Poster Abstracts

COMPARATIVE AND FUNCTIONAL GENOMICS

1) Closely related fungi employ diverse enzymatic strategies to degrade plant biomass

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In natural biotopes, plant biomass is the predominant carbon source for most fungi. Plant biomass consists largely of polymeric compounds of which polysaccharides are the main components. Fungi cannot take up the intact polysaccharides, but need to degrade them extracellularly to monomeric and small oligomeric compounds. Therefore, fungi produces enzymatic mixtures that are tailored specifically to the available polysaccharides. A recent study showed significant differences in the polysaccharide degrading ability of three Aspergilli, but only small differences in their growth on plant polysaccharides [1]. This suggests that related fungal species may have developed different approaches to plant biomass degradation. A better understanding of these strategies will not only increase our insight in fungal biodiversity, but will also help in designing more efficient industrial processes for plant biomass degradation. In this study we have compared the plant biomass degrading potential and strategy of 8 Aspergilli and demonstrate that they have developed a highly diverse approach. [1] Coutinho PM et al. 2009. Fung Genet Biol 46: S161S169.

2) Decifering the mechanisms of aflatoxin formation through functional genomics in *Aspergillus flavus*

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Sequencing of *A. flavus* NRRL3357 showed that its 36-Mb genome contains 13,488 genes including predicted 55 secondary metabolite gene cluster. We sequenced cDNA fragments obtained from Poly(A)-enriched total RNA samples extracted from mycelium grown under 3 conditions: (i) PMS medium, 30 C, 24h, no toxin; (ii) GMS medium, 30 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. 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. This study demonstrates that the aflatoxin pathway gene cluster consisting of 30 genes are tightly regulated. High temperature turns down aflatoxin gene transcription by turning down transcription of the two regulatory genes, the *aflR* and *aflS* (old name: *aflJ*). Further, the change in gene expression ratio of *aflS* to *aflR* renders *aflR* non- functional for activation of aflatoxin pathway gene transcription.

*3) Identification of protein kinase A target genes of *Aspergillus fumigatus* by functional genomics

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A. fumigatus, as both saprophytic mould and medically important human pathogen, needs efficient mechanisms to sense environmental signals and transduce them intracellularly to survive in its entirely different habitats. One of these signal transduction pathways is the cAMP dependent protein kinase A (PKA) pathway. For *A. fumigatus*, components of this generally well conserved signaling cascade have been characterized in detail and its significance for virulence was shown. To identify target genes of PKA, we performed microarray analyses using a mutant strain overproducing the PKA catalytic subunit in comparison to the corresponding wild type. Following this approach, we were able to find 282 up and 343 down regulated genes involved in different cellular processes like carbon and nitrogen metabolism, cell cycle regulation and ribosome biogenesis. Among these genes potentially regulated by PKA, 23 transcription factors were found of which 15 were deleted and the mutant phenotypes were characterized under different cultivation conditions. A C6 finger domain protein that shows highest upregulation of all identified transcription factors is located in a potential secondary metabolite gene cluster. Because a gene deletion resulted only in minor phenotypical changes, an overexpression mutant of this transcription factor was created to gain deeper insights into its function.

*** Posters presented by students**

4) Comparative analysis of Aspergilli to facilitate novel strategies in fungal biotechnology

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Aspergillus is not only one of the most important fungi in biotechnology, it is also one of the most commonly found groups of fungi in environments worldwide and one of the most severe opportunistic human pathogens. Due to this, Aspergillus has one of the largest research communities in the fungal field. This has resulted in it being one of the most intensively studied fungi with respect to genomics. The availability of >10 genomes in combination with the tools developed for Aspergillus genomics (e.g. AspGD and CADRE) enables comparative genomics at a high level. In 2011 the JGI approved sequencing of an additional 8 Aspergilli and Penicillia, to advance comparative genomics in these fungi. This project include more industrial species, but also species distantly related to those for which genome sequences are already available. A large consortium of researchers has been established to perform comparative genomics on the new and already available Eurotiales genomes (32 in total). In addition, this analysis will be supported with experimental data to validate the differences found through bioinformatics. Details on the project and current status will be presented.

5) Annotation of 8 *Aspergillus* genomes derived by the multi-genome Gnomon pipeline.

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Accurate annotation of genomes is still a challenge. Large-scale sequencing projects usually provide additional experimental data (EST, full-length cDNA) that can be utilized in the annotation process to improve the quality of gene models. More recently sequencing efforts are concentrated on pathogens and model organisms from Fungi and Protozoa and are focused on sequencing of genomes of closely related organisms for evolution, genetics and comparative studies. These genomes are relatively small but often lack additional transcript or protein data. Using comparative multi- genome approach can greatly improve the accuracy of gene prediction compared to single genome method. The multi-genome Gnomon approach allows utilizing the transcript and protein data from closely related organisms in a single multi-genome annotation run. This method starts from a single genome Gnomon gene prediction and then uses a comparative analysis among multiple genomes to gradually improve the annotation through an iterative process. At each iteration the best models are selected and used as a training set and evidence for the next step. Transcript and protein alignments are used to guide gene model predictions. The most recent version of Gnomon can utilize RNA-Seq data giving more support to the splice junctions. Eight *Aspergillus* genomes have been annotated simultaneously using this method. Four of these genomes have RNA-Seq data available. The resulting annotation has proven to be more consistent across the genomes than the annotation of the individual genomes.

6) Characterization of Fumonisin B_2 biosynthetic gene cluster in *A. niger* and *A. awamori*.

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A set of 43 *Aspergillus niger* and *A. awamori* strains isolated from grapes cultivated in Mediterranean area was tested to evaluate eventual genetic differences between fumonisin B_2 (FB_2) producing and non-producing strains. The ability to produce FB_2 was correlated to *fum* genes occurrence, evaluated by PCR assyas, using primer sets designed to amplify fragments of *fum1, fum3, fum6, fum7, fum8, fum10, fum13, fum14, fum15*, and some relative intergenic regions. The *A. niger* and *A. awamori* FB_2 producing strains arose amplicon for all tested *fum* genes, while the FB_2 non-producing strains arose amplicon only for few of tested *fum* genes in *A. awamori*, and for all of them in *A. niger*. Maximum parsimony analysis based on the calmodulin gene sequences indicated that the presence/absence of *fum* genes in the isolates is not correlated with phylogenetic relationship among strains. This is the first report correlating the presence of multiple fumonisin biosynthetic genes with fumonisin production in *A. niger*. The results suggest that the absence of FB_2 production in *A. awamori* can result from the absence of at least one gene of the cluster, while in *A. niger* should involve other regulator gene/s probably out of the cluster, or concern variations in a regulatory sequence essential for cluster expression.

7) Genome mining of secondary metabolites in *Aspergillus nidulans*

Clay Wang, University of Southern California.

Genome sequencing of *Aspergillus nidulans* has revealed a large number of polyketide synthases involved in the biosynthesis of secondary metabolites. Our lab in collaboration with the Oakley and Keller labs have been interested in identifying the products of these cryptic genes. Recent progress in our collaborative efforts will be presented. This work is funded by a Program Project grant GM084077 from the National Institute of General Medicine.

8) Recent Developments at the *Aspergillus* Genome Database

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The *Aspergillus* Genome Database (AspGD, http://www.aspgd.org) is a freely available, web-based resource for researchers studying the molecular biology of these fungi.Â The interfaces, navigation functionality, and database are recently upgraded, providing streamlined, ortholog-based navigation of the annotation for multiple species concurrently. We have now completed manual curation of the entire published literature about multiple species, including *A. nidulans*, *A. fumigatus*, and *A. oryzae*.Â We also provide resources to foster interaction and dissemination of community information, tools, and data.Â We collect and provide large-scale datasets with a full-featured genomics viewerÂ to facilitate comparative genomics analysis.Â AspGD is funded by grant R01 AI077599 from the National Institute of Allergy and Infectious Diseases. We welcome, encourage, and appreciate your questions or suggestions, to curators can be reached at aspergillus-curator@lists.stanford.edu

9) Novel approaches for solving bottlenecks and improving recombinant protein production by *Aspergillus*

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The filamentous fungus Aspergillus niger is an important micro-organism used for large scale industrial production of enzymes. As a cell factory it combines a large intrinsic protein production capacity with a long history of safe use. Enzyme production in A. niger has been optimized in many ways. Classical strain improvement, optimization of expression cassettes and gene copy number increase are relevant approaches to achieve high protein productivity levels. Last decade, functional genomics studies have led to the identification of host genes that can be modified to boost protein expression capacity. To optimize gene designs we have developed algorithms that bring single-codon usage as well as codon-pair usage in line with the usage detected in highly expressed genes. An in-depth comparison of the compositional, physiological and structural features of proteins that are poorly secreted and the corresponding features of proteins that are well- secreted has led to a method to predict if an over-expressed protein will successfully be produced or not. Moreover, the same information has been used to design and produce enzyme variants with adapted amino acid features that have an improved secretion while maintaining their catalytic activity.

10) New resources for functional analysis of omics data for the genus *Aspergillus*

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Detailed and comprehensive genome annotation is a prerequisite for effective analysis and interpretation of omics data. As such, Gene Ontology (GO) annotation has become a well accepted framework for functional annotation. The genus *Aspergillus* comprises fungal species that are important model organisms, plant and human pathogens as well as industrial workhorses. However, GO annotation based on both computational predictions and extended manual curation has so far only been available for one of its species, namely *A. nidulans* . Based on protein homology, we mapped 97% of the 3,498 GO annotated A. nidulans genes to at least one of seven other Aspergillus species: *A. niger* , *A. fumigatus* , *A. flavus* , *A. clavatus* , *A. terreus* , *A. oryzae* and *Neosartorya fischeri* . GO annotation files compatible with diverse publicly available tools have been generated and deposited online. To further improve their accessibility, we developed a web application for GO enrichment analysis named FetGOat and integrated GO annotations for all *Aspergillus* species with public genome sequences. Both the annotation files and the web application FetGOat are accessible via the Broad Institute's website. To demonstrate the value of those new resources for functional analysis of omics data for the genus *Aspergillus* , we performed two case studies analyzing microarray data recently published for *A. nidulans* , *A. niger* and *A. oryzae* .

11) Using RNA-Seq data to improve the gene structure annotation of Aspergillus species

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The correct structural annotation of genes is fundamental to downstream functional genomics approaches. Genes undetected by gene prediction algorithms, misplaced or missing exons and wrongly merged genes can jeopardize attempts to produce a comprehensive catalog of an organisms metabolic capabilities. We are currently working toward generating alternative and improved structural annotation of Aspergillus species relevant to food industry and human health. Our approach consists of reconstituting transcripts from RNA-Seq and aligning those against their respective genomic loci. Potential structural modifications are then validated by sequence conservation across related species. In addition, novel algorithms were developed to deal with the challenges intrinsic to non-strand-specific RNA- Seq, which represent the bulk of data available for the Aspergilli; and downstream analyses leveraging the newly defined UTR were performed. The improved gene structure will be available through the Aspergillus genome database site (www.aspergillusgenome.org).

GENE REGULATION

12) Microbial communication comes to *Aspergillus fumigatus*: Activation of a fungal silent secondary metabolite gene cluster by *Streptomyces rapamycinicus

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Aspergillus fumigatus is the most important air-borne human fungal pathogen. The genome of this filamentous fungus exhibits far more gene clusters predicted to encode secondary metabolites than compounds known. Because these unidentified metabolites could have important biological functions and possibly represent drug candidates, it is desirable to activate their often silent biosyntheses. Our aim was to mimic physiological conditions under which secondary metabolite gene clusters could be activated. Previously, we demonstrated activation of a silent secondary metabolite gene cluster of *Aspergillus nidulans* by co-cultivation with *Streptomyces rapamycinicus* which led to formation of orsellinic and lecanoric acid. Interestingly, as shown here, the bacterium is also able to activate silent gene clusters in the human-pathogenic fungus *A. fumigatus*. Co-culturing of *A. fumigatus* with this streptomycete triggered the specific activation of a so far silent fungal secondary metabolite gene cluster leading to the production of a novel secondary metabolite.

13) G protein-coupled receptors of the human-pathogenic fungus *Aspergillus fumigatus

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The filamentous fungus *Aspergillus fumigatus* is present in diverse habitats and therefore confronted with a wide variety of environmental stimuli. Due to its ability to grow on numerous nutrients, *A. fumigatus* is also able to colonise the human body and provoke life-threatening infections. Thus, to be able to sense and respond to changing environmental conditions during infection, *A. fumigatus* contains a large array of sensing and signaling mechanisms, including G protein-coupled receptors (GPCRs). Until now, little is known about the stimuli and signal transduction mechanisms of the 15 GPCRs predicted to be encoded by the genome of *A. fumigatus*. To understand their impact on fungal growth, development and pathogenicity, it is of major importance to investigate their function in detail and to identify their possible contribution to pathogenicity. We created a collection comprising single knock-out strains of almost all *A. fumigatus* GPRCs and started to investigate their phenotypes. First results show that some mutant strains show reduced growth and production of conidia, which gave first hints on the importance of functional signaling pathways for survival of *A. fumigatus*. The ongoing analysis will define the function of different GPCRs in a filamentous fungus.

14) Cross-talk between nitric oxide and light for the regulation of development

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In Aspergillus nidulans, light is the main signal that influences the decision if cells undergo asexual (conidiation) or sexual (cleistothecia) development. In all eukaryotes nitric oxide (NO) is an important signalling and defence molecule and we have shown previously that the short-lived nitrogen oxide radical is generated during the nitrate assimilation process, and detoxified by flavohemoglobin proteins FhbA and FhbB. Here we report that the metabolism of NO is additionally regulated by light. We found that the expression of the flavohemoglobin gene fhbB is induced by light and that this regulation depends on the photoreceptor complex. Our data show that conidiation is gradually repressed by increasing NO levels and at the same time formation of cleistothecia is promoted. We also found that other metabolic genes which potentially affect NO formation or consumption are regulated by light and thus may participate in the fine-balanced regulation of developmental decisions in A. nidulans.

15) Redundant Nuclear Localization Signals Mediate Nuclear Import of the *Aspergillus nidulans* Transcription Activator of Nitrogen Metabolic Genes AreA.

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The *Aspergillus nidulans* GATA transcription factor AreA activates transcription of nitrogen metabolic genes. AreA accumulates in the nucleus during nitrogen starvation but not in the presence of nitrogen sources. AreA contains five putative classical nuclear localization sequences (NLSs) and one putative non-canonical bipartite NLS. We used two approaches to identify the functional NLSs. First, we constructed Green Fluorescent Protein (GFP)-AreA NLS fusion genes and introduced them into *A. nidulans*. The bipartite NLS strongly directs GFP to the nucleus, one of the classical NLSs weakly directs GFP to the nucleus and the other four classical NLSs collaborate to direct GFP to the nucleus. Second, we constructed epitope- tagged gene replacement *areA* mutants affected in individual NLSs or combinations of NLSs to identify sequences required for nuclear localization. Deletion of all five classical NLSs did not affect utilization of nitrogen sources and did not prevent AreA nuclear localization. Mutation of the bipartite NLS conferred inability to utilize alternative nitrogen sources but did not prevent AreA nuclear localization. Combinations of mutations of the six NLSs indicate redundancy among the AreA NLSs.

***16) Characterization of *Aspergillus niger* cellulose mutants**

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The ascomycete *Aspergillus niger* is a soil saprobe ubiquitously found in the environment. The capability of this filamentous fungus to secrete a wide array of hydrolytic and oxidative enzymes involved in the breakdown of plant lignocelluloses is widely used in green and white biotechnology (ie biofuels, pulp and paper). However, *A. niger* grows poorly on cellulose as a sole carbon source as compared to other industrial fungi such as *Trichoderma reesei*. Recently we isolated a mutant in our lab that shows improved growth on cellulose and increased cellulolytic activity compared to the wild type. Recently the gene encoding the Nox regulator in *Podospora anserina* (PaNoxR) was identified and shown to negatively affect cellulase production (Brun et al. 2009). We identified a homologue of this gene in the *A. niger* genome and aimed to study whether the effect on cellulase production was also present in this species. Analysis of micro array data of the cellulose mutant demonstrated that expression of this gene was down- regulated in the mutant compared to the wild type. A knock-out strain for *A. niger noxR* was made and compared to the cellulose mutant and the wild type. Results from this study will be presented. Ref: Brun et al 2009 Mol Microbiol 74:480496

17) Discovery of novel basic helix-loop-helix (bHLH) transcription factors regulating development in *Aspergillus oryzae*
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The basic helix-loop-helix (bHLH) family of proteins comprises a group of transcriptional factors that are often important in development and differentiation. In our previous report, we identified a *sclR* gene encoding a basic helix-loop-helix (bHLH) transcription factor. We constructed *sclR*-disruptant and *sclR*-overexpressing strains, finding that there was hardly any sclerotium-like body to be observed in the *sclR*- disruptant strain, whereas *sclR*-overexpressing strain produced less conidia and more sclerotium-like body on the malt agar medium. In this study, we identified another bHLH transcription factor-encoding gene, *ecdR*. The *ecdR* gene disruptant hardly produced conidia. Conversely, the overexpression of *ecdR* resulted in the formation of a large number of conidia at an early stage. Additionally, when serially diluted conidia were spread-cultivated onto malt agar medium, we found that conidial number of the control strain depended on the cultivated conidium density, while the *ecdR*-overexpressing strain showed no significant change in conidiation. These phenotypes of the *ecdR*-disruptant and *ecdR*-overexpressing strains are partially similar to those of the *sclR*-overexpressing strain and *sclR*-disruptant, respectively. Yeast two-hybrid assays indicated that EcdR interacted with SclR to form heterodimer and simultaneously they could also form homodimer. From these results, we concluded that EcdR and SclR have opposite roles in development. By competitively interacting with each other, they form heterodimer and may result in a mutual inhibition of function.

18) Functional characterization of *Aspergillus nidulans* RpdA: Identification of complex partners and subcellular localization.
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In eukaryotic organisms DNA is compacted into an elaborate structure called chromatin, thus enabling regulation of transcription by controlling the accessibility of the genetic information for transcription factors. Among the key players involved in the regulation of chromatin structure are histone acetyltransferases and histone deacetylases (HDACs) enzymes establishing distinct acetylation patterns in the N-terminal tails of core histones. In filamentous fungi only little is known about the biological functions of these enzymes; nevertheless recent studies have shown that class 2 HDACs affect the regulation of genes involved in stress response and secondary metabolite production. Depletion of RpdA, a class 1 HDAC of *Aspergillus nidulans*, leads to a drastic reduction of growth and sporulation. Functional studies revealed that a short C-terminal motif unique for RpdA- type proteins of filamentous fungi is required for catalytic activity and consequently cannot be deleted without affecting the viability of *A. nidulans*. In order to further characterize RpdA we have started to analyze complex formation and localization of the protein with respect to this motif by expressing TAP- and GFP-tagged RpdA versions. First results indicate that both tagged full-length proteins are functional and suggest a role of the C- terminal motif for proper subcellular localization.

19) Post-transcriptional suppression against potential transposable elements by cryptic splicing and premature polyadenylation 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 [1]. A stress-fluctuation cDNA browser with DOGAN- DB was constructed to survey transposon-like genes such as *Crawler*. Full length of DNA sequences encoding transposable elements were frequently identified. Among them, a novel element homologous to *Tan1* from *A. niger* was identified and tentatively designated *AoTan1* that shows multiple characteristics of class II transposon [2]. Changes of the transcripts from several transposable elements were analyzed under extreme stress conditions such as $CuSO_4$ or heat shock by the method of RT-PCR and 3'-RACE. The mRNA analyses revealed that cryptic splicing occurred in the mRNA from *gag*-like elements in a retrotransposn *AoLTR1* and from a deduced DNA transposon(AO090023000251) homologous to *implala* under the normal culture condition. In the case of *AoTan1*, cryptic splicing could not be detected, whereas premature polyadenylations were observed within coding region of the transposase. By the stress treatments, the increasing in mature mRNA molecules from those elements was caused, allowing the full-length to be produced. These results suggested that *A. oryzae* might possess a common defense system against the potential transposable elements by post-transcriptional regulation such as cryptic splicing or premature polyadenylation as observed in the active transposon *Crawler*. 1)H. Ogasawara *et al*. *Fungal Genet. Biol.* , **46**, 441-449 (2009) 2)H. Ogasawara *et al*. 26th *FGC Abstract Book*, p148 (2011)

20) Withdrawn

21) Expression analysis of aflatoxin biosynthesis genes in *Aspergillus flavus* grown on almond medium at different conditions of water activity.

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Almonds, like other nuts, could be infected by *Aspergillus flavus*, the major responsible of aflatoxin contamination. Water activity is one of the most significant environmental factors influencing aflatoxin production. *Aspergillus flavus* was grown on almond based medium at 28C under different aw conditions (0.99, 0.96, 0.93, 0.90) and the production of aflatoxin B1 (AFB1) was monitored during ten days. The relative expression of the regulatory genes, aflR and aflS, and of the structural genes, aflD and aflO, belonging to the biosynthetic cluster, were analyzed during the early days of growth. The lowering of aw levels causes a delay in the fungal growth and a decrease in the accumulation of AFB1. At favorable conditions for AFB1 production (0.99 aw and 0.96 aw), the regulatory genes were transcribed at their highest levels before the structural ones. The expression profile of structural genes correlated to the accumulation trend of AFB1 better than the regulatory genes. Generally, the structural genes were strongly upregulated in respect to their basal level just before AFB1 accumulation peak, and in a greater degree as compared with regulatory genes. At lower aw when only low amounts of mycotoxin were produced, all the genes were activated in a lesser extent, mainly the structural ones. This research was supported by EU-FPVII project MYCORED (KBBE-2007- 222690).

22) A Molecular genetic analysis of the AreA-NmrA interaction in *Aspergillus nidulans*

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The nitrogen metabolite repression system of *Aspergillus nidulans* allows the expression of genes required for the utilisation of non-preferred nitrogen sources to occur only in the absence of the preferred ammonium or glutamine. The transcriptional activation ability of the primary positive regulator of this system, AreA, is modulated through many processes including differential *areA* mRNA stability, regulation of nuclear import/export and competitive binding with the negative acting AreB. AreA function is also modulated by interaction with the co-repressor NmrA and co- activator TamA. The co-repressor NmrA is able to bind to the GATA Zinc- Finger DNA binding region of AreA, but *in vivo* experiments have shown that this binding is not preferential to AreA binding to GATA containing DNA, so the exact mechanism with which NmrA represses the activity of AreA is unknown. This study aims to investigate this interaction with and repression of AreA through a mutagenic approach. Since overexpression of *nmrA* can prevent the activity of AreA, resulting in an inability to grow on non-preferred nitrogen sources, a screen has been set up to obtain mutants that are insensitive to NmrA activity. The sequence changes in these mutants and the predicted effects on NmrA structure have been determined.

23) Pleiotropic effects of *farA* disruption in *Aspergillus oryzae*

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This study reports on the pleiotropic effects of *farA* disruption- regulation of genes that at first sight do not seem to be influenced by *farA* gene in *Aspergillus oryzae*. It has already been reported that FarA is a Zn(II) $_2$Cys$_6$ transcription factor which up-regulates genes required for growth on fatty acids in *Aspergillus nidulans* (Hynes et al. 2006) and it is also known to regulate cutinase genes in *Fusarium solani* and *A. oryzae* (Li et al. 1997; Li et al. 2002; Garrido et al, 2012). However, its effect on the regulation of other metabolic genes in *A. oryzae* has not yet been reported. Microarray analysis was conducted between the wild-type (WT) and the *farA* disruptant induced with oleic acid. Results confirmed by qRT-PCR showed that a number of genes encoding ribosomal proteins such as 60S ribosomal proteins L18 and L19, and 40S ribosomal proteins L6 and S16 were down- regulated in the disruptant as compared to the WT. It could be implicated that ribosomal gene expressions during fatty acid metabolism may be FarA dependent and *farA* disruption affects directly or indirectly the ribosomal biogenesis. Furthermore, metabolic genes up-regulated in the disruptant and other metabolic genes affected only by oleic acid are also discussed in this study. Results are relevant for understanding more the complexity of transcriptional regulatory networks of filamentous fungi.

24) The bZIP-type transcription factor FlbB: A versatile regulator of *Aspergillus nidulans* asexual development.
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The early stages of asexual development in the model fungus *Aspergillus nidulans* are controlled at the molecular level by a discrete number of regulatory proteins that includes the bZIP-type transcription factor (TF) FlbB. Vegetative hyphae contain two main pools of FlbB, one at the tip and the other at the most apical nucleus. The apical pool requires the interaction with the positional regulator FlbE at or in the proximity of the Spitzenkrper. This interaction requires in FlbB a functional bZIP domain, specific central regions and a highly conserved Cys residue. Nuclear FlbB is renewed after each mitotic cycle and under appropriate conditions, activates the cMyb-type TF FlbD. Both factors, in turn, jointly activate the expression of the conidiation-specific TF *brlA*. A 2D-PAGE screening of proteins in wild type and Δ*flbB* strains showed that the concentration of specific stress-response proteins was controlled through FlbB. *gmcA*, a previously uncharacterized glucose- methanol-choline oxidoreductase coding gene, shows miss-scheduled expression in a Ä*flbB* genetic background and the derived protein is required during development under alkaline pH conditions. Sequencing of mRNA from both vegetative and asexual samples provides for a wide overview on the genes and pathways under the hypothetic transcriptional control of FlbB activity. Preliminary results obtained in the functional characterization of some of these genes are also presented. Overall, the functional versatility of FlbB provides for a new outlook on morphogenetic change and focuses our future work on the study of the molecular mechanisms through which this TF regulates different cellular processes during development.
Keywords: *Aspergillus nidulans*, asexual development, conidiophore, transcription factor, autoregulators.

25) Comparative mRNAs expression patterns between vegetative growth and asexual development in *Aspergillus nidulans*.
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Using the high resolution RNA-Seq, we have analyzed the different mRNA expression patterns between vegetative and asexually developing mycelia of *Aspergillus nidulans*. The pathways and genes that are activated and repressed during vegetative hyphal growth and conidiophore production have been identified. We also describe a substantial number of novel transcripts that are controlled by Upstream Developmental Activators (UDAs, especially FlbB) at different stages of development. Genes involved in secondary metabolism (like polyketide synthases, see poster Rodrguez-Urra *et al*.), increased oxidoreductase activity and/or transcription factors (principally binuclear zinc clusters) are examples of groups regulated by FlbB. Our analysis indicates that during asexual development the 4% of the transcriptome is modified comparing with vegetative growth, including more genes than previously were anticipated. Of these, FlbB regulates the 60% directly or indirectly. These results may provide a blueprint for further study of the *Aspergillus nidulans* development.
Keywords: *Aspergillus nidulans*, asexual development, conidiophore, transcription factor, mRNA sequencing.

26) Regulatory proteins in *Aspergillus fumigatus*
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Aspergillus fumigatus is a saprophytic fungus that can invade immuno-compromised patients through the lung and disseminates via the bloodstream. There is little known about how the fungus can regulate its gene expression to adapt to harsh conditions in blood. We are searching for genes that are up-regulated during blood infection. To study this process we designed an in vitro model to investigate the transcriptome of the fungus. In this setting mycelium is incubated in blood and then analyzed in microarray, transcriptome sequencing and quantitative Realtime-PCR. Differentially expressed genes were identified. Functional enrichment analysis revealed that at a late stage during blood incubation genes are up-regulated in the FunCat categories virulence, disease factors, toxins and detoxification by degradation. During early time points of blood incubation we detect up- regulated genes that belong to a family of regulators in filamentous fungi. The velvet gene family consists of the four proteins veA, velB, velC, and vosA, which exhibit regulatory functions during development in *Aspergillus nidulans*. Together with laeA, the velvet proteins seem to undergo various interactions in *A. nidulans* in order to respond to changed environmental conditions and stress. These features make them interesting candidates for involvement in the adaption process of *A. fumigatus* in blood. Functional deletions of velB, velC, vosA, and laeA were generated and characterized and will be studied in the in vitro blood model.

27) Genome-wide transcriptome and proteome analysis of *Aspergillus oryzae* in the hypoxic stress condition
Ryutaro Jo, Minori Kouno, Ryouko Hamada, Masafumi Tokuoka, Kazuhiro Iwashita (National Research Institute of Brewing, Hiroshima, Japan e-mail: iwashitact@nrib.go.jp)

In the process of making the Japanese traditional fermented foods, *Aspergillus oryzae* is exposed to hypoxic condition. In this study, we analyzed the effect of hypoxic condition on the physiology of *A. oryzae* using multi-omics analysis. Solid-state cultivation under hypoxic condition effect on morphology of *A. oryzae*, whereas hydrolytic enzyme activities were not significantly different except for glucoamylase. Transcriptional profiling revealed that expression of genes involved in glycolysis and ethanol fermentation were up-regulated under hypoxic (4% O_2) condition, which is supported at the protein level by proteomic analysis. On the other hand, expression of proteins involved in TCA cycle were decreased under hypoxic condition, which is consistent with the observation in the metabolite analysis where the amounts of organic acids in TCA cycle were increased in hypoxic condition. These results suggested that *A. oryzae* adapts to hypoxic condition by activation of glycolysis at transcriptional level and suppression of aerobic respiration at protein level. In addition, we found that gene expression level of BrlA involved in the conidiation was decreased under hypoxic condition. The BrlA over-expression mutant did not exhibit delayed conidiation, suggesting that atmospheric oxygen concentration effects on conidiation through BrlA gene expression. Our results provide the first report on the global physiological response of *A. oryzae* against hypoxia.

CELL BIOLOGY

28) Actin Precedes Myosin in Formation of Contractile Rings in *Aspergillus nidulans*
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By screening chemically mutagenized strains of the model fungus *Aspergillus nidulans*, we have identified several septation-impaired isolates with mutations occurring in loci different from those of previously- identified *A. nidulans* chr(34)*sep*chr(34) mutations. Here we report that the lesion in one of these strains (RCH2) occurs in a gene that encodes a fungal homologue of mammalian myosin-II (MyoB; AN4706). Sequencing of the *MyoB* allele in the RCH2 strain identifies a point mutation predicted to result in a glycine-to-aspartate amino acid substitution at residue 843 in the myosin-II converter domain. This residue is conserved in all fungal, plant, and animal myosin-II sequences that we have examined. The mutation does not prevent localization of the MyoB protein to contractile rings, but it does block ring constriction. Wild type MyoB colocalizes with myosin light chain (MLC; AN6732), tropomyosin (TpmA; AN5686), and alpha-actinin (AcnA; AN7707) in contractile rings. Down- regulation of wild-type MyoB expression under control of the *A. nidulans AlcA* promoter blocks septation and localization of MLC to pre-septal rings, but not localization of actin or TpmA. Similarly, ring targeting of AcnA is blocked by the RCH2 mutation. Conversely, ring targeting of MyoB, AcnA, MLC, and TpmA are all blocked by disruption of filamentous actin using Latrunculin B. We propose, therefore, an chr(34)actin-firstchr(34) model for the relationship between actin and myosin-II in formation of contractile rings.

29) The role of flotillin for the formation of apical Sterol-Rich membrane Domains (SRDs) and visualization of SRDs by Photoactivated Localization Microscopy (PALM).
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Apical sterol-rich plasma membrane domains (SRDs), which can be viewed using the sterol-binding fluorescent dye filipin, are gaining attention for their important roles in polarized growth of filamentous fungi. The microdomain scaffolding protein flotillin was thought to be a good candidate involved in the formation of SRDs. We analyzed the function of the flotillin orthologue FloA by gene deletion and protein localization in the maintenance of SRDs and polarity. SRDs are known to be necessary for the localization of some components of the growth machinery. To investigate deeply the relation of lipid membrane domains and protein localization, the distribution of microdomains in SRDs are analyzed by super-resolution microscope technique, Photoactivated Localization Microscopy (PALM). Raft membranes and non-raft membranes were visualized by each marker protein tagged with photoconvertible fluorescent protein mEosFP for PALM. The size of SRDs is around a few μm, whereas the size of lipid rafts ranges in general between 10-200 nm. In recent years, super-resolution microscope techniques have been improving and breaking the diffraction limit of conventional light microscopy whose resolution limit is 250 nm. In this method, a lateral image resolution as high as 20 nm will be a powerful tool to investigate membrane microdomains.

***30) Contamination of peripheral venous catheter associated fungal biofilms**

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In the hospital, the use of venous catheters for the administration of drugs exposes patients to increased risk for fungal contamination. Yeasts can form biofilms on the surface of catheters. These properties give them resistance to antifungal agents. In an attempt to integrate this clinical reality, we undertook a study in the neonatal unit of EHS(Hospital) in Tlemcen, to isolate *Candida* yeasts from venous catheters directly after excision from newborns hospitalized. The approach is to test the ability of yeasts isolated to form biofilms and to test their resistance against amphotericin B. From 281 samples, 3 strains of *Candida albicans* , one strain of *Candida parapsilosis* and 4 strains *Cryptococcus neoformans* were isolated. The biofilms formed by these yeasts were tested by reduction of tetrazolium salts in polystyrene microplates with 96 wells. The results indicate that yeasts of *Candida* species and *Cryptococcus neoformans* in the biofilm were more resistant to amphotericin B than their planktonic counterparts .

31) Proteins necessary for proper COG (conserved oligomeric Golgi) complex function in *Aspergillus nidulans*.

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The COG (conserved oligomeric Golgi) complex is associated with tethering of vesicles undergoing retrograde transport within the Golgi apparatus. Studies in animals and yeast revealed that the COG complex is composed of eight protein subunits which form a bi-lobed structure. In both models, the lobe containing COG1-4 is essential for proper COG function. Little is known of the COG complex in the filamentous fungi. Two temperature sensitive mutants have been discovered in *Aspergillus nidulans*, named *swoP*1 (swollen cell) and *podB*1 (polarity defective). Both mutants display abnormal spore swelling and polarity when grown at a restrictive temperature. Genes complementing the mutations of *swoP*1 and *podB*1 have sequence homology to COG4 (ANID7462.1) and a conserved hypothetical protein, likely COG2 (ANID8226.1), respectively. To study the role of these proteins in the COG complex, an AlcA promoter replacement strategy was performed. When grown on AlcA-suppressive media, the AlcA-promoter COG4 and AlcA-promoter COG7 strains displayed wild type growth at a restrictive temperature, while the AlcA-promoter COG1 and AlcA-promoter COG2 strains displayed swollen cells and abnormal polarity similar to that observed in the *swoP*1 mutant phenotype at a restrictive temperature. This data suggest that COG1 and COG2 are necessary for proper function of the COG complex in *A. nidulans* while COG4 and COG7 are not.

***32) Subcellular localization of AREA and AREB under different carbon and nitrogen regimes.**

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Nitrogen metabolite repression modulates the expression of target genes participating in utilization of alternative nitrogen sources, resulting in transcription only when glutamine or ammonium levels are limiting. In *Aspergillus nidulans* this regulatory mechanism depends on GATA transcription factors AREA and AREB. Both these factors function as a repressor of arginine catabolism genes under nitrogen repressing conditions.The activities of AREA and AREB are differentially regulated by the carbon regime: AREA being necessary for the ammonium repression these genes under carbon repressing conditions, while AREB is primarily involved under carbon-limiting conditions. To invastigate how a subcellural localistion of these two regulators depends on carbon and nitrogen regimes, *A. nidulans* strains expressing AREA and AREB fusions with fluorescent proteins were made and localisation of these two proteins detected under different nitrogen and carbon conditions. Bimolecular Fluorescent Complementation (BiFC) system was also used to determine interactions of AREA and AREB. We also transformed *A. nidulans areB* deletion mutant with *areB* gene from plant pathogen *Fusarium fujikuroi* to check if both proteins are functional homologues.

33) Shuttling of entire MAPK module from membrane to nuclear envelope links fungal development to secondary metabolism
Oezlem Sarikaya Bayram[1], Oezguer Bayram[1], Yasar Luqman Ahmed[1], Jun Ichi Maruyama[1], Oliver Valerius[1], Ralf Ficner[1], Stefan Irniger[1], Gerhard H. Braus[1,2] [1]Institute of Microbiology & Genetics, Georg August University, Goettingen, Germany [2]Goettingen Graduate School for Neurosciences and Molecular Biosciences

The sexual Fus3 MAP kinase module of yeast is highly conserved in eukaryotes and transmits external signals from plasma membrane to nucleus. The module of the filamentous fungus *Aspergillus nidulans* consisting of the AnFus3 MAP kinase, the upstream kinases AnSte7 and AnSte11 together with AnSte50 adaptor lacks the membrane interacting Ste5 scaffold homolog of yeast. The entire MAPK module interact with each other at the plasma membrane as in yeast. We find a different molecular mechanism how the MAPK signal is transmitted in the filamentous fungus: not only Fus3 but the entire complex of four physically interacting proteins migrates from plasma membrane to nuclear envelope. AnFus3 is the only subunit with the potential to enter the nucleus from the nuclear envelope. AnFus3 phosphorylates the conserved nuclear transcription factor AnSte12 to initiate sexual development and the conserved fungal velvet domain protein VeA to coordinate development with secondary metabolite production. Our data define the nuclear envelope as an additional critical control point for signal delivery of a MAP kinase pathway from the cellular surface through the cytoplasm to target regulators located in the nucleus.

***34) The *Aspergillus nidulans* Kinesin-3 Tail Is Necessary And Sufficient To Recognize Modified Microtubules**
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Posttranslational microtubule modifications are numerous; however, the biochemical and cell biological roles of those modifications remain mostly an enigma. The *Aspergillus nidulans* kinesin-3 UncA uses preferably modified MTs as tracks for vesicle transportation. Here, we show that a positively charged region in the tail of UncA (amino acids 1316 to 1402) is necessary for the recognition of modified MTs. Chimeric proteins composed of the kinesin-1 motor domain and the UncA tail displayed the same specificity as UncA, suggesting that the UncA tail is sufficient to establish specificity. Interaction between the UncA tail and alpha-tubulin was shown using a yeast two-hybrid assay and in *A. nidulans* by bimolecular fluorescence complementation (BiFC). Our data show that specificity determination depends on the tail rather than the motor domain, as has been demonstrated for kinesin 1 in neuronal cells. In a non-targeted Y2H approach interaction partners of this region were identified, because they are most likely involved in the recognition of MT subpopulations. Several candidates were confirmed using BiFC. Two are associated with vesicles; one is a predicted siderophore uptake transmembrane transporter and the other one was previously shown to be involved in ER to Golgi vesicle-mediated transport. The deletion of another fished interactor with similarity to Phosphatidylinositol 3- & 4-kinase family showed strongly reduced growth.

***35) *Aspergillus fumigatus* counteracts nitric oxide stress**
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Aspergillus fumigatus is a saprophytic mold that can cause life- threatening infections in immunocompromised patients. In the lung, inhaled conidia are confronted with immune effector cells, i.e., macrophages and neutrophil granulocytes. After recognition, the conidia are phagocytosed and attacked by host-derived reactive oxygen species and antimicrobial proteins. In recent studies it was shown that macrophages and neutrophils produce nitric oxide intermediates that are putatively involved in killing of the fungus. *A. fumigatus* produces several enzymes potentially involved in RNI detoxification, two flavohemoglobins, FhpA and FhpB, and the S-nitrosoglutathion reductase GnoA. To elucidate the role of these enzymes in detoxification of RNI, deletion mutants of FhpA, FhpB and GnoA encoding genes were generated. Mutant strains exhibited enhanced sensitivity against the NO donor DETA-NO. To investigate the role of RNI and its detoxification in fungal pathogenicity, virulence of the delta*gnoA* mutant was analysed in a cortisone acetate murine infection model for invasive aspergillosis. However, no difference in pathogenicity was detectable compared to the wild type and complemented strains. Therefore, the ability to detoxify host-derived RNI does not have a major influence on virulence of *A. fumigatus*.

36) Transcript of heterologous gene is stabilized by codon optimization of premature polyadenylated region in *A. oryzae*
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By using the mite allergen Der f 7 as a model, we have previously revealed that the transcript of AT-biased heterologous gene was polyadenylated prematurely within the coding region in *A. oryzae*. In addition, we have also revealed that this premature polyadenylation was prevented by codon optimization of the Der f 7 cDNA, resulting in the increase in its steady-state mRNA level. In this study, we examined the stability of transcription products derived from the heterologous gene in *A. oryzae* by using 1,10-phenanthroline as a transcription inhibitor. The transcript product of native Der f 7 cDNA fused to glucoamylase gene (*glaA*) was degraded rapidly, and half-life of this mRNA was approximately 13 min. On the other hand, half-life of codon-optimized Der f 7 mRNA fused to *glaA* was approximately 43 min, and this half-life was equal to that of endogenous *glaA* mRNA. These results indicated that Der f 7 mRNA is significantly stabilized by codon optimization. In addition, Der f 7 mRNA was stabilized by codon optimization of only the 3'-half region, in which premature polyadenylation sites were exclusively situated, and half-life of this chimeric Der f 7 mRNA was approximately 39 min. This suggested that destabilization of native Der f 7 mRNA is mainly triggered by premature polyadenylation within the coding region. To our knowledge, this report provides the first experimental evidence that heterologous mRNA is stabilized by codon optimization in eukaryotes.

***37) Functional analysis of Hsp70 family protein SsaA in *A. oryzae*.**
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A. oryzae exhibits azole drug resistance through overexpression of ABC transporter genes such as *atrA* , *atrF*, and *atrG*. Expression of these transporter genes is regulated by a Zn(II)Cys6 transcription factor, AtrR. Although it was suggested that AtrR shows different responses dependent on azole drug species, the detailed mechanism underlying regulation of AtrR in expression of the ABC transporter genes has not been elucidated. Since in *Saccharomyces cerevisiae* the AtrR counterpart Pdr1/Pdr3 are associated with Mediator protein complex, we examined whether or not there are Mediator-like proteins or co-activators interacted with AtrR in *A. oryzae*. To identify such proteins, a tandem affinity purification (TAP) moiety was fused to AtrR, with which candidate proteins prepared from the fungal mycelium were co-immunoprecipitated. The tandem mass spectrometry analysis showed that one of proteins enriched in the co-immunoprecipitated fraction was Hsp70 family protein, an ortholog of yeast Ssa1 (SsaA). In *S. cerevisiae* Pdr1 is positively regulated by Hsp70 protein Ssz1 and Pdr3 is negatively regulated by Ssa1/2. We constructed an overexpression strain of *ssaA* in *A. oryzae* and examined the involvement of the gene in the drug resistance. Overexpression of *ssaA* resulted in a slight decrease in azole drug resistance. In addition, this strain also showed a decrease in the expression of ABC transporter genes. These results suggested that AtrR is negatively regulated by SsaA in *A. oryzae*.

38) *gcsA*, an ARF-GAP-Encoding gene in *A. fumigatus.*
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Aspergillus fumigatus is one of the most important airborne pathogenic fungi, which has the potential to cause Invasive Pulmonary Aspergillose (IPA). Sphingolipids are the major component of the eukaryotic plasma membrane and are involved in the cell wall integrity and virulence. The ADP-ribosilation factor (ARF) family of proteins belongs to the Ras superfamily of small GTPases. The hydrolysis of ARF GTP-bound is mediated by GTPase-activating proteins (GAPs). ARF-GAPs are required for vesicular coat formation in endocytic pathway and have been related to hyphal growth, drug resistance and virulence in *C. albicans*. In this work we identified *gcsA*, the *C. albicans age3* ortholog gene in *A. fumigatus. gcsA* null mutant has normal hyphal growth, and decreased cell polarization in the presence of Myriocin, an inhibitor of serine palmitoyltransferase, the first step in sphingolipids biosysthesis. The absence of *gcsA* gene shows no differences in sensitivity to members of antifungal classes. The null mutant was not able to form biofilm, and was attenuated in virulence in an IPA mouse model. Thus, despite the *gcsA* null mutant has shown a normal sensitivity to antifungal agents, it has presented a role at the sphingolipids biosinthesis and virulence attenuation, suggesting a probable distinct function of this gene in *A. fumigatus*. Acknowledgments: FAPESP

39) Galactofuranose biosynthesis in *Aspergillus niger* provides new opportunities for industrial applications in the field of red and white biotechnology

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Galactofuranose-containing glycoconjugates are present in numerous microbes, many of which are pathogenic for humans. Metabolic aspects of the monosaccharide biosynthesis have proven difficult to elucidate, because galactofuranose metabolites and glycoconjugates are relatively unstable during analyses. Recent advances with genetic approaches have facilitated a better understanding of galactofuranose metabolism. Galactofuranose (Galf) the five-ring isomer of galactopyranose (Galp), is an essential component of the cell wall and required for a structural integrity [1-2]. Recently it has been postulated that Galp bound to UDP, is converted to Galf by a UDP- galactopyranose mutase (UGMA) and subsequently transported into the Golgi by a Galf-transporter named GlfB [3] for the further biosynthesis of e.g. galactomannan, galactoaminogalactan and cell wall glycoproteins (galactomanno-proteins) [4-6].

40) Peroxisomal localization of siderophore biosynthesis in *Aspergillus ssp.*

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Virtually all organisms require iron as an essential nutrient. Siderophores, low molecular mass, iron-specific chelators, play a central role in iron acquisition, iron storage and virulence of various phyto- and animal- pathogenic fungi. However, the subcellular localization of siderophore biosynthesis is unknown. *A. fumigatus* and *A. nidulans* produce two major siderophores: extracellular triacetylfusarinine C (TAFC) for iron acquisition and intracellular ferricrocin (FC) for iron storage. Interestingly, two TAFC biosynthetic enzymes, SidH (cis-anhydromevalonyl CoA-hydratase) and SidF (N5-hydroxyornithine:cis--anhydromevalonyl CoA-N5 transacylase), possess putative peroxisomal targeting signals type 1 (PTS1), which are highly conserved in their orthologs of Aspergillus ssp. . Using N-terminal GFP- tagging of SidH and SidF, we could show that the TAFC biosynthesis is, in part, localized in peroxisomes. Additionally SidH were localized in peroxin mutant strains of A. nidulans to confirm PTS1 dependent import. Peroxins are proteins critical for peroxisome biogenesis (e.g. PexC) or protein targeting into peroxisomes (e.g. PexE). Furthermore peroxin mutant strains were compared to the wild type with respect to siderophore biosynthesis and growth rate during iron-replete and iron depleted conditions to show the role of peroxisomes in iron acquisition. This is the first description of peroxisomal localization of siderophore biosynthetic steps.

BIOCHEMISTRY AND METABOLISM

41) The Interplay of Vacuolar and Siderophore-mediated Iron Storage in the Opportunistic Fungal Pathogen *Aspergillus fumigatus

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Iron is an essential element for all eukaryotes but its excess is deleterious. Iron homeostasis results from tight regulation of iron acquisition and iron storage. *A. fumigatus* produces the extracellular siderophores triacetylfusarinine C (TAFC) and fusarinine C (FSC) for iron uptake and the intracellular siderophores ferricrocin (FC) and hydroxyferricrocin for iron distribution and storage. Siderophore biosynthesis is important for the adaptation to iron starvation and therefore crucial for virulence. Intracellular iron excess has been shown to increase the content of FC-chelated iron and the expression of AFUA_4g12530, termed CccA, which shows similarity to the vacuolar iron importer Ccc1 of *S. cerevisiae*. These data indicate a role of both the vacuole and FC in iron detoxification. Green fluorescence protein-tagging confirmed localization of CccA in the vacuolar membrane. During high iron conditions genetic inactivation of CccA impaired growth, in particular in combination with derepressed iron uptake due to deficiency in the iron regulator SreA. In contrast, overproduction of CccA increased iron resistance. Inactivation of FC biosynthesis did not affect iron resistance. Lack of FC, CccA and in particular both, increased the cellular content of iron chelated by FSC/TAFC breakdown products. A delayed release of iron from FSC/TAFC degradation products might represent another iron detoxifying mechanism. Our data indicate that vacuolar rather than FC-mediated iron storage is the major iron detoxifying mechanism of *A. fumigatus*.

***42) A naphthopyrone synthase-like PKS from *Aspergillus terreus* produces phytotoxins**

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Aspergillus terreus is a saprophytic filamentous fungus with its natural habitat in soil, compost or associated with decaying fruit. *A. terreus* has a large potential to produce a wide variety of different secondary metabolites. However, it lacks a polyketide synthase (PKS) gene conserved in all related Aspergillus species that produces a naphthopyrone derivative responsible for colouration of conidia. Here, we discovered that in *A. terreus* the PKS most closely related to naphthopyrone synthases produces a phytotoxin. Analysis of HPLC profiles from a PKS deletion mutant revealed that it is required for the synthesis of at least 15 different metabolites, among them the major metabolite terrein. This well-known phytotoxin is a strong antioxidant that shows weak toxicity to mammalian cells but potently harms the surface of several fruits. Using a beta- galactosidase reporter strain we observed a weak expression of the gene cluster on minimal media and moderate activation on complex media. Interestingly, expression strongly increased in presence of plant derived compounds such as malt extract or different fruit juices. This indicates a specific recognition of yet unknown plant compounds resulting in phytotoxin production. Further analyses of the gene cluster and its metabolites are under investigation.

43) Signaling the induction of sporulation involves the interaction of two secondary metabolites in *Aspergillus nidulans*

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When growing *Aspergillus nidulans* hyphae encounter the atmosphere they initiate a morphogenetic program leading to the production of spore- bearing structures called conidiophores. Mutants defective in the *fluG* gene fail to initiate asexual sporulation because they lack an endogenous diffusible factor that purportedly accumulates on aerial hyphae, thus signaling development. Culture extracts from a wild type strain can, however, complement this defect when added exogenously. Through a bioassay- guided purification of culture extracts of a wild type strain, a factor that reverted the non-sporulating phenotype of a *fluG* deletion mutant was purified and identified as dehydroaustinol. This meroterpenoid was only active in fractions containing the orsellinic acid derivative diorcinol. This compound interacts with dehydroaustinol to form an adduct, detected by HRMS in a LC-MS experiment, which prevented dehydroaustinol crystal formation, facilitating its access to the putative receptor. This is, to our knowledge, the first instance in which a signaling compound requires the presence of an assisting molecule to facilitate its mode of action. Keywords: *Aspergillus nidulans*, asexual development, conidiophore, autoregulators.

***44) Effects of AOX gene knockout in antioxidant enzyme transcripts of *Aspergillus fumigatus* exposed to menadione**

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Alternative oxidase (AOX) plays an important role in the protection against macrophage ROS-mediated killing in *A. fumigatus*. In order to evaluate the interaction between cytoplasmic antioxidant enzymes and AOX within oxidative environment, we quantified the transcript production of antioxidant enzymes in AOX-knockouted (dAOX) strains treated with menadione in both germinant and hyphal phases of development. Transcripts were quantified with TaqMan qPCR, comprising 12 genes, including those related to thyolic, superoxide dismutase (SOD), catalase (Cat), glutathione reductase and cytochrome C peroxidase enzymes. Hyphae presented higher average levels (60%) of mRNA than germinants relative to untreated dAOX strain, suggesting their higher sensitivity to prooxidant. For both development phases, SOD2 showed the largest relative ammounts of transcripts (at least 10 times higher than control). Other relevant enzyme transcripts include thioredoxin (25.8 times) and Cat1 (12.9 times) for germinants, and glutaredoxin (11.9 times) for hyphae. The transcript production of those enzyme seems to be positively modulated by the AOX knockout. When menadione-treated CEA17 strain (positive for AOX gene) are used as control, thiolic enzyme transcripts are particularly abundant in germinants (1.73 times), whereas Cat1 showed the highest levels of transcripts in hyphae (2.25 times). These results suggest distinct mechanisms of antioxidant compensation during early and late phases of fungus development. The results were partially corroborated by a specific assay of catalase activity with total proteic extracts. The activity was significantly higher in hyphae than in germinants ($p < 0.003$), suggesting its high correlation with fungus development. Financial Support: FAPESP, CNPq, CAPES

***45) Mannitol-1-phosphate dehydrogenase is essential for the development of extreme stress resistant fungal ascospores**.
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Ascospores of *Neosartorya fischeri* exhibit extreme stress resistance. They survive extreme drought (down to 0.5% rh), high temperature (20 minutes at 85^{o}C), high pressure (6000 Bar) and various chemical stresses (e.g. pH and salt stress). The spores are constitutively dormant and can survive several years in a dormant state. Exit of dormancy, and subsequent germination, can be realized by a short heat flash at 85^{o}C. While much research focussed on the characterization of spores, not a lot is known about the process of ascospore development. During maturation ascospores become more heat resistant; this is accompanied with an increase of micro-viscosity and compatible solutes. A remarkable observation is the high concentration of mannitol in young spores, which slowly decreases during maturation of the spores. To evaluate the role of mannitol in development of ascospores, two genes involved in the mannitol metabolism (MPD & MDH) are deleted. The MPD mutant is not producing fully developed ascospores, while the formation of ascomata and asci is not affected. Within conidia, mannitol is thought to play a role in stress resistance and dormancy. We hypothesize a different role of mannitol. High mannitol concentration could result in an osmotic pressure, attracting water and nutrients to the ascocarp. Ongoing research on the promoter of the two mannitol synthesis genes and qPCR will give us more information about when and where MPD is transcribed.

***46) Characterization of Aspergillus niger chitinases involved in aging identifies a novel activity in fungal GH18 chitinases**
J.M. van Munster[1], B.M. Nitsche[2], A.F.J. Ram[2], R.M. van der Kaaij[1], L. Dijkhuizen[1], M.J.E.C. van der Maarel[1] [1] *Microbial Physiology , University of Groningen, The Netherlands.* [2]*Molecular Microbiology, Leiden University, The Netherlands* J.M.van.Munster@rug.nl Aging and stress conditions during industrial fermentations of *Aspergillus niger* may lead to the initiation of sporulation and autolysis, during which glycoside hydrolases (GH) can modify the cell wall structure. A full understanding of processes taking place during aging and nutrient starvation contributes to the identification of strategies to increase fermentation efficiency. Using transcriptome analysis, we identified glycoside hydrolases of *A. niger*produced by aging and nutrient starved mycelium. Two enzymes, putative chitinases, were heterologously expressed, purified and characterised to gain more insight in their physiological and metabolic function. CfcA releases mainly chitobiose from the non-reducing end of chitin oligosaccharides and from chitin present in the fungal cell wall. CfcI is capable of hydrolysing chitotriose and longer chitin oligosaccharides. CfcI functions by cleaving off monomers, possibly in a processive mode, acting on the reducing end of the oligosaccharide substrates. To the best of our knowledge, this activity has not been reported before for fungal chitinases of glycoside hydrolase family 18. We acknowledge AgentschapNL for funding IOP Genomics project IGE07008

47) Inhibition of ochratoxin A production in *Aspergillus carbonarius* by hydroxycinnamic acids from grapes
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Hydroxycinnamic acids: caffeic, coumaric and ferulic acid (HCAs) are endogenous components of grapes and have been reported to inhibit growth of a variety of organisms including fungi and bacteria. They are mostly represented in berry skin and pulp by caftaric, coutaric and fertaric acid, the tartaric esters. Ferulic acid has been proposed recently as a new antifungal agent against the *Dekkera*, spoilage of wines. In this respect, ochratoxin A (OTA), a nephrotoxin with carcinogenic properties, produced by *Aspergillus* and *Penicillium*, is considered the main contaminant of wines, relevant for human health risk. In addition, studies on phenolics and flavonoid compounds have showed the effect of some of them on growth and OTA biosynthesis in *A. carbonarius*. We investigated the activity of the free hydroxycinnamic acids, caffeic, p-coumaric and ferulic, on growth and ochratoxin A production by *A. carbonarius* in artificial and natural substrate. Interestingly, ferulic acid resulted the most effective in reducing OTA biosynthesis already at 0,3 mg/ml without any reduction of fungal growth. The effect on genes involved presumably in OTA biosynthetic pathway has also been investigated. This research was supported by Ministerial project ALISAL-DM 11008/73

***48) A proteome reference map of *Aspergillus nidulans* and new putative targets of the AnCF complex**

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The mould *Aspergillus nidulans* is a well suited model organisms for filamentous fungi and is closely related to many *Aspergillus* species of industrial and medical interest. With the completion and publication of the *A. nidulans* genome it is feasible to study gene expression and protein production on a global scale. A variety of transcriptome studies have been already carried out for *A. nidulans*. By contrast, only little information is available about the dynamic changes of the proteome of *A. nidulans* upon environmental changes, stress conditions or genetic modifications. Furthermore, no proteome map for has been published so far. For this reason, we established the first 2-D reference map for the intracellular protein fraction of *A. nidulans* strain TNO2A7. After 2D-gel electrophoretic separation, visualisation of proteins by Coomassie staining and image analysis with Delta 2D, 435 spots representing 364 different proteins were identified by MALDI-TOF-MS/MS analysis. Quantitative proteomic analysis of a *hapC* deletion mutant revealed many proteins with difference in abundance in comparison to the wild type. Two proteins, a conserved hypothetical protein and a guanine nucleotide dissociation inhibitor, were found to be putative, so far uncharacterised targets of the AnCF complex. Knock-out and double knock-out strains of the corresponding genes are in progress.

***49) Expression Response Of *Aspergillus oryzae* To Different Nitrogen Sources In Batch Cultivations**

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The filamentous fungus *Aspergillus oryzae* is widely used as a microbial cell factory for large scale heterologous protein production. *A. oryzae* is also known as a natural organic acid producer. However, the metabolism and regulation of organic acid production in *A. oryzae* are poorly characterized. Furthermore, the media composition has a major impact on the performance of this organism and the economic feasibility of an industrial fermentation process. We therefore evaluated the global expression response towards and the utilization of different nitrogen sources by *A. oryzae* in batch fermentations. In this study, we aim for a deep investigation of the cellular mechanisms of the utilization of different nitrogen sources. Firstly, we performed batch cultivations with two strains (NRRL3488 and DSM1862) on defined and complex nitrogen sources using di-ammonium sulphate and peptone, respectively. In addition, transcriptome analysis was performed on samples from these fermentations to analyze the gene expression under exponential growth conditions (mid-exponential phase; 6h) and in nitrogen starvation (stationary phase; 30h) to further identify key-players in the metabolism and regulation of gene expression. Cluster analysis helped to identify regulatory sequences among the co- expressed genes. Furthermore the expression data pointed towards malic acid production as a response to nitrogen starvation stress.

50) The Role of Ornithine Supply in Siderophore Biosynthesis in *A. fumigatus

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Iron is an essential nutrient required for a wide range of cellular processes. However, excessive iron accumulation is toxic. Therefore, microorganisms evolved fine-tuned iron uptake and storage mechanisms, such as the siderophore system. The opportunistic fungal pathogen *Aspergillus fumigatus* produces siderophores to acquire, store and distribute iron. Past studies indicated coordination of siderophore biosynthesis with supply of its precursor ornithine. The role of mitochondrial ornithine production in siderophore biosynthesis of *A. fumigatus* was characterized by analysis of the phenotypic consequences of genetic inactivation of the putative mitochondrial ornithine exporter, AmcA (Afu_8g02760). Consistent with a role in mitochondrial ornithine export, inactivation of AmcA resulted in a decrease in the cellular ornithine content as well as a decrease in extra- and intracellular siderophore production. In the presence of the iron chelator bathophenanthroline disulfonate, which inhibits siderophore- independent iron uptake, AmcA-deficiency decreased conidiation, indicating increased iron starvation. In contrast to siderophore production, AmcA- deficiency didn't affect the cellular content in polyamines, which are also derived from ornithine via the ornithine decarboxylase. Nevertheless, AmcA-deficiency increased the susceptibility of *A. fumigatus* to eflornithine, an inhibitor of the ornithine decarboxylase, most likely due to the decreased ornithine pool. Siderophore biosynthesis is mainly fueled by mitochondrial production of ornithine, rather than by conversion of arginine to ornithine in the cytoplasm. There exists coordination between siderophore biosynthesis and its precursor supply. This study also indicates a prioritization of ornithine flux into synthesis of polyamines compared to siderophores, emphasizing the essentiality of polyamines.

51) Closely related fungi employ diverse enzymatic strategies to degrade plant biomass

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In natural biotopes, plant biomass is the predominant carbon source for most fungi. Plant biomass consists mainly of polysaccharides. Fungi cannot take up the intact polysaccharides, but need to degrade them extracellularly to monomeric and small oligomeric compounds using enzymatic mixtures that are tailored specifically to the available polysaccharides. A recent study shoed significant differences in the polysaccharide degrading ability of three Aspergilli, while only small differences were detected in growth on plant polysaccharides [1]. This suggests that they may have developed different approaches to plant biomass degradation, using different enzymes. A better understanding of these strategies will increase our insight in fungal biodiversity and help to design more efficient industrial processes. In this study we compared plant biomass degrading potentials and strategies of 8 Aspergilli and showed that they developed highly diverse approaches to using complex carbon sources. Although all species contain the main regulators involved in plant polysaccharide degradation (AmyR, XlnR, AraR, InuR) the enzymatic sets produced by them differs significantly, suggesting a species specific fine-tuning of plant biomass degradation. [1] Coutinho PM et al. 2009. Fung Genet Biol 46: S161S169.

52) Lactose catabolism in *Aspergillus nidulans*

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Lactose is intracellularly hydrolysed by A. nidulans. Results from early studies enabled the in silico identification of clustered, divergently transcribed intracellular beta-galactosidase (bgaD) and putative lactose permease (lapA) genes. bglD and lapA were co-expressed in response to D-galactose, lactose or L-arabinose, while no transcription was detectable in the co-presence of glucose. By contrast, creA loss-of-function mutants featured derepression of both genes to a basal level under non-inducing conditions. However, lactose- and D-galactose induction only occurred in the absence of glucose, indicating a prominent role for CreA-independent repression in the system's regulation. To confirm lactose permease function, the lapA gene was deleted. Gene-deleted strains grew in liquid lactose media, albeit at a much lower rate than wild-type controls. On the other hand, strains that carried more than one copy of lapA progressively grew faster, showing that transport is the limiting step in lactose catabolism. The effect of lapA gene deletion on lactose uptake was exacerbated at lower concentrations, putting to evidence the existence of a second component of lower affinity for the disaccharide in A. nidulans.

53) A new subfamily of ABC transporters mediates excretion of extracellular siderophores in *A. fumigatus*

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The opportunistic human pathogen *Aspergillus fumigatus* produces extra- and intracellular siderophores for acquisition, storage, and intracellular distribution of iron; siderophore biosynthesis is essential for its virulence. Here we report the functional characterization of three iron regulated ABC transporters, termed AbcB, AbcC, and AbcD. Two of them, AbcB and AbcC, are encoded by genes located within iron-regulated gene clusters and are involved in the excretion of the extracellular siderophores fusarinine C (FsC) and triacetylfusarinine C (TAFC). Inactivation of AbcB blocked excretion of the siderophore fusarinine C (FsC) and increased intracellular accumulation of FsC degradation products. Enhanced green fluorescent protein (EGFP)- tagging localized AbcB in the plasma membrane. Consistently, AbcB- deficiency impaired the growth rate during iron depleted but not iron-replete conditions. Deletion of AbcC decreased TAFC excretion but increased excretion of its precursor FsC. Inactivation of AbcD reduced siderophore excretion only in an AbcB- or AbcC-deficient background, implying compensatory functions of this subset of ABC-transporters. Moreover siderophore excetion could not be abolished in a triple null-mutant over time, assuming alternative siderophore excretion bypasses.

54) The impact of ornithine and arginine biosynthesis on siderophore production of *Aspergillus fumigatus
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The opportunistic fungal pathogen *Aspergillus fumigatus* produces extracellular siderophores for iron uptake and intracellular siderophores for storage and distribution of iron. Moreover, *A. fumigatus* employs a second high-affinity iron acquisition system, reductive iron assimilation (RIA). Siderophore biosynthesis (SB) but not RIA is essential for virulence. The main precursor of siderophores, ornithine, can be produced from glutamate in the mitochondria or cytosolic hydrolysis of ornithine-derived arginine. Here, the impact of inactivation of mitochondrial ornithine biosynthesis (*ΔargEF* mutant lacking N-acetylglutamate kinase/ N-acetylglutamylphosphate reductase) and cytosolic arginine biosynthesis (*ΔargB* mutant lacking ornithine transcarbamoyl transferase) on siderophore production was studied. Both *ΔargEF* and *ΔargB* are arginine auxotrophic. Growth of *ΔargEF* but not *ΔargB* is partially rescued by ornithine supplementation. Blocking RIA by ferrous iron chelation inhibited growth of *ΔargEF* but not *ΔargB*. Siderophore production of *ΔargEF* decreased while that of *ΔargB* increased with declining arginine availability. Taken together, these data indicate that the siderophore system is mainly fueled by mitochondrial rather than cytosolic ornithine production and that mitochondrial ornithine biosynthesis is feedback inhibited by arginine. In agreement with the SB defect, *ΔargEF* displayed a dramatically reduced cellular ornithine content. In contrast, the arginine and polyamine contents were wild type-like, indicating prioritization of the later two biosynthetic pathways over SB. Consistent with cellular balancing of SB and arginine metabolism, arginine was recently identified to allosterically activate the ornithine monooxygenase SidA and consequently SB-mediated ornithine consumption.

POPULATION AND EVOLUTIONARY GENETICS

55) Benefits of sex and ascospore production in *Aspergillus nidulans*
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Aspergillus nidulans can reproduce by asexual or sexual means, producing green conidiospores or red-purple ascospores respectively, the latter produced in dark-purple globose cleistothecia which are surrounded by Hulle cells. The species has a homothallic (self fertile) sexual breeding system. Given the extra metabolic costs associated with sexual compared to asexual reproduction it would be predicted that ascospore production would confer evolutionary benefits. However, due to the homothallic breeding system there is very rarely any increased genetic variation in ascospore offspring and traditionally conidia and ascospores are considered to be equally environmental resistant. We therefore examined in detail whether conidia and ascospores might exhibit as yet undetected differences in spore viability when subjected to certain environmental stressors. Spores from four strains of *A. nidulans* (comprising wild-type and *KU* mutants) were exposed to various levels of temperature (50 ^{o}C - 70 ^{o}C for 30 min) and UV (350 nm for 10 - 60 min) stress. We detected that under certain exposure levels ascospores have significantly increased resistance compared to conidia. The increased environmental resistance of ascospores might be a key factor explaining the persistence of sexuality in this homothallic species, and reasons for differential survival are suggested.

OTHER TOPICS

***56) Secondary metabolism and fungal-insect interactions**
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Fungi synthesize an astonishing variety of secondary metabolites. Even though little is known about the benefit of these metabolites, the ability to regulate secondary metabolism might be seen as an evolutionary adaptation. Presumably fungi regulate secondary metabolites in response to confrontation with natural competitors like insects to guarantee efficient exploitation of environmental resources (1-3). In order to enlighten the biological function of these secondary metabolites with reference to chemical defence reactions of insect-fungal interactions, we utilized complementary approaches of experimental ecology and functional genomic techniques. A further aspect was to investigate the influence of these competitors at trophic interactions. In our research *Drosophila melanogaster* and its natural antagonist *Aspergillus nidulans* are used as an ecology model system. To analyse fungal up- or down regulated target genes in the interaction of *A. nidulans* with *Drosophila* larvae microarray analysis was performed. Quantitative RT-PCR confirms up- regulation of the global regulator *laeA* as well as of *aflR*. Moreover several other genes are up-regulated under competing conditions. Candidate genes are being used for reporter gene analysis and RNAi constructs are being used for competition experiments. 1.Rohlfs M. et al. (2007) Biol Lett 3, 523-25 2.Kempken, F., and Rohlfs, M. (2010) Fungal Ecol 3, 107-14 3.Rohlfs M. et al. (2009) in chr(34)The Mycota XV. Physiology and Genetics: Selected Basic and Applied Aspectschr(34) (Anke, T., Ed.), Springer, 131-51

57) Revision of the genus *Aspergillus*, problems and prospects

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Aspergillus is a broad paraphyletic group of species, and its teleomorphic states are very different among three major clades. Since a one one name system will soon be implemented, either one has to use *Aspergillus* for all species, or one can for example neotypify *Aspergillus* with *Aspergillus niger* and use teleomorphic names for some clades of *Aspergillus sensu lato* and use known teleomorphic names for the rest: *Eurotium* for sections *Aspergillus* and *Restricti*, *Neosartorya* for sections *Fumigati* and *Clavati* , *Emericella* for sections *Versicolores*, *Aenei*, *Nidulantes* and *Sparsi*, and *Aspergillus* for the rest, i.e. sections *Nigri*, *Flavi*, *Candidi*, *Circumdati*, *Terrei*, *Flavipedes*, *Cremei* and *Cervini*. Each solution has advantages and problems.This is still a matter of international agreement, and a meeting in April 2012, One fungus which name will be held in Amsterdam to discuss these issues. Taxonomically a species like *Penicillium inflatum* will be transferred to *Aspergillus*, while *Aspergillus paradoxus*, *A. crystallinus* and *A. malodoratus* will be transferred to *Penicillium*, making a purely morphologically based genus circumscription very difficult. It is recommended to use a polyphasic approach to *Aspergillus* taxonomy.

58) Multiple gene expression system by means of Cre/*loxP*-mediated marker recycling with mutated *loxP* sites in *Aspergillus oryzae*.

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We have previously reported that Cre/*loxP*-mediated marker recycling system in which Cre enzyme is directly introduced into *Aspergillus oryzae* cells to rescue the marker gene flanked by *loxP* sites [1]. In this study, we developed the Cre/*loxP*-mediated marker recycling system with mutated *loxP* sequences to introduce multiple genes, such as secondary metabolite biosynthetic genes, into *A. oryzae*. The selectable marker, *Neurospora crassa pyr4*, located between mutated *loxP* sequences, *loxP66* and *loxP71*, was introduced into *A. oryzae pyrG*$^{-}$. Then Cre enzyme was directly introduced into the *pyrG*$^{+}$ transformants by protoplast fusion, but no candidates that the marker was excised were isolated, although the marker-rescued strains were obtained from the transformants containing the marker flanked by intact *loxP* sites. This would be caused by less susceptibility of mutated *loxP* sequences to Cre-mediated recombination, and thus we attempted to express Cre recombinase in the cell by introduction of the expression vector harboring the *cre* gene under the *thiA* gene promoter. In this attempt we succeeded in efficient excision of the marker gene in the transformant with mutated *loxP*. [1] Mizutani et al. 26th Fungal Genetics Conference, P617

59) Characterisation of the SAGA/ADA complex in *Aspergillus nidulans* by tandem affinity purification

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The recent finding that the histone acetyltransferase (HAT) complex SAGA/ADA mediates the response of the fungus *Aspergillus nidulans* to the bacterium *Streptomyces rapamycinicus* (1) opens up a number of questions. It was shown that the SAGA/ADA complex is involved in the regulation of the orsellenic/ lecanoric acid biosynthesis gene cluster, as deletion of its HAT-encoding gene *gcnE* resulted in the lack of *orsA* transcription during co-cultivation. In order to investigate the SAGA/ADA complex in *A. nidulans*, the complex subunits GcnE and AdaB were tagged and purified by tandem affinity purification (TAP). This method is especially suited for the purification of protein complexes under native conditions. Therefore, the TAP-tag method represents an appropriate system for the investigation and analysis of the SAGA/ADA complex composition under various conditions. The TAP-tag constructs were assembled by fusion PCR and transformed directly into *A. nidulans* via homologues recombination. Western blotting was performed to monitor the purification procedure. For AdaB-TAP and GcnE-TAP bands of the expected size were detected. However, further optimisation of the purification procedure is required. (1) Nuetzmann et al. (2011) *PNAS*

60) Vacuolar H^+-ATPase plays a key role in cell wall biosynthesis of *Aspergillus niger*

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The identification of suitable targets is crucial for the discovery and development of new antifungals. Since the fungal cell wall is an essential organelle, the identification of genes involved in cell wall biosynthesis is expected to help discover new antifungal targets. We selected a thermosensitive, osmotic-remediable mutant with decreased resistance to SDS for complementation analysis. This mutant was complemented by a gene encoding a protein with high sequence similarity to subunit D of the eukaryotic Vacuolar-H^+-ATPase (VmaD). Genetic analysis revealed that the mutant allele encodes a protein that lacks 12 amino acids at the C-terminus. Deletion of the entire gene resulted in very poor growth. The conditional mutant displayed several phenotypes that are typical to V-ATPase mutants, including increased sensitivity to zinc ions and reduced acidification of the vacuole. Furthermore, genes involved in cell wall reassembly like fksA, agsA and phiA are clearly up-regulated in the conditional mutant. Our results indicate that the ATP-driven transport of protons and acidification of the vacuole is crucial for the strength of the fungal cell wall and that reduced activity of the V-ATPase induces the cell wall stress response pathway.

61) Spatially resolving the secretome within the mycelium of the cell factory *Aspergillus niger*

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Hyphae at the periphery of a fungal colony are exposed to unexplored organic material, whereas the center of the colony experiences a utilized substrate. This suggests that enzymes that are secreted to degrade organic material are different in the zones of a colony. *Aspergillus niger* is an important cell factory for the industrial production of enzymes. Here, we determined with stable isotope dimethyl labeling the secretome of 5 concentric zones of 7-day-old xylose-grown colonies of *A. niger* that had either or not been treated with cycloheximide. As expected, cycloheximide blocked secretion of proteins at the periphery of the colony. Unexpectedly, protein release was increased by cycloheximide in the intermediate and central zones of the mycelium when compared to non- treated colonies Electron microscopy indicated that this is due to partial degradation of the cell wall. Apparently, a major part of the proteins are associated with the cell walls. Besides this, the composition of the secretome in each of the 5 concentric zones differed. Taken together, cycloheximide can be used to obtain a (near) complete secretome of *A. niger*. This study thus describes spatial release of proteins in *A. niger*, which is instrumental in understanding how fungi degrade complex substrates in nature.

62) Co-cultivations of fungi: microscopic analysis and influence on protein production

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During their natural life cycle most fungi encounter other microorganisms and live in mixed communities with complex interactions, such as symbiosis or competition. Industrial fermentations, on purpose or by accident, can also result in mixed cultures. Fungal co-cultivations have been previously described for the production of specific enzymes, however, little is known about the interactions between two species that are grown together. *A. niger* and *A. oryzae* are two of the most important industrial fungi worldwide and both have a long history of strain improvement to optimize enzyme and metabolite production. Co-cultivation of these two *Aspergilli* with each other and with the ascomycete phytopathogen *Magnaporthe grisea*, and the basidiomycete white rot fungus *Phanerochaete chrysosporium*, has recently been described by our group (Hu *et al*, 2011). Total secreted protein, enzymatic activities related to plant biomass degradation and growth phenotype were analyzed from cultures on wheat bran demonstrating positive effects of the co-cultivation compared to the individual cultivations. In a follow-up study the morphology and mechanism of the interaction is addressed using microscopy and proteomics. Data from this study will be presented. Reference Hu *et al*. International Biodeterioration & Biodegradation 65 (2011)

63) New promoters to improve heterologous protein production by *Aspergillus vadensis*

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Aspergillus species have always been used as producers of homologous and heterologous proteins. Although *Aspergillus niger* is most commonly used, a close relative of this species, Aspergillus vadensis, has been suggested as a better candidate As this species produces very low levels of extracellular proteases and does not acidify the medium [1]. It has also been shown that this species can produce higher levels of *A. niger* FaeB than *A. niger* itself [2] and can produce a basidiomycete esterase that could not be produced by *A. niger* [3]. To further improve protein production in *A.vadensis*, six new constitutive promoters have been selected from *A. niger* and tested in *A.vadensis*. in comparison to the *gpd*A promoter [4]. selected gene encoding a secreted arabinofuranosidase was used as a reporter for protein production. Based on activity all seven promoters activate the expression of the abf, some at higher levels than *gpd*A. [1] de Vries et al. Appl Environ Microbiol 2004, 70: 3954-3959 [2] Alberto et al. Lett Appl Microbiol 2009, 49: 278282 [3] uranov et al. Arch Microbiol 2009, 191: 133-140 [4] Punt et al. GENE. 1990, 93 :101-109

64) Chemically Induced Haploinsufficiency Screens to Identify Drug Mechanism of Action in *Aspergillus Fumigatus*

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Current options for the treatment of Aspergillus infections are limited and suffer from a variety of shortcomings. Despite the discovery of numerous promising drug targets, few lead compounds have been discovered by target based approaches. This can be explained, in part, by the druggability of a target as some compounds which demonstrate promising activity against an enzyme are not active against the whole cell or are toxic. A solution to this problem is to employ techniques to identify gene targets from compounds that already show antifungal activity and have clean toxicity profiles. Chemical genetic profiling aids identification of drug mechanism of action as diploid strains lacking a single copy of a drugs target are hypersensitive to that drug. Heterozygote *S. cerevisiae* and *C. albicans* libraries have been used to identify the mechanism of action of several promising compounds; however, this has been hindered in *A. fumigatus* by the complexity in generating an adequate set of heterozygous strains. A high- throughput targeted gene KO method for *A. fumigatus* has been established by employing fusion-PCR to generate targeted gene disruption cassettes, optimizing the common transformation protocol for *A. fumigatus* high-throughput gene disruption, and utilising a diploid *Ku80*$^{-}$*/Ku80*$^{-}$ mutant to facilitate more reliable homologous recombination. Preliminary efforts have produced 46 heterozygous KO strains and subsequently, the feasibility of chemical genetic haploinsufficiency studies in filamentous fungi has been demonstrated. This enables high-throughput methods for surveying the genome of *A. fumigatus* for new drug targets and supports unveiling the mechanisms of action of antifungal drugs.

65) The *Vader*-transposon: a molecular tool for random mutagenesis in *Aspergillus niger*

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Transposons are repetitive and mobile elements found in all eukaryotic genomes, which have a huge impact on gene expression. Their ability to cause major mutations within the genome or single genes makes them an interesting tool for random mutagenesis. Using transposable elements in filamentous fungi would aid gene characterization for instance in pathogenic or biotechnological important strains (1, 2). At current we are employing the *Vader* transposon from *Aspergillus niger* (3). This transposable element was inserted between the promoter and the open reading frame of the hygromycin phosphotransferase (*hph*) gene and transformed into *A. niger*. To identify the transposition events we use hygromycin selection and observed a *Vader* excision frequency of about 1 in 2.2 x 10^5 spores. The analyzed colonies showed an excision event on the DNA level and *Vader* footprints were found. The reintegration sites of 21 independent excision events were determined using TAIL-PCRs. *Vader* mostly integrates within or very close to genes, thus it appears to be a useful tool for transposon-mediated mutagenesis in *A. niger* (4). At current we are improving the *Vader* tool and analyze its function in the heterologous host *A. nidulans*. 1. Kempken F (2003) In: Arora DK, Khachatourians GG (eds) Applied Mycology and Biotechnology, Vol. 3 Fungal Genomics, Elsevier Science Annual Review Series, pp83-99 2. Poeggeler S, Kempken F (2004) In: Kueck U (ed) The Mycota II, Genetics and Biotechnology, 2nd edition, Springer Verlag, Heidelberg, New York, Tokyo, pp165-198 3. Braumann I, van den Berg M, & Kempken F (2007) Fungal Genet Biol 44(12):1399-1414. 4. Hihlal E, Braumann I, van den Berg M, Kempken F (2011) Appl Environment Microbiol, 77: 23322336

66) Efficient expression system for production of natural products in *Aspergillus oryzae

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pTAex3 is an expression vector used for heterologous gene expression in *Aspergillus oryzae*. This vector has previously been modified by insertion of a GATEWAY destination module into the strong, starch-inducible amyB expression cassette to produce pTAex3GS. This facilitates directional transfer of genes such as fungal polyketide synthases (PKS) and hybrid polyketide synthase-non-ribosomal peptide synthases (PKS-NRPS) into the expression site. To simplify plasmid construction for whole-pathway expression pTAex3GS was first converted to a yeast-*E. coli* shuttle vector, pTAYA.GS. An EST database was used to identify genes expressed at a high level under the culture conditions we use for heterologous gene expression in *A. oryzae*, and the promoters of three of them, P*adh* (alcohol dehydrogenase), P*eno* (enolase) and P*thia* (thiazole synthase), were evaluated. *A. oryzae* transformants expressing eGFP from P*adh* and P*eno* exhibited intense green fluorescence. We used homologous recombination in yeast to combine P*adh* and P*eno* together with the strong constitutive *A. nidulans* promoter P*gpdA* in pTAYA.GS-Page, a novel multiple gene expression vector which has *Asc*I sites downstream of each promoter. The system was tested by reconstructing and expressing the *Beauveria bassiana* tenellin and *Aspergillus nidulans* aspyridone synthesis pathways, each of which comprises a hybrid PKS-NRPS together with an enoyl reductase and one or more cytochrome P450s, in *A. oryzae*. Yeast recombination between the *Asc*I-cut vector and three PCR products simultaneously placed the tailoring genes downstream of the promoters, creating pTAYA.GSargTen and pTAYA.GSargAsp. Subsequent introduction of the PKS-NRPS gene by GATEWAY recombination created pTAYAargTenellin and pTAYAargAspyridone. Reconstruction of the tenellin and aspyridone biosynthetic pathways proved the multiple gene assembly concept, and chemical analysis showed that 5 of the 11 pTAYAargTenellin transformants analysed produced tenellin, pretenellin B and prototenellin A. Similarly 13 of 14 pTAYAargAspyridone transformants analysed produced aspyridone A and preaspyridone. The results show that our system allows the rapid and simple reconstruction of whole (small) biosynthetic pathways for heterologous expression from a single plasmid in *A. oryzae*. Further development of the system has included replacement of the arginine selectable marker with basta- and phleomycin-resistance genes to allow expression of biosynthetic pathways of up to 12 genes by co-transformation of *A. oryzae* with just 3 plasmids.

67) The Contribution of Melanin to Spore Surface Characteristics in *Aspergillus niger

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Fungi grow on a great variety of organic and anorganic materials. Usually spore adhesion to solid surfaces comprises the first step of colony establishment or biofilm formation. In liquid culture, many filamentous fungi grow as hyphal aggregates or pellets, a process depending on cell-to-cell interactions of spores and/or hyphae. Pellet formation has been described as two-step processes, comprised of initial aggregation of ungerminated conidia followed by further attachment of spores, germ tubes and hyphae. To test the contribution of the initial aggregation/adhesion of ungerminated spores to pellet and biofilm formation in *Aspergillus niger*, we altered the physical and chemical surface characteristics of conidia by inactivating melanin biosynthesis. Albino mutants were constructed by the deletion of the *alb1* gene, encoding a polyketide synthase essential for pigment biosynthesis. Δ*alb1* conidia exhibit an altered surface structure and changed physiochemical properties. Spore aggregation in liquid culture differs significantly in a pH dependent manner between wild type and mutant. However, further pellet formation and enzyme productivity is unaffected, suggesting a minor role of initial spore adhesion in pellet formation. In contrast, under biofilm promoting conditions, Δ*alb1* mycelia adhere more stably to polymer surfaces, suggesting that initial conidial adhesion promotes sessile growth. Enzyme productivity of biofilms was significantly increased compared to pellet cultures, prompting us to further analyze biofilm formation in *A. niger*

***68) Recent Development In The Taxonomy And Phylogeny Of *Aspergillus* And *Penicillium*: Implications For Full Genome Sequence Initiatives**

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The family of *Trichocomaceae* harbors various economically important genera, such as *Aspergillus*, *Penicillium* and *Paecilomyces*. The importance of these genera is illustrated by the high number of undertaken full genome sequencing projects. Recently, new insights in the taxonomy of *Aspergillus* and *Penicillium* have led to numerous new species and name changes of existing species. In this study, the phylogeny of the full genome sequenced strains is investigated using the protein coding genes RPB1, RPB2, Cct8 and Tsr1. Furthermore, the impact of recent developments in the taxonomy of these strains is addressed. Phylogenetic analysis shows a close relationship between the full genome sequenced strains (or representatives of the same species) of *Aspergillus*, *Penicillium sensu stricto*, *Monascus* and *Xeromyces*. *Talaromyces stipitatus* and *Penicillium marneffei* appear to be distantly related to *Aspergillus* and *Penicillium s. str.* As a consequence, *Talaromyces* is re-defined and the combination *Talaromyces marneffei* (= *P. marneffei*) is proposed, leaving *P. chrysogenum* as the sole full genome sequenced species in *Penicillium*. Furthermore, *Talaromyces emersonii* is transferred to the new genus *Rasamsonia* and *Talaromyces thermophilus* will be transferred to *Thermomyces*. The new insights in the relationship among *Aspergillus*, *Penicillium* and related genera will help to interpret the results generated with comparative genomics studies or other studies dealing with evolution of e.g. mating type loci, virulence genes and secondary metabolite biosynthetic gene clusters.

69) The genetic basis of conidial pigmentation in *Aspergillus niger

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A characteristic hallmark of *Aspergillus niger* is the formation of black conidiospores. We have identified four loci involved in spore pigmentation of *A. niger* by using a complementation approach. First, we characterized a newly isolated color mutant, *colA*, which lacked pigmentation resulting in white conidia. Pigmentation of the *colA* mutant was restored by a gene (An12g03950) which encodes the *A. niger* ortholog of the 4-phosphopantetheinyl transferase protein (PptA). The loci giving rise to fawn, olive, and brown color phenotypes were identified by complementation. The fawn mutant was complemented by the polyketide synthase A protein (PksA, An09g05730), the *olvA* mutant by An14g05350 (OlvA) and the *brnA* mutant by An14g05370 (BrnA), the respective homologs of *PksP/alb1*, *ayg1* and *abr1* in *A. fumigatus*. Targeted disruption of the *pptA, pksA, olvA* and *brnA* genes confirmed the complementation results. The different color genes are expected to function in a linear pathway producing the black melanin. To determine the epistasis for the fawn, olive and brown mutants, double mutants were constructed in all possible combinations. As expected, *pksA* is epistatic over both *olvA* and *brnA*, and *olvA* is epistatic over *brnA*.

70) Phenotyping the genus *Aspergillus*

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The genus *Aspergillus* is one of the largest known groups of ascomycetous fungi containing more than 300 species. Most species are commonly found in soil and other environments and are able to grow on a wide range of substrates. The genus includes several species used in biotechnology (*A. niger, A. oryzae, A. sojae*) but also opportunistic human pathogens (e.g. *A. fumigatus*) and mycotoxin producers (e.g. *A. flavus*). As part of our initiative to generate a database (www.fung-growth.org) of comparative physiology of filamentous fungi, we have initiated growth profiling of the complete *Aspergillus* genus. Comparison of the first 35 growth profiles has already revealed significant differences between the species, both with respect to degradation of polymeric carbon sources as well as catabolism of monomeric carbon sources. Highlights of this study will be presented.

71) Modification of regulators in *Aspergillus niger* to produce enzyme mixtures that completely degrade all available plant polysaccharides

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Plant biomass is a major substrate for many industries, such as food and feed, paper and pulp, and biofuel. Fungal enzymes from *Aspergillus niger* have a long history of use in plant-biomass degradation. However, currently available commercial enzyme mixtures are not sufficiently efficient for biofuel pretreatments. While in other applications only part of the biomass needs to be hydrolyzed, complete hydrolysis of all available plant polysaccharides (*i.e.* cellulose, hemicellulose, and pectin) is required for cost-effective biofuel production. The main reason for the absence of a suitable enzyme mixture is that enzyme production by *A. niger* is tightly regulated by its environment. For instance, transcriptional regulator CreA is causing strong repression in the presence of easily metabolisable carbon sources, such as glucose and xylose. Also, the activation of transcriptional activators such as XlnR, AraR, and AmyR are necessary for the production of plant-polysaccharide degrading enzymes. In this project, we aim to modify the production of biomass-degrading enzyme mixtures by genetic engineering of transcription factors in *A. niger*. For instance, initial results showed that deleting CreA in combination with constitutive activation of XlnR enables production of cellulolytic and hemicellulolytic enzymes on a wide variety of carbon sources. Importantly, enzymes are also produced during the initial and later culture phases. Extending this strategy with other regulators is expected to generate enzyme mixtures that contain a broader range and higher level of enzyme activities to achieve complete hydrolysis of all available plant polysaccharides.

72) Revision of the genus Aspergillus, problems and prospects

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Aspergillus is a broad paraphyletic group of species, and its teleomorphic states are very different among three major clades. Since a "one fungus one name" system will soon be implemented, either one has to use *Aspergillus* for all species, or one can for example neotypify *Aspergillus* with *Aspergillus niger* and use teleomorphic names for some clades of *Aspergillus sensu lato* and use known teleomorphic names for the rest: *Eurotium* for sections "*Aspergillus*" and *Restricti*, *Neosartorya* for sections *Fumigati* and *Clavati*, *Emericella* for sections *Versicolores, Aenei, Nidulantes* and *Sparsi*, and *Aspergillus* for the rest, i.e. sections *Nigri, Flavi, Candidi , Circumdati, Terrei, Flavipedes, Cremei* and *Cervini.* Each solution has its advantages and problems. This is still a matter of international agreement, and a meeting in April 2012, "One fungus which name" will be held in Amsterdam to discuss these issues. Taxonomically a species like *Penicillium inflatum* will be transferred to *Aspergillus*, while *Aspergillus paradoxus, A. crystallinus* and *A. malodoratus* will be transferred to *Penicillium*, making a purely morphologically based genus circumscription very difficult. It is recommended to use a polyphasic approach to *Aspergillus* taxonomy and include both physiological, morphological, chemical and sequence based features in all taxonomies.

73) Pleiotropic effects of *farA* disruption in *Aspergillus oryzae

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This study reports on the pleiotropic effects of *farA* disruption-regulation of genes that at first sight do not seem to be influenced by *farA* gene in *Aspergillus oryzae*. It has already been reported that FarA is a $Zn(II)_2Cys_6$ transcription factor which up-regulates genes required for growth on fatty acids in *Aspergillus nidulans* (Hynes et al. 2006) and similar functions in fatty acid utilization with its other orthologs in *Fusarium oxysporum*, *Candida albicans*, and *Yarrowia lipolytica* (Martínez-Rocha et al. 2008; Poopanitpan et al. 2010; Ramírez and Lorenz 2009). It is also known to regulate cutinase genes in *Fusarium solani*and *A. oryzae* (Li et al. 1997; Li et al. 2002; Garrido et al, 2012). However, its effect on the regulation of other metabolic genes in *A. oryzae* has not yet been reported. Microarray analysis was conducted between the wild-type (WT) and the *farA* disruptant induced with oleic acid. Results confirmed by qRT-PCR showed that a number of genes encoding ribosomal proteins such as 60S ribosomal proteins L18 and L19, and 40S ribosomal proteins L6 and S16 were down-regulated in the disruptant as compared to the WT. Each gene contains the cis-element $5'$-CCTCGG-3′ (complement, 5′-CCGAGG-3′) of FarA transcriptional factor in their 5′ upstream regions. It could be implicated that ribosomal gene expressions during fatty acid metabolism may be FarA dependent and farA disruption affects directly or indirectly the ribosomal biogenesis. Furthermore, metabolic genes up-regulated in the disruptant and other metabolic genes affected only by oleic acid are also discussed in this study. Results are relevant for understanding more the complexity of transcriptional regulatory networks of filamentous fungi.

74) The transcriptional regulator RhaR of *Aspergillus niger* is involved in L-rhamnose catabolism and in degradation of Rhamnogalacturonan-I. BS Gruben (1,2), M Zhou (1), A Wiebenga (1), J Ballering (2), RP de Vries (1,2). (1) CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; (2) Microbiology & Kluyver Centre for Genomics of Industrial Fermentation, Utrecht University, The Netherlands.

The *Aspergillus niger* genome contains a broad set of pectinolytic genes, encoding enzymes that act on the different substructures and linkages of pectin. Previous studies demonstrated a complex regulation of these genes, but so far none of the regulators involved in this process have been identified.

We identified the transcriptional activator RhaR is described that is mainly regulates the expression of genes involved in degradation of Rhamnogalacturonan-I. Micro-array analysis revealed down-regulation of genes encoding exorhamnogalacturonases, α-rhamnosidases, rhamnogalacturonan acetylesterases, an unsaturated rhamnogalacturonan hydrolase and a rhamnogalacturonan lyase in the Δ*rhaR* strain compared to the reference strain on L-rhamnose. In addition, a gene encoding a putative pectin acetyl esterase, two genes encoding putative β-1,4-galactosidases and one gene encoding a feruloyl esterase were also down-regulated in the disruptant. RhaR also appears to regulate L-rhamnose catabolism as growth of *rhaR* disruptant strains on L-rhamnose was abolished and two genes encoding putative L-rhamnose catabolic enzymes were down-regulated in the Δ*rhaR* strain.