes encode valuable new food enzymes. One of those new products is *A. niger* asparaginase, an enzyme effective in mitigation of acrylamide formation in food products. To improve efficiency of *A. niger* asparaginase in application, we applied directed evolution to optimize the pH-activity profile of the enzyme. Several new variants were identified, which show improved performance both in vitro and in small scale application tests.

35) Real time qPCR-Based Assay For The Early Detection Of Aflatoxigenic Fungi On Maize Kernels

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The aim of this work is to set up a SYBR-green real time qPCR method, based on the use of specie-specific primers for the early detection and quantification of potential aflatoxigenic fungi *Aspergillus flavus* and *Aspergillus parasiticus* on whole maize kernels. A primer pair was used for amplifying a 352 bp fragment of aflR, gene regulator of the aflatoxin biosynthesis gene cluster. DNA amplification was achieved only with DNA extracted from fungal strains of *A. parasiticus* and *A. flavus* and from maize kernels inoculated with *A. flavus* or *A. parasiticus*, never with DNA of the other fungal species. Amplification was evident in maize artificially inoculated with *A. flavus* 3357 starting from 6 hours of incubation after inoculation, when mycelium was not visible by stereomicroscope analysis as yet. This real time qPCR method could be a real, effective method for the early detection and quantification of the most important aflatoxin-producing fungi in food commodities. The method proposed in this work represents a useful tool to evaluate the quality of raw material at different critical points of the food chain. It could be used to predict potential risk for the presence of potentially aflatoxigenic strains. The combination of this approach with the more expensive and laborious conventional chemical analysis of toxin, could be a real, effective alternative respect to traditional diagnostic methods for the early detection and quantification of aflatoxigenic fungi in food commodities.

36) Formation of Beta-fructofuranosidase by *Aspergillus niger* in Submerged Cultivations

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Although *A. niger* -a versatile and efficient cell-factory in industrial bioprocesses- shows a high production capacity and secretion efficiency, obtainable yields of recombinant proteins are considerably lower than those of homologous proteins. Therefore current research is focussed on the optimisation of cultivation processes resulting in an increased, controlled and tailored formation of desired products. The contribution displays the influence of pH-value, volumetric power input and inoculum concentration on the observed morphology and the formation of homologous recombinant beta-fructofuranosidase under a constitutive promoter as model product. Batch cultivations are monitored and every step of the protein formation path is shown: The expression of the beta-fructofuranosidase gene as well as of genes, which show significant expression levels within the bioprocess, are quantified via real-time PCR. Intra- and extracellular enzyme activities are measured and related to gene expression levels and observed morphology, pellet size and concentration. In conclusion, the protein formation in batch processes is linked to defined cultivation conditions to reveal bottlenecks within the complex production path from gene to product. Therefore the shown results indicate targets for improving, optimising and controlling industrial bioprocesses.

37 A new method for the production of peptides: insertion and isolation of peptides from ankyrin repeat proteins

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Peptides are widely used in the pharmaceutical industry and are also gaining importance as food ingredients. However, the current state-of-the-art production of oligopeptides is via chemical synthesis, which is expensive especially for long peptides. In addition, peptides can be produced by means of fermentation, wherein multimeric genes encoding tandem repeats of the peptide are fused to a carrier protein. After production of the fusion protein peptides are cleaved from the carrier protein. However the yield of the process is generally low and the fusion protein generally accumulates in inclusion bodies. We developed a new approach in which peptides are inserted in the variable loops of ankyrin repeats. Ankyrin repeat domains are conserved structures and consists of tandem repeats of a 33 amino acid ankyrin repeat unit. Interestingly, in between two adjacent ankyrin units inserts up to 47 amino acids has been observed in nature. We have shown in *E.coli* and *Aspergillus niger* that peptides can be inserted in between two adjacent ankyrin repeat units and peptides can be isolated from the protein. In *Aspergillus niger*, ankyrin repeat proteins comprising peptides were fused to truncated glucoamylase. The chimeric repeat protein comprising peptide was successfully expressed and secreted by *Aspergillus niger*. In conclusion, production of peptides by inserting peptides in ankyrin repeat proteins is a successful new approach for fermentative peptide production.

38) Determination of Viable Spores in Seeding Cultures Using Fluorescent Dyes

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Spores are used in inoculum development or directly as starting culture in submerged cultivation processes. While the influence of process conditions during cultivation is well-known, the influence of the spore quality in seeding cultures has not been investigated in depth. However the spore viability in seeding culture is an important criterion for product quantity. The contribution displays the investigation of the transferability of fluorescence based rapid screening assays, developed for bacterial cells, to study spore quality. The results of this method show the applicability to determine the proportion of viable and defect spores in seeding cultures of filamentous fungi. Additionally, good correlations to germination characteristics in submerged cultivation are demonstrated and allow fast, reliable and quantitative distinction between viable and dead spores. Furthermore a biochemical method, based on enzyme activity, has been verified to characterize the spore viability in seeding cultures. The assay allows the characterization of the growth stadium of the filamentous fungi during the sporulation process. Furthermore the enzyme based assay enables the determination of biomass activity in the early phase of submerged cultivation and reveals first information about the feasibility of the cultivation. In conclusion the presented investigations display the possibility of assessing the ratio of viable to defect spores in seeding cultures, whereby a routine method prior to large scale cultivations could be applied.

39) Impact of Grape Berry Resveratrol on the growth of *A. carbonarius* and on the biosynthesis of ochratoxin A

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The objective of this research was to investigate whether *A. carbonarius* contamination induces resveratrol production in grape berry. A possible correlation between OTA production and resveratrol biosynthesis has also been considered. In this study, Vitis vinifera berries were infected, during ripening, by a conidial suspension of *A. carbonarius* and incubated for 6, 12, 24, 48, 120 hours at 30°C. After incubation, each berry was analyzed, at each time interval considered, for quantifying *A. carbonarius*, OTA and resveratrol in grapes. Real Time PCR method with specie-specific primers (Acpk), designed on the basis of the OTA-related polyketide synthase sequences, was carried out quantifying the fungal development in grapes. Our results show a correlation between the growth of the fungus and biosynthesis of OTA and resveratrol content into grape berries, leading to hypothesize that some grapevine cultivars are more capable of self-protection against fungal contamination

40) Development of a novel inducible expression system for the production of heterologous proteins in *Aspergillus*: successful production of lignolytic enzyme.

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For industrial applications and (medical) scientific research efficient(heterologous) production of compounds like, enzymes, antibodies, viral epitopes, chemical compounds and antibiotics is of great importance. For these purposes *Aspergillus* functions as a suitable production host. Over the years all kinds of commercially available expression systems have been developed. A well established expression system is the one based on the protein Glucoamylase (GlaA). However, in the case of heterologous protein production the efficiency of these systems is still very depending on the protein to be produced. Recently, we identified the *inuE* gene, encoding for the exo-inulinase protein in *A. niger*, as the most strongly induced gene in the presence of inulin and sucrose. Characteristics of the system were studied by placing *gfp* behind the *inuE* promoter. This reporter strain showed that the *inuE* gene is highly expressed when grown on inulin and sucrose. No expression was observed when grown on glucose, fructose and xylose indicating a tight control on different carbon sources. This tight control can be a benefit if the heterologous protein to be produced can be a disadvantage for fungal growth. Finally, different peroxidases (ArP and MnP) and a laccase were successfully produced in *Aspergillus* using this novel inducible expression system.

41) Gliotoxin- exploiting its anti-fungal effects

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Aspergillus fumigatus biosynthesizes gliotoxin, yet little is known about the effect of gliotoxin on *A. fumigatus*. Exposure of *A. fumigatus* to gliotoxin (14 microg/ml) for 3 hours results in the de novo expression of Cu,Zn SOD, a short chain dehydrogenase and a eukaryotic translation elongation factor (beta1 subunit). Exposure also significantly altered the expression (up and down-regulated > 2x) of 27 other proteins including (i) upregulated: GliT (AFUA_6G09740; reported elsewhere), adenine phosphoribosyl transferase and allergen Asp f3 and (ii) down-regulated: catalase and a peroxiredoxin. Targeted deletion of gliT resulted in hypersensitivity of *A. fumigatus* delta gliT to gliotoxin (reported elsewhere). Re-introduction of gliT into *A. fumigatus* delta gliT, using a split marker approach, at a different locus (ctsD;AFUA_4G07040) with selection on gliotoxin-containing media, resulted in deletion of ctsD. Absence of ctsD expression was accompanied by restoration of gliT expression (RT-PCR), and resistance to gliotoxin. Significant (P < 0.001) in *A. nidulans*, *A. terreus*, *A. niger*, *Cochliobolus heterostrophus* and *Neurospora crassa* was observed at 5 microg/ml gliotoxin. Growth of *A. flavus*, *Fusarium graminearum* and *A. oryzae* was significantly inhibited (P < 0.001) at 10 microg/ml gliotoxin. Potentially, all of these fungal species are suitable for transformation with gliT to facilitate transformant selection on gliotoxin.

42) Utilization Of Exogenous Heme By *Aspergillus niger*

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The incorporation of heme as a cofactor is a putative limiting factor in the overproduction of heme-containing fungal peroxidases in *Aspergillus* species. Addition of hemin to growth medium has been reported to improve the production of peroxidase. However, hemin uptake and the effect of hemin addition on the transcriptional regulation of the heme biosynthesis pathway genes have hardly been studied in *Aspergillus*. To gain more insight into the heme biosynthesis pathway, the genes encoding the eight different enzymes in the pathway were identified in the *A. niger* genome. Individual deletion of four genes in the pathway (*hemA*, *hemB*, *hemF* or *hemH*) showed that all four are essential. Growth of the *hemA* deletion mutant could be restored by addition of 5'-aminolevulinic acid (ALA). Supplementation with hemin alone did not restore growth. The inability to grow directly on hemin is likely due to the lack of siroheme. Deletion strains of *hemF* and *hemH*, located after this branch point in the heme biosynthesis pathway, could be partially rescued by the addition of hemin. Growth of these mutants can be improved by additional supplementation of Tween80. A detailed characterization of the deletion strains is currently ongoing. These results strongly indicate that *A. niger* is capable of sequestering heme from its environment and utilize this heme for cellular processes.

43) Enzymatic Hydrolysis of Lignocelluloses: Identification of Novel Cellulases Genes from Filamentous Fungi

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Lignocellulosic materials form a huge part of the plant biomass. Cellulose can be degraded to simple sugar components by means of enzymatic hydrolysis. However, due to its complex, crystalline structure it is difficult to break it down and the cooperative action of a variety of cellulolytic enzymes is necessary. Fungi are known to have potential in production of a variety of cellulolytic enzymes. The aim of this work is to discover new thermostable and robust cellulolytic enzymes for improved enzymatic hydrolysis of biomass. For this purpose two screening methods are applied in different fungal strains with high cellulolytic activities: an expression-based method using suppression subtractive hybridization and a targeted genomic screening approach using degenerate PCR. Suppression subtractive hybridization facilitates identification of genes encoding cellulolytic enzymes that are expressed when cultivating a fungal strain in medium with cellulose as the carbon source. By means of degenerate PCR, specific genes, homologous to the genes of previously classified glycoside hydrolases from CAZY database, are searched for in selected strains of *Aspergillus* sp., *Trichoderma* sp. and *Penicillium* sp. Both methods are anticipated to facilitate identification of target genes which subsequently will be cloned and expressed in a relevant fungal host for further characterization of the expressed enzymes. The goal is to introduce new enzymes to industrial processes.

44) Biochemical producing fungi

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We are in the process of developing a biorefinery concept for the use of selected plant biomasses for production of high value biochemicals aiming at replacing chemicals produced from fossil fuels. One aspect will be the engineering of efficient biomass converting fungal strains with the ability to produce high amounts of specific organic acids. *Aspergillus niger* producing citric acid is a classical example of industrial application of fermentative processes in a filamentous fungal strain. We would like to utilize both the large potential for secretion of hydrolytic enzymes and the organic acid producing machinery of the filamentous fungi for further genetic engineering. We anticipate to initiate the engineering by manipulating central pathways in carbon metabolism i.e. glycolysis to increase the funnelling of sugars to acid production. By initial screening of a large collection of fungal strains isolated from natural habitats we have identified isolates with high production and excretion of organic acids. Among these are several *Aspergillus* species and one *Penicillium* sp. with higher excretion of TCA-cycle intermediates, and these strains have been chosen for genetic engineering.

45) *Aspergillus niger*: Mapping fungal specific zinc-finger transcription factors to gene co-expression networks

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Aspergillus niger is a saprophytic filamentous fungus with a long history in industrial production of organic acids such as citric and gluconic acid as well as various enzymes like glycoamylases and pectinases. Furthermore, *A. niger* is an important multi-cellular model organism to study for example the establishment of cell polarity or protein secretion and it is used for the discovery of new anti-fungal drugs. *A. niger* can easily be genetically modified and cultivated under defined growth conditions in bioreactors. DSM published its 33.9 million base pair genome in February 2007 and an Affymetrix DNA microarray platform with more than 14.000 annotated open reading frames (ORFs) and genetic elements was designed. Among the predicted ORFs, over 300 fungal specific zinc-finger transcription factors (TFs) have been annotated. The function of most of these TFs remains unknown. In the current study we, have built gene co-expression networks from a dataset of about 100 Affymetrix microarrays covering more than 30 different growth conditions including those which induce stress related to secretion, maintenance of polarity, cell wall integrity, carbon-source utilization and starvation. Exemplarily for a wide range of applications, we show mapping of putative TFs with unknown functions to these networks. Allocation of TFs to functionally enriched gene clusters can serve as an indication for their regulatory role and thereby give valuable leads for further experimental studies.

46) A systems biology approach towards itaconic acid production in *Aspergillus*

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The black filamentous fungi *Aspergillus niger* has a long tradition of safe use in the production of enzymes and organic acids, and is widely used in biotechnology as host for the production of food ingredients, pharmaceuticals and industrial enzymes. Besides, *Aspergillus niger* grows on a wide range of substrates under various environmental conditions. In our research we have addressed the production of one of the commercially interesting building-block organic acids, itaconic acid. To unambiguously identify the itaconic acid biosynthetic pathway several parallel approaches were taken using *Aspergillus terreus* as parental host strain. Using a combination of controlled fermentation design, reversed genetics and transcriptomics approaches the pathway specific cis-aconitate decarboxylase (CAD) encoding gene was identified. More specifically, data analysis from the transcriptomics study show that the cis-aconitate decarboxylase (CAD) gene and its clustered genes (Class I) are the most highly expressed ones related to itaconate production. Expression of the CAD gene in *E.coli* proved that this gene encodes cis-aconitate decarboxylase. Furthermore, expression of the CAD gene in *Aspergillus niger* resulted in the production of itaconate in the fermentation medium. Further genetic modifications of the itaconic acid metabolic pathway and fermentation medium improvement were initiated to improve itaconate levels.

47) Investigating the Regulation of GliA Expression in *Aspergillus fumigatus*

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Gliotoxin, a member of the epipolythiodioxopiperazine (ETP) class of toxins, is important to virulence in certain host models. Most genes involved in gliotoxin production and transport are located on a gene cluster, which is co-transcribed. Deletion of an essential gene within the gliotoxin biosynthetic pathway, *gliP*, led to a lack of gliotoxin production within *A. fumigatus*, as well as a significant reduction in virulence in a steroid treated host. Reduced virulence was a result of the presence of neutrophils within the host, as other labs using neutropenic mouse models did not see this trend. In microarray studies, *gliA*, the gliotoxin efflux pump, is induced over 30-fold in the presence of neutrophils. Growth of *A. fumigatus* in medium containing sodium nitrate also significantly induces expression of *gliA*. Although many studies have been done to elucidate the effects of gliotoxin on host cells, little is known about the expression of the gliotoxin cluster. To identify cis-acting regulatory elements in the *gliA* promoter, we examined expression from promoter deletion mutants fused to *lacZ*. Several positive and negative regulatory elements were identified that altered expression in a nitrogen source dependent manner. Using a *gliA* promoter *lacZ* fusion reporter construct, we screened for activators of *gliA* expression and identified candidate plasmids that activate the reporter. We are conducting additional experiments to investigate this regulation. Elucidating the genes that are responsible for the regulation of *gliA* will lead to a greater understanding of gliotoxin synthesis and transport, which is important to the pathogenesis of *A. fumigatus*.

48) Cloning, expression, and characterization of beta-glucosidase genes from a black *Aspergillus* spp.

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Through a broad cellulolytic activity screening of fungi isolated from various locations, a very promising fungus, identified as a black *Aspergillus*, was selected for further studies. An enzyme extract of this fungus was obtained through solid state fermentation and tested to profile its beta-glucosidase activity. Four beta-glucosidase genes from this fungus have been cloned through PCR with degenerate primers designed from conserved motif regions of known beta-glucosidase genes from *Aspergilli* followed by genome walking strategies. The goal is to have these genes expressed in *Trichoderma reesei*. An expression system was constructed with the constitutive ribosomal promoter RP27 for expression of the his-tagged beta-glucosidase genes and with the *hygB* gene as selection marker. Analysis of the different transformants is currently on-going and subsequently the different beta-glucosidase proteins will be purified for detailed analysis of specific activity, Km, sugar tolerance, thermostability as well as their ability to break down shorter chained oligosaccharides.

49) Examination of species of *Aspergillus* Section Nigri for fumonisin production and presence of the fumonisin biosynthetic gene *fum8*.

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Fumonisin is a mycotoxin associated with cancer and several other serious diseases in humans and animals. Production of the mycotoxin has been reported for over two decades in *Fusarium* species, but has been reported only recently in strains of *Aspergillus niger*. In addition, a homologue of the fumonisin biosynthetic gene (FUM) cluster, originally identified in *Fusarium verticillioides*, has been identified in the genome sequence of *A. niger*. Here, we examined seven species in *Aspergillus* Section Nigri that occur on grapes for fumonisin production and presence of the fumonisin biosynthetic gene *fum8*, which served as a marker for the FUM cluster. Fumonisin B2 (FB2) production was detected in nine of 32 *A. niger* strains examined, but not in any strains of *A. brasiliensis*, *A. carbonarius*, *A. foetidus*, *A. japonicus*, *A. tubingensis*, and *A. uvarum* that were examined. In addition, PCR and Southern blot analyses provided evidence for the presence of *fum8* in 11 *A. niger* strains but not in strains of the other species examined. These findings indicate that the discontinuity of fumonisin production in grapes isolates of *A. niger* likely results from absence in some isolates of at least part of the FUM cluster. The results also confirm the taxonomic complexity of *A. niger* from grapes and provide a possible explanation for previously observed variability in FB2 contamination of grapes and wine.

50) RNA-Seq Analysis of Aflatoxin Gene Expression in *Aspergillus flavus*

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A. flavus is the major producer of aflatoxin, which is responsible for millions of dollars in losses in the world and for significant health issues in developing countries, and is the second leading cause of aspergillosis in immunocompromised individuals. Sequencing of *A. flavus* NRRL3357 showed that its 36-Mb genome contains 13,488 genes including the aflatoxin gene cluster. Here we describe our efforts to use the RNA-Seq technology to characterize the entire transcriptome of the species under conditions conducive to aflatoxin production. To that end, we sequenced cDNA fragments obtained from Poly(A)-enriched total RNA samples extracted from fungal mycelium grown under 3 conditions: (i) PMS medium, 29 C, 24h, no toxin; (ii) GMS medium, 29 C, 24h, make toxin; and (iii) GMS medium, 37 C, 24h, no toxin. Two cDNA libraries from each treatment were sequenced using the Illumina (SOLEXA) short-read technology. Over 5 Million 100 nt reads were sequenced for each cDNA prep, which were combined to generate a powerful high resolution map of the *A. flavus* transcriptome. In addition, we used the RPKM analysis to determine transcript abundance in the 3 mRNA samples. The analysis detected expression in at least 50 % of the genes for each condition and contributed to our understanding of the genetic basis of the aflatoxin regulation.

Student authors have their name underlined.