The Thirteenth International *Aspergillus* Meeting

Asperfest 13

April 3, 2016

EUROSITES La Chapelle, PARIS

Aspergillus insulicola
Photo courtesy CBS-KNAW

Aspergillus Genomes Research Policy Group (AGRPG)

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

2015 AGRPC

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The Thirteenth International *Aspergillus* Meeting
Asperfest 13
Sunday, April 3, 2016
EUROSITES La Chapelle, 69 ter, rue de la Chapelle, 75018 PARIS

8:30 Registration and poster hang up

9:00 Welcome, introductions and announcements Michelle Momany
University of Georgia, USA

9:15 Session I Vera Meyer
Berlin University of Technology, Germany

Highly efficient CRISPR mutagenesis by microhomology-mediated end joining in *Aspergillus fumigatus*
Ling Lu, College of Life Sciences, Nanjing Normal University, China

Regulation of transporter endocytosis, membrane traffic and turnover in *Aspergillus nidulans*: The UapA paradigm
George Diallinas, *Aspergillus nidulans* Molecular Genetics Lab, Faculty of Biology, Athens University, Greece

Aspergillus terreus: Terrein production as adaptation to its environmental niche
Matthias Brock, Fungal Genetics and Biology Group, School of Life Sciences, Nottingham, UK

10:15 - 10:45 Coffee Break

10:45 Session II Mikael R. Andersen
Technical University of Denmark

Sequencing 200 million years of Aspergillus evolution - how far have we come?
Tammi Vesth, Technical University of Denmark

FungiDB: online resource for fungal data integration and *in silico* experimentation
Omar Harb, University of Pennsylvania

Building a diamond-level assembly of the *Aspergillus nidulans* genome
Berl Oakley, University of Kansas

12:00 - 13:00 Lunch

12:30-14:00 Novozymes Poster Session David Cánovas
University of Seville, Spain

14:00 Community directions discussion; Elections Michelle Momany
University of Georgia, USA

14:30 Session III: Talks from Abstracts Paul Dyer
University of Nottingham, UK

The regulatory network of the *Aspergillus nidulans* transcription factor TamA reveals a role in metabolic precursor homeostasis
Richard Todd, Kansas State University, USA

How aspergilli rearrange secondary metabolic gene clusters to generate new compounds
Sebastian Theobald, Technical University of Denmark

High resolution visualization and exoproteomics of plant biomass colonization and degradation by *Aspergillus niger* wild type and regulatory mutants
Joanna Kowalczyk, CBS-KNAW Fungal Biodiversity Centre, The Netherlands

Genetic interactions of *A. fumigatus* RlmA with components of the cell wall integrity pathway and its relationship with production of fumiquinazolines
Iran Malavazi, Universidade Federal de São Carlos, Brazil

15:30 Pontecorvo Lecture (sponsored by BMS) Isabelle Benoit
CBS-KNAW, Netherlands

The time tunnel has a lot of traffic
Miguel Angel Peñalva Soto, Centro de Investigaciones Biologicas (CSIC), Spain

16:15 Election results; Novozymes student poster prizes; other discussion items

17:00 Buses to ECFG reception

Abstracts

*Denotes student presenting poster

1*. Tip-to-nucleus communication and control of conidiation in vegetative hyphae of *Aspergillus nidulans*

Elixabet Perez-de-Nanclares-Arregi[a], Ion Luis Abad[a], Eduardo A. Espeso[b], Unai O. Ugalde[a] and Oier Etxebeste[a]

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Abstract

FlbB is a bZIP-type transcription factor (TF) involved in the induction of asexual development in the filamentous fungus A. nidulans, which is localized at the tip and the apical nucleus [1]. Tip-to-nucleus migration of FlbB was demonstrated using the photoconvertible protein Dendra2 [2,3]. We observed that tip localization required functional C-terminal and the N-terminal bZIP transcriptional regulatory domains, while a nuclear localization signal was crucial for nuclear accumulation. The dimerization domain within the bZIP mediated the apical interaction of FlbB with a related asexual development regulator, FlbE. In the absence of flbE, FlbB lost its apical localization and also the competence to induce conidiation [2]. In silico analyses predicted the presence of six functional domains within FlbE, which could have a role in signal recognition, protein-protein interaction, targeting to subcellular compartments and post-translational modification. Four conserved cysteine residues within the central region of FlbB have been postulated to form disulfide bonds with a C-terminal cysteine, leading to a change in spatial conformation and the consequent nuclear accumulation, as described in the H2O2 sensor Pap1 in S. pombe [4]. This work describes the characterization of the above mutants and the role of the corresponding FlbB/E domains in their apical localization or modification.

References

[1] O. Etxebeste et al. (2008) Eukaryotic Cell, 7, 38-48.[2] E. Herrero-García et al. (2015) Molecular Microbiology, 98, 607-624.[3] E. Perez-de-Nanclares-Arregi and O. Etxebeste (2014) Fungal Genetics and Biology, 70, 33-41.[4] E. Castillo et al. (2002) Molecular Microbiology, 45, 243-254.

Keywords
tip-to-nucleus communication, flbB, asexual development, Dendra2, cysteine, conidiation

2. The response of *Aspergillus niger* to lignocellulose is sequential and varies due to the compositional changes generated by pre-treatments

Jolanda van Munster[a], Paul Daly[a], Martin Blythe[a], Roger Ibbett[a], Matt Kokolski[a], Kerrie Barry[b], Christopher Petzold[c], Mikko Arvas[d], Blake Simmons[c] and David Archer[a]

[a] University of Nottingham, UK; [b] DOE Joint Genome Institute, USA; [c] Joint BioEnergy Institute, USA; [d] VTT, Finland

Abstract

Second-generation biofuel production requires efficient enzymes for lignocellulose digestion. To aid production of better and cheaper enzymes, we investigated how the biotechnology workhorse Aspergillus niger responds to untreated and pre-treated substrates.We applied genome-wide transcriptome analysis to provide a 5 day time-course of lignocellulose degradation and are the first to report the fungal response to ionic liquid pre-treated substrates. We mapped transcript levels to a metabolic model,aiding identification of substrate-independent as well as pre-treatment affected metabolic responses.Early time-points featured time-dependent clustering of transcript abundances, and were hallmarked by increased lipid metabolism. Pre-treatment and substrate dependent responses dominated mid time-points. Induction and repression of genes in cultures with ionic liquid pre-treated substrates

was dynamic and correlated with pentose sugar concentrations. Hydrothermal pre-treatment of substrates resulted in reduced levels of transcript CAZy gene transcripts, proteins and enzyme activities, correlating to removal of parts of the lignocellulose. Sporulation and starvation were hallmarks of the later time-points. Secondary metabolism was induced mainly in cultures on ionic liquid pre-treated substrates.We show that pre-treatment, substrate and time each have major influence on the fungal responses to lignocellulose.
Keywords

Aspergillus niger, lignocellulose, biomass, pre-treatment, biofuels, CAZymes, glycoside hydrolases, time-course, genome-wide transcriptome analysis, metabolic model

3*. Conditional Gene Expression in *Aspergillus niger* - Perspectives and Limitations of Inducible Expression Systems

Simon Boecker, Franziska Wanka, Roderich Süßmuth, Mark Arentshorst, Arthur Ram, Vera Meyer

Abstract

Synthetic biology tools allowing precise regulation of gene expression are powerful tools to study gene functions in vivo and to streamline metabolic pathways. Inducible, tuneable and metabolism-independent gene switches are of special interest as they can be easily controlled and shut on or off at any stage during the life cycle of the organism of interest. Recently, we established a tetracyclin-dependent expression system for the industrial platform organism Aspergillus niger that can either be used to switch the expression of a certain gene on (the Tet-On system) or off (the Tet-off system) upon addition of the inducer tetracycline or its derivative doxycyclin.[1,2] However, if more genes have to be controlled independently, this system reaches its limits. We therefore aimed to design additional inducible expression systems functioning in A. niger and other filamentous fungi. We tested the performance and efficacy of two conditional expression systems responding to the antibiotics inducer erythromycin or to the hormone analog diethylstilbestrol. We used luciferase as a reporter gene and applied a MTP-based assay to evaluate and compare the systems with the established Tet-On and Tet-off systems [1,2]. Corresponding results will be shown.
References

[1] V. Meyer et al. (2011) Appl. Environ. Microbiol., 77, 2975–2983.[2] F. Wanka et al. (2015) Fungal Genet. Biol., in press.
Keywords

Aspergillus niger, expression systems, Tet-On/Tet-Off, erythromycin expression system

4*. Components of the Bud Site Selection System and the Septation Inititation Network affect arthrospore formation in *Acremonium chrysogenum*

Janina Kluge, Miriam Lutomski, Ulrich Kück

Abstract

The filamentous fungus Acremonium chrysogenum is the primordial producer of the β-lactam antibiotic cephalosporin C. This β-lactam antibiotic is of great biotechnological and medical relevance due to its antibacterial activity against gram-positive and -negative bacteria. Continuous and directed improvement of industrial strains is required to optimize cephalosporin C production efficiently. A typical morphological feature of A. chrysogenum is the fragmentation of vegetative mycelium into arthrospores. These are uni- or binuclear cells, which develop during a prolonged cultivation under limited nutrient supply. Due to the known correlation of cephalosporin C production and arthrospore formation, we are interested in the identification of specific regulatory factors affecting both, cephalosporin C biosynthesis and morphological development. Current analyses focus on septation because constriction of arthrospores occurs from septa. Here, we present functional characterizations of components of the bud site selection system and the septation initiation network. We performed microscopic analysis of arthrospore formation in different time intervals, growth tests under various stress conditions and localization studies to verify the direct regulation of arthrospore formation by this regulatory network. To further investigate this developmental process, we established the visualization of filamentous actin with the live cell marker Lifeact.

Keywords

Acremonium chrysogenum, cephalosporin C, arthrospores

5*. Secreted aegerolysins and MACPF domain-containing proteins in the filamentous fungus *Aspergillus niger*

Maruša Novak[a], Nada Kraševec[b], Urška Čepin[c], Toshihide Kobayashi[d], Peter Maček[a], Gregor Anderluh[b] and Kristina Sepčić[a].

[a] University of Ljubljana, Biotechnical Faculty, Department of Biology, Ljubljana, Slovenia; [b] L11, National Institute of Chemistry, Ljubljana, Slovenia; [c] BioSistemika Ltd and National Institute of Biology, Ljubljana, Slovenia; [d]Lipid Biology Laboratory, RIKEN, 2-1, Hirosawa, Wako-shi, Saitama 351-0198, Japan

Abstract

Aegerolysins and MACPF-proteins (Pfam06355 and 01823, respectively) are found in various kingdoms of life. In Basidiomycota, they seem to be involved in the development, while in filamentous fungi they might act as virulence factors. Fungal members of both protein families were shown to interact with lipid membranes, either sole or in combination with one another. It appears that roles of these proteins are pleiotropic and adapted to fungal life-style.In the genome of the saprophytic, filamentous fungus Aspergillus niger, we identified two nucleotide sequences encoding aegerolysins, and two encoding MACPF-proteins. Using qPCR, we showed that the increase of expression of all four target genes coincides with the beginning of fungal conidiation, and that prevention of conidiation alters the expression profiles and significantly downregulates their expression. Deletion of either of the aegerolysin genes did not affect conidiation rate, growth dynamics and fungal development. Using antibodies against recombinant aegerolysin (AspHS), we confirmed that A. niger aegerolysins are secreted out of the fungus despite their lack of corresponding signal peptide. Surface plasmon resonance and vesicle sedimentation assays showed that AspHS protein binds to liposomes composed of ceramide-phosphoethanolamine and cholesterol, but does not permeabilize them. Furthermore, fluorescently labelled AspHS can bind to membranes of insect cells that contain considerable amounts of ceramide-phosphoethanolamine, but is not toxic to these cells.Our results suggest that aegerolysins and MACPF-proteins are produced during conidiation of A. niger, but are not actively involved in this process, indicating that their role(s) might be related to some other physiological processes in the fungus, e.g. defence mechanisms

Keywords

Aspergillus niger, gene expression, non-conventional secretion, lipid membranes

6. Nitrate reductase-dependent synthesis of nitric oxide boosts during development in *Aspergillus*

Ana T. Marcos[a], María S. Ramos[a], Jose F. Marcos[b], Lourdes Carmona[b], Joseph Strauss[c], and David Cánovas[a,c]

[a]Department of Genetics, Faculty of Biology, University of Seville, Spain; [b] Department of Food Science, Institute of Agrochemistry and Food Technology (IATA), Valencia, Spain; [c] Fungal Genetics and Genomics Unit, Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences (BOKU) Vienna

Abstract

Nitric oxide (NO) is a signalling molecule involved in many biological processes in bacteria, plants, and mammals. However, little is known about the role and biosynthesis of NO in fungi. Our data show that the nitrate assimilation pathway contributes to the biosynthesis of NO in Aspergillus. This route is more active during development, requires a functional nitrate reductase gene (niaD), and is active under repressive conditions in the presence of ammonium. Quantification of NO during the transition from vegetative growth to development revealed that NO production is regulated at the early stages of development. The niaD and the two flavohaemoglobins (fhbA and fhbB) genes are involved in this process. fhbB expression is low during the early stages of the transition from vegetative growth to conidiation but strongly induced 24 h after the initiation of conidiation. niaD and fhbA are temporarily induced immediately after the induction of conidiation. However, induction of fhbA but not niaD requires the addition of nitrate to the media. Increasing the levels of NO reduced conidiation without affecting the expression of the conidiation regulator brlA, but induced the formation of cleistothecia by a mechanism that involves the expression of the sexual regulator nsdD. The nitrate-independent and nitrogen metabolite repression-insensitive transcriptional upregulation of niaD during conidiation suggests a novel role for nitrate reductase in linking metabolism and development.

Keywords

Nitric oxide, conidiation, brlA, flavohaemoglobin, cleistothecia, nitrate reductase, niaD

7. The regulatory network of the *Aspergillus nidulans* transcription factor TamA reveals a role in metabolic precursor homeostasis

Richard Todd, Damien J. Downes

Abstract

The Aspergillus nidulans Zn(II)2Cys6 transcription factor TamA is widely conserved in Ascomycetes. TamA regulates the key nitrogen assimilation gene gdhA directly via its DNA binding domain, while acting as a coactivator of the global nitrogen GATA transcription factor AreA independent of its DNA-binding motif at other nitrogen utilization genes. gdhA encodes NADP-glutamate dehydrogenase, which catalyzes formation of glutamate from 2-oxoglutarate and ammonium. To determine the physiological role of TamA, and to identify the TamA DNA binding and coactivator genome-wide regulatory networks we used RNA-Seq to compare global gene expression in tamAΔ complete loss-of-function and tamAC90L loss-of-DNA binding mutants. We show that TamA affects expression at some promoters as a DNA binding transcription factor, at some promoters independent of its DNA binding domain, and at others via both modes of action. Gene ontology networks analysis reveals that TamA regulates directly or indirectly genes for amide metabolism, nitrogen assimilation, carboxylic, oxo- and organic acid metabolism, transport, iron homeostasis, and secondary metabolism, as well as oxidoreductase genes. The oxidoreductase and nitrogen assimilation pathway targets suggest that TamA transcriptionally modulates levels of 2-oxoglutarate, which is an oxygen donor or co-substrate for oxidoreductases and the carbon skeleton for nitrogen assimilation to glutamate. In addition, TamA transcriptionally regulates multiple carbon metabolism genes involved in metabolism of precursors for biosynthesis of amino acids. Our data suggests that TamA coordinates homeostasis of carbon skeletons and amino donation from glutamate for biosynthesis of amino acids and compounds produced from amino acid precursors including iron siderophores and certain secondary metabolites.

Keywords

Aspergillus; nitrogen metabolism; gene regulation

8. Investigating gene expression patterns of *Aspergillus niger* to deduce global and gene-specific regulation networks for secondary metabolite gene clusters

Min Jin Kwon, Paul Schäpe, Benjamin Nitsche, Vera Meyer

Department Applied and Molecular Microbiology, Institue of Biotechnology, TU Berlin, Germany

Abstract

Aspergillus niger is a multi-purpose cell factory used in biotechnology for the production of organic acids and hydrolytic enzymes. To further rationally expand its product portfolio, we are currently investigating its potential as expression host for homologous and heterologous secondary metabolites (SMs). The genome of A. niger is predicted to encode 78 SM gene clusters consisting of 81 key genes including, e.g. 38 polyketide synthases, 17 non-ribosomal peptide synthetases and four PKS-NRPS hybrids [1,2]. So far, only eleven gene clusters were linked to their SMs and many clusters still remain to be elucidated for their biological functions and activities.Gene co-expression network analysis is a powerful approach for the functional annotation of uncharacterized genes and to predict regulatory proteins controlling gene expression in a pathway-specific or global manner. It aims to find genes with a consistent, correlated expression pattern across phenotypically diverse samples or experimental conditions. We have therefore screened our transcriptomic database that stores about 350 high-throughput microarray data for A. niger CBS 513.88, its cognate and mutant strains. The database includes 155 different cultivation conditions reflecting different carbon and nitrogen sources, starvation and stress conditions, conditions related to temporal and spatial stages during its life cycle, different cultivation concepts and many more. Using Bioconductor, pairwise correlation coefficients were calculated and pairs with a Spearman score higher than 0.5 were considered to be significantly co-expressed. The resulting gene co-expression network was investigated for genes co-expressed with 81 SM key enzymes and specifically scrutinized for predicted regulatory proteins including transcription factors and histone modifying genes. These in silico analyses uncovered that the expression of regulatory proteins known from other Aspergilli such as orthologs of VelC (velvet complex), VipC (methytransferase), GcnE (acetyltransferase) highly correlated with the SM key genes. Also, many so far unknown potential regulators were correlated with up to nine different SM key enzymes, whose function is currently being studied.

References

[1] Sanchez et al (2012) Nat Prod Rep 29:351-371.[2] Inglis et al (2013) BMC Microbiol 13:91.

Keywords
Aspergillus niger, secondary metabolite

9*. Sulfate assimilation influences cephalosporin C production in *Acremonium chrysogenum*

Dominik Terfehr, Ulrich Kück

Abstract

The filamentous fungus Acremonium chrysogenum is the main industrial producer of the β-lactam antibiotic cephalosporin C, which is an valuable antibiotic due to its broad activity against Gram-positive and -negative bacteria. Therefore, it is relevant to gain deeper insight into the biosynthetic processes involved in cephalosporin C production. One essential part of the biosynthetic process is the availability of building blocks. Cysteine, one of the building blocks required for the first step in β-lactam biosynthesis, is synthesized by incorporation of assimilated sulfur or by conversion of other sulfur metabolites. The conversion, especially the reverse transsulfuration outgoing from methionine, was described as being important for cephalosporin C production. However only little is known about the contribution of the assimilatory pathway. Two important enzymes within this pathway are the cysteine synthase CysB and the homocysteine synthase CysD, which incorporate the penultimate product of the assimilation path to form cysteine or homocysteine. In other filamentous fungi the pathway via CysB is described as the main route.We generated cysB and cysD single and double deletion strains to elucidate the role of these pathways for primary and secondary metabolism. The deletion strains show an altered production of antibiotic compounds, morphology and tolerance towards oxidative stress, which mark the importance of the assimilatory pathway.

Keywords
Acremonium chrysogenum, cephalosporin C biosynthesis, functional genomics, sulfur metabolism

10. Condition-dependent outcrossing in the filamentous fungus *Aspergillus nidulans*

N.O. Rode[a,b] , D. Arbuthnott[a,c] , S. Schoustra[d] , H. Rundle[a] & R. Kassen[a]

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Abstract

The relative importance of sexual reproduction in the adaptation of fungi to their environment is largely unknown. A recent study [1] using laboratory strains of Aspergillus nidulans suggests that in the absence of a partner, the rate of selfing (and hence the investment in sexual reproduction) is condition-dependent in this species. We investigated if, in the presence of a partner, the relative investment in outcrossing vs. selfing was also condition-dependent. We used both wild isolates and mutation accumulation lines (derived from a wild isolate) and measured the relative investment in outcrossing, selfing and asexual reproduction, along with growth rate and competitive fitness. Our results showed that outcrossing is the highest when the two partners have similar growth rates. In contrast, selfing was the highest when one strain was growing at a faster rate compared to the other. Our results unravel the complex interactions between resource allocation to different modes of reproduction and fitness in this species.

References

[1] S. Schoustra et al. (2010). Curr. Biol., 20, 1350–1355.

Keywords
fitnesss-associated outcrossing, wild isolates, mutation accumulation lines, cleistothecium

11. The hypoxia-induced dehydrogenase HorA is required for coenzyme Q10 biosynthesis, azole sensitivity and virulence of *Aspergillus fumigatus*

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Abstract

During infection A. fumigatus has to adapt rapidly to oxygen-limiting conditions when the fungus grows in inflammatory or necrotic tissue. In a previous proteome analysis, we identified a mitochondrial protein to be highly up-regulated during the early phase of hypoxic adaptation. Here, this protein was found to represent the novel oxidoreductase HorA. Localization and activity studies indicated that HorA is localized in mitochondria and mediates respiratory activity and function of complex I. In Saccharomyces cerevisiae a homologous protein was shown to play a role in biosynthesis of coenzyme Q. Reduced ubiquinone content in the generated horA deletion mutant of A. fumigatus also indicated a respective function in coenzyme Q10 biosynthesis. Consistent with the fact that ubiquinone is involved in maintaining cellular redox homeostasis we detected an impaired redox homeostasis, an accumulation of NADH and subsequently a major change of the fungal physiology by proteome analysis and growth assays. A delay in germination, reduced growth on sugars and an increased resistance against antifungal azole drugs was observed in the ΔhorA mutant. Interestingly, all phenotypes were completely reversed by the addition of the synthetic electron carrier menadione. The ΔhorA mutant showed significantly attenuated virulence in two murine infection models of invasive pulmonary aspergillosis. Therefore, the biosynthesis of coenzyme Q and, particularly, the fungal specific protein HorA play a crucial role in virulence of A. fumigatus. Since HorA and orthologous proteins do not occur in mammals, HorA may be an attractive target for the development of new antifungal compounds.

Keywords

Aspergillus fumigatus, hypoxia, coenzyme Q, virulence

12. Speciation over 200 million years – What makes an *Aspergillus* species

Jane L. Nybo, Tammi C. Vesth[a], Sebastian Theobald[a], Inge Kjærbølling[a], Jens C. Frisvad[a], Thomas O. Larsen[a], Igor V. Grigoriev[b], Scott E. Baker[b], Mikael R. Andersen[a]

[a] Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark; [b] Joint Genome Institute, Walnut Creek, CA, USA; [c] Joint Bioenergy Institute, Berkeley, CA, USA

Abstract

The study of speciation, how new species arise, diverge and remain separate, has a central role in evolutionary biology. Partly because it embraces so many disciplines, including population genetics, behavioural sciences, comparative genomics, biodiversity, biogeography and ecology.We try to answer the questions surrounding speciation in Aspergillus because of the diversity of its genus. It holds species relevant to plant and human pathology, food biotechnology, enzyme production, model organisms, and extremophiles.Speciation is nearly impossible to study and in most cases, we know very little about the genetic basis of species formation. But in this project we look at approximately 300 newly sequenced Aspergilli across an evolutionary span of 200 million years. This is, in evolutionary terms, a high number of species per million years, which allows us to approach the genes and functions that defines the Aspergillus genus and its pan, core, section and clade genomes. But we also aim to identify the genes that are involved in speciation and those unique to the individual species. To identify potential evolutionary events, we group the Aspergillus genomes into functional similar families (homologs), by using a novel homologous grouping method based on protein sequence similarity and functional domain prediction that maps the homologous families' genotypes to phenotypes.From this we can highlight previously unknown incidents that can affect speciation, such as horizontal gene transfers between closely or distantly related species, chromosomal rearrangements, gene duplications, creations and losses and propose genome-founded hypotheses on which types of genes drive speciation in Aspergillus.

Keywords

Aspergillus, comparative genomics, diversity, speciation, core, pan, genome, sequencing, genomics, homologs, genotype, phenotype, horizontal gene transfer, HGT, gene losses

13. aspMine - online comparative analysis of the *Aspergillus* genus

Tammi Vesth (1), Jane L. Nybo (1), Sebastian Theobald (1), R.P. de Vries (4), Igor V. Grigoriev (3), Scott E. Baker (2), Mikael R. Andersen (1)

1) Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark; 2) Joint Bioenergy Institute, Berkeley, CA, USA; 3) Joint Genome Institute, Walnut Creek, CA, USA4) Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands

Abstract

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

Keywords

genomics, Aspergillus, bioinformatics

14*. Comparative genomics of four *Aspergillus* species with focus on identification of specific secondary metabolite gene clusters

Inge Kjærbølling, Tammi C. Vesth[a], Jens C. Frisvad[a], Jane L. Nybo[a], Sebastian Theobald[a], Igor Grigoriev[b], Scott E. Baker[c], Thomas O. Larsen[a] and Mikael R. Andersen[a]

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Abstract

In order to examine the genetic diversity of the Aspergillus genus, and to establish new reference genomes for our ongoing project of sequencing all +300 species of the Aspergillus genus, a set of four diverse Aspergillus species (A. campestris, A. novofumigatus, A. ochraceoroseus and A. steynii) have been whole genome sequenced.Using comparative genomics, the selected species have been compared to a group of eight Aspergillus species with sequenced genomes to determine the level of genetic diversity. In examining the unique genes for each species, we have found that the most common function for these unique genes were involved in regulation. Another common function for the unique genes is often associated with secondary metabolism. These results show that parts of regulation and secondary metabolism is very species specific and important for the differentiation between species. We also hypothesize that these traits are particularly transferable from other organisms.Here, we have also demonstrated that comparative analysis of whole genome sequences can be used to identify and couple specific secondary metabolites to their respective gene clusters. In order to make the coupling, biological and chemical knowledge has to be combined with the genome sequences and prediction algorithms. Depending on the knowledge of the metabolite and the biosynthesis various approaches can be used. We have developed four strategies for this purpose and using these strategies it was possible to identify putative secondary metabolite clusters for aflatoxin, chlorflavonin, novofumigatonin and ochrindol in A. ochraceoroseus, A. campestris, A. novofumigatus and A. steynii respectively.Lastly, A. novofumigatus has been compared to a close relative, the pathogenic species A. fumigatus, to

get a better understanding of the mechanisms of pathogenicity and virulence. The A. fumigatus genes known to be involved in pathogenicity/virulence were located in A. novofumigatus and orthologs identified.The genome sequences presented here illustrate the large diversity found in the Aspergillus genus and highlights the potential for discovery of new and beneficial secondary metabolites. It also shows how biological, biochemical and sequence information can be combined to identify genes involved in specific functions, thereby aiding the future experimental design involved in further investigations.

Keywords
Aspergillus, whole genome sequenced, comparative genomics, diversity, secondary metabolite (SM), gene cluster, pathogenicity

15*. Highly active promoters for protein production during extremely low growth rates in *Aspergillus niger*

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Abstract

The concept of perfusion or retentostat cultivations attracts renewed interest for cultivations of mammalian and microbial cells. A perfusion cultivation mode refers to a continuous inflow and outflow of medium, while the cells are retained within the bioreactor. As a result, the growth rate of the culture can decrease down to a level of almost zero. The major advantage of the perfusion mode is high cell number and high productivity in small-scale bioreactors. Another advantage of this cell retention cultivation mode is the continuous removal of toxic products and/or the production of unstable products, which cannot remain stable in a batch or fed-batch culture due to inherent sensitivities against proteases or other degradative enzymes.We have recently shown that retentostat cultivations of the microbial cell factory Aspergillus niger can be kept stable for at least two weeks, adjusts almost zero-growth rates under which A. niger undergoes asexual reproduction and induces a transcriptional program which suggests that A. niger becomes specifically adapted to produce secondary metabolites and cysteine-rich proteins (Jørgensen et al., 2010, AEM 76). In order to exploit this phenomenon, we screened the transcriptome of A. niger for genes highly up-regulated under zero-growth conditions. Two were selected (anafp, hfbD) and their promotor activities studied using luciferase (mluc) as intracellular reporter gene as well as the biotechnological relevant antifungal protein AFP as secreted, cysteine-rich protein, which is toxic for A. niger. Expression cassettes were constructed and used to replace the endogenous anafp and hfbD genes, respectively. The expression of mluc and afp were investigated in all four strains at the level of mRNA and protein, which demonstrated that the anafp promoter mediates a very high peak-like expression profile, whereas the hfbD promoter mediates a constant but moderate high protein expression during 14 days of cultivation. These findings open new perspectives for the perfusion cultivation mode of A. niger for high production of heterologous proteins with industrial relevance.

Keywords
perfusion fermentation, zero growth rate, antifungal protein, hydrophobin, promoter

16. How Aspergilli rearrange Secondary Metabolic Gene Clusters to Generate New Compounds

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Abstract

The WHO is reporting a rising number of multidrug resistant strains every year, increasing the need for new drug development. However, the current methods for natural product discovery rely on a large amount of experimental work, making them unable to keep up with this demand .In the aspMine project, we are sequencing and analyzing more than 300 genomes of Aspergillus species, partially to tackle this problem and increase the speed of natural product discovery using comparative genomics.Our comparative approach relies on a database setup (MySQL), the Secondary Metabolite Unique Regions Finder (SMURF) algorithm, BLASTP and clustering analysis to process the huge amount of data. Finally, the analysis of this large dataset enables us to examine gene cluster diversity and dynamics in Aspergilli. In the previous studies we have shown that Aspergilli harbour up to 60 secondary metabolic gene clusters (SMGC), many of them of unknown function, promising possible solutions for the demand in natural products.In this study we are focusing on the SMGCs that have been combined and rearranged into larger SMGC. We use knowledge based filtering on tailoring domains of SMGCs to define their borders in syntenic block analysis and detect cases of SMGC insertion. Verification of rearrangement events is done by analysis of tailoring domains. With this study, we describe SMGC dynamics throughout the Aspergillus genus and give insights into rearrangement of SMGC for new natural products. Our results will be helpful in the development of new natural compounds and tackle the problem of antibiotic resistance.

Keywords

Aspergillus, secondary metabolism, comparative genomics, genetic diversity

17. Oligonucleotide directed mutagenesis of Aspergilli genomes using CRISPR-Cas9 technology

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Abstract

The CRISPR-Cas9 genome editing technology has recently been adapted for several species of filamentous fungi, including various Aspergilli species, Trichoderma reesei, Neurospora crassa, and Pyricularia oryzae among others. CRISPR-Cas9 induces specific DNA double strand breaks (DSBs) in the genome using a small specific RNA molecule as a guide. These breaks can then be used to destroy selected genes by relying on error-prone DNA repair by the non-homologous end-joining (NHEJ) to introduce mutations, or by increasing the efficiency of gene targeting in NHEJ proficient strains. Although elimination of a gene is an efficient tool towards understanding the function of the encoded protein, it is often advantageous to introduce small specific mutations to dissect the functionality of the protein in more detail. For example, it is possible to address the importance of individual amino-acid residues in protein function by changing single codons in the gene. Similarly, by introducing subtle changes in a multi-domain protein it is possible to understand the contribution of individual domains in the overall function of this protein. In applied sciences, site directed mutagenesis can be used to optimize enzyme function by protein engineering. Here we demonstrate a simple strategy for the generation of seamless point mutations, using short synthetic single stranded oligonucleotides and a CRISPR-Cas9 system in Aspergillus nidulans, and explore the parameters for efficient gene targeting, using this type of gene targeting substrate. We show that even in fungi with a well-established genetic toolbox CRISPR-Cas9 can still be a valuable addition, opening up new genetic engineering strategies.

Keywords

Aspergillus, CRISPR-Cas9, point mutations

18*. Genetic interactions of *A. fumigatus rlmA* with components of the cell wall integrity pathway and its relationship with production of fumiquinazolines

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Abstract

Aspergillus fumigatus is an allergen of mammals and an important opportunistic pathogen that causes invasive pulmonary aspergillosis in immunosuppressed individuals. The ability to handle different stress conditions is essential to the survival and virulence of the pathogen inside the host. Therefore, environmental changes are sensed by invading microorganisms and transduced through signaling transduction pathways that lead to cell adaptation strategies. The Cell Wall Integrity Pathway (CWIP) is a signaling cascade primarily activated in fungal cells under conditions of synthesis and/or remodeling of the cell wall. In S. cerevisiae, CWIP is launched by the activation of the Protein Kinase C (PKC1), which interplays with a MAP kinase cascade (MAPK) leading to the phosphorylation of the associated RLM1 transcription factor. We have demonstrated that A. fumigatus rlmA homologue plays an important role in the maintenance of the cell wall and the ΔrlmA strain is avirulent in a mouse model infection of invasive pulmonary aspergillosis. Here, we observed that rlmA deletion led to increased TNF-α production by bone marrow derived macrophages and higher detection of β-1,3 glucan in the fungal cell wall in comparison to the wild-type and complementing strain, suggesting that the ΔrlmA mutant had altered cell wall organization. In addition, we analyzed the genetic interactions of the A. fumigatus rlmA with the other components of the CWIP in A. fumigatus, i. e., pkcAPKC1 and mpkAMPK1 during the cell wall stress. Our results indicate that there is an epistatic relationship between these genes indicating that the CWIP organization in A. fumigatus resembles that of S. cerevisiae. Additionally, we verified that rlmA are involved in the production of gliotoxin and fumiquinazoline C since the ΔrlmA mutant presented lower concentration of these secondary metabolites. Consistently, the expression of the genes in the fumiquinazoline cluster (fmqA-E) was down regulated in ΔrlmA mutant during the asexual development. Our results show that canonical CWIP is conserved in this pathogen and plays an important role in virulence, host recognition and production of secondary metabolites. Financial support: FAPESP and CNPq, Brazil

Keywords

Aspergillus fumigatus , cell wall, Genetic interactions

19. ROLE OF A SECONDARY METABOLISM GENE CLUSTER IN THE PATHOGENIC INTERACTION BETWEEN *Aspergillus flavus* AND *Zea mays*

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Abstract

Aspergillus flavus is an opportunistic and saprophytic crops pathogen mostly known as an effective mycotoxins producer. Starting from previous studies aimed to identify gene clusters encoding for secondary metabolites, involved in pathogenicity of A. flavus, we focused on Cluster32 and specifically on a Zn2Cys6 transcription factors, present inside the cluster. Our purpose is to understand its role in the regulation of Cluster32 expression and to clarify finally its significance within the process of pathogenesis.To achieve this, we designed a knockout mutant for Zn2Cys6 via the TOPO cloning method: we have assembled a construct containing the argD gene, coding for the enzyme ACETYL ORNITHINE AMINOTRANSFERASE, flanked by 3_R and 5_R, regions homologue to Zn2Cys6. Once obtained, we used the deletion construct to transform AFC-1, a double auxotroph mutant incapable of producing Arginine and Uracil. Simultaneously, to characterize better the metabolic profile related to the cluster 32, we produced overexpression mutants of Zn2Cys6 fused to GFP. Thus, mutants were screened by fluorescence emission.Such mutant, have been tested to assay pathogenicity and fitness in different environmental conditions, compared to the wild type

Keywords: Aspergillus flavus, argD, cluster 32

20*. High resolution visualization and exoproteomics of plant biomass colonization and degradation by *Aspergillus niger* wild type and regulatory mutants

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Abstract

Aspergillus niger is an ascomycete fungus able to secrete multiple extracellular enzymes that break down plant polymers. This ability has been extensively researched by scientists and industrial companies for many industrial applications. The production of these enzymes is tightly regulated by several regulators, such as the carbon catabolite repressor CreA and the (hemi-)cellulolytic activators XlnR and AraR. While the effects of regulator deletions on the production of the enzymes has been studied, how this affects the colonization and degradation of plant biomass by A. niger has so far not been studied in detail.In this study we have addressed this topic by detailed visualization of the colonization and degradation of wheat bran by A. niger wt and mutants for the regulators mentioned above using high-tech microscopy of the DOE-EMSL institute (Helium Ion Microscope (HIM), ETEM, liquid cryoTEM, super resolution and standard confocal fluorescence microscopy, FISH). To explore the molecular basis for the differences observed between the strains we also performed exo-proteome analysis on these cultures. This way we could link the reduction in degradation efficiency of the mutants to the absence of subsets of enzymes. This study for the first time unearths the correlation between enzyme production and substrate colonization and reveals which enzymes play an essential role in this process. Highlights from this study will be presented.

Keywords

Aspergillus niger, plant biomass degradation, transcription factors, regulation, colonization

21*. Production of novel synthetic natural products by engineering of fungal PKS-NRPS hybrids

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Abstract

Filamentous fungi are prolific producers of a large number of bioactive and structurally diverse secondary metabolites. These include compounds of mixed biosynthetic origin such as cytochalasin E, where the PKS-NRPS encoding gene ccsA from Aspergillus clavatus has been shown to be involved in the biosynthesis of the core backbone of the molecule.1 Here, we will present our efforts towards biocombinatorial synthesis of novel natural products through engineering of the cytochalasin E pathway. First, co-expression of CcsA with a trans-acting enoyl reductase CcsC encoded in the same A. clavatus gene cluster resulted in a Diels Alder derived product when expressed in A. nidulans. Secondly, we have identified a compound structurally similar to the CcsA/CcsC product by co-expression of the PKS-NRPS Syn2 with the enoyl reductase Rap2 from Magnaporthe oryzae. With the goal of synthesizing novel synthetic natural products, we constructed a CcsA-Syn2 chimeric enzyme and successfully produced the expected new product of mixed polyketide-nonribosomal origin. Thus, swapping of the entire CcsA NRPS module with the corresponding NRPS module from Syn2, led to the production of a compound with the CcsA-specific polyketide backbone attached to the tryptophan residue provided by the Syn2 NRPS. The reciprocal cross (Syn2 PKS and CcsA NRPS) also led to production the expected chimeric product. Furthermore, we have demonstrated that the length and amino acid sequence of the inter-modular linker are not crucial for preserving PKS-NRPS function.

References

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Keywords Aspergillus nidulans, Aspergillus clavatus, Magnaporthe oryzae, PKS-NRPS

22. Heterologous production of immunosuppressant mycophenolic acid in *Aspergillus nidulans*

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Abstract

Filamentous fungi are well-known producers of a wide range of valuable secondary metabolites, which can be advantageously exploited e.g. in the pharmaceutical industry. One of the most prominent examples is mycophenolic acid (MPA). MPA inhibits inosine-5'-monophosphate dehydrogenase (IMPDH), which catalyzes the rate limiting step in the guanine nucleotide synthesis. Since B- and T-lymphocytes rely entirely on de novo purine synthesis, MPA is used as an immunosuppressant during organ transplants. We have recently identified the mpa gene cluster in Penicillium brevicompactum1 and have subsequently verified several steps in the MPA biosynthetic pathway2,3,4. However, the role of four genes remained to be characterized. We have therefore heterologously expressed the mpa cluster in a stepwise manner in Aspergillus nidulans and established a cell factory for MPA production. Using this strategy, we have demonstrated that MpaA possesses prenyl transferase activity and catalyzes the conversion from 5,7-dihydroxy-4-methylphtalide to 6-farnesyl-5,7-dihydroxy-4-methylphtalide (FDHMP). We have also shown that MpaG catalyzes the last enzymatic step in the biosynthesis of MPA in vivo, resulting in the production of MPA. Interestingly, one of the intermediates (demethyl-MPA) can be formed from FDHMP via an unknown enzymatic activity present in A. nidulans. Lastly, we also found exciting examples of cross chemistry in A. nidulans, which resulted in the production of MPA variants, In conclusion, we have successfully characterized the biosynthetic pathway of the top-selling drug, MPA and we have demonstrated that A. nidulans is a suitable cell factory for its heterologous production.

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Keywords cell factory, Aspergillus nidulans, mycophenolic acid, secondary metabolism

23*. A comparative proteomic approach to investigate secondary metabolite gene cluster expression in various *Aspergillus nidulans* cell types

Benedict Dirnberger (1), Oliver Valerius (1), Enikö Fekete Szücs (1), Cindy Meister (1), Ines Teichert(2), Jennifer Gerke (1), Danielle Troppens (1), Gerhard H. Braus(1)

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Abstract

A. nidulans sexual and asexual differentiation are linked to significant differences in formation of secondary metabolites and the formation of various distinct cell types in the different developmental programmes (Bayram et al., 2016; Fung. Genet. Biol. 87, 30-53). A comparative approach to investigate differences in the proteomes of fungal cells of different developmental programmes was initiated. 1981 proteins could be identified in the vegetative mycelium, 1874 proteins were identified in hyphae where sexual development was induced (dark conditions) and 1889 proteins in hyphae where asexual development was induced (illumination). Hülle cells are unique cell types of Aspergilli and are linked to the sexual programme. These globose thick-walled cells presumably support the formation of cleistothecia which are closed sexual fruiting bodies. A protocol for the enrichment of Hülle cells was established where 963 proteins could be identified. An enzyme of the monodictyphenone cluster as well as the dimethylallyltransferases XptB/XptC are specifically expressed in sexual mycelium and in Hülle cells. The current status of this proteomic comparison will be presented.

Keywords secondary metabolism gene clusters, development, protein expression, proteomics

24. Molecular signaling mechanisms of bacteria-induced fungal silent gene clusters

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Abstract

Beyond being an important airborne fungal pathogen of humans, Aspergillus fumigatus harbours extensive secondary metabolism potential. Most of the secondary metabolite gene clusters, however, remain silent under common laboratory cultivation conditions. Natural environment mimicry represents a means of activating the biosynthesis of natural products in A. fumigatus. Co-cultivation of the fungus with a soil-dwelling bacterium Streptomyces rapamycinicus leads to the activation of a cryptic pathway, yielding fumicyclines, a group of short-lived meroterpenoids. The interdomain communication appears to be mediated through close proximity of the two organisms, whereby the actinomycete attaches to the fungal hyphae. A. fumigatus rapidly responds to the stimulus by activating several signaling cascades that interconnect during the process. Among these, the cell wall integrity signaling pathway, and in particular its final effector, a MAP kinase MpkA contributes crucially to the signal transmission. The objective of the present project is to further elucidate the mechanism of bacteria-induced signal transduction, using the combination of molecular microbiology and analytical chemistry methods.

Keywords

A. fumigatus, S. rapamycinicus, secondary metabolism

25. The ER protein BsdA is a HulA_'Rsp5 adaptor necessary for vacuolar turnover of a misfolded version of the UapA transporter via selective autophagy

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Abstract

Transmembrane proteins translocate co-translationally in the endoplasmic reticulum (ER) membrane and traffic as vesicular cargoes, via the Golgi, in their final membrane destination. Misfolding in the ER leads to protein degradation basically through the ERAD/proteasome system. Here, we use a specific mutant version of the purine transporter UapA (ΔR481) to show that misfolded versions of plasma membrane cargoes undergo vacuolar turnover prior to localization in the plasma membrane. We show that non-endocytic vacuolar turnover of ΔR481 is absolutely dependent on BsdA, an ER transmembrane adaptor of HulARsp5 ubiquitin ligase. We obtain in vivo evidence that BsdA interacts with HulARsp5 and ΔR481, primarily in the ER. Importantly, ΔR481 is shown to co-localize, in a BsdA-dependent manner, with the key selective autophagy marker Atg8, in the proximity of the ER and in vacuoles. Genetic block of autophagy (atg9Δ, rabOts) reduces, but does not abolish the non-endocytic vacuolar turnover of ΔR481, suggesting that in this case a fraction of the misfolded transporter might be redirected for vacuolar degradation via the Golgi. Our results support that multiple routes along the secretory pathway operate for the detoxification of Aspergillus nidulans cells from misfolded membrane proteins, and that BsdA is key factor for marking specific misfolded cargoes.

Keywords

membrane traffic/turnover/transporter/selective autophagy/Aspergillus/protein misfolding

26. The *Aspergillus fumigatus* leucine biosynthesis-regulator LeuB is crucial for adaptation to iron starvation and virulence in *Galleria mellonella*

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Abstract

The mould Aspergillus fumigatus is the most common airborn fungal pathogen of humans causing allergic reactions and severe invasive diseases in immunocompromised patients. In order to identify potential novel targets for antifungal therapy, we investigated the mechanisms involved in biosynthesis and regulation of the amino acid leucine, which represents an essential amino acid for humans. Therefore, we generated three A. fumigatus mutant strains lacking either the leucine biosynthetic enzymes LeuA (□-isopropylmalate isomerase, Afu2g11260) or LeuC (isopropylmalate synthase, Afu1g15000), or the leucine regulatory transcription factor LeuB (Afu2g03460). Deficiency in either LeuA (strain ΔleuA) or LeuC (strain ΔleuC) resulted in leucine auxotrophy, whereby the ΔleuC mutant required significantly higher leucine supplementation for growth than the ΔleuA mutant. Deficiency in LeuB (strain ΔleuB) resulted in partial leucine auxotrophy, i.e. the mutant was able to grow without leucine supplementation but required leucine supplementation for full growth. Interestingly, the ΔleuB mutant displayed significantly decreased resistance to iron starvation. In the Galleria mellonella infection model, deficiency of LeuA, LeuB and particularly LeuC attenuated virulence of A. fumigatus. In conclusion, these data demonstrate that leucine metabolism is a virulence determinant of A. fumigatus and reveal an unprecedented crosstalk between leucine and iron metabolism.

Keywords

leucine, Aspergillus fumigatus,Galleria mellonella,iron

27. Lactose uptake is mediated by differentially regulated permeases in *Aspergillus nidulans*, one of which is also involved in cellobiose catabolism

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Abstract

In Aspergillus nidulans, uptake rather than hydrolysis is the rate-limiting step of lactose catabolism. Deletion of the lactose permease A (lacpA) gene reduces the growth rate on lactose while its overexpression enables faster growth than wild-type strains are capable of. We have identified a second physiologically relevant lactose transporter, LacpB. Mycelia from mutants deleted for lacpB appear to take up only minute amounts of lactose, while lacpA/lacpB double deletion strains are unable to produce new biomass from lactose. Although transcription of both permease genes was strongly induced by lactose, their inducer profiles differ markedly. lacpB responded also strongly to beta-linked glucopyranose dimers cellobiose and sophorose, while these inducers of the cellulolytic system did not provoke any lacpA response. On the other hand, lacpA but not lacpB expression was consistently high in D-galactose cultures. In a lacpA-negative background, lacpB was overinduced by cellobiose in comparison to wild type; consequently, cellobiose uptake was faster and biomass formation accelerated in lacpA deletants. In contrast, in lacpB knockout strains, growth rate and cellobiose uptake were considerably reduced relative to wild type, indicating that the cellulose- and lactose catabolic systems employ common elements. Nevertheless, our permease mutants still grew on cellobiose which suggests that its uptake in A. nidulans prominently involves hitherto unknown transport systems.

Keywords

Aspergillus nidulans, Lactose, Cellobiose, Transporter

28. The Monothiol Glutaredoxin GrxD is Essential for Growth and Iron Homeostasis in *Aspergillus fumigatus*

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Abstract

Efficient adaptation to iron starvation is an essential virulence determinant of the most common airborne fungal pathogen Aspergillus fumigatus. In the current study we characterized the role of the A. fumigatus monothiol glutaredoxin ortholog GrxD (Afu2g14960), orthologs of which mediate cellular transport and sensing of iron in S. cerevisiae and S. pombe.Heterokaryon rescue technology proved that grxD is an essential gene in A. fumigatus, which contrasts S. cerevisiae and S. pombe. Conditional expression of grxD demonstrated that GrxD deficiency can be partially compensated by high iron supplementation. Taken together with the transcriptional upregulation of grxD during iron starvation, these data are in agreement with a role of GrxD in cellular iron transport. In a shift from GrxD-inducing to -repressing conditions under iron starvation, the grxDc strain displayed increased accumulation of protoporphyrine IX (PpIX), the iron-free precursor of heme. This is consistent with a role of GrxD in iron sensing as PpIX accumulation is a hallmark of deficiency in HapX, the major transcription factor required for adaptation to iron starvation. However, deregulation of iron-regulated genes at the transcriptional level was not observed. Moreover, we identified a strain, which carries a grxDc suppressor mutation enabling growth in standard medium. Transcriptional analysis combined with genetic mapping revealed that the suppressor phenotype is caused by derepression of iron uptake via inactivation of sreA, which encodes a transcriptional repressor of siderophore biosynthesis and reductive iron assimilation.

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Keywords grxD, iron

29. Prediction of secondary metabolite encoding genes based on chemical structure analysis

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Abstract

Dereplication of the secondary metabolite profile from the filamentous fungus Aspergillus brasiliensis, by High Performance Liquid Chromatography coupled with Diode Array Detection and High Resolution Mass Spectrometry lead to the discovery of a novel biomarker having a unique UV spectrum and elemental composition. Structural elucidation based on Nuclear Magnetic Resonance spectroscopy of the pure compound revealed an apolar polyketide or fatty acid derived secondary metabolite, possibly assembled from two entities, a C8 and a C12 chain, fused via a Claisen-like condensation and subsequent cyclisation to form a core lactone ring structure. Despite the apolar nature of the compound initial bioassay investigation have demonstrated antibacterial activity against methicillin-resistant Staphylococcus aureus MB5393.The metabolite was also identified in strains of A. carbonarius and A. tubingensis, setting the scene for comparative bioinformatics analysis of the three genomes. Four candidate gene clusters have been selected for construction of knock out mutants using CRISPR/Cas9 in A. brasiliensis. This poster will summarize our efforts towards characterization of the biosynthetic pathway of this new compound that we have named brasenol.

Keywords
brasiliensis, natural product, secondary metabolite, structural elucidation, nmr, biosynthesis

30*. The use of polycistronic gene expression to produce secondary metabolites in *Aspergillus niger*

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Abstract

Aspergillus niger is used for the production of various primary metabolites, proteins and enzymes. In order to establish *A. niger* as expression host for secondary metabolites, we are currently investigating its potential to produce homologous and heterologous secondary metabolites. However, controlled co-expression of a set of genes or entire pathways is technically challenging. One approach to conquer this problem might be the adaptation of polycistronic gene expression, the prevalent mode in prokaryotes. Using the P2A peptide, this can also be achieved in eukaryotes, as shown for the penicillin gene cluster in *A. nidulans* (Unkles et al. 2014).
To adapt and evaluate this system for *A. niger*, we constructed a polycistronic gene cassette under the tet-on system using the P2A peptide. The cassette contained three genes: two genes essential for the heterologous production of the cyclodepsipeptide enniatin from glucose (*esyn1*, nonribosomal peptide synthetase and *kivR*, ketoisovalerate reductase from *Fusarium oxysporum,* Richter et al. 2014) and one gene encoding luciferase (*luc*) as reporter gene. To analyse and compare whether the position of a gene within the polycistronic construct has an effect on protein activity, luciferase was placed at different positions and all expression cassettes were integrated as single copy at the *pyrG* locus of *A. niger*. Our results show that polycistronic gene expression did indeed allow expression of all three proteins and the formation of enniatin.

Keywords
Aspergillus niger, Enniatin, secondary metabolites, polycistronic gene expression

31. A transcriptome meta-analysis proposes a novel biological role of the antifungal protein AnAFP in *Aspergillus niger*

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Abstract

Although Aspergillus (A.) niger is used since decades in industrial biotechnology for production of organic acids and proteins, it largely depicts a black box and we are far from understanding how most of the internal cellular processes work on the molecular level. However, the availability of its genome sequence and hundreds of microarray data for this fungus make it now feasible to shed light into this black box.We have established a database including 377 high-throughput microarray data from 158 different cultivation conditions for A. niger. Results of a transcriptome-meta analysis of this database suggest a novel prominent biological role of AnAFP. Remarkably, its expression is apparently regulated in a non-defense manner. Instead, upon carbon starvation, anafp is strongly upregulated. Its expression profile resembles that of genes involved in nutrient mobilization and with a predicted role for autophagy. In addition, anafp expression strongly increases when the mycelium becomes committed to asexual development. Compared to the wild type, its expression is more than two- to tenfold upregulated in both a ΔbrlA or ΔflbA background, respectively. As the flbA mutant depicts an autolytic phenotype, we propose AnAFP has a function during the asexual life cycle of A. niger and is somehow linked to autophagic processes during normal development.Our in-house transcriptomic database depicts a valuable tool which enabled us to zoom into the gene expression networks and physiological processes of A. niger. Further analysis of this database will help to increase our knowledge of the complex regulation of A. niger's gene network.

Keywords
Antifungal protein, AnAFP, A. niger, Transcriptome analysis, Autophagy, Growth, Autolysis

32*. Rational domain swapping in NRPS-like genes to expand the chemical space of its products

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Abstract

Fungi are a vast source of natural products and the ongoing efforts to sequence the genome of more species allows extensive genome mining for new compounds with potentially therapeutic effects. Another way to discover or generate new compounds is to genetically engineer the biosynthetic genes involved in product formation. This research involves a class of biosynthetic genes: a non-ribosomal peptide synthase (NRPS) -like family of enzymes that consists of three separate domains and new compounds could be made by swapping around homologous domains. The adenylation domain activates amino acid derivates, the thiolation domain anchors monomers, and the thioesterase domain facilitates cyclization of two monomers and releases the product. The focus lies on those thioesterase domains and how they affect heterocycle formation. All the engineering is done in Aspergillus nidulans which is a model fungus for heterologous expression that allows us to easily modify existing genes or introduce exogenous genes, often without the need to remove introns. This work will provide deeper insights in secondary metabolite biosynthesis in fungi and the extent to which we can manipulate it to make the drugs of the future.

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Keywords
fungi, Aspergillus nidulans, thioesterase domain swapping, engineering

33*. Characterization of *Aspergillus fumigatus* isolated from air and surfaces of the International Space Station

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Abstract

Fungi are a great source of natural products that are of use for humankind. A wide range of bioactive compounds produced by fungi is used in pharmaceutical and food industries, agriculture, and beyond. On the other hand, some fungi are plant, animal, and human pathogens. Fungal pathogens may cause serious health complications in immunocompromised human populations. Pathogenic fungi produce a range of secondary metabolites (SMs) that influence their virulence (melanins, siderophores, species-specific toxins) and immunologic potential. Aspergillus fumigatus is a saprophytic, filamentous fungus that is ubiquitous outdoors (soil, decaying vegetation) and indoors (hospitals, simulated closed habitats, etc.). Aspergillus fumigatus can adapt to various environmental conditions and form airborne conidia that are the inoculum for a variety of diseases (e.g. non- and invasive pulmonary infections, allergic bronchopulmonary aspergillosis, etc.) in immunocompromised hosts. In an on-going Microbial Observatory Experiments on the International Space Station (ISS) molecular phylogeny and radiation resistance of several fungal isolates were characterized. Two strains, ISSF 21 and IF1SW-F4, were isolated from the HEPA filter and the surface of the Cupola of the ISS, respectively. Using primers targeting the internal transcribed spacers ITS1 and 2, both isolates were identified as A. fumigatus. The whole genome sequence analysis of ISSF 21revealed increased number

of single nucleotide polymorphisms (SNPs) when compared to the reference A. fumigatus 293. SM profiles of both ISS isolates were compared to the reference (Af293) on 11 different media but no significant differences were observed. Finally, knowing that A. fumigatus is an opportunistic pathogen and microgravity highly influences the antibiotic susceptibility and pathogenicity of microorganisms, we examined pathogenicity of both ISS isolates using the zebrafish larval model. The ISSF 21 was more virulent than two clinical strains (Af293 and CEA10) the virulence of the second isolate IF1SW-F4 is being tested. Proteomics and transcriptomics of these ISS isolates might reveal the molecular mechanisms of the increased virulence. Subsequently, if the enhanced virulence is attributed to the microgravity, NASA needs to develop countermeasures to protect the astronaut's health whose immune system is reported to be compromised under microgravity.

Keywords
Aspegillus fumigatus, ISS, UV resistance

34*. Multi-omic analysis of *Aspergillus niger* isolated from the International Space Station

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Abstract

Fungal secondary metabolites (SMs), such as the antibiotic penicillin and the cholesterol-lowering statin lovastatin, have had a tremendous impact on humankind over the years because of their diverse bioactivities. Recent advances in genome sequencing of filamentous fungi suggests that only 10-20% of the natural products that these species can produce have been identified, because the majority of fungal SM clusters are silent under standard laboratory conditions. Expression of these genes often requires exposure to a specific condition, and therefore culturing fungi in different conditions often results in different SM profiles. The SMs produced by these "cryptic" pathways are a promising source for new drug discovery.This project involves the investigation of a fungal strain isolated from the International Space Station (ISS) US Lab surface, identified as Aspergillus niger. The filamentous fungus A. niger is a workhorse for the biotech industry. It is used industrially as a production host for citric acid and enzymes. We hypothesized that because the ISS's microgravity and enhanced radiation conditions are potentially stressful for fungal growth, the ISS-isolated A. niger strain may produce novel SMs that the citric acid-producing A. niger strain (ATCC 11414) does not. To investigate this hypothesis, liquid chromatography-mass spectroscopy (LC-MS) was used to compare the SM profiles produced by these strains after growth in various conditions. The data indicated that the ISS-isolated A. niger strain produces compounds not produced by the A. niger ATCC 11414 strain.In the interest of drug discovery and other industrial applications, we are determining the structures of these compounds. This process involves large-scale cultivation of the ISS-isolated A. niger strain, followed by extraction, purification, and characterization of each compound by nuclear magnetic resonance (NMR) spectroscopy. We have sequenced the genome of the ISS-isolated A. niger strain and are using a combination of genomics, transcriptomics, and proteomics to investigate the nature of these secondary metabolite differences. In addition to providing a unique and novel drug discovery opportunity, this project provides insight into the metabolic phenotypes selected for by environments of microgravity and enhanced radiation. Supported by 2012 Space Biology NNH12ZTT001N grant #19-12829-27.

Keywords
secondary metabolites, Aspergillus niger

35. Carbon catabolism of *Aspergillus niger* grown on sugar beet pulp

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Abstract

Degradation of plant biomass to fermentable sugars is of critical importance for the use of plant materials for biofuels and in bio based economy. Filamentous fungi are ubiquitous organisms and major plant biomass degraders. Single colonies of some fungal species can colonize massive areas as large as five soccer stadia. During growth, the mycelium encounters heterogeneous carbon sources. We assessed whether substrate heterogeneity is a major determinant of spatial gene expression in colonies of Aspergillus niger. We analyzed whole genome gene expression in five concentric zones of colonies utilizing sugar beet pulp. Growth, protein production and secretion occurred throughout the colony. Analysis of genes involved in carbon catabolism, genes encoding plant biomass degrading enzymes and their regulatory aspect will be presented.

Keywords

Aspergillus niger; pectin, zonal gene expression

36. Localisation and function of the GTPase ArfA in *Aspergillus niger*

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Abstract

A comprehensive understanding of the secretory pathway of filamentous fungi and its interplay with polar growth and cell wall integrity is still lacking in industrial fungi such as Aspergillus niger. GTP-binding proteins such as members of the Arf family are major switches controlling coated vesicle formation during intracellular trafficking in eukaryotes. The function of all Arf family proteins have been studied in S. cerevisiae, whereas only SarA of the seven predicted ones have been examined in A. niger so far. In the current study, we have investigated the function of ArfA, the orthologue of the S. cerevisiae ARF1 and ARF2 genes in A. niger, as the encoding gene is specifically up-regulated in A. niger under conditions that lead to high secretion of glucoamylase (Jørgensen et al 2009). ARF1 and ARF2 have multiple roles within the secretory pathway of S. cerevisiae and the closet human orthologue was recently shown to be a key factor ensuring the hypersecretion phenotype of neuroendocrine tumor cells (Münzberg et al 2015). The GTP-binding protein ArfA seems to be an essential protein for A. niger as deletion of the arfA gene (An08g03690) turned out to be lethal. We thus generated a conditional arfA mutant with the help of the Tet-On expression system (Meyer et al 2007) in a fluorescently labelled v-SNARE background strain (GFP-SncA; Kwon et al. 2013). This approach allowed us to follow the effect of arfA downregulation and over-expression on post-Golgi cargo distribution (v-SNARE) and glucoamylase secretion in a single isolate. We could also show that ArfA is able to complement the lethal phenotype of a S. cerevisiae arf1 arf2 double null mutant.

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Keywords

ArfA, A. niger

37. Analysis of cargo-dependent post-Golgi vesicles flux in *Aspergillus niger*

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Abstract

Comprehensive understanding on how fungal growth and branching are intertwined with the secretory flux of proteins towards the hyphal tip is still missing. To gain deeper insights into these processes, we established a fluorescently labelled v-SNARE reporter strain (GFP-SncA) in Aspergillus niger (Kwon et al. 2013). This strain was used to replace the homologous glaA gene coding for glucoamylase, which is the most abundant secretory protein of A. niger, with a gene copy being under control of the metabolism-independent Tet-on promoter. This modification was also done in a GFP-SncA reporter strain deleted for racA. RacA is a Rho GTPase GTPase which is key for polarity establishment and maintenance of A. niger hyphae and its deletion is known to provoke a hyperbranching phenotype (Kwon et al. 2013). The availability of both strains now enable us to study the link between cargo load, amount of secretory vesicles and number of hyphal tips.

References

Kwon et al (2013) Plos One 8, 7 e68946

Keywords
A. niger, post-Golgi cargo, hyperbranching

38*. Re-routing central metabolism in *Aspergillus nidulans*

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Abstract

Plant biomass is the most abundant and renewable carbon source for many fungal species. The composition of biomass consists of about 40-45% cellulose, 20-30% hemicellulose, and 15-25% lignin and varies among plant species. In the bio-based industry, Aspergillus species are used for the production of lignocellulolytic enzymes to pretreat agricultural waste biomass (e.g. wheat bran). The enzymes are expensive to produce and purify. In this study, our aim was to create an Aspergillus strain that do not metabolize the hexoses fermented by yeast to produce biofuels, but still secrete extracellular enzymes. Therefore, several metabolic mutants were generated that were (partially) impaired in glycolysis, by deleting the hexokinase (hxkA) and glucokinase (glkA) genes. To prevent repression of enzyme production due to the accumulation of hexoses, strains were generated in which these mutations were combined with a mutation in CreA, the repressor involved in carbon catabolism. Phenotypic analysis revealed that the growth of the ΔhxkAΔglkA mutant was particularly reduced on wheat bran. However, hexoses did not accumulate during growth of the mutants on wheat bran, suggesting that glucose metabolism is re-routed towards alternative carbon catabolic pathways.Deletion of creA combined with blocking the glycolysis results in an increased expression of pentose catabolic and phosphate pathway (PCP and PPP) and xylanolytic genes. This indicates that the reduced ability to use hexoses as carbon sources has resulted in a shift towards the pentose fraction of wheat bran as a major carbon source to support growth.

Keywords
Aspergillus nidulans; wheat bran; glycolysis; metabolic mutants

39*. Molecular Genetic Characterization of Terreic Acid Pathway in *Aspergillus terreus*

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Abstract

Terreic acid is a natural product derived from 6-methylsalicylic acid, and as potential cancer therapeutics, it can selectively inhibit the catalytic activity of Bruton's tyrosine kinase. A compact gene cluster of eight genes for its biosynthesis was characterized in Aspergillus terreus by individually knocking out each gene within the gene cluster. Isolation of the intermediates and shunt products from the mutant strains, combined with bioinformatic analyses, allowed for the proposition of a biosynthetic pathway for terreic acid.

Keywords

terreic acid; 6-methylsalicylic acid; Aspergillus terreus; biosynthesis

40. Omnispective systematics and phenotypic characters indicate that *Aspergillus* is one large polythetic class of species

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Abstract

A fully polythetic genus is one in which no character or feature is present in all species, but after all present in most of the species. It is the unique combination of all anagenetic extrovert characters that will circumscribe each species and cladogenetic characters based on nucleotide sequences will then help suggesting a cladonomy and hopefully phylogeny of the species in the genus. Use of anagenetic characters (autapomorphies) will pave the way for paraphyletic taxa, but for the time being there is a consensus in mycology that holophyletic taxa should be used. *Aspergillus* is thus at present a holophyletic but polythetic class of species. Concerning anagenetic characters, differentiational morphology has been emphasized in taxonomy, but other fundamental differentiational characters based on small and large molecule extrolites, nutrition and ecophysiology should also be used in an omnispective taxonomy. Many small molecule extrolites, such as sterigmatocystin, pseurotin, emodin, and YWA1 are present in different sections of *Aspergillus*, and even when the exact same extrolites are not present in different sections, heteroisoextrolites show a relationship between species. An example of heteroisoextrolites is gliotoxin in section *Fumigati* which has the analogues acetylaranotin in section *Terrei*, aspirochlorin in section *Flavi* and emestrin in section *Nidulantes*. There are similar exoenzyme examples across *Aspergillus*, and full genome sequencing of all species in *Aspergillus* will help elucidate this. Phylogenetic species recognition based on concordance of multiple gene genealogies, as suggested by Taylor et al [1] is thus insufficient for use in taxonomy.

References

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41. Identification of Δ9-desaturase as the Target of a Novel Potent Antifungal Agent in *Aspergillus fumigatus*

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Abstract

The health risk of fungal infections is still heavily underestimated. Annually around 1.5 million deaths are caused by fungal infections, which exceeds the number of people being killed by malaria and tuberculosis. The azoles represent the gold standard for treatment of infections caused by the fungal pathogen *Aspergillus fumigatus*. Resistance to this class is growing due to prolonged exposure of patients to these drugs and the widespread use of azoles in agriculture. The mortality rate for individuals who are infected with a resistant isolate exceeds 85%. Novel antifungal drugs are urgently needed.

In this study we identify and validate the molecular target of a novel and potent antifungal agent. A variety of genetic, genomic as well as biochemical approaches including chemical genomics, transcriptional profiling and, phenotypic profiling were used to identify Δ9-desaturase (*sdeA*) as the likely drug target. We confirmed the association between the antifungal compound and *sdeA* with both targeted gene expression analysis and quantification of the product:substrate ratio (oleic acid:stearic acid) of Δ9-desaturation in cells exposed to the compound.

To further analyse the suitability of Δ9-desaturase as potential drug target in *A. fumigatus*, a tetracycline/doxycycline inducible *sdeA* strain ($sdeA^{TET}$) was created. The $sdeA^{TET}$ isolate was unable to grow in the absence of the inducer doxycycline indicating that *sdeA* is essential for viability. Supplementation with oleic acid only partially reversed the growth defect of the strain. $sdeA^{TET}$ was avirulent in both an insect (*Galleria melonella*) and a murine model of infection. This lack of virulence was reversed by doxycycline treatment.

Taken together, this study shows that *sdeA* is the target of the novel antifungal compound and demonstrates the target is critical for virulence.

42. SrbA and the CCAAT binding complex – Novel Regulatory Mechanisms for Ergosterol Biosynthesis and Azole Resistance in Aspergillus fumigatus

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Antifungal azole drugs have been the cornerstone of systemic antifungal therapy for the last 30 years. However, resistance to the azoles, particularly in the major human mould pathogen *Aspergillus fumigatus*, is emerging and reaching levels that have prompted some centres to move away from azoles as a sole birst line therapeutic. One particular family of pan-azole resistant isolates dominates. Strains with TR34/L98H, a combination of a 34 base pair tandem repeat in the *cyp51A* 5' non-translated region with a mutation of the coding sequence resulting in the amino acid alteration L98H, have been found globally. Isolates harboring the TR34 mutant allele display increased *cyp51A* expression levels however, the precise mechanism underlying upregulation of the gene remained unclear. Two transcriptional regulators have been associated with modibied azole tolerance in *A. fumigatus*, the sterol regulatory element SrbA, and the CCAAT binding complex (CBC). SrbA acts a positive regulator of ergosterol biosynthesis and promotes azole tolerance. Hence loss of SrbA activity results in an increase in azole susceptibility. Modibication of the HapE subunit of the CBC (to HapEP88L) has been shown promote azole resistance. Here we demonstrate that the 34 *mer* duplicated in TR34 is bound by both SrbA and the CBC. We show that CBC acts complementary to SrbA as a negative regulator of ergosterol biosynthesis and show that lack of CBC activity results in increased sterol levels via transcriptional derepression of multiple ergosterol biosynthetic genes including those coding for HMG-CoA-synthase, HMG-CoAreductase and sterol 14-alpha demethylase. We reveal that the P88L substitution within HapE signibicantly impairs the binding afbinity of the CBC to its target site resulting in derepression of *cyp51A* and other genes in the ergosterol

biosynthetic pathway. We identify that the mechanism underpinning TR34 driven overexpression of *cyp51*A results from duplication of SrbA but not CBC binding sites and show that deletion of the 34 *mer* results in lack of *cyp51A* expression and increased azole susceptibility similar to a *cyp51A* null mutant. Finally we show that strains lacking a functional CBC are severely attenuated for pathogenicity in a pulmonary systemic model of aspergillosis indicating that, although null mutants are resistant to azoles, they would be unlikely to be able to sustain an infection.

43*. Versatility of the Tet-On Gene Expression System: Unique Approaches to Induce Metabolite Production in *Aspergillus terreus* and *Penicillium canescens*

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Significant advancements in fungal genome sequencing have revealed many silent core biosynthetic genes presumed to be involved in cryptic secondary metabolite production. There are major incentives to develop innovative technologies to uncover the identity of these cryptic metabolites. The tetracycline-expression system is an inducible, tunable and metabolism-independent gene expression system, shown to be a versatile tool to control and fine-tune eukaryotic gene expression. While first established in fungi using Aspergillus niger (Meyer et al., 2011), here we report the development of the Tet-on system as an effective gene activation tool in two different species of filamentous fungi, A. terreus and Penicillium canescens. The Tet-on system was applied in A. terreus to induce the expression of a cryptic nonribosomal peptide synthetase (NRPS)-like gene, dbgA. Dox-induced expression of dbgA alone was sufficient to produce the new metabolite, 3-hydroxyl-dibenzylidenglycolid. To validate the versatility of this system in a different genera of filamentous fungi, the Tet-on system was established in P. canescens to produce an overexpression strain of a known metabolite, Pseurotin A (PsoA). Here we demonstrate the successful Tet-on promotor replacement of the PsoA, cluster-specific, Zn(II)2Cys6 transcription factor, FapR. In dox-induced media, overexpression of the cluster-specific FapR, was confirmed by the significant increase in PsoA metabolite production as detected by LCMS.

Keywords Penicillium canescense, Metabolic Engineering, Natural Product, Secondary Metabolites, Pseurotin A, Aspergillus Terreus, TetOn, Doxycycline

44*. Can heterokaryosis drive persistent azole resistance in *Aspergillus fumigatus*?

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Abstract:
Aspergillus fumigatus causes a range of diseases in humans, some of which are characterized by fungal persistence for many years. *A. fumigatus* may persist by adapting to the human lung environment, through physiological and genomic changes. The fungus can adapt to this environment through genetic diversity that is generated by spontaneous mutations or recombination and subsequent selection of the fittest genotypes. In addition, aspects of the fungal lifecycle, such as sexual and asexual spore formation, probably crucially contribute to the adaptation potential of aspergillus. *A.fumigatus* may also persist as mycelia for many years in a human body and develop patient-acquired azole resistance during therapy in hospitals. Such mycelia may become heterokaryotic by mutation of one of the nuclei or by anastomosis of hyphae from genetically different mycelia. Here we study how such a heterokaryon adapts to a changing azole environment. We formed heterokaryons and diploids from *A. fumigatus* strains of different levels of resistance. When exposed to various azole environments, the heterokaryons revealed remarkable shifts in the nuclear ratio, and the resistance level of most heterokaryons exceeded that of the corresponding heterozygous diploids. Our results indicate the relevance of heterokaryosis for azole-resistance in colonies of *A. fumigatus*. Moreover, these analyses help us to understand the dynamics of azole resistance in *A. fumigatus* both in the field and in patients.

45. FlbC is involved in the transcriptional regulation of *Aspergillus oryzae* glucoamylase and protease genes in solid-state culture

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Aspergillus oryzae produces a large amount of secreted proteins in solid-state culture, and some proteins such as glucoamylase (GlaB) and acid protease (PepA) are specifically produced in solid-state culture, but rarely in submerged culture. From the disruption mutant library of *A. oryzae* transcriptional regulators, we successfully screened a disruption mutant showing an extremely low production level of GlaB but a normal level for α-amylase production. This strain was a disruption mutant of the C_2H_2-type transcription factor, FlbC, which is reported to be involved in regulation of conidiospore development. The disruption mutants of other upstream regulators comprising a conidiation regulatory network had no apparent effect on GlaB production in solid-state culture. In addition to GlaB, the production of acid protease in solid-state culture was also markedly decreased by *flbC* disruption. Northern blot analysis revealed that transcription products of *glaB* and *pepA* were definitely decreased in the *flbC* disruption strain. These results suggested that FlbC is involved in transcriptional regulation of genes specifically expressed under solid-state cultivation conditions, possibly independently of the conidiation regulatory network.

46*. Polysaccharides composition of the cell wall of *Aspergillus oryzae* disruptants of genes involved in carbon catabolite repression

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Aspergillus oryzae produces large amounts of amylolytic enzymes in the presence of maltooligosaccharides. In the presence of glucose, however, their production is repressed by carbon catabolite repression system. In filamentous fungi, it has been proposed that glucose repression is regulated by the transcription factor CreA and the ubiquitin processing protease CreB. We generated the gene deletion mutants of *creA* and *creB* in *A. oryzae*, and observed that the wild-type (WT) and Δ*creB* strains grew in compact hyphal pellets, whereas the Δ*creA* strain grew in pellets of smaller size or a pulpy-like morphology in submerged culture (1). In this study, to elucidate the cause of morphology change in submerged culture, we examined the effect of *creA* and *creB* deletions on cell wall components. Cell wall components of each strain were fractionated by alkali treatment and carbohydrate composition of each fraction was determined by high-performance anion-exchange chromatography (HPAC). The weight of glucose derived from α-1,3-glucan of Δ*creA* strain was higher than that of WT. Furthermore, the expression level of major α-1,3-glucan synthase gene *agsB* and putative α-amylase gene *amyD*, which is proposed to be involved in α-1,3-glucan synthesis, were much higher than those of WT and Δ*creB* strains. In contrast, the weight of glucose derived from α-1,3-glucan of Δ*creB* strain decreased compared with that of WT even though the morphology was not changed by *creB* gene deletion. These results suggested that CreA and CreB regulated the α-1,3-glucan synthesis of the cell wall, and the morphology change by *creA* gene deletion was caused by an increase in the ratio of α-1,3-glucan in the cell wall, which resulted in changing the balance of cell wall components.

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

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