10th EUROPEAN CONFERENCE
ON FUNGAL GENETICS

PROGRAMME & ABSTRACTS

Stichting European Conference of Fungal Genetics
Kamer van Koophandel 41173975

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Dear Colleagues

It's with great pleasure that I welcome you to Noordwijkerhout for the 10th European Conference on Fungal Genetics. It marks the return of an ECFG to the Netherlands, following ECFG2 which was held in Lunteren in 1994. With every ECFG we witness further advances in our understanding of fungal biology and ECFG10 is no exception. We have an exciting programme in all aspects of fungal biology and genetics, with a Keynote lecture, fifteen plenary lectures and eight parallel sessions that reflect the most vibrant areas in our research into fungi.

I still know from ECFG2 that the organisation of a major scientific conference requires a major effort from a team of people. Although I chair the organising committee, most of the burden has fallen largely to my colleagues from Leiden University; Arthur Ram, Vera Meyer and Sandra de Weert, and my colleagues from the HAN University; Christien Lokman and Theo Goosen, who together formed the so-called Local Organising Committee. They have been tireless in their organisation efforts behind the scenes. They deserve our thanks because, without their efforts, I don't think that ECFG10 would have been possible. We are also grateful to Celia Lloyd and Intelligent Events for so ably supporting us through the planning process.

In addition to the local organising committee, the Dutch scientific committee, chaired by Arthur Ram, took care of organising a very interesting scientific program. I therefore wish to thank Arthur and his committee who have ensured that we have a programme of speakers embracing both leading and emerging scientists. I also wish to thank all of our sponsors and, in particular, NH CONFERENCE CENTRE LEEUWENHORST for their major support and flexibility during the preparation of ECFG10. We have attracted more than 400 delegates to the conference and over 250 posters which will be presented by their authors in two poster sessions. However, all posters will be on display throughout the conference. We gratefully acknowledge DSM for making available 5 poster prizes. These prizes, of € 200,00 each, will be awarded to the best posters by young scientists. I anticipate that ECFG10 will be scientifically and socially a memorable conference in Noordwijkerhout. I hope you enjoy it and return to your laboratories invigorated with new ideas and links with fellow scientists.

Cees van den Hondel
Chair of the Organising Committee
Leiden, March 2010

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PL – Plenary Lectures
PS – Parallel Symposia
PR – Poster Abstracts

Programme
Monday 29 March 2010

14.00-18.00	Registration and poster setup	
		room: Rotondo
18.00-18.05	Welcome Prof. Dr. Cees van den Hondel	Chair of the Organizing committee
18.05-18.15	Dr. R. W. van Leen, MBA	Chief Innovation Officer DSM
18.15-18.25	Opening Prof. Dr. S. M. Verduyn Lunel, Dean of the Faculty of Science, Leiden University	
18.25-19.25	Key note Lecture Arturo Casadevall, Albert Einstein College of Medicine, USA **(KL1)** Thoughts on the origin of virulence of pathogenic fungi	
19.25-23.00	Welcome reception	

Tuesday 30 March 2010 — room: Rotondo

Plenary Lectures I: Fungal Diversity and Evolution — Chair: Pedro Crous

09.00-09.30 François Lutzoni, Duke University, Durham, NC, USA **(PL1.1)**
Macroevolution as a unifying framework for integrative studies of fungal symbiotic systems

09.30-10.00 Christina Cuomo, Broad Institute of MIT and Harvard, Cambridge, MA **(PL1.2)**
Genome insights into early fungal evolution and global population diversity of the amphibian pathogen *Batrachochytrium dendroabatidis*

10.00-10.30 Francine Govers, Wageningen University, Wageningen, The Netherlands **(PL1.3)**
Effector diversity and gene innovations in *Phytophthora*

10.30-11.00 Coffee break

11.00-11.30 Jan Stenlid, Swedish University of Agricultural Sciences, Uppsala, Sweden **(PL1.4)**
What have we learnt about pathogenicity from sequencing the *Heterobasidion* genome?

11.30-12.00 Igor Grigoriev, DOE Joint Genome Institute, Walnut Creek, CA, USA **(PL1.5)**
Genomic encyclopedia of fungi: Bioenergy prospective

12.00-14.00 Lunch

Parallel session 1 — room: Asamblea

Phylogeny and Fungal Tree of Life — Chairs: Pedro Crous and Joey Spatafora

14.00-14.25 Joey Spatafora, Oregon State University, USA **(PS1.1)**
Pan-orthologs, phylogenetic informativeness and the fungal tree of life

14.25-14.45 Marcel Zamocky, BOKU-University, Vienna, Austria **(PR1.1)**
Horizontal gene transfer of *katG* genes from bacteroidetes into ascomycetes

14.45-15.05 Evy Battaglia, Utrecht University, The Netherlands **(PR1.5)**
Analysis of regulation of pentose utilization in *Aspergillus niger* reveals evolutionary adaptations in the Eurotiales

15.05-15.30 Jennifer Wortman, University of Maryland School of Medicine, Baltimore, USA **(PS1.2)**
AspGD comparative genomics pipelines and visualization tools

15.30-16.00 Coffee break

16.00-16.20 Robert Proctor, US Department of Agriculture, Illinois, USA **(PR1.8)**
Variation in sequence and location of the fumonisin mycotoxin biosynthetic gene cluster in *Fusarium*

16.20-16.40 Gerrit HJ Kema, Plant Research International, Wageningen, The Netherlands **(PR1.10)**
The draft genome sequence of *Mycosphaerella fijiensis*, the black sigatoka pathogen of banana

16.40-17.05 Berend Snel, Utrecht University, The Netherlands **(PS1.3)**
The evolution of pathways and gene functions: lessons from phylogenomics in fungi

Parallel session 2 **room: Sorbon**

Fungal-host biology Chairs: Gillian Turgeon and Paul Verweij

14.00-14.25 Li-Jun Ma, The Broad Institute, USA **(PR2.21)**
Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium oxysporum*

14.25-14.45 Elena Perez-Nadales, University of Cordoba, Spain **(PS2.1)**
Role of a mucin-like membrane protein in signalling and pathogenicity of *Fusarium oxysporum*

14.45-15.05 Nicole Gerardo, Emory University, Atlanta, USA **(PS2.2)**
The evolution and maintenance of pathogen specialization in the fungus-growing ant symbiosis

15.05-15.30 Duur Aanen, Wageningen University, The Netherlands **(PS2.3)**
High symbiont relatedness stabilizes mutualistic cooperation in fungus-growing termites

15.30-16.00 Coffee break

16.00-16.20 Pietro Spanu, Imperial College London, UK (**PR2.29**)
Genome expansion in powdery mildews is caused by loss of immunity against genomic parasites

16.20-16.40 Jens Heller, Westf. Wilhelms-Universität, Germany (**PR2.18**)
The use of redox-sensitive GFP2 as a biosensor of the redox status in *Botrytis cinerea*

16.40-17.05 Hubertus Haas, Innsbruck Medical University, Austria (**PR2.10**)
HAPX is involved in maintenance of iron homeostasis and virulence of *Aspergillus fumigatus*

Parallel session 3 **room: Rotondo**

Regulation of Gene Expression at the Genome Level Chairs: Han de Winde and Cees Sagt

14.00-14.25 Heinz Osiewacz, Johann Wolfgang Goethe-University, Frankfurt, Germany **(PS3.1)**
Molecular pathways controlling the lifespan of the ascomycete *Podospora anserina*

14.25-14.45 Linda Johnson, AgResearch Limited, New Zealand (**PR3.24**)
Mutualism versus pathogenesis: fungal endophyte, friend or foe ?

14.45-15.05 Peter Punt, TNO Quality of Life, The Netherlands (**PR3.31**)
Constructed *Aspergillus niger* gene co-expression networks relate to biological processes

15.05-15.30 Tobias Schafmeier, Heidelberg University Biochemistry Center, Germany **(PS3.2)**
Frequency-modulated nucleo-cytoplasmic shuttling cycles are the basis for circadian activity and abundance rhythms of the *Neurospora* clock transcription factor WCC

15.30-16.00 Coffee break

16.00-16.20 Julio Rodriguez-Romero, Karlsruhe Institute of Technolgy, Germany (**PR3.28**)
Light-dependent gene regulation in *Aspergillus nidulans* is mediated through binding of a phytochrome white-collar light regulator complex

16.20-16.40 Philipp Wiemann, Westfälische Wilhelms-Universität Münster, Germany (**PR3.6**)
Components of a velvet-like complex in *Fusarium fujikuroi* affect differentiation, secondary metabolism and virulence

16.40-17.05 Graeme Garvey University of Wisconsin-Madison, USA (**PS3.3**)
Functional characterization of LaeA

18.00-19.30 Dinner

19.30-20.30 Poster Session 1a. Poster categories presenting: 1, 2, 3, 4 (even posters)

20.30-21.30 Poster Session 1b. Poster categories presenting: 1, 2, 3, 4 (uneven posters)

Wednesday 31 March 2010 **room: Rotondo**

Plenary Lectures II: Fungal Way of Living Chair: Paul Tudzynski

09.00-09.30 Han Wösten, Utrecht University, The Netherlands **(PL2.1)**
Micro- and macro-colonies of *Aspergillus niger* are heterogenic with respect to gene expression and secretion

09.30-10.00 Michael Feldbrügge, University Düsseldorf, Düsseldorf, Germany **(PL2.2)**
Microtubule-dependent mRNA transport in *Ustilago maydis*

10.00-10.30 Miguel A. Peñalva, Centro de Investigaciones Biológicas CSIC, Madrid, Spain **(PL2.3)**
Membrane traffic in *Aspergillus nidulans*

10.30-11.00 Coffee break

11.00-11.30 Paul S. Dyer University of Nottingham, Nottingham, UK **(PL2.4)**
Sex change in fungi: revealing secrets

11.30-12.00 John W. Taylor University of California, Berkeley, USA (**PL2.5)**
Unexpected population structure in *Neurospora crassa* from the Caribbean basin

12.00-18.00 Pick-up lunch and excursion to the Keukenhof

18.00-20.00 Dinner

Parallel session 4 **room: Rotondo**

Fungal Physiology and Biochemistry Chairs: Merja Penttilä and Christien Lokman

20.00-20.25 Gerhard Braus, Georg-August-Universität Göttingen, Germany **(PS4.1)**
Coordination of fungal development and secondary metabolism in *Aspergillus nidulans*

20.25-20.45 Jerica Sabotic, Jožef Stefan Institute, Ljubljana, Slovenia **(PR4.20)**
Clitocypin and macrocypins cover different mushroom defences

20.45-21.05 Carol Davis, NUI Maynooth, Ireland **(PR4.24)**
A glutathione S-transferase, GliG, may mediate thiol incorporation in gliotoxin biosynthesis and is not involved in auto-protection against gliotoxin

21.05-21.30 Valeria Mapelli, Chalmers University of Technology, Sweden **(PS4.2)**
Metabolism of selenium in *Saccharomyces cerevisiae* and improved biosynthesis of bioactive organic Se-compounds

21.30-22.00 Coffee break

22.00-22.20 Vivian Geogakopoulos, University of Adelaide, Australia **(PR4.1)**
Components of the SAGA complex are involved in acetate repression in *Aspergillus nidulans*

22.20-22.40 Michael Blatzer, Medical University Innsbruck, Austria **(PR4.3)**
SidL, an acetyltransferase involved in biosynthesis of the intracellular siderophore ferricrocin in *Aspergillus fumigatus*

22.40-23.05 Bernhard Seiboth, TU Vienna, Austria **(PS4.3)**
Arabinan and L-arabinose metabolism in *Trichoderma reesei*

Parallel session 5 **room: Asamblea**

Fungal Way of Living: Sex and other encounters Chairs: Paul Tudzynski and Pierre de Wit

20.00-20.25 Gerrit Kema, PRI, Wageningen, The Netherlands **(PS5.1)**
The dodge of blotch: saving sex in *Mycosphaerella graminicola*

20.25-20.45 Céline O'Gorman, Ruhr-Universitaet Bochum, Germany **(PR5.4)**
Penicillium chrysogenum: is it another shy sexual Ascomycete?

20.45-21.05 Jan A.L. van Kan, Wageningen University, The Netherlands **(PR5.6)**
Mating type loci of *Botrytis cinerea*

21.05-21.30 Sébastian Duplessis, INRA, France **(PS5.2)**
Chasing effectors in the genomes of fungal symbionts and pathogens of trees

21.30-22.00 Coffee break

22.00-22.20 Gillian Turgeon, Cornell University, USA **(PR5.9)**
Sex, virulence, stress, and histidine kinase response regulator proteins

22.20-22.40 Erika Kothe, Friedrich-Schiller-University, Jena, Germany **(PR5.12)**
Mushroom forming basidiomycetes: Mating and more

22.40-23.05 Nicholas J. Talbot, University of Exeter, UK **(PS5.3)**
Investigating the genetic control of infection-related development in the rice blast fungus *Magnaporthe oryzae*

Parallel session 6 **room: Sorbon**

Fungal Way of Living: Cell Biology Chairs: Vera Meyer and Ben Cornelissen

20.00-20.25 Steven Harris, University of Nebraska, USA **(PS6.1)**
Ancestral homologs of the yeast bud site selection proteins regulate septum formation and development in filamentous fungi

20.25-20.45 Alexander Lichius, University of Edinburgh, United Kingdom **(PR6.23)**
Tip-focused Rho GTPase activity and the actin cytoskeleton regulate directional growth of *Neurospora crassa* germlings

20.45-21.05 Mojca Bencina, National institute of Chemistry, Ljublijana, Slovenia **(PR6.8)**
Calcium and pH homeostasis in *Aspergillus*: small molecules under control

21.05-21.30 Gregory Jedd, The National University of Singapore, Singapore **(PS6.2)**
Biogenesis and evolution of the fungal woronin body

21.30-22.00 Coffee break

22.00-22.20 Sara Gremillion, Armstrong Atlantic State University **(PR6.18)**
Mutations in two Golgi apparatus COG proteins affect growth and glycosylation in *Aspergillus nidulans*

22.20-22.40 Corby Kistler, University of Minnesota **(PR6.9)**
Fusarium graminearum as a model for human Niemann-Pick type C disease

22.40-23.05 Ida van der Klei **(PS6.3)**
The role of microbodies in penicillin production

Thursday 1 April 2010 **room: Rotondo**

Plenary Lectures III: Fungal Physiology and Gene Expression Chair: David Archer

09.00-09.30 Joseph Strauss, Austrian Institute of Technology, Vienna, Austria **(PL3.1)**
Chromatin-level regulation of metabolic gene clusters in *Aspergillus*

09.30-10.00 Matthew Sachs, Texas A&M University, USA **(PL3.2)**
Gene regulation through the control of ribosome movement

10.00-10.30 Jürg Bähler, University College London, UK **(PL3.3)**
Dynamic repertoire of the fission yeast transcriptome surveyed at single-nucleotide resolution

10.30-11.00 Coffee break

11.00-11.30 Pascale Daran-Lapujade, Delft University of Technology, The Netherlands **(PL3.4)**
Regulation of the glycolytic activity in *Saccharomyces cerevisiae*: a systems biology approach

11.30-12.00 Cees van den Hondel, Leiden University, The Netherlands **(PL3.5)**
Filamentous-fungal biotechnology: Veni, Vidi, Vici?

12.00-13.00 Lunch and poster session

13.00-14.00 Poster Session 2a. Poster categories presenting: 5, 6, 7, 8 (even posters)

14.00-15.00 Poster Session 2b. Poster categories presenting: 5, 6, 7, 8 (uneven posters)

Parallel session 7 **room: Sorbon**

Fungal and Oomycete Effectors Chairs: Guido Van den Ackerveken and Marc-Henri Lebrun

15.00-15.25 Marc-Henri Lebrun, CNRS-UCB-INSA-BCS, CRLD Bayer Cropscience, France **(PS7.1)**
Fungal secondary metabolites as effectors of pathogenicity

15.25-15.45 Tim Friesen, USDA-ARS, Fargo, USA **(PR7.21)**
Cloning and characterization of SnTox1, a novel virulence effector gene important in the wheat-*Stagonospora nodorum* interaction

15.45-16.10 Martijn Rep, University of Amsterdam, The Netherlands **(PS7.2)**
Effectors of *Fusarium oxysporum*

16.10-16.35 Regine Kahmann, Max Planck Institute, Marburg, Germany **(PS7.3)**
The effectors of *Ustilago maydis* and related smut fungi

16.35-17.00 Coffee break

17.00-17.20 Rahim Mehrabi, PRI, Wageningen University, The Netherlands **(PR7.8)**
Genome mining and functional genomics of small secreted proteins (SSPs) in *Cladosporium fulvum*, *Mycosphaerella graminicola* and *M. fijiensis*

17.20-17.40 Isabelle Fudal, UMR Bioger-CPP, INRA, Grignon, France **(PR7.19)**
Crystal structure of the avirulence gene avrlm4-7 of *Leptosphaeria maculans* illuminates its evolutionary and functional characteristics

17.40-18.05 Stephan Wawra, University of Aberdeen, United Kingdom **(PS7.4)**
Unraveling the mechanism of RxLR mediated translocation of Oomycete effector proteins

Parallel session 8 **room: Rotondo**

Fungal Biotechnology Chairs: Hein Stam and Peter J. Punt

15.00-15.25	Roel Bovenberg, DSM, The Netherlands (**PS8.1)** Fungal Biotechnology: lessons learned from *Penicillium* strain improvement
15.25-15.45	An Li, TNO Quality of Life, Zeist, The Netherlands **(PR8.22)** A systems biology approach towards itaconic acid production in *Aspergillus*
15.45-16.10	Lena Nilsson, Aalborg University, Denmark **(PR8.45)** Biochemical producing fungi
16.10-16.35	Jamie Ryding, Verenium Corporation, USA **(PS8.2)** Fungal enzyme expression as a unit operation in the production of cellulosic ethanol
16.35-17.00	Coffee break
17.00-17.20	Astrid Mach-Aigner, Vienna University of Technology, Austria **(PR8.15)** Engineering an N-acetylneuraminic acid synthesis pathway into *Trichoderma*
17.20-17.40	Hans Visser, Dyadic Nederland, Wageningen, The Netherlands **(PR8.5)** Development of a low-cellulase background *Chrysosporium lucknowense* C1 strain.
17.40-18.05	Randy Berka, NOVOzymes Inc, USA **(PS8.3**) Genomic and transcriptomic analysis of *Thielavia terrestris* – a thermophilic ascomycete of biotechnological interest
19.30-late	Conference Dinner and Farewell Party

KEYNOTE AND PLENARY LECTURES

Monday March 29

Keynote Lecture

KL1

Thoughts on the origin of virulence of pathogenic fungi

Arturo Casadevall
Albert Einstein College of Medicine, USA
casadeva@aecom.yu.edu

Of the more than 1.5 million fungal species only about 150-300 are pathogenic for humans, and of these, only 10-15 are relatively common pathogens. In contrast, fungi are major pathogens for plants and insects. These facts pose several fundamental questions including the mechanisms responsible for the origin of virulence among the few pathogenic species and the high resistance of mammals to fungal diseases. This talk will explore the origin of virulence among environmental fungi with no obvious requirement for animal association. Dr. Casadevall will develop the hypothesis that interactions with non-animal hosts such as protista selected for traits that, in certain circumstances like weakened immunity, can allow invasion of mammalian hosts. Furthermore, the presentation will discuss recent evidence that vertebrate endothermy and homeothermy create a restricted environment for the overwhelming majority of fungal species and speculate that pressures from fungal diseases contributed to both the extinctions at the end of the cretaceous that resulted in the demise of the dinosaurs and to the great mammalian radiation that followed in the tertiary era. Finally, Dr. Casadevall will comment on the possibility that climate warming will erode the thermal difference between mammalian and environmental temperatures, an event that could potentially usher in new fungal diseases in the late 21st century.

Tuesday March 30

Plenary Session I

Fungal Diversity and Evolution

PL1.1

Macroevolution as a unifying framework for integrative studies of fungal symbiotic systems

François Lutzoni
Duke University, Department of Biology, Durham, NC, USA 27708.
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Major advancements in the study of symbiotic systems ranging from parasitism to mutualism are more likely to be attained through a coordinated multidisciplinary approach, involving the interplay of theoretical and empirical research including a reciprocal illumination process between cell and molecular biology and organismal biology. A synthesis of the results from evolutionary, genetic/genomic, and ecological studies centered on lichen symbiosis and endophytic systems will be presented as examples. Phylogenetics provides a logical and primary framework for this integrative research. A phylogenetically based reconstruction of the past sheds new light on our understanding of current biological trends. The testing of hypotheses derived from this more comprehensive knowledge has a greater potential to yield major discoveries in all fields of biology. Large-scale phylogenetic studies are essential to this endeavor and necessitate data management pipelines with adequate bioinformatic tools.

PL1.2

Genome insights into early fungal evolution and global population diversity of the amphibian pathogen *Batrachochytrium dendroabatidis*

Christina Cuomo[1], Sharadha Sakthikumar[1], Jason Stajich[2], Nick Inglis[3], Bernard Henrissat[4], James Galagan[1], Manfred Grabherr[1], Robert Lintner[1], Matthew Pearson[1], Chinnappa Kodira[1], Antonis Rokas[5], Alan Kuo[6], Jeremy Schmutz[6], Chad Nusbaum[1], Michel Leroux[3], Joyce Longcore[7], Igor Grigoriev[6], Timothy James[8], and Bruce Birren[1]

[1]Broad Institute of MIT and Harvard, Cambridge, MA [2]Univ. of California Riverside, Riverside, CA [3]Simon Fraser University, Burnaby, BC, CANADA [4]CNRS, Universités Aix-Marseille I & II, Marseille, FRANCE, [5]Vanderbilt Univ., Nashville, TN [6]Joint Genome Institute, Walnut Creek, CA [7]University of Maine, Orono, ME [8]University of Michigan, Ann Arbor, MI
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Batrachochytrium dendrobatidis (*Bd*) is a fungal pathogen of amphibians implicated as a primary causative agent of amphibian declines. The genome sequence of *Bd* was the first representative of the early diverging group of aquatic fungi known as chytrids. We have sequenced and assembled the genomes of two diploids strains: JEL423, isolated from a sick *Phylomedusa lemur* frog from Panama and JAM81, an isolate from Sierra Nevada, CA. By identifying polymorphisms between these two assemblies with survey sequence from additional global isolates, we have characterized the genome-wide pattern of variation, and used conservation patterns to model the recent evolution of *Bd* strains. By comparing the predicted proteins of *Bd* to that of other fungi and eukaryotes, we identified gene families expanded in *Bd*, some with potential roles in pathogenesis. The recent sequence of two additional chytrid genomes allows more specific characterization of such gene families within chytrids, and better delineation of expansions in the lineage leading to *Bd*. We have also characterized a set of genes conserved only with non-fungal eukaryotes, some of which play a role in flagella and centrosome structure in those species. Comparative analysis with the additional chytrid genomes will strengthen this basal vantage point for genomic comparisons across the fungi as well as with the sister animal clade and other eukaryotes.

PL1.3

Effector diversity and gene innovations in *Phytophthora*

Francine Govers
Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands
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Phytophthora literally means plant destroyer, a name coined by the founding father of mycology, Anton de Bary, when he proved that a microorganism was the causal agent of the devastating potato late blight disease. The genus *Phytophthora* belongs to the oomycetes, a distinct lineage of fungus-like eukaryotes within the supergroup Chromalveolates and related to brown algae and diatoms. The ~ 240 Mb genome of *Phytophthora infestans* is the largest and most complex in the chromealveolate lineage and its sequence reveals features that illuminate its success as a pathogen. Comparison to other *Phytophthora* genomes showed rapid turnover and massive expansion of specific families encoding effector proteins, including the host-translocated effectors sharing an RXLR motif. These fast-evolving effector genes are localized to highly dynamic and expanded regions of the *P. infestans* genome and may attribute to the rapid and successful adaptability of this pathogen to host plants. Other hallmarks reminiscent of a dynamic genome are copy number variations and gene innovations, the latter resulting in proteins with oomycete-specific domain combinations several of which probably have a function in signal transduction.

PL1.4

What have we learnt about pathogenicity from sequencing the Heterobasidion genome?

Jan Stenlid
Swedish University of Agricultural Sciences
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Annosum root rot is one of the most devastating diseases in conifer forests. It is caused by the necrotrophic basidiomycete *Heterobasidion annosum s.l.* consisting of a species complex with partly overlapping geographic distributions and host ranges. Recently, the genome of *H. annosum* has been sequenced and annotated. We have found several key traits such as signaling pathways and transcription factors associated with pathogenicity in other pathogens also to be present in the *H. annosum* genome. The analysis of the gene content also showed presence of the basic gene sets necessary for wood decomposition. We have constructed a genetic linkage map, recently transferred to the physical gene map, and identified several QTLs that are associated with e.g. pathogenicity, wood decomposition, growth rate and fungal interactions, these also give candidate genes for host interactions. Interestingly, the density of evolutionary young transposable elements is high within the QTLs for pathogenicity. Transcriptome analysis resulted in gene sets that are significantly associated with growth as a pathogen, indicating that coping with oxidative stress, producing secondary metabolites, degrading wood components and detoxifying host defense reactions are part of the arsenal activated in contact with living host tissue. A subset of these genes is located within the QTLs for pathogenicity. Studies are on the way to silence or knock out candidate genes in the fungus to verify the importance of several of the indicated candidate genes. We have also conducted a resequencing of a population of 24 isolates of *H. annosum.* This allows us to study population genomics in terms of size of linkage disequilibrium, signs of selection etc. and from there infer recent selective events. Gene models found in these genome areas are plausible target candidates for active selection.

PL1.5

Genomic encyclopedia of fungi: bioenergy prospective

Igor Grigoriev
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Already sequenced fungal genomes are biased towards organisms of medical importance and mostly represent a single phylum. At the same time, fungi are very important in many other areas of life including bienergy. Better understanding pathogens and symbionts is critical for a sustainable growth of feedstock plants. Fungi are most efficient organisms in degrading biopolimers such as lignocellulose. Since future biorefineries will rely on fungi that efficiently secrete cellulolitic enzymes and ferment sugars, discovery of new metabolic processes and enzymes is essential and depends on sequencing a broader spectrum of fungal genomes.

The *Genomic Encyclopedia of Fungi* project is aimed at sampling phylogenetic breadth and ecological diversity of fungi. Developing systematic approaches to sequencing and analysis of well designed groups of organisms rather than individual genomes should help to decode fungal inventions related to plant pathogenicity, symbiosis, cellulose degradation and fermentation as well as improve our understanding of fungal diversity and established industrial organisms.

Wednesday March 31

Plenary Session II

Fungal Way of Living

PL2.1

Micro- and macro-colonies of *Aspergillus niger* are heterogenic with respect to gene expression and secretion

Han A.B. Wosten, Charissa de Bekker, G. Jerre van Veluw, Pauline Krijgsheld, Fengfeng Wang, Robertjan Bleichrodt, Arman Vinck

Microbiology and Kluyver Centre for Genomics of Industrial Fermentations, Utrecht University, The Netherlands

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The filamentous fungus *Aspergillus niger* forms centimeter-scale macro-colonies on solid media, whereas (sub)millimeter micro-colonies are formed within liquid shaken cultures. Previously, it has been shown that zones within macro-colonies of *A. niger* are heterogenic with respect to gene expression and protein secretion. Recently, we have shown by COPAS technology that gene expression is also heterogenic between micro-colonies of a liquid shaken culture of *A. niger*. Quantitative PCR on RNA isolated from laser-dissected zones of micro-colonies of *A. niger* showed that RNA distribution is also heterogenic between zones of these colonies. In fact, using GFP as a reporter it has been shown that even within a particular zone of a macro- or micro-colony gene expression is heterogenic. These data show that a colony of *A. niger* is not a mass of identical hyphae, not even when such colonies are smaller than 1 millimeter. Our results imply that protein production of *A. niger* can be improved in industrial fermentations by reducing the heterogeneity within the culture.

PL2.3

Membrane traffic in *Aspergillus nidulans*

Miguel A. Peñalva, Juan F. Abenza, Antonio Galindo, América Hervás-Aguilar and Areti Pantazopoulou
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In hyphal tip cells of *Aspergillus nidulans*, intracellular distances between apical and basal regions and between the different nuclei within the same cytoplasm are large. Secretion, which predominates in the apex, is spatially coupled to compensatory endocytosis.

The *A. nidulans trans*- and *cis*-Golgi is formed by a dynamic network of rings, tubules and fenestrated structures that is strongly polarised. Polarisation of the Golgi appears to be mediated, at least in part, by polarisation of the ERES. Thus, the burning and as yet standing question is what determines polarisation of the transitional ER. The organisation of both the *cis*- and the *trans*-Golgi is dramatically but reversibly affected by brefeldin A, possibly acting at two different levels. Brefeldin arrests apical extension but growth is restored after washing out, correlating with recovery of the normal Golgi organisation. One important and unexpected feature of the secretion machinery is that whereas ERES and *cis*-Golgi elements reach the apical dome, the *trans*-Golgi seems to be actively excluded from this region. Our data underscore our very poor understanding of the complexities of membrane traffic in filamentous fungi, in spite of its major economic impact in Biotechnology and Medicine.

PL2.4

Sex change in fungi: revealing secrets

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Fungi are able to reproduce by both sexual and asexual means. The majority of species are able to reproduce sexually, and many species are capable of producing both sexual and asexual spores. However, perhaps 15-20% of all fungi are known only to reproduce by asexual means. Where sexual reproduction occurs some species exhibit 'heterothallic' obligate-outbreeding mating systems, whereas others exhibit 'homothallic' self-fertile breeding systems. Previous work has suggested that heterothallic sexual reproduction is the ancestral state. A fundamental question in evolutionary biology is therefore what genetic modifications occur to result in 'sex change' leading to homothallism and asexuality? Unravelling these changes also has applied significance for potential exploitation of the sexual cycle. Work will be described involving filamentous ascomycete species where a combination of genomic and experimental approaches has been used to investigate the nature of sex change in fungi. Research has focussed in particular on the role of mating-type (*MAT*) and pheromone-signalling pathway genes in mediating sexuality. In model *Aspergillus* and *Botrytis* species it appears that transitions between heterothallism and homothallism can be achieved by a variety of *MAT* locus rearrangements and other genomic changes, demonstrating the flexible nature of fungal genomes. Meanwhile, some supposedly 'asexual' species are revealed to have secretive sexual tendencies – as shown by the recent discovery of a functional sexual cycle in *Aspergillus fumigatus* (teleomorph *Neosartorya fumigata*).

PL2.5

Unexpected Population Structure in *Neurospora crassa* from the caribbean basin.

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We used solexa sequencing of mRNA to simultaneously identify Single Nucleotide Polymorphisms (SNPs) and quantify gene expression for more than sixty isolates of Neurospora crassa from the Caribbean Basin. Through population genomic analysis of the SNP data, we find strong support for two recently diverged populations, one endemic to Louisiana and the other distributed through Florida, Haiti and the Yucatan. We also identify a subset of genes that show the signature of positive selection and a subset that are differentially expressed between species. Based on the evidence for a recent divergence time and the presence of gene flow between these populations, we argue that this represents an ideal dataset for the study of the early stages of speciation.

Thursday April 1

Plenary Session III

Fungal Physiology and Gene Expression

PL3.1

Chromatin-level regulation of metabolic gene clusters in *Aspergillus*

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In fungi primary and secondary metabolism genes involved in the same metabolic pathway are often clustered in the genome. This arrangement may facilitate co-regulation of these genes in response to environmental or developmental signals. Chromatin represents both the physiological substrate and a physical barrier for transcription factors to access regulatory elements on gene promoters. By adopting different levels of compaction and the correct architecture of the underlying nucleosomal subunits chromatin regulates gene expression and chromosome function. An epigenetic code defined by covalent modifications of the nucleosomal histone proteins is conserved throughout eukaryotes and defines the state of chromatin. In *Aspergillus* and other fungi, these modifications have been shown to influence metabolic and developmental processes. The examples presented here show that nitrogen metabolism and the production of secondary metabolites are regulated at the level of chromatin structure and accessibility. In the nitrate assimilation and proline degradation gene clusters the activity of the bi-directional promoters are influenced by nucleosome positioning (1). The major transcription factors play a decisive role in this process, e.g. the GATA factor AreA mediates histone acetylation and, in cooperation with the pathway-specific transcription factor NirA, subsequent nucleosome remodelling (2).

The transition from primary to secondary metabolism (SM) in different *Aspergillus* species is also associated with drastic chromatin rearrangements. *A. nidulans* mutants lacking components involved in the formation of strongly repressive heterochromatin (Heterochromatin-protein-1, H3-K9 methyltransferase) show over-expression of genes involved in biosynthesis of several secondary metabolites. LaeA, a conserved principal regulator of SM, is counteracting the decoration of histones by repressive marks (3). Moreover, the inactivation of a COMPASS-complex component (CclA) leads to reduction of repressive H3K9 marks in gene clusters for which metabolites have not been identified before (4). Chromatin restructuring upon entry into SM may be a conserved mechanism in fungi and modification of the chromatin landscape may thus lead to a more complete picture of the secondary metabolome in fungi.

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PL3.2

Gene regulation through the control of ribosome movement

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The regulated translation of mRNA can affect both rates of protein synthesis and mRNA stability. The expression of the small subunit of fungal arginine-specific carbamoyl phosphate synthetase is controlled by the translation of an upstream open reading frame (uORF) present in the 5'-leader region of its mRNA. Translation of the uORF, which specifies the arginine attenuator peptide (AAP), leads to reduced gene expression in response to elevated levels of arginine. Our data, based mainly on studies of *Neurospora crassa arg-2* and *Saccharomyces cerevisiae CPA1*, indicate that, first, translation of this coding region causes ribosomes to stall at the uORF termination codon when the level of the amino acid arginine is high. The stalled ribosome blocks the access of scanning ribosomes to the downstream start codon that is used to initiate synthesis of the biosynthetic enzyme, thus reducing gene expression. We have direct evidence from cell-free translation systems that arginine and related molecules cause stalling by interfering with the activity of the ribosome peptidyl transferase center and indications that these molecules induce a conformational change in the nascent AAP within the ribosome. Second, stalling of the ribosome at the uORF termination codon destabilizes the mRNA through the nonsense-mediated mRNA decay (NMD) pathway. Analyses of mRNA stability through pulse-chase studies in wild-type and nmd^- *N. crassa* strains provide direct evidence that the stability of the *arg-2* mRNA is controlled by NMD. mRNA transcriptomes of wild-type and mutant cells have provided additional insights into NMD-control in *N. crassa*.

PL3.3

Dynamic repertoire of the fission yeast transcriptome surveyed at single-nucleotide resolution

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Recent data from several organisms indicate that the transcribed portions of genomes are larger and more complex than expected, and many functional properties of transcripts are not based on coding sequences but on regulatory sequences in untranslated regions or non-coding RNAs. Alternative start and polyadenylation sites and regulation of intron splicing add additional dimensions to the rich transcriptional output. This transcriptional complexity has been sampled mainly using hybridization-based methods under one or few conditions. We applied direct high-throughput sequencing of cDNAs (RNA-seq), complemented with data from high-density tiling arrays, to globally sample transcripts of *S. pombe*, independently from available gene annotations. We interrogated transcriptomes under multiple conditions, including rapid proliferation, meiotic differentiation and environmental stress, and in RNA processing mutants, to reveal the dynamic plasticity of the transcriptional landscape as a function of environmental, developmental, and genetic factors. High-throughput sequencing proved to be a powerful and quantitative method to deeply sample transcriptomes at maximal resolution. In contrast to hybridization, sequencing showed little, if any, background noise and was sensitive enough to detect widespread transcription in >90% of the genome, including traces of RNAs that were not robustly transcribed or rapidly degraded. The combined sequencing and array data provided rich condition-specific information on novel, mostly non-coding transcripts, untranslated regions and gene structures, thus improving the existing genome annotation. Sequence reads spanning exon-exon or exon-intron junctions gave unique insight into a surprising variability in splicing efficiency across introns and genes. Splicing efficiency was largely coordinated with transcript levels, and increased transcription led to increased splicing in test genes. Hundreds of introns showed regulated splicing during cellular proliferation or differentiation.

PL3.4

Regulation of the glycolytic activity in *Saccharomyces cerevisiae*: a systems biology approach

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The Embden-Meyerhof-Parnas pathway of glycolysis is the key pathway of sugar metabolism in many living organisms, including man. Glycolysis is a central pathway for carbon assimilation and is directly linked to all industrial applications of *Saccharomyces cerevisiae* from biomass to biofuel production. Despite this large economical impact and decades of investigation, the regulation of yeast glycolysis remains elusive and to date, attempts at controlling the glycolytic flux by genetic engineering have consistently failed. To improve our understanding on the mechanisms governing the glycolytic flux in baker's yeast, a systems biology approach was undertaken. Yeast was grown under various conditions at steady-state in chemostat and in dynamic conditions using tightly controlled cultivation tools. The glycolytic system was investigated in a quantitative manner using a systems approach integrating all levels in the gene expression cascade from gene to *in vivo* flux (i.e. transcripts, proteins, active enzymes, metabolites, fluxes). This multi-level approach showed that, at steady-state as in dynamic environment, the local fluxes in glycolysis are largely governed by metabolic regulation, i.e. by *in vivo* activation/inhibition of enzyme activities by metabolites. Conversely, hierarchical regulation (*i.e.* regulation of protein concentration) is only marginally involved in the regulation of the local glycolytic fluxes. In all tested conditions baker's yeast displays a 'glycolytic overcapacity' that may be regarded as a waste of energy (glycolytic proteins can represent up to 20% of the whole cell protein) but could represent a selective advantage for yeast cells evolving in natural environments exposed to circadian cycles and a to variety of stressful conditions. The potential selective advantage during diurnal temperature oscillations will be discussed.

PL3.5

Filamentous-fungal biotechnology: Veni, Vidi, Vici?

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The establishment of genetic transformation of *Aspergillus nidulans* has been the basis for a new set of developments in filamentous-fungal biotechnology. Almost thirty years later, it is interesting to have a short look back at the expectations at that time, the technology development which occurred and the progress which has been made up until now.
Although an enormous increase in understanding basic biological processes which occur in our (biotechnologically important) model filamentous fungi has taken place, clearly a number of bottlenecks remain which hamper the yields which are theoretically achievable. This lecture will focus on the progress made and future directions for research addressing three important bottlenecks: 1. protein folding and secretion of (heterologous) proteins; 2. degradation of secreted proteins in the culture fluid; 3. optimal morphology of filamentous fungi in the bioreactor.
Finally the prospects of filamentous-fungal biotechnology in the future will be discussed.

PARALLEL SESSIONS

Tuesday March 30

Parallel session 1: Phylogeny and Fungal Tree of Life

PS1.1

Pan-orthologs, phylogenetic informativeness and the fungal tree of life

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The Assembling the Fungal Tree of Life (AFTOL) project is a large multi-laboratory collaboration that is focused on analyzing large amounts of genomic sequence data to infer phylogenetic relationships within the Kingdom Fungi. Our current research has identified a set of pan-orthologs that are distributed throughout the Fungi and are of suitable length and variability for phylogenetic analyses. In addition to standard phylogenetic analyses, we have tested the phylogenetic informativeness of these loci and compared them to markers traditionally used in phylogenetic analyses. Major findings from these analyses reveal that there exist numerous orthologous markers that are significantly more informative than standard loci currently in use in molecular phylogenetics and that individual markers vary in their level of informativeness based on the node or region of the phylogenetic tree in question. To expand this sampling to lineages for which genomes have not been sequenced, we are developing high throughput sequencing strategies and bioinformatic tools for mining heterogeneous genomic data for the AFTOL pan-orthologs and their inclusion in phylogenetic analyses. We will outline our bioinformatics pipeline, and present some preliminary data and findings concerning the evolution of plant-associated fungal lineages and major evolutionary trends in the Ascomycota.

PS1.2

AspGD comparative genomics pipelines and visualization tools

Jennifer Russo Wortman[1], Jonathan Crabtree[1], Marcus C. Chibucos[1], Joshua Orvis[1], Martha B. Arnaud[2], Maria C. Costanzo[2], Diane O. Inglis[2], Prachi Shah[2], Marek S. Skrzypek[2], and Gavin Sherlock[2]
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The *Aspergillus* Genome Database (http://www.aspgd.org/) is an online genomic resource for *Aspergillus* genomics and molecular biology, with information curated from the literature and web-based research tools for exploration and analysis of these data. With ten annotated genome sequences from eight *Aspergillus* species publicly available, and more on the way, our goals include leveraging comparative genomics for annotation improvement, and providing sophisticated querying and visualization tools to maximize the utility of these data.

The *Aspergillus* genomes were annotated at different institutions using diverse methods over an extended time frame, during which available tools and datasets have evolved. Common annotation inconsistencies include missed gene calls, gene truncations, failure to predict small exons, and inappropriate gene merges. We are developing a standard, optimized pipeline across all *Aspergillus* genomes, comprehensively incorporating new data as it becomes available and leveraging comparative genomics analyses to improve protein-coding gene prediction. Resulting annotations will be integrated into AspGD, along with ortholog and synteny data linking knowledge across the *Aspergilli*.

Otholog and synteny data are currently available at the AspGD website through data downloads as well as interactively through the Sybil software infrastructure. Sybil is an open source, web-based software package for visualizing and mining comparative genomics data (http://sybil.sf.net). Powered by a Chado relational database (http://www.gmod.org/schema), Sybil provides an extensive set of interfaces for interrogating multi-genome comparisons at varying levels of resolution from the whole genome scale to individual loci.

Sybil allows users to search for genes, clusters or genomic regions and visualize the results in a comparative genomic context, along with syntenic relationships and annotated features of interest. Configurable reports are available through the web interface, supporting the identification of core and accessory genes from all or a subset of available genomes. Reports and displays are interactive and linked, allowing seamless navigation from a chromosome view, to a cluster report, to an individual protein page. All of the data in AspGD are freely available to the public from http://www.aspgd.org/. AspGD is supported by grant RO1 AI077599 from the NIAID at the NIH.

PS1.3

The evolution of pathways and gene functions: lessons from phylogenomics in fungi

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The ever increasing amount of sequenced genomes potentially allows unprecedented insight into pathway evolution. However precise analyzes are hindered by incomplete knowledge on protein interactions and genome dynamics that obfuscate precise orthology. These processes include gene duplications, genome duplications, domain re-arrangements and gene loss. Small scale manual analyzes and careful large scale computational analyzes nevertheless allow for the elucidation of some fascinating evolutionary stories. In this talk I will present two stories that were uncovered using phylogenomic analysis of fungal genome sequences.

Firstly I will discuss the evolution of the pathway surrounding the small GTPase Ras. The genome sequences of primitive fungi show that in contrast to what was previously thought many features of this pathway are not animal specific but were present in the ancestor of the Fungi/Metazoa group. The phylogenetic profiles of individual orthologous groups in this pathway do not match well, but they do match near perfectly on the family level, providing a new twist to the phylogenetic profile method for function prediction and our understanding of the evolutionary forces constraining the co-evolution of interacting proteins. Secondly I will discuss the evolutionary implications of single gene duplications versus genome duplications in the evolution of protein complexes in *S. cerevisiae*.

Tuesday March 30

Parallel session 2: Fungal-Host Biology

PS2.1

Role of a mucin-like membrane protein in signalling and pathogenicity of *Fusarium oxysporum*

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The soilborne fungus *Fusarium oxysporum* causes vascular wilt in a wide range of plant species by penetrating roots, invading the cortex and colonizing the vascular tissue. Fmk1, a mitogen activated protein kinase (MAPK) orthologous to *S. cerevisiae* Fus3 and Kss1, is essential for plant infection. The signalling components upstream of the Fmk1 cascade are currently unknown. In yeast, the membrane mucin Msb2 functions at the head of the filamentous growth MAPK cascade. We identified a gene from *F. oxysporum* whose predicted product has sequence homology with yeast Msb2 and shows a similar domain structure, including an N-terminal signal sequence, a predicted serine-threonine rich mucin region, a transmembrane domain and a short cytoplasmic tail. Western analysis using an HA-tagged Msb2 version showed that *F. oxysporum* Msb2 is an integral membrane protein which is expressed during vegetative growth and tomato root infection. Deletion mutants lacking *msb2* showed reduced phosphorylation levels of Fmk1, suggesting that Msb2 may function upstream of this MAPK. In contrast to D*fmk1* strains, D*msb*2 single and D*fmk1*/D*msb2* double mutants exhibited enhanced sensitivity to the cell wall-targeting compounds Congo Red and Calcofluor White, suggesting that Msb2 also signals in an Fmk1-independent pathway functioning in the cell wall stress response. The D*msb2* strains showed delayed invasive growth across cellophane membranes and significantly reduced virulence on tomato plants. Our results suggest that Msb2 is a mucin-like membrane protein that contributes to invasive growth and virulence of *F. oxysporum* by signalling partly via the Fmk1 MAPK cascade.

PS2.2

The evolution and maintenance of pathogen specialization in the fungus-growing ant symbiosis

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For approximately 50 million years, fungus-growing ants have been cultivating fungi for food. Over the evolutionary history of this ancient agricultural association, the ants have diversified into more than 200 species that are divided into five distinct phylogenetic and ecological groups, each with their own favored fungal crops. The ants' fungal crops are plagued by microfungal pathogens in the genus *Escovopsis*. To combat these pathogens, the ants engage in a mutualism with antibiotic-producing actinobacteria. Bioassays interacting strains of the ants' cultivated fungi with strains of the pathogen demonstrate that pathogen strains are coevolved and specialized, which would likely prevent rampant switching between hosts. This specialization is driven by the ability of the host fungi to inhibit some pathogen strains but not others, and the ability of the pathogens to recognize and grow towards chemical signals of some hosts but not others. The bacteria, however, exhibit less evolutionary specialization in their inhibition patterns towards the pathogen. Elucidation of this intricate system of symbiotic coevolution will be facilitated by ongoing genome sequencing of the ants and their microbial associates.

PS2.3

High symbiont relatedness stabilizes mutualistic cooperation in fungus-growing termites

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It is unclear how mutualistic relationships can be stable when partners disperse freely and have the possibility of forming associations with many alternative genotypes. Theory predicts that high symbiont relatedness should resolve this problem, but the mechanisms to enforce this have rarely been studied. In this presentation, I describe experiments addressing this question for the mutualistic symbiosis between fungus-growing termites and *Termitomyces*. Our experiments show how colonies succeed in propagating only a single heterokaryon of their *Termitomyces* symbiont, despite initiating cultures from genetically variable sexual spores from the habitat at the start of a colony. High inoculation density of asexual heterokaryotic spores in the substrate followed by successful fusion among clonally related mycelia enhances the efficiency of asexual spore production in proportion to strain frequency. This positive reinforcement results in an exclusive lifetime association of each host colony with a single fungal symbiont and hinders the evolution of cheating. Our findings explain why vertical symbiont transmission in fungus-growing termites is rare and evolutionarily derived.

Tuesday March 30

Parallel session 3: Regulation of Gene Expression at the Genome Level

PS3.1

Molecular pathways controlling the lifespan of the ascomycete *Podospora anserina*

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The ascomycete *Podospora anserina* is characterized by a limited lifespan. After a strain specific period of growth the growth rate of a colony decreases, the morphology of the culture changes and finally the hyphal tips die. This 'senescence syndrome' is under control of environmental factors and genetic traits. Recent investigations uncovered a hierarchical network of pathways influencing life and healthspan of this eukaryotic ageing model. These pathways counteract molecular damage which results from natural metabolic processes (e.g. respiration). In particular, pathways involved in the control of a functional population of mitochondria were found to play a major role. At the molecular level different mitochondrial proteases are effective. Among others, PaLON, a matrix protease was found to be important in protecting cultures against the consequences of oxidative stress.

Overexpression of *PaLon* leads to an increased heathspan, the period in the lifespan, in which no vital functions are impaired. The corresponding transgenic strains are more resistant against oxidative stress, are characterized by reduced protein damage and improved mitochondrial function.

Although, this and other quality control pathways are effective, *P. anserina* cultures finally turn to senescence. During this last step in the fungal life cycle pathways leading to programmed cell death are induced. Data about investigations intervening into these pathways will be discussed.

PS3.2

Frequency-modulated nucleo-cytoplasmic shuttling cycles are the basis for circadian activity and abundance rhythms of the *Neurospora* clock transcription factor WCC

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In *Neurospora crassa* the clock transcription factor White collar complex (WCC) controls the rhythmic expression of a large number of genes. The clock protein Frequency (FRQ) regulates WCC activity in a negative feedback loop by mediating its CK-1a dependent phosphorylation. In a positive loop, FRQ-dependent phosphorylation reduces the turnover rate of the WCC, resulting in accumulation of inactive and stable WCC. Rapid degradation of active WCC is a regulatory mechanism preventing an overshoot of WCC dependent transcription. WCC undergoes rapid, sub-circadian cycles of nucleo-cytoplasmic shuttling. These are linked to cycles of FRQ dependent inactivation by phosphorylation and reactivation by PP2A dependent dephosphorylation of WCC in the cytosol. Rhythmically expressed FRQ modulates the kinetics of WCC phosphorylation and shuttling cycles in a circadian manner, producing a daily rhythm of WCC activity and abundance. Hence, phosphorylation of the WCC is the molecular basis underlying both, negative feedback of FRQ on WCC activity and positive feedback of FRQ on WCC stability.

PS3.3

Functional characterization of LaeA

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Here we present the initial findings of a biochemical and genetic investigation into the mechanism of LaeA, a putative methyltransferase that functions as a global regulator of secondary metabolism in *Aspergillus nidulans*. LaeA has been found to be part of a large nuclear velvet complex that is required for secondary metabolite production as well as light regulated morphological development. Preliminary data suggests LaeA may control secondary metabolite gene clusters through chromatin remodeling. However, there is no direct evidence linking the velvet complex to chromatin remodeling. We have initiated a study to functionally characterize LaeA. Several LaeA orthologs were recombinantly expressed in *E. coli* and assayed for solubility. The full length LaeA protein from *A. nidulans* is only soluble as a MBP fusion protein, which has proved to be uninformative for *in vitro* activity assays. A partial proteolysis study was performed to identify soluble domains that could be amenable to *in vitro* analysis. Soluble truncation mutants were identified and have proved useful for *in vitro* methyltransferase activity assays. Validation of the truncated LaeA proteins was carried out through successful *in vivo* complementation of a *ΔlaeA* mutant. These truncation mutants are functionally equivalent to the full-length protein by restoring sterigmatocystin (ST) biosynthesis to wild type levels. Using the truncated LaeA protein, we have confirmed binding of S-adenosyl-L-methionine (methyl group donor) and have identified a methyltransferase activity. Each candidate protein substrate's site of methylation is being mapped by trypsin digestion coupled with LC/MS. The *in vivo* role of LaeA methylation will be evaluated with point mutants for effects on ST biosynthesis. Our findings confirm LaeA has methyltransferase activity and provide the first functional insights into the mechanism of LaeA regulation of secondary metabolism.

Wednesday March 31

Parallel session 4: Fungal Physiology and Biochemistry

PS4.1

Coordination of fungal development and secondary metabolism in *Aspergilus nidulans*

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The homothallic filamentous ascomycete *A. nidulans* is able to form fruitbodies (cleistothecia) either by mating of two strains or by selfing in the absence of a partner. The three-dimensional *A. nidulans* cleistothecium is the most complicated structure this fungus is able to form. Differentiation and secondary metabolism are correlated processes in fungi that respond to various parameters including light, nutrients, aeration or pheromones. Our work on several proteins will be described, which are involved in the crosstalk between developmental regulation and secondary metabolism control in *Aspergillus nidulans.* They include the heterotrimeric *velvet* complex VelB/VeA/LaeA, where VeA bridges VelB to the nuclear master regulator of secondary metabolism LaeA, the eight subunit COP9 signalosome complex controlling protein turnover, and the MAP kinase-related protein kinase ImeB.

PS4.2

Metabolism of selenium in *Saccharomyces cerevisiae* and improved biosynthesis of bioactive organic Se-compounds

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Selenium (Se) is an essential element for many organisms as it is present under the form of Se-cysteine in Se-proteins. 25 Se-proteins are known in humans and are all involved in protection of cells from oxidative stress. The main sources of Se for animals are edible plants able to accumulate Se from the soil in inorganic and organic forms. Some of the Se organic forms bioavailable for animals have been proven to have cancer-preventing effects if regularly introduced into the diet. Since Se content in plants is highly susceptible to environmental factors, the intake of Se is often insufficient to result in beneficial effects. Therefore, the use of Se-enriched yeast as food supplement is made available to avoid Se shortage. The yeast *Saccharomyces cerevisiae* does not require Se as essential element, but is able to metabolise and accumulate Se. Due to the very similar properties of Se and sulphur (S), S- and Se-compounds share the same assimilation and metabolic routes, but the competition is in favour of S-species, as the high reactivity of Se leads to the formation of toxic compounds. Due to the delicate balance between beneficial and toxic effects of Se, the study of Se metabolism in yeast is a crucial point towards the establishment of a yeast cell factory for the production of bioactive Se-compounds. The present study shows how the presence of Se influences cell physiology and metabolism. On this basis, we show how the coupling of metabolic engineering and bioprocess optimization represents a successful strategy towards the production of organic Se-molecule with high anti-cancer potential. The Se-metabolome has been carefully mapped.

PS4.3

Arabinan and L-arabinose metabolism in *Trichoderma reesei*

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The efficient use of complex plant material as carbon source for the production of different bio-based products requires an improved transformation of the different plant cell wall constituents. The saprotrophic fungus *T. reesei* (*Hypocrea jecorina*) has been well established for the biotechnological production of cellulases and xylanases and for the degradation of the respective polymers. However, the enzymes and their regulation involved in the degradation of other plant carbohydrate polymers including the L-arabinose polymer arabinan are less well understood.

In the genome sequence of *H. jecorina* four genes including three α-L-arabinofuranosidase genes (*afb1*, *afb2*, *afb3*) and a β-xylosidase with a separate α-l-arabinofuranosidase activity (*bxl1*) are found but no endoarabinanase. The resulting degradation product L-arabinose is taken-up and further degraded by a fungal specific degradation pathway which is interconnected with the D-xylose pathway. The following sequence of enzymes was established starting with an L-arabinose reductase, followed by an L-arabinitol dehydrogenase LAD1, an L-xylulose reductase LXR1, a xylitol dehydrogenase XDH1 and a xylulokinase XKI1. The L-arabinose reductase step in *T. reesei* is catalyzed by the aldose reductase XYL1 which is also involved in the degradation of D-xylose. Cloning of a fungal LXR1 enzyme responsible for NADPH dependant reduction of L-xylulose to xylitol was previously reported but our analysis revealed that LXR1 is not involved in L-arabinose catabolism. We have therefore tested different other LXR candidates and have identified one LXR whose deletion reduces the growth on L-arabinose and L-arabitol. Growth on arabinan, and its monomer L-arabinose requires the operation of the general cellulase and xylanase regulator XYR1. This impairment of growth in the *xyr1* deleted strain can be overcome by constitutive expression of the aldose reductase XYL1. Transcriptional analysis reveals that *abf1-3* and *bxl1* are induced by L-arabinose and L-arabinitol. Transcription of *abf2* and *bxl1* is dependent on XYR1 and cannot be compensated for by constitutive expression of XYL1. Induction of all four arabinofuranosidases is strongly enhanced in a *lad1* deleted strain and severely impaired in the *xyl1* deleted strain. We conclude that the transcription of the arabinofuranosidase genes requires an early pathway intermediate (L-arabinitol or L-arabinose), the first enzyme of the pathway XYL1, and in the case of *abf2* and *bxl1* also the function of the cellulase regulator XYR1.

Wednesday March 31

Parallel session 5: Fungal Way of Living: Sex and Other Encounters

PS5.1

The dodge of blotch: Saving sex in *Mycosphaerella graminicola*

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Mycosphaerella graminicola is the causal agent of septoria tritici blotch, currently the most important disease of wheat in Europe. Despite the recent identification of 15 resistance genes and their potential application in plant breeding, disease control is currently achieved mainly by fungicides. However, fungicide resistance development in natural *M. graminicola* populations frequently occurs and is a serious concern. Depending on the fungicides this may develop gradually, such as with resistance to azoles, or much more rapidly as was observed for strobilurin fungicides. In order to understand this rapid spread of resistance we have performed a range of crossing experiments that demonstrate that external stress factors hamper disease development but cannot prevent sexual development. As *M. graminicola* is a heterothallic bipolar pathogen, sexual development requires two mating partners - carrying different mat alleles (mat1-1 or mat 1-2) - that both produce female and male organs. We use an in planta crossing protocol that reliably enables the isolation of segregating/mapping populations. The first stress factor that we used was host resistance. Various crosses on a range of cereal hosts indicated that sex always takes place as long as one of the mating partners is virulent. Thus, even an avirulent isolate that does not establish a compatible interaction with the host plant is perfectly able to enter into the sexual process resulting in viable ascospores. As a consequence the genes of such an avirulent isolate are transmitted to subsequent generations. This is fundamentally different from many other host-pathosystems where avirulent isolates - and their genes - are lost in subsequent generations. We used strobilurin fungicides as a second stress factor by crossing sensitive and resistant isolates under various strobilurin concentrations (3-200%). Although strobilurins prevent disease development of sensitive isolates, and as a consequence minimize biomass, abundant sexual development occurred under all conditions, thus irrespective of the applied strobilurin concentration. Moreover, our results showed that the 'stressed' mating partner – the sensitive parent – acted as the preferred paternal partner. Thus, external stress factors on avirulent or sensitive isolates do not preclude the production of *M. graminicola* spermatia that effectuate viable ascospore production. The fact that the sensitive isolates are preferred paternal donors – and consequently the resistant strains are maternal donors – in the sexual process resulted in major shifts in strobilurin resistance in the segregating populations as the target site for strobilurins is on the mitochondrial genome. A minimal dose of 6% strobilurin already rendered entire populations resistant to these compounds. This explains the rapid pan-European spread of strobilurin resistance in M. graminicola, likely in temporally and geographically independent occasions, with no loss of nuclear genetic variation. The recently discovered genome plasticity of *M. graminicola* may contribute to its ability to overcome environmentally adverse conditions.

PS5.2

Chasing effectors in the genomes of fungal symbionts and pathogens of trees

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After the completion of the *Populus trichocarpa* genome sequence (Tuskan et al, Science, 2006), the Joint Genome Institute (JGI, Department of Energy, USA) sequenced several genomes of microbes interacting with poplar trees. This program comprises fungi with different lifestyles (symbionts and pathogens) and aims at understanding their role in forest ecosystems and molecular mechanisms underlying tree-microbe interactions. Analysis of the coding space of genomes of the ectomycorrhizal basidiomycete *Laccaria bicolor* (sequenced by the JGI, Martin et al, Nature, 2008) and the ectomycorrhizal ascomycete *Tuber melanosporum* (the gourmet black truffle, sequenced by the french Genoscope) revealed that the two fungi have derived different 'symbiosis molecular toolkits' to associate with their hosts. Interestingly, *L. bicolor* genome encodes numerous small secreted proteins that share motifs with effectors recently described in fungal and oomycete pathogens. Analysis of the genome of *Melampsora larici-populina*, the basidiomycete responsible for the poplar rust disease (sequenced by JGI) also revealed the presence of a large repertoire of small secreted protein encoding genes that likely contains putative effectors required to establish successful colonization of plant tissues. Examples from these fungal genomic projects will illustrate how genomic analyses combined with transcriptomic approaches helped in identifying candidate effectors and ongoing functional characterization of candidates in both *L. bicolor* and *M. larici-populina* will be presented.

PS5.3

Investigating the genetic control of infection-related development in the rice blast fungus *Magnaporthe oryzae*

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Magnaporthe oryzae is the causal agent of rice blast, one of the most serious economic problems affecting rice production. The availability of genome sequences for *M. oryzae* and its host, *Oryza sativa*, has provide the means to investigate this fungal-plant interaction in great detail and develop a system biology approach to understanding plant disease. During plant infection, *M. oryzae* develops a differentiated infection structure called an appressorium. This unicellular, dome-shaped structure generates cellular turgor, that is translated into mechanical force to cause rupture of the rice cuticle and entry into plant tissue. My research group is interested in determining the molecular basis of appressorium development and understanding the genetic regulation of the infection process by the rice blast fungus. We have shown that development of a functional appressorium is linked to the control of cell division. Blocking completion of mitosis by generation of a temperature-sensitive *monimA* mutant, for instance, prevents appressorium morphogenesis and a similar phenotype occurs when MobimE mutants are analysed. Furthermore, following mitosis, conidia undergo cell collapse and programmed autophagic cell death. The absence of non-selective autophagic cell machinery in *M. oryzae* is sufficient to prevent the fungus from being able to cause disease. These findings indicate that appressorium morphogenesis requires completion of mitosis and initiation of autophagic recycling of the contents of the fungal spore to the appressorium. Appressorium formation is also associated with an oxidative burst that requires NADPH oxidases that a virulence determinants of *M. oryzae*. To study appressorium physiology and function in greater detail we have used proteomics to define the major changes in protein abundance associated with plant infection by *M. oryzae* and metabolite fingerprinting by electrospray ionisation mass spectrometry and GC-ToF-MS to define major metabolic changes in both the fungus and its host during the onset of rice blast disease. This is linked to our study of the physiology of turgor generation and the role of glycerol, trehalose and glycogen metabolism to the production of infection-competent apppressoria.

Wednesday March 31

Parallel session 6: Fungal Way of Living: Cell Biology

PS6.1

Ancestral homologs of the yeast bud site selection proteins regulate septum formation and development in filamentous fungi

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The defining feature of fungal cells is polarized growth, whereby cell wall deposition is confined to a discrete location on the cell surface. The annotation of multiple fungal genome sequences has revealed that the signaling modules and morphogenetic machinery involved in polarized growth are largely conserved across the fungal kingdom. Nevertheless, fungal cells exhibit a diverse variety of shapes that are largely based on two growth patterns: hyphae and yeast. We suggest that these different patterns reflect variation in the mechanisms that spatially and temporally regulate cellular morphogenesis. To test our hypothesis, we are characterizing ancestral homologues of the yeast bud site selection proteins. In particular, we have found that the yeast axial bud pattern markers Bud3, Bud4, and Axl2 are weakly conserved in the Pezizomycotina. Functional studies in Aspergillus nidulans implicate Bud3 as a guanine nucleotide exchange factor (GEF) that regulates septation in hyphae by activating the GTPase Rho4. Bud4 is also involved in septum formation; genetic interactions suggest that it might facilitate septin organization. In addition, our studies reveal roles for Bud4 and Axl2 in cytokinesis during conidiophore development. Notably, Bud4 localizes to all septa in conidiophores, whereas Axl2 is only found at the junction between spores and their subtending phialide. Our observations support the existence of a phialide-specific morphogenetic program that might be unique to the Aspergilli and related Eurotiomycetes. Furthermore, they also provide insight into the ancestral functions of the yeast bud site selection system in the filamentous fungi.

PS6.2

Biogenesis and evolution of the fungal Woronin body

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Woronin bodies are peroxisome-derived organelles that evolved approximately 500 million years ago in a common ancestor of filamentous Ascomycetes where they perform an adaptive function supporting the hyphal syncytium. These organelles are centered on the HEX protein, which self-assembles to produce micrometer scale protein assemblies that bud from the peroxisome to produce a second organelle with a distinct composition and cellular localization. Forward genetic screens in *Neurospora crassa* have identified two new genes, *wsc* (Woronin sorting complex) and *leashin*, which encode key components of the WB biogenesis machinery. WSC functions by forming membrane associated oligomers that envelop HEX assemblies to promote budding. In a second function, WSC engages the cytoplasmic tethering protein Leashin, which mediates cell cortex association in a step that is essential for organelle inheritance. This work defines a biogenesis pathway in which the dual function of WSC acts to coordinate organelle morphogenesis and inheritance. I will conclude my talk with a discussion of mechanisms that control Woronin body abundance and speculation on the nature of genetic innovation that fostered Woronin body evolution.

PS6.3

The role of microbodies in penicillin production

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The filamentous fungus *P. chrysogenum* is the industrial producer of the important β-lactam antibiotic penicillin. The initial steps of the penicillin biosynthetic pathway are localized in the cytosol, namely the non-ribosomal peptide synthetase δ-(L-aminoadipyl)-L-cysteinyl-D-valine synthetase (ACVS), which produces the tripeptide ACV, and isopenicillin N synthetase (IPNS), which catalyses the formation of isopenicillin N (IPN). The last steps of penicillin biosynthesis occur in specialized organelles, the microbodies (peroxisomes). These organelles contain the enzymes isopenicillin N:acyl CoA acyltransferase (IAT) and phenylacetyl-CoA ligase (PCL), which catalyze the conversion of IPN into penicillin G.

The peroxisomal localization of IAT and PCL in peroxisomes is essential for efficient penicillin production, because mutants defective in peroxisome formation show reduced penicillin production levels. Moreover, a close correlation seem to exist between penicillin production and the volume fraction of peroxisomes per cell [1]. Additionally, artificial overproduction of a single peroxisomal membrane protein, Pex11p, resulted in increased penicillin production levels together with massive proliferation of peroxisomes. In this strain the level of the penicillin biosynthetic enzymes was not altered [2]. Recently we introduced the penicillin biosynthesis pathway into the yeast *Hansenula polymorpha*. In this organism penicillin was produced and efficiently secreted in the medium. Also in this heterologous host peroxisome deficiency resulted in strongly decreased penicillin production levels [3].

References:

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Thursday April 1

Parallel session 7: Fungal and Oomycete Effectors

PS7.1

Fungal secondary metabolites as effectors of pathogenicity: role in the complex interplay between rice and *Magnaporthe grisea*

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Functional analyses of fungal genomes are expanding our view of the metabolic pathways involved in the production of secondary metabolites. These genomes contains a significant number of genes encoding key biosynthetic enzymes such as polyketides synthases (PKS), non-ribosomal peptide synthases (NRPS) and their hybrids (PKS-NRPS), as well as terpene synthases (TS). *Magnaporthe grisea* has the highest number of such key enzymes (22 PKS, 8 NRPS, 10 PKS-NRPS, and 5 TS) among fungal plant pathogens, suggesting that this fungal species produce a large number of secondary metabolites. In particular, it has 10 hybrid PKS-NRPS that likely produce polyketides containing a single an amino-acid. Three of them (*ACE1, SYN2* and *SYN8*) have the same expression pattern that is specific of early stages of infection (appressorium-mediated penetration), suggesting that the corresponding metabolites are delivered to the first infected cells. *M. grisea* mutants deleted for *ACE1* or *SYN2* by targeted gene replacement are as pathogenic as wild type Guy11 isolate on susceptible rice cultivars. Such a negative result could result from a functional redundancy between these pathways. However, *ACE1* null mutants become specifically pathogenic on resistant rice cultivars carrying the *Pi33* resistance gene compared to wild type Guy11 isolate that is unable to infect such rice cultivars. Introduction of a Guy11 wild type *ACE1* allele in *Pi33* virulent *M. grisea* isolates restore their avirulence on Pi33 resistant rice cultivars, showing that *ACE1* behaves as a classical avirulence gene (AVR). *ACE1* differs from other fungal *AVR* genes (proteins secreted into host tissues during infection) as it likely controls the production of a secondary metabolite specifically recognized by resistant rice cultivars. Arguments toward this hypothesis involve the fact that the protein Ace1 is only detected in the cytoplasm of appressoria and is not translocated into infectious hyphae inside epidermal cells. Furthermore, *Ace1-ks0*, an *ACE1* allele obtained by site-directed mutagenesis of a single amino acid essential for the enzymatic activity of Ace1, is unable to confer avirulence. According to this hypothesis, resistant rice plants carrying *Pi33* are able to recognize its fungal pathogen *M. grisea* through the perception of one fungal secondary metabolite produced during infection. The map based cloning of the *Pi33* rice gene was initiated and this gene maps at a locus rich in classical NBS-LRR resistance genes. Further work is ongoing to identify which gene is *Pi33*. In order to characterize the secondary metabolite produced by *ACE1*, this gene was expressed in a heterologous fungal host such as *Aspergillus oryzae* under the control of an inducible promoter. The removal of the three introns of *ACE1* allowed the expression of the enzyme in *A. oryzae*. Characterization of the novel metabolite produced by *Ace1* is in progress.

PS7.2

Effectors of *Fusarium oxysporum*

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The tomato xylem-colonizing fungus *Fusarium oxysporum* f.sp. *lycopersici* (Fol) secretes effectors into xylem sap of its host. Three of the eleven small secreted proteins that we identified trigger effector-mediated immunity: Avr1, Avr2 and Avr3 are recognized by the resistance proteins I, I-2 and I-3, respectively. Interestingly, Avr1 suppresses I-2 and I-3-mediated resistance.

Several Fol effectors were shown through gene knock-out to contribute to virulence towards susceptible plants. We aim to uncover the molecular mechanisms through which effectors of Fol trigger susceptibility (suppression of resistance) and immunity (activation of R proteins). We also focus on clarification of genetic processes underlying evolution of host-specific pathogenicity in the Fo species complex.

We found that effector genes in Fol reside on 'pathogenicity chromosomes'. These chromosomes can be transferred between clonal lines, conferring host-specific pathogenicity to the recipient. Transfer of pathogenicity chromosomes explains the emergence of new pathogenic clonal lines of Fo. This process may also contribute to the evolution of compatibility with novel hosts through recombination between mobile chromosomes resulting in new effector repertoires.

PS7.3

The effectors of *Ustilago maydis* and related smut fungi

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The basidiomycete fungus *Ustilago maydis* is a biotrophic maize pathogen that does not use aggressive virulence strategies and needs the living plant tissue for completion of its life cycle. The *U. maydis* genome codes for a large set of novel secreted effector proteins. Many of the respective genes are clustered in the genome and are upregulated during pathogenic development. About half of these gene clusters have crucial roles during discrete stages of biotrophic growth. We have now determined which of the clustered effector genes are responsible for the virulence phenotype. We also show that most effectors also exist in related smut fungi, but are poorly conserved, suggesting their involvement in the arms race with the host. *U. maydis* is eliciting distinct defense responses when individual effector clusters/genes are deleted. Maize gene expression profiling and the identification of interacting proteins allowed us to classify the response to individual mutants and to obtain leads to where the fungal effectors might interfere. Using localization, binding and uptake studies we provide evidence that some effectors function in the apoplast while others are likely to have a cytoplasmic function.

PS7.4

Unraveling the mechanism of RxLR mediated translocation of Oomycete effector proteins

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Several Prokaryotic and Eukaryotic microbial pathogens have evolved intriguing mechanisms to translocate proteins into their host cells. The translocated proteins are called effectors as they can modulate molecular processes in their hosts in order to establish an infection and/or suppress their immune response. For example, certain types of bacteria possess a needle like injection system, the type III secretion system (T3SS), which allows a direct translocation of effector proteins into the cells under attack. Whilst the bacterial translocation machineries are well described, little is known about how effectors from Eukaryotic pathogens are delivered into their host cells. The early stage of infection caused by the eukaryotic oomycete pathogen *Phytophthora infestans* involves a biotrophic phase. In this early interaction stage the secretion of oomycete RxLR effectors takes place via haustoria, which are structures formed by the pathogen that are in intimate contact with the extra haustorial membrane produced by the plant. The mechanism by which oomycetes direct their RxLR effectors into host cells is as yet unknown and is the main focus of our research. It has been postulated that endocytosis processes or protein transporters are responsible. Here we present our latest results, which give insight into the mechanism of the oomycete RxLR-EER protein translocation system.

Thursday April 1

Parallel session 8: Fungal Biotechnology

PS8.1

Fungal biotechnology: lessons learned from *Penicillium* strain improvement

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Production of penicillin by Penicillium chrysogenum is a classic hallmark of fungal biotechnology. Since its famous discovery by Fleming and initial production during the Second World War penicillin production has increased enormously as a result of strain and process improvements. Using metabolic pathway engineering high yielding penicillin strains were also converted in efficient cephalosporin producers. Recently, we determined the full genome sequence of Penicillium and developed post genomic tools to study the Penicillium strain lineage for mutations acquired in the long optimization process. In addition basic studies on fungal metabolism, microbody formation and transporters have increased our knowledge on penicillin formation significantly. The presentation will cover both basic and applied aspects of the research done.

PS8.2

Fungal enzyme expression as a unit operation in the production of cellulosic ethanol

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Verenium is a leader in the development of next-generation cellulosic ethanol as well as the development of Specialty Enzyme Products. Verenium's cellulosic ethanol process utilizes on-site, fungal enzyme production to supply the enzymes for the saccharification of the cellulose fiber stream in a continuous saccharification-fermentation process. Fungal enzyme production is a significant unit operation within the facility and successful integration of this unit operation into the overall process presents a number of challenges and opportunities. These experiences will be discussed in the context of the operation of Verenium's 1.4 MGY demonstration plant in Jennings, LA. In addition, the data obtained from Demonstration Plant operation are helping to guide the development of Verenium's process-optimized lignocellulosic enzyme cocktail. This high performance fungal enzyme cocktail is anticipated to become a key differentiating component of Verenium's commercial cellulosic ethanol process.

PS8.3

Genomic and transcriptomic analysis of *Thielavia terrestris* – a thermophilic ascomycete of biotechnological interest

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Thielavia terrestris (anamorph = *Acremonium alabamense*) is a thermophilic ascomycete that is of interest as a potential source of thermostable enzymes for biotechnological applications such as biomass decomposition. A high-quality draft genome sequence of *T. terrestris* NRRL 8126 was recently completed at the Joint Genome Institute. Subsequent mining, editing, and annotation efforts are in progress by an international team of collaborators. The genome assembly comprises eight scaffolds (231 contigs) spanning 36.9 Mbp (sequence coverage = 10.15x). The overall G+C content, excluding mitochondrial DNA, was approximately 58%. The presence of telomeric repeats [(TTAGGG)n/(CCCTAA)n] at both ends of scaffolds 1,3, 4 and 6, and at one end of scaffolds 2, 5, 7 and 8 suggests that the assembly contains nearly complete chromosome sequences. Among the 9815 predicted protein-coding genes in the *Thielavia* genome, >750 transposases were identified on the basis sequence identity with *Aspergillus nidulans* transposons, and a sizeable proportion of these appear to be degraded by RIP. Approximately 6-8% of the gene models are predicted to encode secreted proteins such as oxidoreductases, peptidases and a variety of glycoside hydrolases. Compared to the well-studied cellulolytic fungus *Trichoderma reesei*, an obvious expansion of genes encoding family GH61 proteins was noted. Nimblegen expression arrays were deployed in a preliminary investigation to compare the transcription profiles of *T. terrestris* cells grown on several substrates (*e.g.*, glucose, cellulose, xylan, soy flour), and induction of genes predicted to encode cellulases and hemicellulases was observed on cellulose and xylan, respectively. A comparison of transcriptome data for cells grown in glucose medium at 34°C and 45°C suggested that growth of *T. terrestris* at the higher temperature may induce expression of genes encoding membrane proteins, sterol biosynthetic enzymes, heat shock proteins/chaperones and components of the ubiquitin proteasome pathway.

Poster Abstracts

Poster Category 1:

Phylogeny and Fungal Tree of Life

PR1.1

Horizontal gene transfer of *katG* genes from bacteroidetes into Ascomycetes

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Bifunctional catalase-peroxidases (KatGs) are encoded in widely spread *katG* genes, useful markers for the reconstruction of the evolution of resistance against reactive oxygen species (ROS). They are present in the genomes of archaea, bacteria and fungi with bacterial KatGs being more abundant and most ancient. In fungi their physiological function still remains unclear or hypothetical. The present phylogenetic analysis reveals that *katG* genes were transferred from the genomes of bacteroidetes into ascomycete fungi by a single horizontal gene transfer (HGT) event. Moreover, detailed sequence analysis clearly shows the presence of two distinct groups of fungal catalase-peroxidases with *katG1* genes encoding intracellular proteins (KatG1) and *katG2* genes having a signal sequence thus encoding secreted peroxidases (KatG2). So far a single *katG1* gene was found in the genome of the basidiomycete *Ustilago mayidis*, probably transferred from ascomycetes. All other known *katG1 and katG2 genes* are present selectively in the ascomycete genomes. Catalase-peroxidases of the first group (KatG1) are abundant mainly among Eurotiomycetes and Sordariomycetes. They are involved in removal of intracellular peroxides occurring as by-products of metabolism. On the other hand KatG2s are present in phytopathogenic fungi of the class Sordariomycetes. They may be involved in defence of phytopathogenic fungi against oxidative burst induced by the plant host after fungal invasion. Current investigation of structure-function relationship of heterologously expressed fungal *katG1 & katG2* genes will shed light on their actual physiological role.

PR1.2

Mapping QTLs in multiple phenotypes by linkage analyses

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Saccharomyces cerevisiae strains exhibit a large genotypic and phenotypic diversity, which makes this organism an attractive model for mapping quantitative trait loci (QTLs). So far only a few studies have used natural isolates to dissect complex traits and the underlying natural variation. The *Saccharomyces* Genome Resequencing Project (SGRP) has released genome sequence data (1 to 4X coverage) of 72 strains of *S. cerevisiae* and its closest known relative *S. paradoxus.* Half of the *S. cerevisiae* strains sequenced fall into five distinct clean lineages, whereas the others have mosaic recombinant genomes. Four strains representative of different clean lineages were chosen for generating a grid of six crosses. We generated 96 segregants from each cross (total of 576) and genotyped 170 loci evenly spaced along the genome (a marker every ~80 kb). Preliminary results for crossing over indicate the presence of conserved recombination hotspots between the crosses and a general reduction in recombination events in two of the crosses. All the segregants were extensively phenotyped under several conditions in order to perform linkage analysis and major QTLs were mapped for most of the phenotypes tested. Among these, high temperature growth (40ºC) and NaAsO2 (5 mM) resistance, showed the highest number of QTLs detected. For high temperature growth, several QTLs were found in specific pair combinations or shared between all crosses and little overlap was found between QTLs identified here and previously reported ones. This set of segregants will be useful to obtain a more complete picture of the genetic mechanisms underlying natural phenotypic variation.

PR1.3

Next-generation sequencing of the 40 Mb genome of the ascomycete *Sordaria macrospora*

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Next-generation sequencing techniques have revolutionized the way genome sequencing is done today. However, de novo assembly of eukaryotic genomes still presents significant hurdles due to their large size and stretches of repetitive sequences. Filamentous fungi usually have genomes of 30-50 Mb with few repetitive regions; therefore, their genomes are suitable candidates for *de novo* sequencing by next-generation sequencing techniques. Here, we present a draft version of the *Sordaria macrospora* genome obtained by a combination of Solexa paired-end sequencing and 454 sequencing. Paired-end Solexa sequencing of genomic DNA in libraries of 300 bp (four lanes) and 500 bp (three lanes) and an additional 10x coverage with 454 sequencing resulted in ~4 Gb of DNA sequence. The reads were assembled to a 39 Mb draft version with an N50 size of 117 kb using the Velvet assembler. By comparative analysis with the genome of *Neurospora crassa*, the N50 size was increased to 498 kb. Based on gene models for *N. crassa*, ~10000 protein coding genes were predicted. Comparison of the *S. macrospora* genes with that of other fungi showed that *S. macrospora* harbors duplications of several genes that are single-copy genes involved in self/nonself-recognition in other fungi. Furthermore, *S. macrospora* contains more polyketide biosynthesis genes than its close relative *N. crassa*, some of which might have been acquired by horizontal gene transfer. These data show that for filamentous fungi, *de novo* assembly of genomes from next-generation sequences alone is possible and the resulting data can be used for comparative studies to address questions of fungal biology.

PR1.4

Evolutionary relationships in the anthracnose pathogen *Colletotrichum acutatum*

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Colletotrichum acutatum is an important pathogen causing economically significant losses of temperate, subtropical and tropical crops. Globally, *C. acutatum* populations display considerable genotypic and phenotypic diversity. The overall objective is to understand the evolutionary relationships within the species with particular reference to the pathogen populations associated with the strawberry production systems in the UK.

More than 150 *C. acutatum* isolates related to different hosts worldwide have been assembled. Phylogenetic analysis of sequence data from the rRNA gene block-ITS region, HMG-box of the Mat1-2 gene and the beta-tubulin 2 gene led to the identification of eight distinct genetic groups within *C. acutatum*. The subsets of isolates represented within these genetic groups corresponded to the groups A1 - A8 identified previously based on the ITS marker. Almost all of the isolates capable of homothallic sexual reproduction, both in culture and in nature, comprise a single genetic group A7. Isolates representing populations capable of heterothallic sexual reproduction belong to two distinct genetic groups A3 and A5. Moreover, the eight genetic groups representing the global *C. acutatum* populations form at least two distinct clusters. Molecular characterisation of *C. acutatum* populations representing the introduction and spread of the pathogen in the strawberry production systems in the UK showed the presence of at least three genetic groups A2, A3 and A4. Overall, our results suggest the existence of *C. acutatum* populations potentially undergoing speciation processes, related to their reproductive behaviour and host association patterns. Further molecular and phenotypic characterisation is in progress.

PR1.5

Analysis of regulation of pentose utilization in *Aspergillus niger* reveals evolutionary adaptations in the eurotiales

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D-xylose and L-arabinose are highly abundant components of plant biomass and therefore major carbon sources for many fungi. Fungi produce extracellular enzymes to release these sugars, which are subsequently taken up into the cell and converted through the pentose catabolic pathway. In Aspergilli and most other filamentous ascomycetes, D-xylose release and the pentose catabolic pathway are regulated by the transcriptional activator XlnR. In *Aspergillus niger*, we recently described the transcriptional activator AraR, which controls L-arabinose release and the pentose catabolic pathway. In this study we performed a phylogenetic analysis of the genes of the pentose catabolic pathway as well as the two transcriptional activators (AraR and XlnR) to identify evolutionary changes in the utilization of these sugars. This analysis showed that AraR is only present in the Eurotiales and appears to have originated from a gene duplication event (from XlnR) after this order split from the other filamentous ascomycetes. XlnR is present in all filamentous ascomycetes with the exception of members of the Onygenales. As this order is part of the same subclass, Eurothiomycetidae, as the Eurotiales, this indicates that strong adaptation of the regulation of pentose utilization has occurred at this evolutionary node. In the Eurotiales a unique two-component regulatory system for pentose release and metabolism has been evolved, while the regulatory system has become absent in the Onygenales. In contrast, homologues for most genes of the L-arabinose/D-xylose catabolic pathway are present in all filamentous fungi, irrespective of the presence of XlnR and/or AraR, indicating that the evolutionary changes mainly affect the regulatory system and not the pathway itself.

PR1.6

A molecular diagnostic for tropical race 4 of the banana

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This study analysed genomic variation of the translation elongation factor 1α (*TEF-1*) and the intergenic spacer region (IGS) of the nuclear ribosomal operon of *Fusarium oxysporum* f. sp. *cubense* (Foc) isolates, from different banana production areas, representing strains within the known races, comprising 20 vegetative compatibility groups (VCG). Based on two single nucleotide polymorphisms present in the IGS region, a PCR-based diagnostic tool was developed to specifically detect isolates from VCG 01213, also called tropical race 4 (TR4), which is currently a major concern in global banana production. Validation involved TR4 isolates, as well as Foc isolates from 19 other VCGs, other fungal plant pathogens and DNA samples from infected tissues of the Cavendish banana cultivar Grand Naine (AAA). Subsequently, a multiplex PCR was developed for fungal or plant samples that also discriminated *Musa acuminata* and *M. balbisiana* genotypes. It was concluded that this diagnostic procedure is currently the best option for the rapid and reliable detection and monitoring of TR4 to support eradication and quarantine strategies.

PR1.7

Development of the *Aspergillus oryzae* comparative fungal genome database

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By the recent advancement of genome sequencing technology, the numerous numbers of genome sequences have been reported in several industrial and pathogenic fungi. The comparative genomic of these fungi will supply us huge noble information for phylogenetic study, identification of specie specific genes cluster, identification of genes function and etc. Several genome database was established including *Aspergillus species* and published. The genome sequence of *Aspergillus oryzae* was also deposited and published in these databases. However, available information is not sufficient in the point of comparative genomics, especially, comparison of well studied genome, such as *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Neurospora crassa,* and *Aspergillus nidulans*. Thus, we developed *Aspergillus oryzae* comparative fungal genome database (NRIB CFGD) in this study. First of all, we enriched the CDS information of *A. oryzae* genes, such as the result of motif and domain analysis, blast analysis against KOG, COG, and Swissprot, assign of EC number and expression profile of this fungi. Furthermore, we compared the *A. oryzae* genome against 13 other fungal genomes. Among them, authologous genes were clustered by bidirectional best-hit analysis. These information were supplied with graphical interface and user can browse locus, transcripts, CDS, expression profiles, and result of authologous gene analysis very easily. In the comparative genome site, user can browse dot blot analysis, differential analysis and the comparison of synteny of genes. This database will be opened soon and waiting for your access.

PR1.8

Variation in sequence and location of the fumonisin mycotoxin biosynthetic gene cluster in *Fusarium*

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Several *Fusarium* species in the *Gibberella fujikuroi* species complex (GFSC) and rare strains of *F. oxysporum* can produce fumonisins, a family of mycotoxins associated with multiple health disorders in humans and animals. In *Fusarium*, the ability to produce fumonisins is governed by a 17-gene fumonisin biosynthetic gene (*FUM*) cluster. Here, we examined the cluster in *F. oxysporum* strain O-1890 and nine other species (e.g. *F. proliferatum* and *F. verticillioides*) selected to represent a wide range of the genetic diversity within the GFSC. Flanking-gene analysis revealed that the *FUM* cluster can be located in one of four genetic environments. Comparison of the genetic environments with a housekeeping gene-based species phylogeny revealed that *FUM* cluster location is correlated with the phylogenetic relationships of species; the cluster is in the same genetic environment in more closely related species and different environments in more distantly related species. Additional analyses revealed that sequence polymorphism in the *FUM* cluster is not correlated with phylogenetic relationships among some species. However, cluster polymorphism is associated with production of different classes of fumonisins in some species. As a result, closely related species can have markedly different *FUM* gene sequences and can produce different classes of fumonisins. The data indicate that the *FUM* cluster has moved within the *Fusarium* genome during evolution of the GFSC and further that sequence polymorphism was sometimes maintained during the movement such that clusters with markedly different sequences are now located in the same genetic environment.

PR1.9

Relationships among *Lasiodiplodia theobromae* isolates associated with tropical fruit plants inferred from the analysis of ITS and EF1-α gene

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Lasiodiplodia theobromae is a phytopathogenic fungus causing gummosis, a threatening disease for cashew plants in Brazil. A collection of isolates of *L. theobromae* obtained from cashew plants and also from others tropical fruit plants was studied on the basis of sequence data from the ITS regions and EF1-α gene. Sequence data and ITS-RFLP patterns indicate a substantial genetic variability among isolates from cashew plants showing symptoms of the disease and also from others tropical fruit plants, such as lemon, *Spondia* sp., passion fruit and graviola plants. However, no difference was found among *L. theobromae* isolates from symptomatic cashew plants and from symptomless cashew plants colonized by the fungus, indicating that possibly the same specie that endophytically colonize the cashew plants with no apparent symptoms is also responsible for the disease. Further studies based on detection of Single Nucleotide Polymorphisms are being carried out and have potential utility for detection of L. theobromae strains in cashew plant seedlings.

PR1.10

The draft genome sequence of *Mycosphaerella fijiensis*, the black sigatoka pathogen of banana

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Mycosphaerella fijiensis (anamorph: *Paracercospora fijiensis*) is a hemibiotrophic fungal pathogen of banana and the causal agent of the devastating Black Sigatoka or black leaf streak disease. Its control requires weekly fungicide applications when bananas are grown under disease-conducive conditions, which mostly represent precarious tropical environments. We started a multidisciplinary research program on *M. fijiensis* that is aiming at pesticide reduction. The first goal was to collect genomic data and to develop tools for molecular analysis of this pathosystem. Analyses of electrophoretic karyotypes on DNA extracted from protoplasts of *M. fijiensis* showed that chromosome sizes range between 500 kb and ~12 Mb. An extraordinarily high level of chromosome length polymorphism is observed among the *M. fijiensis* strains coming from both different global populations as well as within a single field population. The results suggest that sexual recombination and chromosome size polymorphisms are important in the evolution of *M. fijiensis*. A genetic linkage map comprising 19 linkage groups covering 1417 cM containing 235 Diversity Array Technology markers, 87 microsatellite (SSR) and three minisatellite (VNTR) markers was calculated using high LOD scores (LOD >10). All markers were sequenced and aligned to the draft 7.8x whole genome shotgun Sanger sequence of *M. fijiensis* CIRAD86. In addition more than 30,000 ESTs from three in vitro libraries were sequenced. The latest whole genome assembly of the shotgun reads was constructed with the JGI Arachne assembler and coordinated with the aforementioned genetic linkage map. The genome has an estimated size of 74 Mb and is now assembled into 56 scaffolds covering more than 99% of the genome. The largest scaffold is 11.8 Mb in length and 28 scaffolds (99.8. %) are larger than 50 Kb. The genome size of *M. fijiensis* is 80% larger than that of *M. graminicola* mostly due to the presence of additional repeated sequences. The current draft release, version 1.0, includes a total of 10,327 gene models predicted and functionally annotated using the JGI annotation pipeline. The availability of the *M. fijiensis* genome will greatly assist future studies aimed at the control of black leaf streak disease as well as genomic comparisons with many other agronomically important Dothideomycetes fungi that currently are being sequenced through the Fungal Genome Program at the U.S. Department of Energy's Joint Genome Institute.

PR1.11

CADRE: An update on web services and data

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The Central *Aspergillus* Data Repository (C*A*DRE; http://www.cadre-genomes.org.uk) is a public resource for viewing assemblies and annotated genes arising from various world-wide *Aspergillus* projects. We currently house data for nine genomes, including the most recent annotation contributed by the Eurofungbase *Aspergillus nidulans* project. We have continued to manually annotate this genome and have shared it with other relevant resources such as AspGD (http://www.aspergillusgenome.org) and Ensembl Genomes (http://www.ensemblgenomes.org). These collaborations have helped to further improve gene structures and naming within *A. nidulans* annotation and, more importantly, to provide consistency across resources.

Ensembl Genomes is a new resource that seeks to complement the current Ensembl collection (predominantly vertebrate) by including other taxonomic groups. With limited expertise, this can only be done with the support of specific research communities. Therefore, as representative data of the *Aspergillus* community, C*A*DRE has been integrated into Ensembl Genomes and is maintained by both teams. This collaboration has allowed us to further embellish annotation and to perform comparative analyses across eight of the genomes. Towards the end of this integration project, we were also able to submit the *A. nidulans* Eurofungbase annotation to EMBL with post-project contributions from C*A*DRE, AspGD and Ensembl Genomes.

PR1.12

Genetic biodiversity of *Trichoderma* from Poland

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Towards assessing the occurrence and genetic diversity of *Trichoderma*, we have used 222 isolates originated from different region and ecological niches of Poland. Isolates were identified at the species level by sequence analysis of their internal transcribed spacer ITS1 - ITS2 of the rDNA cluster, the four and five intron of translation elongation factor 1- alpha (*tef1*) (using program TrichOKEY v 2 and TrichoBLAST identification tools) and phylogenetic analysis. Fifty three strains were positively identified as *Trichoderma viride,* fifty two as *T. harzianum*, twenty five as *T. koningii,* eighteen as *T. citrinoviride,* sixteen as *T. atroviride,* fifteen as *T. viridescens,* thirteen as *T. hamatum,* eight as *T. virens,* five as *T. aggressivum,* four as *T. asperellum,* three as *T. longibrachiatum*, three as *T. longipile,* two as *T. koningiopsis* and single strains of *T. cremum, T. gamsii, T. tomentosum, H. hunua, H. parapilulifera.*

Finally, we identified 18 species among 222 isolates. These data suggest a relatively low genetic diversity of *Trichoderma* species in Poland.

PR1.13

The KP4 gene family

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Microbial communities can play a critical role in agricultural ecosystems. In fungal-fungal interactions, an organism can produce metabolites that elicit a physiological response in other organisms that can affect the outcome of the interaction. Understanding this communication process maybe critical to maximize crop disease control and therefore crop production. Small, cysteine-rich proteins, synthesized by plants, fungi, viruses and bacteria, can serve as antimicrobial peptides and can be an integral part of their defense system. KP4, produced by the *Ustilago maydis* P4 virus, is one of these proteins and inhibits growth of other fungi, including *Fusarium* and *Aspergillus*, by blocking calcium ion channels. The mature KP4 protein is 105 amino acids and contains 10 cysteine residues. Here, analysis of publicly available genomic sequence databases identified 36 KP4-like genes from a range of Ascomycota, a Basidiomycota, and the moss *Physcomitrella patens*. Six of the KP4-like genes encode a protein with two KP4 domains. Sequence comparison and phylogenetic analysis of the corresponding proteins/domains has provided insight in to the evolutionary history of the KP4 family and provided evidence for lateral gene transfer between kingdoms. The data also suggest that duplication to form a KP4 dimer occurred independently in different lineages of the Ascomycota. Understanding the nature and function of KP4 proteins in mycotoxin-producing species of *Fusarium* may help to limit plant diseases and increase food safety and food production.

Poster Category 2:

Fungal-Host Biology

PR2.1

Glycolytic function is necessary for *Cryptococcus neoformans* virulence

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Cryptococcus neoformans is an important fungal pathogen of immunocompromised individuals, with a close relative - *C. gattii* - emerging as a serious threat for the immunocompetent. During active infection, *C. neoformans* colonizes the airspaces of the lungs resulting in pneumonia, and subsequently migrates to the central nervous system (CNS). The fungus persists in the cerebrospinal fluid (CSF), and causes meningoencephalitis that is fatal if untreated. Since *C. neoformans* colonizes these fundamentally different niches within the host, we sought to understand fungal carbon utilization during infection, and in particular the role of glycolysis in this model fungal pathogen. We created mutants at either end of the glycolytic metabolic pathway, which are restricted for growth on glucose. A pyruvate kinase mutant (*pyk1Δ*) and a hexose kinase I & II double mutant (*hxk1Δ/hxk2Δ*) were made and evaluated for virulence in both rabbit-CSF and murine-inhalation models of cryptococcosis. Results show that both mutations blocking glucose utilization result in complete attenuation of disease in both animal models. Since the *pyk1Δ* mutant cannot utilize lactate for growth when glucose is present, we made a *pyk1Δ/mig1Δ* double mutant lacking the glucose catabolite repressor *MIG1*. This double mutant should be able to utilize all carbon sources available, yet it exhibited greater reduction in CSF persistence in the rabbit model compared to the *pyk1Δ* single mutant and was also attenuated in the mouse model. These data suggest that energy production from glucose in various host contexts is crucial for virulence in *C. neoformans*.

PR2.2

Insights into calcium signal transduction – functional characterization of calcineurin, calcipressin and two putative calcium channels in *B. cinerea*

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For gaining more insights into the host-pathogen interaction and cell differentiation of the phytopathogen *B. cinerea*, we are interested in studying the role of the Ca^{2+}-signal transduction pathway and its arrangement in the intracellular signalling network.

The Ca^{2+}/calmodulin-dependent phosphatase calcineurin is a conserved protein that plays a critical role in Ca^{2+} signalling and stress response. Contrary to our previous expectation that the deletion of calcineurin is lethal, we recently succeeded with the generation of calcineurin A (*bccnA*) deletion mutants. They are strongly impaired in growth and non-pathogenic, as strains grow very slowly as small, compact colonies. However, improved growth was observed on media containing high sugar concentrations or sodium chloride.

Recently, a new class of conserved calcineurin regulators, named calcipressins, has been identified in yeast. The deletion of the single calcipressin homologue in *B. cinerea* (*bcrcn1*) affects vegetative growth on all tested media and showed reduced virulence on living bean plants.

In addition to these components of the Ca^{2+} signalling pathway, the cellular impact of external Ca^{2+} has been investigated by deletion of two genes encoding the putative Ca^{2+} channels BcCCH1 and BcMID1. Both mutants and the double knockout mutant exhibit similar phenotypes, as there are generally not impaired in growth, morphology or virulence. However, under high salt conditions and with the Ca^{2+}-chelator agent EGTA vegetative growth is affected.

PR2.3

The role of *Botrytis cinerea* Ryp1-like protein (Rlp), a putative transcriptional regulator, during pathogenesis

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Botrytis cinerea, which causes gray-mold rot, attacks a wide range of plant species. Due to this wide host range and the severe damage to agriculture, efforts have been made to understand the infection process and to identify genes involved in pathogenicity. In this study, the role of *BcRLP*, which shows similarity to the morphological switch regulators *Candida albicans WOR1* and *Histoplasma capsulatum RYP1*, in pathogenicity was analyzed. Gene knock-out and complementation studies revealed that *RLP* is required for pathogenicity. The *rlp* mutant is able to penetrate plant tissue, but is not able to cause necrotrophy. In addition, the mutant is blocked in conidia formation, is sensitive to oxidative stress and is severely reduced in the production of the toxin botrydal. We speculate that Rlp is required for the necrotrophic growth phase.

PR2.4

The role of small GTPases in growth and virulence of *Claviceps purpurea*

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The biotrophic plant pathogen *Claviceps purpurea* which infects monocotyledonous plants, among them important crops like rye, is an interesting model organism for research in plant-pathogen interactions. The strict polar growth in early infection stages of *C. purpurea* in rye ovaries is of particular interest, as the fungus is not recognized as a pathogen possibly due to its pollen tube-like growth. Small GTPases and their downstream effectors are known to be involved in polarity. Therefore the investigation of the effects of these factors is crucial for a better understanding of polar growth in filamentous fungi.

Knockout strains of the small GTPases Rac and Cdc42 and the p21 activated kinase Cla4 as a GTPase downstream effector have been generated. Deletion of Cdc42 lead to a hyperbranching phenotype with increased sporulation[1] whereas deletion of Rac and Cla4 respectively caused a phenotype with severe impairment in sporulation, branching and growth[2]. Thus, an antagonistic relationship between the GTPases Rac and Cdc42 as well as an involvement of Rac and Cla4 in the same pathway can be concluded. Furthermore the effects of introduced constitutive active forms of Cla4 and Rac in *C. purpurea* have been investigated. Additionally, functional studies of the guanine nucleotide exchange factor Cdc24 were started.

References

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PR2.5

Treatment of *Trichophyton rubrum* ex vivo skin infection with a new antifungal agent, ambruticin, leads to the activation or repression of several genes, including a putative mitochondrial protein and a subtilisin protease

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During host infection and treatment with cytotoxic agents, microorganisms must be able to remodel metabolic pathways to scavenge nutrients and to respond to the stress in order to survive in the host milieu. The identification of the mechanisms involved in fungal adaptation to host microenvironment and stress response is essential to understand the pathogenic process of fungal infection, enabling the establishment of new therapeutic measures. The anthropophilic dermatophyte *Trichophyton rubrum* is one of the most prevalent fungi isolated from cutaneous infections; however, the molecular mechanisms involved in its pathogenicity remain unclear. Moreover, antifungal drugs commonly used in the treatment of fungal infections act upon a limited number of cellular targets, presenting numerous side effects. In this work, an *ex vivo* human skin infection model was used to identify *T. rubrum* expressed genes during interaction with human epidermal cells and treatment with the antifungal agent ambruticin, which supposedly interfere with the osmoregulation system. The results show that this drug presents antifungal activities against this dermatophyte, leading to a swelling of the hyphal tip and consequently fungal death, also modulating the expression of several genes. The *ex vivo* human skin infection model followed by suppressive subtraction hybridization allowed the identification of genes involved in cellular transport and biogenesis of cellular components that were activated or repressed in response to ambruticin. The activation of the gene coding for a mitochondrial protein with unknown function, homolog to the *Saccharomyces cerevisiae* Tar1p, or repression of the gene coding for a subtilisin protease 5, during treatment of *T. rubrum ex vivo* skin infection suggests that they might be important for the maintenance of this dermatophyte in the host tissue, especially in the presence of an inhibitory agent.

PR2.6

Comparing *Fusarium graminearum* gene expression during infection of three monocot hosts

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Fusarium graminearum is a broad host pathogen which has a major impact on cereal crops worldwide, causing fusarium head blight in small grain cereals (wheat, barley, oats) and gibberella ear rot in maize. To examine whether *F. graminearum* responds differently to diverse hosts, we compared the *Fusarium* gene expression profile during early infection of maize kernels and wheat and barley heads under the same environmental conditions. A single *F. graminearum* strain was used for the inoculation of all three hosts. Custom 4X44K Agilent microarrays designed to represent the *F. graminearum* gene set (13,918 predicted ORFs) were used to obtain transcriptome profiles of tissues 1d, 2d and 4d post-inoculation. Cross-hybridizing plant sequences were eliminated by conducting duo-dye hybridizations, comparing mock-inoculated and *Fusarium*-inoculated tissues directly. An analysis of the gene expression profiling will be presented.

PR2.7

What large-scale T-DNA insertional mutagenesis tells us about pathogenicity? a functional genomics analysis in the dothideomycete *Leptosphaeria maculans*

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The Dothideomycete phytopathogen *Leptosphaeria maculans* is capable to alternate saprophytic, hemibiotrophic, endophytic and necrotrophic life styles during a single infectious cycle on its host plant, *Brassica napus*. However, little is known about the determinants of such plasticity. A reverse genetic strategy was developed, and a large-scale T-DNA insertional mutagenesis project was conducted resulting in: i) a collection of 5000 transformants phenotyped when interacting with plants, ii) a collection of 170 pathogenicity altered mutants, and iii) a set of 400 T-DNA flanking sequences. In addition, the *L. maculans* genome was sequenced and annotated, and whole genome Gene Ontology (GO) analysis was performed. Here we present combined analyses of the genomic pattern of 318 T-DNA insertion events (T-IEs), and the functional pattern of 279 T-DNA targeted genes (T-TGs). T-IEs analyses showed that: i) T-IEs favoured regulatory regions of gene-rich euchromatic genomic regions, and ii) T-IEs density correlated with CG skew near the transcription initiation site. These results are consistent with the T-DNA intranuclear targeting model, relying on gene expression machinery. T-TGs analyses showed that: i) T-IEs targeted 48.9% of the biological processes that were identified by whole-genome GO analysis, and that ii) T-IEs favoured biological processes that are consistent with the cellular state of a germinating conidia. Functional analysis of T-TGs in pathogenicity altered mutants will be presented.

PR2.8

Host-parasite interaction in the *Brassica napus/Verticillium longisporum* system

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The first leaky auxotrophic mutant for aromatic amino acids of the near-diploid fungal plant pathogen *Verticillium longisporum* (VL) has been generated. VL enters its host *Brassica napus* through the roots and colonizes the xylem vessels. The xylem contains little nutrients including low concentrations of amino acids. We isolated the gene *Vlaro2* encoding chorismate synthase by complementation of the corresponding yeast mutant strain. Chorismate synthase produces the first branch point intermediate of aromatic amino acid biosynthesis. A novel RNA-mediated gene silencing method reduced gene expression of both isogenes by 80% and resulted in a bradytrophic mutant, which is a leaky auxotroph due to impaired expression of chorismate synthase. In contrast to the wild type, silencing resulted in increased expression of the cross-pathway regulatory gene *VlcpcA* (similar to *cpcA/ GCN4*) during saprotrophic life. The mutant fungus is still able to infect the host plant *B. napus* and the model *Arabidopsis thaliana* with reduced efficiency. *VlcpcA* expression is increased in planta in the mutant and the wild-type fungus. We assume that xylem colonization requires induction of the cross-pathway control, presumably because the fungus has to overcome imbalanced amino acid supply in the xylem.

PR2.9

***Colletotrichum graminicola* globally accelerates senescence but locally induces photosynthetically active green islands on aging maize leaves**

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Typically, pathogenesis of the hemibiotrophic fungus *Colletotrichum graminicola* and defense responses of its host, *Zea mays*, are studied by inoculation of young leaves. Equivalent studies have not been performed with leaves undergoing senescence, a situation which may be epidemiologically relevant in the field. We compare the infection of *C. graminicola* on senescing and mature leaves. We discovered that in contrast to the anthracnose symptom known from young and mature leaves green islands, reminiscent to those from obligate biotrophs, are formed on senescing leaves. Microscopy revealed that the fungus grows in both symptoms from the epidermal layer towards the bundle sheath that was used for longitudinal spreading. In green islands host tissues remained intact for a more extended period. Imaging PAM fluorescence analyses at high spatial resolution revealed that photosynthesis is transiently maintained at green islands but declined in tissue surrounding the infection site. In contrast, in younger green leaves photosynthesis is reduced only at the infection sites. Further support for the local modification of host physiology comes from qRT-PCR experiments analyzing gene expression at high spatial resolution. Decreased transcript levels of senescence markers corroborated a pathogen-induced delay of senescence. Expression of several genes encoding proteins involved in photosynthesis was strongly reduced after fungal infection. In contrast, the transcript levels of a cell wall invertase were strongly increased at green islands suggesting that *C. graminicola* induced a new carbon sink in the senescing tissue.

PR2.10

Hapx is involved in maintenance of iron homeostasis and virulence of *Aspergillus fumigatus*

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Consistent with iron playing a crucial role in virulence, we have previously shown that biosynthesis of siderophores (iron chelators involved in uptake, storage and intracellular distribution of iron) is essential for virulence of *A. fumigatus*. The characterization of the fungal iron metabolism might aid improvement of diagnosis and treatment of fungal infections.

During iron-replete conditions, siderophore biosynthesis is repressed by the GATA factor SreA. Here we report the characterization of a second iron regulator, the bZIP transcription factor HapX. SreA and HapX are interconnected by a regulatory feedback loop: SreA repressed expression of *hapX* during iron sufficiency and, *vice versa*, HapX repressed expression of *sreA* during iron starvation. During iron starvation, inactivation of HapX resulted in derepression of iron-dependent pathways (e.g. the mutant strain displayed accumulation of the iron-free heme precursor protoporphyine IX) but reduced production of extra- and intracellular siderophores. Moreover, the *hapX* deletion mutant displayed significantly reduced virulence in a murine model of aspergillosis.
This study demonstrates the crucial role of HapX in iron regulation and virulence of *A. fumigatus*. Deleterious consequences of inactivation of SreA and HapX are strictly confined to iron replete and -depleted conditions, respectively. Consequently, attenuation of virulence by inactivation of HapX, but not of SreA, underlines that *A. fumigatus* faces iron-limited conditions during mammalian infection.

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PR2.11

Characterization of a novel regulatory gene involved in virulence in the phytopathogen *Fusarium graminearum*

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F. graminearum is a pathogenic fungus that infects cereal crops. It produces a trichothecene mycotoxin, deoxynivalenol (DON), which is positively associated with virulence in the fungus and causes toxicity in humans and livestock. A study performed by Alexander et al. suggested *Tri15* may be negatively regulating some of the genes in the trichothecene biosynthetic pathway in *F. sporotrichioides.* In contrast, disruption of *Tri15* in *F. graminearum*, neither affected its ability to synthesize 15-ADON nor its pathogenicity. This study explores the role of *Tri15alt*, a homologue of *Tri15*. *Tri15alt* encodes for a protein that has three zinc fingers, two of which are highly homologous to the zinc fingers found in Tri15. Targeted disruption of *Tri15alt* in *F. graminearum* did not compromise the biosynthesis of 15-ADON. However, pathology studies performed on a susceptible variety of wheat (Roblin) revealed that *Tri15alt* disrupted strain is more virulent than the wildtype strain. We have performed microarray analyses on this mutant and results will be presented to identify genes involved in virulence.

Alexander N.J., S.P. McCormick, T.M. Larson and J.E. Jurgenson. 2004. Expression of Tri15 in *Fusarium sporotrichioides*. Curr Genet 45: 157-162

PR2.12

Crosstalk between *Claviceps purpurea* and rye during early stages of pathogenesis

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The ergot fungus *Claviceps purpurea* is a phytopathogenic fungus with a broad host range including economically important species like wheat, rye, barley and rice. Nevertheless the infection with *C. purpurea* is highly conserved and organ specific as only young ovaries of flowering grasses are infected on a defined pathway probably mimicking pollen tube growth. During infection *C. purpurea* shows directed and polar growth comparable to axon differentiation, pollen tube growth and root hair formation (Tudzynski and Scheffer 2004). Thus we are currently comparing *in planta* growth of *C. purpurea* during infection with pollen tube growth. Apart from striking differences in growth rates the fungus deviates from the pollen tube pathway after early infection stages. To investigate the host reaction in detail a microarray analysis is underway. The deletion of the two known small GTPases Cdc42 and Rac have different but severe effects on the polar and directed growth of *C. purpurea*. In other fungi, their temporal and spatial regulation has shown to be of major importance for their functions. Therefore we are analyzing the functions of the scaffold protein Bem1 in *C. purpurea*. A *bem1* homolog was detected in *Claviceps* and completely sequenced. Protein interaction studies and a gene replacement analysis of *bem1* are in progress.

PR2.13

Selection pressures exerted by apple scab resistance QTL on *Venturia inaequalis* co-inoculated strains

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Pathogen adaptation to major resistance genes has often been reported. Partial resistance conferred by QTL is expected to be more durable. However adaptation to this type of resistance is not well documented. Partial resistance erosion can be seen as a multiple step breakdown if several QTL are successively overcome by the pathogen. This process can be driven by the differential selection exerted by the QTL on the pathogen population. In this study we will evaluate selection pressures exerted by QTL on a mixed inoculum of *V. inaequalis*, the apple scab causal pathogen. Broad-spectrum and isolate-specific scab resistant QTL were previously identified in a F1, partially resistant, apple progeny independently challenged with 6 contrasted monoconidial strains. In this study, we equally mixed these 6 strains, inoculated them on the same F1 progeny, scored sporulation severity and then collected leaves on each individual in order to identify sporulating strains. A QTL analysis performed on the AUDPC revealed that only broad spectrum QTL were detected, underlying the usefulness of mixed inoculum for broad spectrum QTL detection. Quantification of the relative proportion of each strain on each individual by pyrosequencing to evaluate selection pressures exerted by QTL alone or in combination is underway. A QTL analysis will be performed on these genotyping data and compared to the previous QTL analyses. This study should help us to better understand how partial resistance filters a mixture of contrasted strains according to the number, effect, and spectrum of action of the involved QTL.

PR2.14

Fungal virulence and host susceptibility genes in the *Fusarium oxyporum-Arabidopsis* interaction

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The interaction of the root infecting fungal pathogen *Fusarium oxysporum* with Arabidopsis is a highly tractable system for a molecular analysis of fungal virulence and host susceptibility and immunity. We have completed a rigorous analysis of 6868 T-DNA insertion mutants of Arabidopsis Col-0 ecotype, selected lines with altered disease phenotype (P=<0.01) and retested these to identify mutants with significant & reproducible increased resistance or susceptibility. Second allele insertions are currently being tested to provide certainty on specific gene functions. These studies have identified ~100 novel genes with previously unidentified roles in immunity and susceptibility to this pathogen. To complement this we have identified a small range of fungal mutants with altered pathogenicity and virulence. One of these includes mutants in the SIX4 gene which is required for full virulence. Experiments are underway to attempt to match putative functions in the host that are necessary for susceptibility with functions in the pathogen required for virulence. Initial experiments are focusing on the role of host jasmonate signalling in susceptibility and how the pathogen may intervene in this.

PR2.15

The G protein-coupled receptor Gpr1 is required for host recognition and mycoparasitism in the biocontrol agent *Trichoderma atroviride*

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Mycoparasitic *Trichoderma* species are applied as biocontrol agents in agriculture to guard plants against fungal diseases. During mycoparasitism, *Trichoderma* interacts with phytopathogenic fungi which is preceded by recognition of the host and results in its disarming. In various fungal pathogens including mycoparasites, G protein signaling regulates pathogenicity-related functions. Nevertheless, the corresponding G protein-coupled receptors (GPCRs) involved in the recognition of host-derived signals are largely unknown. Functional characterization of Gpr1 from *T. atroviride* revealed a prominent role of this GPCR in host recognition and regulation of mycoparasitism-associated processes. *gpr1* silencing led to altered vegetative growth, conidiation and antifungal metabolite production. When confronted with host fungi, the mutants exhibited an avirulent phenotype resulting from their inability to recognize and attach to host hyphae and undergo mycoparasitism-related infection structure formation. In addition, mutants were unable to respond to living host fungi with the expression of chitinase- and protease-encoding genes. Interestingly, their ability to produce chitinases was not impaired in liquid culture in the presence of colloidal chitin or N-acetyl-glucosamine, ruling out that these are ligands of Gpr1. Exogenous cAMP was able to restore infection structure formation in the mutants but had no effect on mycoparasitic overgrowth. A search for targets of the signaling pathway(s) involving Gpr1 resulted, amongst others, in the isolation of a gene encoding a member of the cyclin-like superfamily. Additionally we found evidence for Gpr1 to be constitutively active. Our data highlight the fundamental role of Gpr1 during sensing of environmental signals and transduction to intracellular regulatory targets during the antagonist-host interaction.

PR2.16

***Arthroderma benhamiae* secretome analysis and pathogenicity factor characterisation**

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Arthroderma benhamiae, a zoophilic pathogen causing inflammatory Dermatophytosis in humans and rodents, was selected to serve as a model organism for elucidation of general pathogenicity mechanisms of Dermatophytes. Proteins secreted by the fungus are believed to have important functions during infection. After growth on keratin, a major component of the host's skin, proteins secreted by the fungus were analysed using 2D-PAGE and mass spectrometry for protein separation and identification, respectively. *A. benhamiae* secretes a large number of proteolytic enzymes from different protease families. Beside these, numerous proteins of carbon hydrate and lipid metabolism as well as functionally uncharacterised proteins lacking conserved domains have been found. This work presents the first comprehensive secretome analysis for *A. benhamiae*. In extracts of purified mycelial cell walls two major proteins were identified. The hydrophobin Hyp1 is released by hydrogen fluoride/pyridine treatment whereas a secreted but functional uncharacterised protein was also present in SDS extracts. Its appearance was highest on keratin, it was absent upon growth on casamino acids and therefore termed keratin-induced protein 1 (Kip1). These two proteins are located at the cell surface, where they could directly contact host structures during infection. To elucidate the putative role of these proteins in pathogenicity, gene deletion mutants (*Δkip1*, *Δhyp1*) as well as a strain constitutively expressing *Kip1* were constructed and are currently analysed.

PR2.17

Role of mannitol in pathogenic determinism in *Alternaria brassicicola*

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Mannitol is the most abundant polyols occurring in nature and is usually the most abundant of all the soluble carbohydrates within the mycelium. This compound is known to play different roles in the cell including carbohydrate storage and stress tolerance. The ability of mannitol to quench reactive oxygen species (ROS) was demonstrated (Voegele et al., 2005), leading to the hypothesis that this polyol could play an antioxydant role in host-pathogen interactions. This study focused on the role of mannitol in the infectious process of the nécrotrophic pathogen *Alternaria brassicicola* responsible for the black spot disease of *Brassicacae*. The production and secretion of mannitol by *A. brassicicola* were induced in the presence of host-plant extract suggesting its involvement in the pathogenic process. Genes coding two essential enzymes of the mannitol metabolism, mannitol dehydrogenase (MDH) and mannitol-1-phosphate dehydrogenase (MPD), were characterized and were separately or additionally disrupted by insertional mutagenesis. Enzyme assays confirmed the lack of enzymatic activity for each enzyme in corresponding null mutants. Double-mutant, mutants deficient in MPD or MDH alone produced 15%, 50% and 100% respectively, of the mannitol synthesized by the wild type. Pathogenicity assays revealed the disruption of mannitol metabolism genes affected aggressiveness of *A. brassicicola*. All mutants showed increased sensitivity to hydrogen peroxide compared to wild type. Ab∆*mpd* and Ab∆*mpd*∆*mdh* also exhibited a reduced growth at lower matric potential whereas Ab43∆*mdh* was more tolerant. Data throw new light on specifics functions of mannitol metabolism in necrotrophic metabolic strategy of *A. brassicicola*.

PR2.18

The use of redox-sensitive GFP2 as a biosensor of the redox status in *Botrytis cinerea*

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The necrotrophic plant pathogen *Botrytis cinerea* is the causal agent of grey mould disease in more than 200 plant species. There has been evidence that *B. cinerea* has to cope with oxidative stress during the infection process caused by an early plant defence reaction, the oxidative burst. However, it was shown recently that the AP1-transcription factor Bap1 as main transcriptional regulator of H_2O_2-scavenging proteins is not essential for the virulence of *B. cinerea*. In fact *Botrytis* even seems to enhance the ROS level *in planta* by producing its own ROS during infection. To answer the rising question if *Botrytis* really suffers oxidative stress during the infection process, we are in need of tools to measure its redox status *in vivo*. The ratiometric redox-sensitive GFP2 (roGFP2) reversibly responds to redox changes induced by incubation with H_2O_2 or DTT *in vitro* and specifically senses the glutathione redox potential (E_{GSH}) after expression in *Arabidopsis thaliana*. Our aim is to establish roGFP2 in the filamentous fungus *B. cinerea* to investigate the redox status of *Botrytis* during infection. Here we show that roGFP2 is functional in *Botrytis* hyphae and can be used to determine changes in E_{GSH} *in vivo*. Since we also want to compare the stress-induced redox changes of different signalling mutants in real time using roGFP2, we need a defined integration site of the reporter gene construct. Analyses of the gene *bcniaD,* encoding for a nitrate reductase*,* show that the *bcniaD*-locus is suitable as site of integration for reporter gene constructs in *B. cinerea*.

PR2.19

Characterization of mannosyltransferase mutants of the pathogenic mold *Aspergillus fumigatus*

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The fungal cell wall comprises different carbohydrates either as part of glycoconjugates, like glycolipids and glycoproteins, or as constituents of its core structure. During infection the cell wall of pathogenic fungi has to be robust enough to withstand the stress applied by the host immune response. Moreover, distinct cell wall carbohydrates are likely to be recognized by host pattern recognition receptors, a key event in the innate immune response to fungal infections. We currently analyze a set of mannosyltransferases of the pathogenic mold *Aspergillus fumigatus* that by homology to their *Saccharomyces* counterparts are supposed to catalyze key steps in the formation of glycoconjugates. GDP-mannose:inositol-phosphorylceramide (MIPC)-derived glycosphingolipids are important pathogen-associated molecular patterns (PAMP) of *Candida albicans* and have recently been discussed as relevant PAMPs of *A. fumigatus.* We identified MitA as the only MIPC transferase in *A. fumigatus*. A Δ*mitA* mutant lacks MIPC and MIPC-derived glycosphingolipids and accumulates the precursor IPC. The mutant grows normally, shows no defects in cell wall or membrane organization and a normal resistance to various stressors. However, it is delayed in germination and sensitive to high Ca^{2+} concentrations. The Δ*mitA* mutant is not significantly impaired in its virulence or ability to trigger a cytokine response in macrophages, arguing against a role of MIPC-derived glycosphingolipids as important *A. fumigatus* PAMPs. We have also analyzed two mutants that lack key enzymes in the synthesis of O- and N-linked glycans, namely Mnt1 and OchA. Data on their fitness, pathogenicity and interaction with innate immune cells will be presented.

PR2.20

Molecular interaction between *Trichoderma virens* and tomato roots

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The ability of *Trichoderma* spp. to antagonise plant pathogens, to induce plant resistance and to promote growth in plants has been widely described. Endo-PG produced by *Trichoderma* spp. can assist root penetration and play a pre-eliciting role in systemic resistance (SIR), a beneficial effect detected in plants colonised by *Trichoderma*.
Two endo-PG genes (*eptv1* and *eptv2*) have been identified and partially characterised in a *T. virens* isolate, I10, previously investigated for its antagonistic ability in several biological systems.
A GFP transformant, I10GFP, derived from the same isolate was exploited to monitor the tomato root colonisation process and to define suitable timing for further experiments.
An expression analysis has pointed out a different regulation of those genes. The *eptv1* gene resulted induced when the fungal isolate was grown in liquid cultures supplemented by pectin or plant cell walls and when it was applied to tomato roots in growth chamber. Expression times were comparable in both systems. The *eptv2* gene resulted constitutively expressed in all conditions tested, including controls.
Root exudates showed a promoting effect both on mycelial growth and on conidia germination of *T. virens* I10.
Further mechanisms of the molecular communication between tomato roots and *T. virens* mycelium are under investigation.

PR2.21

Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium oxysporum*

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Fusarium species are among the most important phytopathogenic fungi, having significant impact on crop production and animal health. Distinctively, strains of *F. oxysporum* exhibit wide host range and are pathogenic to both plant and animal species, reflecting remarkable genetic adaptability. To understand the mechanism underlying such genetic plasticity and rapid pathogenic development, we compared the genomes of three economically important and phylogenetically related, yet phenotypically distinct phytopathogenic species, *F. graminearum, F. verticillioides* and *F. oxysporum* f. sp. *lycopersici.* Comparative analysis revealed greatly expanded lineage-specific (LS) genomic regions in *F. oxysporum* that include four entire chromosomes and account for more than one-quarter of the genome. These regions are rich in transposons and genes involved in host-pathogen interactions, including known effectors, enzymes targeting plant substrates or processes, and genes involved in lipid signalling and gene silencing. In addition, we studied the LS chromosomes among 10 selected *F. oxysporum* strains using both high throughput sequence and optical mapping technologies and confirmed the wide existence of these LS chromosomes in *F. oxysporum* species complex. We also found evidence for the acquisition of the LS chromosomes through horizontal transfer, which may explain the polyphyletic origins of host specificity in *F. oxysporum* and the rapid emergence of new pathogenic lineages in distinct genetic backgrounds.

PR2.22

Loss-of-function of the avirulence gene, SIX4, by transposon-insertion in tomato wilt pathogen *Fusarium oxysporum f. sp. lycopersici*

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Fusarium oxysporum f. sp. *lycopersici* (*FOL*) is the soilborne pathogen of tomato wilt. In the pathogen, three races 1, 2, and 3 have been determined based on the specific pathogenicity to tomato varieties. The compatible or incompatible relationships between races and varieties can be explained by the interactions between the avirulence genes carried by *FOL* and resistance genes carried by tomato varieties according to gene-for-gene theory (Flor, 1956). For example, race 1 carrying *AVR1* is avirulent to tomato cultivars with a resistance gene *I*, and races 2 and 3 carrying no *AVR1* is virulent to the tomato cultivars with *I*. Houterman et al. (2008) reported *SIX4* corresponding to *AVR1* in *FOL* race 1.

In 2008 a strain of *FOL* (KoChi-1), overcoming *I*-mediated resistance, emerged in Japan. Although KoChi-1 is not race 1, PCR revealed that KoChi-1 carried *SIX4*. Sequence analysis showed that *SIX4* ORF in KoChi-1 was truncated by a transposon (759 bp). The inserted transposon is non-autonomous and belongs to *hAT* family (Hua-Van et al., 2000). According to the Genome Databases of Broad Institute, 72 copies of the identical transposon exist in *F. oxysporum*. Integration of an intact *SIX4* derived from a race 1 isolate into KoChi-1 genome complemented avirulence to a tomato cultivar possessing *I*. This is the first report of an avirulence gene truncated by transposon-insertion in *F. oxysporum*.

PR2.23

The role of a signal peptidase component in pathogenicity of *Colletotrichum graminicola* to maize.

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Fungal stalk rot is one of the most economically important diseases of maize, estimated to result in losses worldwide of approximately 5% annually. *Colletotrichum graminicola* causes anthracnose, one of the most common and damaging of the fungal stalk rots. *C. graminicola* also causes a leaf blight disease on maize. We used a random mutagenesis technique (REMI) to identify several genes that are required for pathogenicity of *C. graminicola* to maize stalks and leaves. The sequence of one tagged gene (*Cpr1*) identified it as a conserved component of the endoplasmic reticulum (ER)-localized signal peptidase. Given the essential function of that enzyme complex in protein transport it was surprising that, except for a slightly reduced growth rate, the REMI mutant was almost normal in culture. However, it was completely non-pathogenic to maize leaves and stalks. The REMI mutant was able to colonize senescing or paraquat-treated maize tissues normally. It also infected host cells normally if it was co-inoculated with the wild type. The REMI mutant was more sensitive *in vitro* to thermal stress and to chemically-induced secretion stress. We are testing the hypothesis that *Cpr1* functions specifically in adaptation to secretion stress during establishment of the biotroph, and to facilitate the secretion of effectors that induce susceptibility of host cells.

PR2.24

The role of glycerol metabolism in the *Colletotrichum higginsianum-Arabidopsis* interaction

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Glycerol-3-phosphate (G3P) is an important component of carbohydrate and lipid metabolic processes. Inoculation of Arabidopsis with the hemibiotrophic fungal pathogen *Colletotrichum higginsianum* was associated with an increase in G3P levels in the host. A mutation in the Arabidopsis G3P generating G3P dehydrogenase (G3Pdh, *GLY1*) resulted in reduced levels of G3P and enhanced susceptibility to *C. higginsianum*. Correspondingly, overexpression of *GLY1* increased G3P levels and enhanced resistance to the pathogen. Manipulating endogenous G3P levels by genetic mutations, or by overexpression in transgenic plants of other genes affecting G3P biosynthesis, demonstrated that higher amounts of G3P were always associated with higher levels of resistance. Interestingly, there was a similar effect of G3P levels on pathogenicity in the fungus: a knock out of the *C. higginsianum* G3Pdh resulted in reduced levels of G3P, and a significant reduction in pathogenicity to Arabidopsis. Most intriguing of all, the mutant strain regained its normal pathogenicity to mutant plants containing reduced levels of endogenous G3P. Together, these results suggest a novel and specific link between G3P metabolism in the host and pathogen during pathogenesis.

PR2.25

A shuttling RNA-Binding protein involved in synthesis of natural products regulates fungal development and pathogenicity

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Very little is known about the function of RNA-binding proteins during plant infection in filamentous fungi. The *Magnaporthe oryzae* T-DNA mutant M35 was identified as being defective in plant colonisation. M35 has undergone insertional inactivation of a gene encoding a novel RNA-binding protein (RBP35) with two protein modules, an RRM domain (RNA Recognition Motif) and the Arg-Gly-Gly rich region. The RBP35 homologues are found only in filamentous fungi. The full length cDNA has been isolated of the *RBP35* gene. It contains an unusually large 5'UTR (~930 bp) with several regulatory regions including a conserved intron (211 bp) and several uATGs. A his-tagged version of the RBP35 protein binds specifically poly$(G)_{30}$ RNA homopolymers and not ssDNA or dsDNA. Amino and carboxy RPB35-cherry protein fusion constructs localise both in the nucleus and cytoplasm. Western blots carried out with a specific antibody raised against RBP35 revealed the presence of two protein isoforms, the full length protein (~44 kDa, RBP35a), and a smaller protein (~31 kDa, RBP35b) derived from the proteolytic cleavage of the carboxy end of RBP35a. The RBP35b protein lacks the Arg-Gly-Gly rich region and partially restores the altered phenotype of *Drbp35*. A proteomics comparison between the *Drbp35* mutant and the corresponding wild-type strain showed that several enzymes required for flavonoid and melanin synthesis were up- and down-regulated in *Drbp35*. Proteomic results correlated with the metabolic profiles from the wild-type strain and *Drbp35*. Currently, we are optimising methods to identify mRNAs and proteins interacting with RBP35a and RBP35b. This will allow us to identify the post-transcriptional network regulated by RBP35 and in which step of the RNA processing RBP35 is participating.

PR2.26

Comparative studies of the transcriptome of the leafcutter ant fungal symbiont

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It has been possible by using the method "Serial Analysis of Gene Expression" (SAGE) to show quantitative differences in the enzyme expression in the vertical profile of the fungus *Leucoagaricus gongylophorus,* who lives in symbiosis with the leafcutter ant *Acromyrmex echinatior*. The ants live and nest inside the fungal garden and feed the fungus fresh leaves harvested from the surroundings. The fresh leaves are placed on top of the fungal garden, and the fungus starts degrading the plant material while growing upwards, in the end releasing glucose and nutrients for the fungus and the ants. Due to the slow degradation of plant material the fungal garden is different in structure and in the enzyme production profile in a vertical direction from top to bottom. In this study, the fungus was divided into three layers, top, middle and bottom, and enzyme production in the different layers was investigated at the transcriptome level using SAGE combined with cDNA tag and genome sequencing. The results reflected major differences in plant cell wall degradation among layers, revealed specifically by big differences in the production of laccases, endoglucanases, cellobiohydrolases and xylanases.

PR2.27

Unraveling the complex dikaryotic genome sequence of the soil fungus *Rhizoctonia solani*.

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A consortium consisting of North American and international scientists in the Rhizoctonia community is actively involved in a collaborative project to obtain a high quality complete genome sequence of the soil fungus *R. solani* anastomosis group 3 (teleomorph=*Thanatephorus cucumeris*), strain Rhs1AP. This fungus is a competitive saprobe and an important pathogen of plants in the family Solanaceae. In addition to its economic importance as a plant pathogen, the fungus and its closely related species can often function as beneficial endomycorrhizal symbionts that promote the germination of orchid seeds and growth of orchid seedlings. Sanger, 454 Titanium FLX pyrosequencing, and Illumina (Solexa) methods have been used to generate approximately 2.5 Gb of sequence data that represents approximately 22X coverage of the estimated 90 Mb genome. An optical restriction map of the fungal chromosomes has been developed and there are 23 contigs with both telomeric regions and seven unfinished contigs with one or no telomeric regions. The size of the contigs ranged from 1.85 to 5.85 Mb and the rDNA repeats were identified on the latter contig. A method to generate haploid components of the genome has been developed and will be used to complement the optical map and cDNA library data to better assemble and annotate the dikaryotic genome sequence. The genome sequence of *R. solani* will provide a basis for comparative studies to increase our understanding of the evolution of fruiting body development and modes of trophic behavior in important basal and transitional group of basidiomycete fungi in the Agaricomycotina.

PR2.28

Fungal secondary metabolism and its impact on ecological interactions with insects

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Filamentous fungi and saprophage insects are suspected to be competitors on decaying organic matter (1, 2). Both organisms have equal requirements considering habitat and nutrition. Insect larvae negatively influence mould development (3), but filamentous fungi can be an important cause of mortality of insect larvae (4). These competitions in insect-mould interactions have largely been ignored. First investigations suggest a role of genes for fungal secondary metabolism (1). Using a combination of experimental ecology and functional genomic techniques the function of secondary metabolites (e.g. mycotoxine) as a chemical defence in insect-fungal interactions as well as the influence of these competitors at trophic interactions between insects ought to be investigated. For our research the vinegar fly *Drosophila melanogaster* and its natural antagonist *Aspergillus* were used as a form of ecology model system. Microarrays of *Aspergillus nidulans* have been used to identify fungal target genes up- or downregulated when interacting on festered matter with the antagonistic *Drosophila* larvae. Preliminary test with quantitative RT-PCR of RNA from *A. nidulans* confronted with *D. melanogaster* larvae indicates upregulation of the global regulator *laeA*, as well as *aflR.* Specific down- or upregulation of these target genes will be performed to analyse their importance for competitions in insect-fungal interactions. The consequence on evolutionary fitness of the fungi and insects will be analysed. (1) Rohlfs M, Trienens M, Fohgrub U, Kempken F (2009) In: The Mycota XV, pp131-151 (2) Kempken F, Rohlfs M (2009) *Fungal Ecol*, doi:10.1016/j.funeco. 2009.08.001 (3) Rohlfs M (2005) Mycologia 97:996-1001 (4) Rohlfs M (2005) Frontiers in Zoology 2:2

PR2.29

Genome expansion in powdery mildews is caused by loss of immunity against genomic parasites

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We report that the genome of the powdery mildew *Blumeria graminis* f.sp. *hordei* is ~120Mb, which is much larger than other closely related Ascolycetes. This is due to an extraordinary amplification of retro-transposons which results in > 70% repetitive DNA in the genome. All the three genes encoding enzymes known to be necessary for Repeat Induced Point mutation (RIP) are absent from all powdery mildews analysed. A comparative genome-wide analysis of repetitive DNA in *B. graminis* and other Ascomycetes reveals an absence of RIP-ing in the repetitive DNA. This suggests that the evolution of a larger genome in the Erysiphales has been driven by a massive proliferation of genomic parasites (retro-transposons) allowed by the loss of one of the mechanisms to keep these elements under control. The fact that *B. graminis*, like all powdery mildews, is an obligate biotrophic pathogen whose significant trophic stage is intracellular suggests that genome miniaturisation is not a necessary accompaniment of parasitism observed elsewhere. Indeed other plant pathogenic fungi such as the rusts (Basidiomycetes), *Phytophthora infestans* (Oomycetes) and mycorrhizal fungi such as truffles and the Glomales show similar genome expansion suggests that the phenomenon is connected to their common life style. We speculate that genome expansion driven by retro-transposition may provide a selective advantage for fungi and fungal-like organisms interacting with plants.

Poster Category 3:

Regulation of Gene Expression at the Genome Level

PR3.1

Intracellular NADP and ATP regulate transcription of the yeast GAL regulon

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The interplay between the yeast prototypical transcriptional activator Gal4p and the inhibitor protein Gal80p determines the transcriptional status of the genes needed for galactose utilization in *Saccharomyces cerevisiae*. In this study, we showed that deletion of components responsible for mitochondria and cytocytoplasmic synthesis of NADP including *pos5* and *utr1* impaired the induction of GAL genes. The malfunction of respiratory oxidative phosphorylation was also found to affect the induced transcription of *GAL* genes. Increase in intracellular level of NADP or ATP in these mutants restored the normal induction process with the recovered occupancy of Ser5 phosphorylated RNA polymerase II on the *GAL* promoter. On the other hand, deletion of *dsg1* coding for an F box protein impaired the early-onset expression of a *GAL1-LacZ* reporter. However, the induced transcription of *GAL* mRNA was not affected although a decreased occupancy of RNA polymerase II on the 5' coding region of *GAL* genes occurred. The Dsg1-mediated induction defect was partly complemented by increase in intracellular level of NADP but not by that of ATP. Nonetheless all the induction defects were abrogated by the absence of transcription inhibitor Gal80p. *In vitro* analysis revealed that NADP synergizes with ATP to destabilize the interaction between Gal80 and Gal4. Taken together, these results suggest that interaction status between Gal4p and Gal80p not only determines the on-off of transcription of GAL genes, but may also be involved in the fine tuning of the whole transcription process including the formation of mature mRNAs.

PR3.2

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

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

PR3.3

RRMA, an RNA binding protein involved in regulated mRNA degradation

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

PR3.4

D-xylose: repressor or inducer of xylanase expression in *Hypocrea jecorina* (*Trichoderma reesei*)

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The saprophytic fungus *Trichoderma reesei* (teleomorph *Hypocrea jecorina*) is able to degrade a huge variety of biopolymers such as xylan and cellulose, the predominant compound of plant materials. The produced hydrolytic enzymes have received great industrially importance (e.g. food and feed industry, pulp and paper industry). Before we started our investigations, it was already known that D-xylose is an inducer for hydrolytic-enzymes encoding genes. In this study we show that the degree of induction is dependent on the applied D-xylose concentration. We could demonstrate that high induction of hydrolytic-enzymes encoding genes can be observed using 0.5 or 1 mM D-xylose for 3 hours of cultivation. D-xylose causes never glucose-like repression of transcription of xylanase-encoding genes, even not at high concentrations (66 mM). The investigations show that the transcription factor Carbon catabolite repressor 1 (Cre1) reduces the expression of the Xylanase regulator 1 (Xyr1), the main activator of many hydrolytic enzymes encoding genes, and as a consequence, lower amounts of hydrolytic enzymes are expressed. In this study we can demonstrate that D-xylose has to be metabolized via the Xylose reductase (Xyl1) to achieve induction of xylanase expression. Finally, we show that a strain bearing a constitutively expressed x*yr1* could partly overcome the negative influence of Cre1.

PR3.5

Time course microarray analysis of *ΔPcvelA* and *ΔPclaeA* mutants of *Penicillium chrysogenum*

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The filamentous fungus *Penicillium chrysogenum* is the main industrial producer of the β-lactam antibiotic penicillin. Recently the genome sequence of *P. chrysogenum* was published, thus strain improvement by reverse genetics became feasible. For efficient gene replacements, a *P. chrysogenum* strain lacking non-homologous end joining (NHEJ) was constructed by deletion of the *Pcku70* gene, a homologue of human Ku70 gene essential for NHEJ. Although ΔPcku70 strain lacks any obvious phenotype under native conditions, we were able to identify a stress-related molecular phenotype by conducting a whole genome microarray time course analysis. Strain ΔPcku70 was used to generate the mutants ΔPcvelA and ΔPclaeA. The proteins PcvelA and PclaeA, homologues of the VeA and LaeA proteins from *Aspergillus nidulans*, are part of a multiprotein complex and positively regulate penicillin biosynthesis in *P. chrysogenum*. In order to identify common target genes of PcvelA and PclaeA, a comparative microarray analysis was conducted with transcripts from the deletion mutants ΔPcvelA and ΔPclaeA isolated after 48, 60 and 96 hours of growth. For comparison, we used the data from the time course analysis of the ΔPcku70 strain. Our results clearly demonstrate common as well as distinct functions of PcvelA and PclaeA as regulators of secondary metabolism and fungal development in *P. chrysogenum.*

PR3.6

Components of a velvet-like complex in *Fusarium fujikuroi* affect differentiation, secondary metabolism and virulence

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Besides industrially produced gibberellins (GAs), *Fusarium fujikuroi* is able to produce additional secondary metabolites such as the pigment bikaverin and the mycotoxins fumonisins and fusarins. The global regulation of these biosynthetic pathways is only poorly understood. Recently, the Velvet complex containing VeA and several other regulatory proteins was shown to be involved in global regulation of secondary metabolism and differentiation in *Aspergillus nidulans*. Here we report on the characterization of two components of the *F. fujikuroi* Velvet-like complex, FfVel1 and FfLae1. The gene encoding this first reported LaeA ortholog outside the class of *Eurotiomycetidae* is upregulated in the Δ*Ffvel1* mutant (shown by microarray-studies) and FfLae1 interacts with FfVel1 in the nucleus (shown by BiFC). Deletion of *Ffvel1* and *Fflae1* revealed for the first time that Velvet can simultaneously act as positive (GAs, fumonisins and fusarins) and negative (bikaverin) regulator of secondary metabolism, most likely by interconnecting with the nitrogen regulation network. Furthermore, we show that both components affect conidiation and virulence of *F. fujikuroi* on rice plants. Cross genus complementation studies of Velvet complex component mutants between *Fusarium* and *Aspergillus* support an ancient origin for this complex which has undergone a divergence in specific functions mediating development and secondary metabolism.

PR3.7

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

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

PR3.8

***Trichophyton rubrum* gene expression kinetics during growth on keratin as the sole nutrient source.**

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Trichophyton rubrum is a filamentous fungus that infects human skin and nails, being the most prevalent dermatophyte worldwide. During its growth on keratin a shift on the extracellular pH from acidic to alkaline occurs, which may be an efficient strategy for its successful infection and maintenance in the host. *T. rubrum* responds to environmental pH by derepressing nonspecific proteolytic enzymes and keratinases with optimal activity at acidic pH during the initial stages of infection, probably in response to the acidic pH of human skin. Although several factors contribute to its pathogenicity, successful infection depends on the adherence capacity of the infecting dermatophyte and its ability to sense and overcome the acidic pH of the skin, and usage of the molecules from the host tissue as nutrient source. In this work *T. rubrum* gene expression kinetics during keratin degradation was evaluated by cDNA microarrays to gain a better comprehension of the adaptive responses to molecules presented in the host microenvironment. During *T. rubrum* growth on keratin medium, we observed that germination of the conidia and hyphal formation were accompanied by a gradual increase in the extracellular pH, ranging values from pH 6.5 to pH 8.5. After hybridization of the microarrays, and SAM analysis, 124 genes were found to be differentially expressed throughout the cultivation times. In 24h of cultivation, the transcript enconding a glutamate carboxypeptidase 2 was up-regulated, which must be important for protease release contributing to the clivage of keratin. At 48h, in which the extracellular pH was 8.0, we observed an up-regulation of the genes encoding PalA and PalB proteins, showing the activation of the environmental pH sensing pathway during keratin utilization, suggesting its role during *T. rubrum* pathogenic process. The genes encountered here are involved in several cellular processes, and their regulation during keratin degradation and pH sensing may be important in the initial stages of dermatophyte infection or in its maintenance in the host tissue.

PR3.9

A novel CUCU modification of poly(A) tail signals the end of mRNA

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In eukaryotes differential transcript degradation represents an integral component of gene regulation. Generally the limiting step in mRNA turnover is deadenylation, which triggers translational repression and 5' decapping once the length of poly(A) tail reached about 15 A residues. We have recently shown that in *Aspergillus nidulans* both the Caf1 and Ccr4 orthologues are functionally distinct deadenylases*:* Ccr4 is responsible for basal degradation while Caf1 is required for the regulated degradation of specific transcripts and the variation in Processing (P) body formation, which occurs in response to a wide range of stimuli. Disruption of the Ccr4-Caf1-Not complex leads to premature, poly(A) independent decapping. We have shown that decapping is correlated with a novel transcript modification, the addition of a CUCU sequence. This 3' modification of mRNA occurs precisely at the point decapping is triggered. The addition of the CUCU based sequence tag requires a nucleotidyltransferase CutA, the disruption of which significantly stabilises mRNA and blocks the formation of P-bodies. Intriguingly, the key enzyme complex responsible for deadenylation and therefore degradation, Ccr4-Caf1-Not, also protects mRNA from premature modification and deadenylation independent decapping. We propose that 3' modification of adenylated mRNA, which is likely to represent a common eukaryotic process, primes the transcript for dissociation from ribosomes, decapping and efficient degradation (1).

References:

1. Morozov IY, Jones MG, Razak AA, Rigden DJ and Caddick MX. CUCU modification of mRNA promotes decapping and transcript degradation in *Aspergillus nidulans*. Mol Cell Biol, 2010, V.30 (4) in press.

PR3.10

***Agaricus bisporus* as a model to study effects of chromosomes on complex traits**

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The button mushroom *Agaricus bisporus* has a typical life cycle in that after meiosis-II, most basidia form two spores and each spore receives non-sister nuclei. Only a small portion of the basidia form four spores, each receiving one haploid nucleus. There are indications that most of the present-day commercial hybrids of the button mushroom are derived from Horst U1 via fertile single spore cultures. We have isolated both parental nuclei from one of the "new" commercial lines and compared the genetic make-up with that of the parental lines of Horst U1 using 580 SNPs. Only three recombinations between homologous chromosomes were found and both were completely reciprocal. The redistribution of homologues over both nuclei was also completely reciprocal when compared to Horst U1. Both strains thus have an identical allelic constitution but show, nevertheless, clear phenotypic differences. It indicates that the redistribution of homologues has an influence on phenotypes and this offers an interesting tool for breeding.
SNP markers were also used for segregation analysis using homokaryotic single spore cultures of Horst U1. This particular set of offspring showed hardly any recombination (18 recombinants in 143 individuals). Since all homologues are segregated independently, this offers an opportunity to generate chromosome substitution lines within a limited number of generations.
Agaricus bisporus offers the opportunity to study the effect of individual chromosomes on complex traits either by chromosome substitution or redistribution of homologous chromosomes over the parental nuclei.

PR3.11

Genome sequence of Shiitake mushroom *Lentinula edodes*

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Understanding the genomics and functional genomics of a mushroom allows us to improve its cultivation and quality. Sequencing the genome provides a comprehensive understanding of the biology of the mushroom. We can also develop many molecular genetic markers for breeding and genetic manipulation. We can identify genes encoding various bioactive proteins and pathways leading to bioactive compounds. Our laboratory sequenced the genome of *Lentinula edodes* (Shiitake, Xianggu). The genome size is about 60 Mbases. We are annotating the genes and analyzing the metabolic pathways. In addition, we have been using a battery of molecular techniques to study *Lentinula edodes* development. We used RNA arbitrarily primed-PCR, SAGE, LongSAGE, EST sequencing and cDNA microarray to analyze genes differentially expressed along the developmental stages. We are learning more about the molecular biology and genetics of this important mushroom. We will also know more about mushroom fruiting body development through comparative genomics among the genome-sequenced mushrooms.

PR3.12

Mycoparasitic response of *Trichoderma atroviride* to host fungi of different phyla on the transcriptome level

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Trichoderma spp. are capable to recognize and attack plant-pathogenic fungi of distinct phyla like *Rhizoctonia solani* (Basidiomycete), *Botrytis cinerea*, *Fusarium graminearum* (both Ascomycetes), *Phytophthora* spp, and *Pythium* spp (both Oomycetes). The scope of action involving recognition, secretion on cell wall lysing enzymes, secretion of secondary metabolites, formation of penetration structures, and lysis of the host fungus is commonly summarized as mycoparasitism. In the present work the transcriptome of different stages of interaction of *T. atroviride* IMI206040 with *P. capsici* and *R. solani* were sequenced using pyrosequencing by 454 Life Science Technologies. Due to the distinct cell wall composition of the host-fungi chosen, the aim was to obtain differences in gene expression depending on the presence or absence of chitin in the host cell wall and genes which may be involved in host specificity. A total number of almost 300.000 reads represented more than 2000 and up to 6500 different *Trichoderma* transcripts, depending on interaction stage and plant-pathogenic fungus. Statistical analysis revealed that out of 353 differentially expressed genes only 6 were common during mycoparasitism of *T. atroviride* with *P. capsici* or *R. solani*. One of them was identified as *epl1*, coding for an elucidating plant response-like protein in *Trichoderma*. The remaining 347 transcripts are currently analyzed due to their possible involvement in the host-specific response of *Trichoderma*.

PR3.13

High throughput sequencing of the *A. nidulans* transcriptome

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

PR3.14

RNA-Seq analysis of aflatoxin gene expression in *Aspergillus flavus*

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

PR3.15

Transcriptional analysis of the response to extracellular pH changes in *Fusarium graminearum Pac1* mutants and effect on trichothecenes B accumulation

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Fusarium graminearum infects wheat and maize and produces type B trichothecenes. These mycotoxins cause serious problems when consumed via contaminated cereals. *Tri* genes, located in the "*Tri* cluster", are responsible for the biosynthesis of trichothecenes B. *In vitro*, *Tri* genes of *F. graminearum* strain CBS 185.32 are expressed at day 3 with the toxin starting to accumulate one day later. Strikingly, the induction of *Tri* genes expression always seems concomitant with a sharp pH drop in the media. Acidic pH seems a determinant factor for induction, as neither the toxin nor the *Tri* genes are detectable at neutral pH. The pH regulation of gene expression in fungi is mediated by the Pac1 transcription factor involved in various secondary metabolites regulation. An *FgΔPac1* deletion mutant and a strain expressing a constitutively active form (*FgPac1*^{C}) were constructed in *F. graminearum*. Expression of this constitutive Pac1^{C} factor strongly reduces expression of *Tri* genes and toxin accumulation at acidic pH. Unexpectedly, deletion of *Pac1* does not induce toxin production at neutral pH. However, it causes an earlier *Tri5* induction and toxin accumulation at acidic pH. In order to determine the interference with other *Tri* genes regulatory mechanisms, exploring general transcriptional response to pH variation for mutants and wild-type strains were also performed using microarrays. Preliminary results will be presented.

PR3.16

Molecular analysis of the role of the HOG MAP kinase gene in the response and adaption of the basidiomycete *Heterobasidion annosum* to stress

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The basidiomycete *Heterobasidion annosum (Fr.) Bref*. is a filamentous white rot fungus, considered to be the most economical important pathogen of conifer trees. Very little is known about molecular and biochemical aspects related to this fungus pathogenicity. The role of some signal transduction genes (MAP kinases) as well as their importance in the stress response and adaptation of the conifer pathogen was investigated. To date, no MAP kinases have been characterized in this white rot fungus *H. annosum*. The role of the stress related HOG MAP kinase which is thought to be involved in the fungal osmotic tolerance was studied under different osmotic stress conditions. In the bioinformatic analysis the HOG gene shows a typical MAP kinase domain with high level of similarity among basidiomycetes. Phylogenetic revealed the basidiomycete HOG genes group together in a clade quite separated from the ascomycetes. To assay for functional relevance of the gene during osmotic stress, total RNA was extracted and the expression level of the HOG transcript quantified by qPCR. In a parallel study, the full-length HOG gene was cloned and used for a functional complementation assay in the *S. cerevisiae hog1*Δ mutant strain. The result showed that the fungus displayed a decreased growth when exposed to an increased salt osmolarity conditions. Increased levels of the MAPK HOG gene transcript was observed in high stress conditions. Complementation functional study with full length gene in the yeast *hog1*Δ mutant strain is ongoing and the results will be presented. Taken together these results show the putative role of the HOG gene in the basidiomycete *Heterobasidion annosum* and its importance in the capacity of the fungus to overcome osmolarity stress conditions in the natural environment.

PR3.17

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

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

PR3.18

Up-regulation of ABC transporter genes stimulated by azole drugs dependent on the transcription activator AtrR in *Aspergillus oryzae*

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During the long-term usage of the azole drug for antifungal therapy in aspergillosis, emergence of azole resistant isolates of *Aspergillus fumigatus* has been recently documented. One of the possible mechanisms of azole resistance is the up-regulation of genes encoding drug efflux pumps, mainly belonging to ABC transporters. However, the mechanism that regulates ABC transporter gene expression has not been elucidated in filamentous fungi including *Aspergillus* species. In *Aspergillus oryzae*, until now we have shown that overexpression of a transcription regulator gene *atrR* positively regulates expression of ABC transporter genes (*atrA*, *atrF*, and *atrG*) leading to azole drug resistance. In this study, expression of *atrR* and the three ABC transporter genes was induced by azole drug added in the wild-type strain. Furthermore, addition of azole drug significantly enhanced the expression level of the ABC transporter genes in an overexpression strain of *atrR*, although equal amount of the *atrR* transcript was accumulated in the strain irrespective of addition of azole drug. In contrast, in an *atrR* deletion mutant, no expression of the above mentioned ABC transporter genes was observed regardless of the presence of azole. In *Saccharomyces cerevisiae* and *Candida glabrata*, xenobiotic substrates such as azole drugs have recently been shown to directly bind and activate the transcription regulator Pdr1p via a nuclear-receptor-like pathway. Also in *A. oryzae*, AtrR protein may be activated by azole drugs through a similar mechanism to accelerate the gene expression of ABC transporter genes to survive in such an environment.

PR3.19

Complementation experiments in histone deacetylase-deficient *Aspergillus* strains

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Modifications on the N-terminal tails of core histones highly impact the regulation of many genes in eukaryotic organisms. In filamentous fungi, however, only little is known about the enzymes that modify histones. Nevertheless, it has become increasingly evident that histone acetyltransferases and histone deacetylases (HDACs), which are responsible for a balanced acetylation status of the histone tails, are crucial for the regulation of genes involved in fungal pathogenicity, stress response, production of antibiotics or mycotoxins, and resistance against antifungal drugs. Our work focuses on RpdA, a class 1 HDAC of the model organism *Aspergillus nidulans.* We recently could demonstrate that RpdA is essential for growth and development of the fungus. Now we investigate the ability of complementation of class 1 HDACs from different filamentous fungi, yeasts and human in an *Aspergillus* strain with depleted RpdA activity. The RpdA orthologs of filamentous fungi like *A. fumigatus* or *Cochliobolus carbonum* were able to complement RpdA minus strains, whereas orthologs of yeasts (RPD3/Clr6) or human (HDAC1/HDAC2) were not. These results confirm that RpdA-type enzymes of filamentous fungi comprise distinct motifs that are essential for fungal growth and development. These characteristic features and their significance turn RpdA-type enzymes into promising targets for (fungal specific) HDAC inhibitors (HDACi) with impact on the vitality of these organisms. Several HDACi were recently approved (or are under evaluation) as therapeutic and chemo-preventive agents against cancer, neurodegenerative disorders and transplantation intolerance. In the future, these molecules may also represent attractive canditates for the development of novel antifungal agents, or may have potential as compounds used in combination with drugs administered in classical antifungal therapies.

PR3.20

Investigating the regulation of glia expression in *Aspergillus fumigatus*

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

PR3.21

Genome of *Schizophyllum commune* yields insight into regulation of mushroom formation

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Genome Of *Schizophyllum Commune* Yields Insight Into Regulation Of Mushroom Formation
The wood degrading fungus *Schizophyllum commune* is a model system for mushroom development. The recently sequenced 38.5 Mb genome contains 13,210 genes. We performed whole genome expression analysis using the sequencing-based technique MPSS (Massively Parallel Signature Sequencing) on 4 developmental stages: monokaryon, stage I aggregates, stage II primordia and mushrooms. These data yield insight into the process of mushroom formation in high detail. For example, compared to other stages of development protein production is up-regulated in stage I, gene regulation is up-regulated in stage II and metabolism is down-regulated in mushrooms. Interestingly, 43% of the genes were shown to be also expressed in anti-sense direction, suggesting a role for anti-sense gene regulation during development.

We identified 471 transcription factor genes in the genome, of which 27 were evolutionary restricted to mushroom forming fungi. Moreover, about one third of all transcription factor genes were differentially regulated during development. Eleven transcription factor genes were deleted using recently developed and efficient gene deletion techniques. Inactivation of *reg1*, *reg2* or *reg3* resulted in colonies with only vegetative mycelium. Development stopped in stage I after inactivation of *reg4*, whereas inactivation of *reg5, reg6* or *reg7* resulted in more but smaller mushrooms than wild-type. These are the first strains with a targeted gene inactivation that show an effect on mushroom formation.

Taken together, we conclude that the genome sequence and genetic amenability of *S. commune* strongly contribute to our understanding of mushroom formation.

PR3.22

A novel motif in fungal Class 1 Histone deacetylases is essential for growth and development of *Aspergillus*

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ct" class="mceAdvanced">In eukaryotic organisms DNA is compacted into an elaborate structure called chromatin, thus enabling regulation of transcription by controlling the accessibility of the genetic information for transcription factors. Among the key players involved in the regulation of chromatin structure are histone acetyltransferases and histone deacetylases (HDACs) —enzymes establishing distinct acetylation patterns in the N- terminal tails of core histones. In filamentous fungi only little is known about the biological functions of these enzymes; nevertheless recent studies have shown that class 2 HDACs affect the regulation of genes involved in stress response and secondary metabolite production. Here we report that depletion of RpdA, a class 1 HDAC of *Aspergillus nidulans*, leads to a drastic reduction of growth and sporulation. Functional studies revealed that a short C-terminal motif unique for RpdA-type proteins of filamentous fungi is required for catalytic activity and consequently cannot be deleted without affecting the viability of *A. nidulans.* Thus, the C-terminal extension of RpdA-type proteins represents a promising target for fungal specific HDAC-inhibitors that might have potential as new antifungal compounds with clinical and agricultural applications.

PR3.23

On the presence of N^6-methyl adenine in fungal genomic DNA

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The presence of modified bases in the DNA of almost every organism analysed is known for more than fifty years now. While m^5C (5-methyl-citosine) methylation has been shown and broadly studied in bacteria, fungi, other lower eukaryotes, plants and animals, the investigation on DNA m^6A (6-methyl-adenine) methylation in eukaryotes has been relatively ignored, probably by the thinking of it being absent, irrelevant, or difficult to accomplish. The idea that m^6A has a modest (or not at all), role in the regulation of eukaryotic genomic structure, organisation, and regulation contrasts with what it happens in bacteria, in which more than one important cellular and "epigenetic" mechanisms of regulation are mediated by m^6A methylation.

Anyhow, several lower and higher (human, rat and plant) eukaryotes have been investigated in relation to their m^6A presence and content, with some interesting results. A gap in these studies is the fungal kingdom, which probably could give mixed (and probably relevant), information on what happened from prokaryotes to eukaryotes in this matter. Our first approach to answer this question was based on m^6A sensitive digestion with restriction enzymes, and *in silico* analyses of DNA from more then ten fully-sequenced fungi.

High-molecular weight DNA was obtained from the fungi to be analysed and digested with *Dpn*I, an enzyme that cuts the sequence GA↓TC only when the A in this sequence is methylated. What we have found is that several groups of fungi have in fact an m^6A DNA-methylation system in their genomes. Why? How? What for? These are today questions to be resolved.

These preliminary results only show what happens at the GATC sequence. So, we also analysed *in silico* the fungal genomes looking for N^6-methyl adenine transferases. In some of them, which do not show any significant digestion with *Dpn*I, we have been able to find sequences more than similar to m^6A DNA-methyl-transferases.

The data obtained in these two kind of experiments give us new ideas to find out the presence, importance, and possible function of m^6A DNA-methylation in fungi.

PR3.24

Mutualism versus pathogenesis: fungal endophyte friend or foe?

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We are investigating the molecular mechanisms behind how a mutualistic symbiotic endophyte in its natural host grass, *Elymus spp*. (tribe Triticeae) has become pathogenic on *Triticum spp.* To unravel the switch from mutualism to pathogenesis, we have applied a mycological approach along with the latest transcriptomic technology. We obtained a low (5-10%) systemic infection rate in a New Zealand wheat *cultivar* with resulting plants becoming severely stunted and eventually dying. To specifically perform a high-throughput analysis of fungal differential gene expression, the new generation sequencing method, SOLiD™ is being utilised to compare systemic infections of endophyte with *Elymus spp*. (mutualistic interaction) versus *Triticum spp.* (pathogenic interaction). The Affymetrix 61k Wheat GeneChip has been employed to focus on wheat differentially expressed genes in mock-infected versus endophyte-infected wheat plants at various stages of infection. Initial wheat GeneChip experiments have shown that the low systemic infection rate is a consequence of activation of plant defence responses, along with perturbations in hormonal homeostasis. In conjunction, we have observed meristem re-organisation resulting in trapping of fungal hyphae so that colonisation of the auxiliary buds is impossible.

PR3.25

Transcriptome sequencing and comparative transcriptome analysis of the scleroglucan producer *Sclerotium rolfsii*

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The plant pathogenic basidiomycete *Sclerotium rolfsii* produces the industrially exploited exopolysaccharide scleroglucan, a polymer that consists of β-(1→3) linked glucose with a β-(1→6) glycosyl branch on every third unit. Although the physicochemical properties of scleroglucan are well understood, almost nothing is known about the genetics of scleroglucan biosynthesis. Similarly, the biosynthetic pathway of oxalate, the main by-product during scleroglucan production, has not been elucidated yet. In order to provide a basis for genetic and metabolic engineering approaches, we studied scleroglucan and oxalate biosynthesis in *S. rolfsii* using different transcriptomic approaches.

Two *S. rolfsii* transcriptomes obtained from scleroglucan-producing and scleroglucan-nonproducing conditions were pooled and sequenced using the 454 pyrosequencing technique yielding ~ 350,000 reads. These could be assembled into 21,937 contigs and 171,833 singletons, for which 6,951 had significant matches in public protein data bases. Sequence data were used to obtain first insights into the genomics of scleroglucan and oxalate production and to predict putative proteins involved in the synthesis of both metabolites. Using comparative transcriptomics, namely Agilent microarray hybridization and suppression subtractive hybridization, we identified ~ 800 unigenes which are differently expressed under scleroglucan-producing and non-producing conditions. From these, candidate genes were identified which could represent potential leads for targeted modification of the *S. rolfsii* metabolism for increased scleroglucan yields.

The results provide for the first time genomic and transcriptomic data about *S. rolfsii* and demonstrate the power and usefulness of combined transcriptome sequencing and comparative microarray analysis. The data obtained allowed us to predict the biosynthetic pathways of scleroglucan and oxalate synthesis and to identify important genes putatively involved in determining scleroglucan yields. Moreover, our data establish the first sequence database for *S. rolfsii*, which allows research into other biological processes of *S. rolfsii*, such as host-pathogen interaction.

PR3.26

The black truffle of perigord responds to cold stress with an extensive reprogramming of its transcriptional activity

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Free-living fungi often encounter different kinds of environmental stresses, including changes in temperature, osmolarity, pH, humidity, availability of O2 and nutrients, exposure to toxins, UV or heavy metals, as well as competition with other organisms. To understand the cell adaptation and the survivor in non-ideal conditions, a better comprehension of many basic events is required. *Tuber melanosporum* can be subjected to different stress conditions, considering its life cycle. In this work, the genome sequence of the ectomycorrhizal ascomycete *Tuber melanosporum* was analysed with the aim to identify and characterize genes involved in environmental stress response. As a second step whole genome arrays were used to verify the transcriptional profiling in the presence of a cold shock (4°C for 7 days). In a whole genome microarray (7496 genes/probe), 423 genes resulted significantly differentially expressed (> 2.5 fold; p-value < 0.05) in stressed mycelia compared to the control ones. After 4°C exposure for 7 days the number of up-regulated genes was 187; the down-regulated genes were 236. The 50-60% of the up- or down-regulated transcripts had no KOG classification and were clustered as unclassified proteins, which represent the most abundant category both in up- and down-regulated genes. A gene subset, concerning a range of biological functions, was chosen to validate the microarray experiment using qRT-PCR. Sixteen out of 22 considered genes confirmed the array data. At our knowledge this is the first work, which considers the global gene expression profiling in a filamentous fungus under cold stress condition.

PR3.27

Screening and sequence analysis of zhd101 (zearalenone lactonohydrolase) homologues in *Trichoderma/Clonostachys* sp.

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Zearalenones are economically important group of *Fusarium sp.* mycotoxins, exhibiting estrogenic activity and chemical strucure consisting of a resorcinol moiety fused to a 14-membered macrocyclic lactone. These compounds are converted into a far less estrogenic product by incubation with *Clonostachys rosea* IFO 7063 expressing *zhd101* zearalenone lactonohydrolase gene. In the present study we described screening of *Trichoderma/Clonostachys* combined collection for new strains with functional lactonohydrolase homologues. In the screened samples, we observed degradation reactions in 10 of 79 total *Trichoderma sp.* and *Clonostachys sp.* isolates and have been able to determine new lactonohydrolase homologue sequences with average sequence identity of 90%.

PR3.28

Light-dependent gene regulation in *Aspergillus nidulans* is mediated through binding of a phytochrome white-collar light regulator complex

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Light serves as one important environmental signal to regulate development and metabolism in many fungi. The light response has been studied to great detail in *Neurospora crassa* (1) and *A. nidulans*. *A. nidulans* develops mainly asexually in the light and mainly sexually in the dark. We have discovered phytochrome (FphA) as a red-light sensor (3), and found that the blue-light receptor system proteins LreA (WC-1) and LreB (WC-2) along with VeA form a *light-regulator complex* (LRC)(2). We propose a functional relationship and a cross-talk between both photoreceptors.

In order to identify light-regulated genes, we used a two-colour microarray system. After 30 minutes of illumination about 260 genes (approx. 2.5% of the whole genome) were differentially regulated. 209 genes were up- and 51 down-regulated. Some of those genes display homology to other photo-inducible genes identified previously in *N. crassa* like *ccg-1*, *con-10*, *con-6*, etc. Among the light-inducible genes were also transcription factors probably implicated in secondary metabolism regulation and genes encoding enzymes of the secondary metabolism. Many genes encode proteins involved in stress responses, and a large group represents uncharacterized genes.

The regulation of $conJ^{con-10}$ and $ccgA^{ccg-1}$ has been studied in detail. Differential expression of *conJ* and *ccgA* was confirmed by real time PCR. Using chromatin IP (ChIP), we found that the LRC binds directly to the promoters of *conJ* and *ccgA*. We do have evidence that not only the WC homologues are able to bind DNA but also phytochrome. Phytochrome-DNA interaction occurs probably through the response regulator domain at the carboxy terminus of FphA.

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PR3.29

Transcriptional response is a limiting factor in cellulase overproduction by *Trichoderma reesei*

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Due to its capacity to produce large amounts of cellulases, the tropical ascomycete *Trichoderma reesei* is increasingly being investigated in second generation biofuel production from lignocellulosic biomass. The induction mechanisms of *T. reesei* cellulases have recently been described in some details, but the regulation of the genes involved in their transcription has not been studied thoroughly. Our work reports the regulation of expression of the two activator genes *xyr1* and *ace2*, and the corepressor gene *ace1* during induction of cellulase biosynthesis by the inducer lactose in a low producing strain *T. reesei* QM 9414. We show that all three genes are induced by lactose. *Xyr1* is also induced by D-galactose, but this induction was independent of lactose metabolism. Moreover, *xyr1* and *ace1*, but not *ace2*, were carbon catabolite repressed. For *xyr1,* this repression operated both on the basal and also the induced expression level, while *ace1* was mostly affected at the basal level of expression. Significant differences in these regulatory patterns were observed in the hyperproducer strain *T. reesei* CL847. This suggests that a strongly elevated basal transcription level of *xyr1* and reduced upregulation of *ace1* by lactose may have been important for generating the hyperproducer strain, and are thus major control elements of cellulase production.

PR3.30

Functions for the *Magnaporthe oryzae* Flb3p and Flb4p transcription factors in the regulation of conidiation

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The *M. oryzae* genes *FLB3* and *FLB4*, orthologues of the *A. nidulans* regulators of conidiation FlbC and FlbD respectively were deleted. Analysis of resultant mutants demonstrated that Flb4p is essential for spore formation and that strains lacking this gene have a 'fluffy' colony morphology due to an inability to complete conidiophore formation. Meanwhile Flb3p is required for normal levels of aerial mycelium formation. Using microarray analysis we identified genes dependent on both transcription factors. This analysis revealed that the transcription of several genes encoding proteins previously implicated in sporulation in *Magnaporthe* or in other filamentous fungi are affected by *FLB3* and/or *FLB4* deletion. Additionally the microarray analysis revealed Flb3p may effectively metabolically reprogramme the cell by repressing transcription of genes encoding biosynthetic enzymes and inducing transcription of genes encoding catabolic enzymes. From seven genes whose transcription is controlled by one or both of these transcription factors we identified the Flb4p dependent gene *CON11* as being required for normal levels of sporulation in *M. oryzae*. *CON11*-deleted mutants also exhibited a reduced growth rate and virulence.

PR3.31

Constructed *Aspergillus niger* gene co-expression networks relate to biological processes

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The fungus *Aspergillus niger* has been studied in considerable detail with respect to various industrial applications. Although its central metabolic pathways are relatively well understood, the mechanisms that control the adaptation of its metabolism are understood poorly. In this study, clustering of coexpressed genes has been performed on the basis of DNA microarray data sets from two experimental approaches. In one approach, low amounts of inducer caused a relatively mild perturbation, while in the other approach the imposed environmental conditions including carbon source starvation caused severe perturbed stress. Evolutionary conserved genes were used to construct gene co-expression networks for both the individual and combined data sets.

Comparative analysis revealed the existence of modules, some of which are present in all three networks. In addition, experimental condition-specific modules were identified. Module-derived consensus expression profiles enabled the integration of all protein-coding *A. niger* genes to the coexpression analysis, including hypothetical and poorly conserved genes. Conserved sequence motifs were detected in the upstream region of genes that cluster in some modules, *e.g.*, the binding site for the amino acid metabolism-related transcription factor CpcA as well as for the fatty acid metabolism related transcription factors, FarA and FarB. Moreover, not previously described putative transcription factor binding sites were discovered for two modules: the motif 5'-CGACAA is overrepresented in the module containing genes encoding cytosolic ribosomal proteins, while the motif 5'-GGCCGCG is overrepresented in genes related to 'gene expression', such as RNA helicases and translation initiation factors.

PR3.32

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

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Aspergillus niger is a saprophytic filamentous fungus with a long history in industrial production of organic acids such as citric and gluconic acid as well as various enzymes like glycoamylases and pectinases. Furthermore, *A. niger* is an important multi-cellular model organism to study for example the establishment of cell polarity or protein secretion and it is used for the discovery of new anti-fungal drugs. *A. niger* can easily be genetically modified and cultivated under defined growth conditions in bioreactors. DSM published its 33.9 million base pair genome in February 2007 and an Affymetrix DNA microarray platform with more than 14.000 annotated open reading frames (ORFs) and genetic elements was designed. Among the predicted ORFs, over 300 fungal specific zinc-finger transcription factors (TFs) have been annotated. The function of most of these TFs remains unknown.

In the current study we, have built gene co-expression networks from a dataset of about 100 Affymetrix microarrays covering more than 30 different growth conditions including those which induce stress related to secretion, maintenance of polarity, cell wall integrity, carbon-source utilization and starvation. Exemplarily for a wide range of applications, we show mapping of putative TFs with unknown functions to these networks. Allocation of TFs to functionally enriched gene clusters can serve as an indication for their regulatory role and thereby give valuable leads for further experimental studies.

PR3.33

The *flbF* gene encoding a putative C_2H_2-type transcription factor is involved in the expression of sterigmatocystin genes and asexual development in *Aspergillus nidulans*

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Asexual reproduction cycle is a common mode of reproduction for variety of filamentous fungi. The asexual reproductive cycle in *Aspergillus nidulans* can be divided into two distinct phases : vegetative growth and development. In spite of the extensive efforts and prominent progress in addressing the functions of the genes involved in the fungal differentiation, large numbers of the genes responsible for the developmental process in *A. nidulans* remain to be elucidated. Thus we performed transcriptome profiling during the entire process from spore germination to asexual differentiation using the 70-base-oligomer microarrays and ANURR. The array revealed stage-dependent expression of distinct genes set. The most significantly regulated genes ($p<0.05$) were grouped in five and six clusters based on their expression profiles during vegetative growth and asexual differentiation, respectively. More than 51 genes encoding putative transcription factors were found to be included in the stage-specific clusters. To figure out the function of the genes for the putative transcription factors, we are performing construction of knock-out mutants of the genes.

Here, we report the functional analysis of the *flbF* gene which was expected to encode a potential transcription factor with a C_2H_2 zinc finger DNA binding motif, bipartite nuclear localization signal, and glutamine rich region. Deletion of *flbF* resulted in delay of asexual reproduction and partial fluffy phenotype, also accumulation of brown pigment on media. From these observations, we propose that *flbF* is a putative regulator for initiation of asexual reproduction and secondary metabolism.

This work was supported by grant from Korea Research Foundation

PR3.34

Deciphering the function of FarA transcriptional factor in the expression of lipolytic genes and production of enzymes for the degradation of biodegradable plastics in *Aspergillus oryzae*

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FarA is a Zn_2Cys_6 transcription factor which upregulates genes required for growth on fatty acids in *Aspergillus nidulans*. FarA is also highly similar to the cutinase transcription factor CTFalpha of *Nectria hematococca*. In this study, we examine whether FarA also works in the regulation of genes responsible for the production of lipolytic enzymes like cutinase for the degradation of a biodegradable plastic, poly-(butylene succinate-*co*-adipate) (PBSA), in *A. oryzae*. The wild-type (WT) and the disruptant strains were grown in minimal agar medium with PBSA and the wild-type showed clear zone around the mycelia and the presence of cutinase (CutL1) protein while the disruptant did not. In addition, qRT-PCR revealed that the expression of *cutL1* gene was significantly reduced in the disruptant compared to the WT. Difference of monoacylglycerol lipase gene (*mdlB*) expression was also found between the WT and the disruptant. These results indicated that the FarA transcriptional factor may be implicated in the expression of lipolytic genes and the production of PBSA-degrading enzymes.

Poster Category 4:

Fungal Physiology and Biochemistry

PR4.1

Components of the saga complex are involved in acetate repression in *Aspergillus nidulans*

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Acetate, in *A. nidulans*, is a repressing carbon source that leads to similar levels of CreA mediated repression as glucose. *acdX* was identified in a mutation screen in *Aspergillus nidulans* to identify genes involved in acetate repression but not in glucose repression. The conservation of the amino acid sequence of AcdX of *A. nidulans* and Spt8 of *Saccharomyces cerevisiae* suggests that the SAGA (Spt-Ada-Gcn5-Acetyltransferase) complex may have a role in acetate repression in *A. nidulans*, since Spt8 is a component of the SAGA complex.

The SAGA complex is highly conserved from yeast to humans. In yeast it is involved mostly in the regulation of highly regulated genes that respond to environmental stresses, such as metabolic starvation, DNA damage and heat. SAGA in yeast has been shown to have positive and negative functions on transcription. Bioinformatic analysis indicates that the components of the SAGA complex are also present in *A. nidulans*.

We report results of experiments undertaken to confirm the existence of the SAGA complex in *A. nidulans* and to determine whether AcdX is a component of the complex. The *A. nidulans* homologue of the *S. cerevisiae* SAGA complex Spt3, designated SptC, was N terminally tagged with the TAP tag to allow the purification of the SAGA complex. From these results it is evident that AcdX is a component of a multiprotein complex and that it co-immunoprecipitates with SptC, providing further evidence that the SAGA complex exists in *A. nidulans*, and includes AcdX.

PR4.2

Self-protection against a potent weapon: the dothistromin toxin story

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Dothistromin is a non-host selective toxin that is toxic to most types of cells. It is a close chemical relative of the aflatoxins and has similarities in both genetics and biochemistry to these compounds. *Dothistroma septosporum*, a serious pine needle pathogen, is a prolific producer of dothistromin. *In planta*, dothistromin that accumulates in necrotic disease lesions is visible due to its red colouration and gives rise to the common name of 'red-band needle blight'. Studies with dothistromin-deficient mutants of *D. septosporum* revealed that dothistromin is not required for pathogenicity to the susceptible host *Pinus radiata*. The current hypothesis is that dothistromin has a role in competition against other microorganisms that are known to reside in pine needles, and *in vitro* studies with pine needle endophytes support this hypothesis. The purpose of the current work was to determine how *D. septosporum* is able to protect itself against its own potent toxin. A gene (*dotC*) adjacent to known dothistromin biosynthetic genes is predicted to encode a Major Facilitator Superfamily (MFS) transporter. To determine whether the DotC protein is a membrane-bound transporter that can pump dothistromin out of the cell, *dotC*-deficient mutants, complemented mutants and strains containing DotC-GFP fusions were studied. As predicted, *dotC* mutants secreted less dothistromin than wild type cells and the DotC-GFP fusions indicated a membrane location. However the results also suggested that DotC has an important role in regulating dothistromin biosynthesis and that compartmentalization within the cell may be an important mechanism for self-protection against dothistromin.

PR4.3

SidL, an acetyltransferase involved in biosynthesis of the intracellular siderophore ferricrocin in *Aspergillus fumigatus*

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Virtually all organisms require iron as indispensable cofactor for various metabolic processes. The opportunistic fungal pathogen *Aspergillus fumigatus* produces two major siderophores (low molecular-mass ferric iron chelators): it excretes triacetylfusarinine C for iron uptake and accumulates ferricrocin for intracellular iron storage. Biosynthesis of both triacetylfusarinine C and ferricrocin has previously been shown to be crucial for virulence of *A. fumigatus.*

Here, we report the functional characterization of a new component of the fungal siderophore biosynthetic machinery Afu1g04450, termed SidL. SidL is conserved in siderophore-producing but not non-siderophore producing ascomycetes. The C-terminal half of SidL shows similarity to acetylases involved in bacterial siderophore biosynthesis, e.g. *Escherichia coli* IucB (a hydroxylysine acetylase required for aerobactin biosynthesis) and PvdY (a hydroxyornithine acetylase required for pyoverdin biosynthesis), and the hydroxyornithine:anhydromevalonyl coenzyme A-transacylase SidF that is essential for triacetylfusarine C biosynthesis. Deletion of *sidL* in *A. fumigatus* reduced ferricrocin biosynthesis during iron starvation and blocked ferricirocin biosynthesis during iron-replete growth. Furthermore, *sidL*-deficiency blocked conidial ferricrocin accumulation under strict iron-replete conditions but not when mycelia were transferred from iron-depleted to iron-replete conditions before sporulation. In contrast, SidL-deficiency had no effect on triacetylfusarinine C production. The expression of *sidL* was affected neither by iron availability nor the iron regulator SreA.

Taken together, these data show that SidL is a constitutively expressed hydroxyornithine acetylase involved in ferricrocin biosynthesis. Moreover, the data indicate the existence of a second hydroxyornithine acetylase, the activity of which is induced by iron starvation. This study identified a novel component of the fungal siderophore biosynthetic machinery and revealed unexpected complexity.

PR4.4

GliT protects *Aspergillus fumigatus* against the harmful effects of gliotoxin

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Gliotoxin, an epipolythiodioxopiperazine (ETP)-type toxin (326 Da) containing an essential disulphide bridge, plays a major role in mediating the virulence of the human pathogen, *Aspergillus fumigatus*. Gliotoxin toxicity in mammalian cells is generally enabled by direct inactivation of essential protein thiols as well as redox cycling, leading to hydrogen peroxide formation. In *A. fumigatus*, enzymes involved in gliotoxin biosynthesis are located within a coordinately expressed, multi-gene cluster. Here we report the functional characterisation of a putative thioredoxin reductase encoded by *gliT* within this gene cluster. Expression of *gliT* is subject to regulation by the transcriptional activator GliZ and gliotoxin. Deletion of *gliT* is detrimental for growth only in the presence of exogenously added gliotoxin, which can be cured by supplementation with reduced glutathione. GliT is localised in the cytoplasm and in the nucleus. GliT is not essential for virulence of *A. fumigatus* in larvae of the greater wax-moth *Galleria mellonella*. The potential autoprotective role of GliT was investigated further by heterologous expression of *gliT* in *Aspergillus nidulans*. And indeed, GliT conferred resistance to gliotoxin, making it a valuable tool for transformation of fungi lacking an ortholog of *gliT*.

PR4.5

Involvement of *Trichoderma reesei* (*Hypocrea jecorina*) G-alpha protein GNA1 during mycoparasitsm against *Pythium ultimum*

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Trichoderma reesei (Hypocrea jecorina) is widely used in industry and its potential for use in agriculture as a biocontrol agent against phytophatogenic fungi has just started. We have investigated the involvement of G proteins during mycoparasitism against plant pathogens. Here we described the role of GNA1, a G-alpha protein which belongs to alfa$_i$ group in Cell Wall Degrading Enzymes (CWDEs) production by *T. reesei* during antagonism against *Pythium ultimum*. For that, two mutants were used: Δ*gna1* and *gna1*QL (constitutively activated version of GNA1). The *gna1*QL mutant*, like* the parental TU-6, inhibited the growth of *P. ultimum* in plate confrontation assay and grew faster than the parental TU-6 while the Δ*gna1* did not grow over *P. ultimum*. Scanning electron microscopy showed that the *gna1*QL mutant promoted more morphological alterations of *P. ultimum* cell wall than the parental TU-6 while the Δ*gna1* caused no effects*.* The mutant Δ*gna1* produced less CWDEs than *gna1*QL and TU-6. The *gna1*QL mutant showed a better performance in production of CWDEs such as endochitinase, N-Acetyl-β-D-glucosaminidase (NAGase), β-1,3-glucanase, protease, lipase and acid phosphatase, after 72 hours of incubation. However, the parental TU-6 showed higher cellulase activity than gna1QL and Δ*gna1*. The intracellular content of cAMP in the strains after 72 hours of incubation was: *gna1*QL (79.85 ± 12), Δ*gna1 (*268.65 ± 8.5) and TU-6 (109.70 ± 9.2) pmol/mg protein. We therefore suggest that the production of some CWDEs during mycoparasitism by *T. reesei* against *P. ultimum* can be mediated by GNA1 activity or cAMP levels.

PR4.6

Galacturonic acid catabolism in *Botrytis cinerea*

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D-galacturonic acid (GalA) is the major component of pectin, which can be degraded by plant pathogens; GalA potentially is an important carbon source for microorganisms living on decaying plant material. For bacteria, a catabolic pathway of GalA has been described, which consists of five enzymes converting GalA to pyruvate and glyceraldehyde-3-phosphate. A different catabolic pathway is proposed in filamentous fungi. In *Hypocrea jecorina*, GalA is converted to pyruvate and glycerol via D-galacturonate reductase, L-galactonate dehydratase, 2-keto-3-deoxy-L-galactonate aldolase, and glycerol dehydrogenase.

The *Botrytis cinerea* genome contains a D-galacturonate reductase gene (*BcgaaA*), a L-galactonate dehydratase gene (*BcgaaB*), and a 2-keto-3-deoxy-L-galactonate aldolase gene (*BcgaaC*). The three genes were cloned into a protein expression vector and the enzymatic activity determined for each gene separately. The heterologous simultaneous expression of BcgaaA, BcgaaB, and BcgaaC in an *E. coli ΔuxaC* mutant which cannot grow on GalA is performed to determine whether the catabolic pathway from *B. cinerea* can restore the growth deficiency in *E.coli*. Targeted gene replacement of BcgaaC or both BcgaaA and BcgaaC resulted in *ΔgaaC* mutants and *ΔgaaAC* double knock-out mutants that displayed significantly reduced growth when D-galacturonic acid was used as the sole carbon source. The mutants showed similar virulence as the wild-type stain B05.10 on tomato leaves, indicating that GalA is not the main carbon source for *B. cinerea* growth during infection on tomato leaves. The virulence will be tested on other pectin-rich plants and tissues.

PR4.7

Regulation of D-galactose metabolism in *Aspergillus nidulans*

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D-Galactose is present in hemicelluloses and pectin which are constituents of the plant cell wall. In pectin, β-1,4-linked D-galactose residues are present in galactan or in arabinogalactan side chains. In hemicelluloses, D-galactose residues are present in the form of side residues which are β-linked in to xyloglucan and xylan, but α-linked in to galactoglucomannan.

For the degradation of these structures by filamentous fungi, several enzyme classes are required depending on the linkage. These classes are α- and β-galactosidases and endo- and exogalactanases.

Aspergillus nidulans, a saprobic filamentous fungus, is able to use D-galactose efficiently as a carbon source. *A. nidulans* can convert D-galactose through two pathways: the common Leloir pathway as well as the recently described alternative D-galactose utilization pathway.

Recently two regulators, GalR and GalX were identified that control D-galactose metabolism in *A. nidulans*. The interaction of these regulators, their control of the various genes of the two D-galactose utilization pathways as well as genes encoding extracellular galactose releasing enzymes will be discussed.

PR4.8

Genetic Basis for the 3-ADON and 15-ADON Trichothecene Chemotypes in *Fusarium graminearum*

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In some regions of the world, most strains of the wheat head blight pathogen *Fusarium graminearum* have one of two trichothecene mycotoxin production profiles (chemotypes), which are designated as 3-ADON and 15-ADON. In a defined medium, strains with the 3-ADON chemotype produce a trichothecene (3-acetyldeoxynivalenol) with an acetate at carbon atom 3 (C3) but not at C15, whereas 15-ADON strains produce a trichothecene (15-acetyldeoxynivalenol) with an acetate at C15 but not at C3. Despite this, strains with both chemotypes possess enzymatic activities necessary for production of trichothecenes with acetates at both C3 and C15, e.g. 3,15-diacetyldeoxynivalenol (3,15-diADON), suggesting the chemical modification responsible for the two chemotypes occurs near the end of the trichothecene biosynthetic pathway. Polymorphisms in the trichothecene biosynthetic genes *TRI3*, which encodes a C15 acetyltransferase, and *TRI12*, which encodes a transport protein, are used as genetic markers to distinguish between strains with 3-ADON and 15-ADON chemotypes. However, a causal relationship between *TRI3*/*TRI12* and the chemotypes has not been demonstrated. Sequence analysis has revealed marked differences in the coding sequence of the esterase gene *TRI8* in 3-ADON versus 15-ADON strains. To determine whether these differences can affect trichothecene chemotype, we examined the activity of *TRI8* as well as *TRI3*. The data indicate that differences in activity of the *TRI8* esterase, rather than the *TRI3* acetyltransferase, are the basis of 3-ADON and 15-ADON chemotypes in *F. graminearum*.

PR4.9

Tools for exploration of *Aspergillus* gene, protein, and sequence information at the *Aspergillus* Genome Database (AspGD)

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The *Aspergillus* Genome Database (AspGD) is an online genomic resource designed to facilitate research on Aspergilli and on other medically and economically important fungal pathogens. We provide an online reference for *Aspergillus* genomics and molecular biology, with up-to-date, high-quality information curated from the scientific literature, as well as web-based research tools for exploration and analysis of these data. The Sybil Comparative Genomics tool at AspGD displays alignments of the genomic regions encoding clusters of homologous proteins from ten *Aspergillus* genomes (*A. nidulans*, *A. fumigatus*, *A. flavus*, *A. oryzae*, *A. niger*, *A. clavatus*, *A. terreus*, and *Neosartorya fischeri*), as well as various displays for exploration of syntenic regions among these organisms. The GBrowse Genome Browser supports navigation and searching of genes and chromosomal regions of the *A. nidulans* FGSC A4 and *A. fumigatus* Af293 genomes, and will be extended to other Aspergilli in the future. Additional tools are available for search and retrieval of *A. nidulans* sequence and gene and protein information that has been curated from the scientific literature. The suite of sequence analysis tools includes BLAST, pattern matching, restriction mapping, and primer design. In addition, AspGD offers keyword-based and gene-property-based searches, Gene Ontology (GO)-based analysis of gene lists by function and localization, bulk data query and retrieval, and downloadable files. While AspGD curation has initially focused on *A. nidulans*, we will begin curation of the scientific literature on *A. fumigatus* and other *Aspergillus* species in 2010 and will provide the full suite of AspGD tools for each of these species in the future. We also provide tools for community interaction, including a colleague registry by which *Aspergillus* research community members may share contact information and research interests to facilitate collaboration, and a list of *Aspergillus* research laboratories. Our mission is to be responsive to the needs of the research community, and we welcome your feedback and suggestions, at aspergillus-curator@genome.stanford.edu. All of the data in AspGD are freely available to the public from http://www.aspgd.org/. AspGD is supported by grant RO1 AI077599 from the NIAID at the NIH.

PR4.10

The antifungal protein AFP from *Aspergillus giganteus* inhibits the viability of *Saccharomyces cerevisiae* strains deficient in cell wall integrity

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The antifungal protein AFP, secreted by *Aspergillus giganteus* is able to inhibit the growth of a variety of filamentous fungi (e.g. *A. fumigatus*, *A. niger*, *Fusarium oxysporum*) by causing membrane invaginations and membrane permeabilisations. However, the protein does not negatively affect the growth of yeast, bacteria or mammalian cell types. Our recent work demonstrated that AFP inhibits chitin synthase activities in sensitive fungi which is counteracted by increased activities of the cell wall integrity pathway (CWIP).
In order to understand the resistance mechanism of yeast strains, we have screened *S. cerevisiae* mutants deleted for the components of the CWIP and chitin synthesis. Most of the ~ 70 screened strains remained AFP-resistant, except the knock out mutants *Δ*wsc1, *Δ*tor1, *Δ*vps34 and *Δ*chs1, which became moderate-sensitive towards AFP. The plasma membranes of these mutants became readily permeabilized by AFP. Interestingly, the presence of AFP provoked increased chitin synthesis in these strains, an observation which we also made for the AFP-resistant filamentous fungus *Penicillium chrysogenum* and the moderate-sensitive mutant of *F. oxysporum Δ*chsV.
The obtained results stipulate the hypothesis that moderate-sensitive and resistant filamentous fungi counteract AFP inhibitory effects by strongly increasing their chitin levels, thereby making the cell walls presumably more rigid. However, this response does not occur in AFP-sensitive fungi. Apparently, the classical CWIP is not sufficient to counteract AFP inhibitory effects and seems not to be involved in increased chitin synthesis. Our findings give rise to the assumption that fungal strains which only use the classical CWIP are AFP-sensitive.

PR4.11

Photoreception and the *Neurospora* circadian clock

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The circadian clock creates a temporal structure within cells in animals, plants, fungi and cyanobacteria. One aspect of the clock is a self-sustained oscillation with a period of ~24h in constant conditions. However, circadian clocks in nature are rarely subjected to constant conditions. They are normally exposed to a rhythmic environment, where signals (zeitgebers) such as light and temperature entrain the oscillations to the 24 h day. Blue-light responses in *Neurospora* require the *wc-1* and *wc-2* genes and these proteins also regulate the circadian clock. However, the *Neurospora* genome contains additional genes coding for putative photoreceptors, including a cryptochrome, an opsin, and two phytochrome genes. We obtained knockout mutants for the genes coding for putative photoreceptors from the *Neurospora* Functional Genomics Project and screened them in a variety of protocols for circadian rhythm and entrainment. When assayed under constant conditions *Δcry*, *Δphy-1*, *Δphy-2*, Δ*nop-1* and Δ*orp-1* strains have the same free running period relative to the wild-type control. In cycling conditions, however, all of the knockouts show differences in phase of entrainment compared to the wild-type strain. The most extreme phenotype in our study is the Δ*cry*, Δ*phy-2*, *matA* strain, which is arrhythmic under all conditions tested. In summary, our results show that additional photoreceptors play a role in entraining the *Neurospora* circadian clock. We conclude that *Neurospora* indeed models circadian clocks of in other organisms with respect to light input, because they also show striking contributions of input pathway components on circadian clock behaviour.

PR4.12

Chitinases of *Aspergillus niger* upregulated during autolysis

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The filamentous fungus *Aspergillus niger* is well known for its capacity to secrete high amounts of proteins and metabolites, and is therefore used in industry for the production of enzymes and chemicals. The mycelium of this fungus is highly differentiated. After stationary growth phase, part of the mycelium is degraded in a process called autolysis. Autolysis is characterized by hyphal fragmentation, loss of biomass, ammonia release and the production of enzymes such as proteases and glycoside hydrolases. These glycoside hydrolases could function in degradation of cell wall polymers such as chitin. However, knowledge about the exact mechanism of autolysis is currently limited.

During industrial fermentation processes, autolysis can cause the productive biomass to decrease, causing reduced product yield. A better understanding of autolysis can contribute to the formation of strategies to increase efficiency of fermentations.

In order to increase understanding of the dynamics of the fungal mycelium, a consortium of academic and industrial partners investigates autolysis and differentiation in *Aspergillus niger.* One goal of this project is the identification and characterization of glycoside hydrolases that are involved in autolysis. By using microarrays to monitor transcription levels during growth, we have identified genes that are upregulated during the autolytic phase compared to exponential growth phase. Four of these genes belong to glycoside hydrolyse family 18, which consists mainly of (putative) chitinases. In order to investigate the properties of these enzymes we performed heterologous gene expression in *E. coli* with subsequent purification using affinity tags. The activity of purified proteins is investigated.

PR4.13

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

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The *Aspergillus* Genome Database (AspGD; www.aspgd.org) is a web-based genomics resource for researchers studying the genetics and molecular biology of an important group of fungal microorganisms, the aspergilli. AspGD provides high-quality manual curation of the experimental scientific literature, including gene names, general descriptions, phenotype data, and Gene Ontology (GO) annotations, as well as tools for exploring these data. AspGD is based on the framework of the *Saccharomyces* Genome Database (SGD) and *Candida* Genome Database (CGD), two genomic resources with which many users within the fungal research community are already familiar. The manual annotation of gene information, phenotype data and GO annotations is the focus of this presentation. Initially, we have focused on the manual curation of genomic information for *Aspergillus nidulans*, the best-characterized species of the group. We will expand our efforts to include curation of *A. fumigatus, A. flavus, A. oryzae, A. niger, A. clavatus, A. terreus*, and *Neosartorya fischeri* genomes. The curation process for gene-specific information entails screening of the published literature to identify the relevant journal articles, and manual evaluation of each paper by trained scientific curators, who extract the information for collection into the database using structured vocabularies (such as GO and our phenotype description system) as well as free-text descriptions. The curated information for each gene appears in brief on its Locus Summary page, which links out to details pages that provide additional information, including phenotype and GO details, a history of sequence and annotation that affect the gene, and a comprehensive list of references. We welcome questions, requests for data, comments, or suggestions to help us better serve the needs of the research community, and encourage researchers to contact us at AspGD is supported by grant RO1 AI077599 from the NIAID at the NIH.

PR4.14

Identification of the putative glycerol transporter of the halophilic black yeast *Hortaea werneckii*

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Purpose: *Hortaea werneckii* is an ascomycetous halophilic black yeast naturally inhabiting hypersaline waters of solar salterns. It is remarkable for its growth at a wide range of NaCl concentrations (0 – 5.2 M) and is a novel eukaryotic organism for studying cellular responses to extremely elevated environmental salinity. We have found previously that *H. werneckii* adapts to high NaCl concentration by accumulating glycerol and erythritol. The purpose of this study was to search for and identify a glycerol transporter of the halophilic black yeast *Hortaea werneckii*. Methods: The partial gene sequence was obtained by suppression subtractive hybridization and extended by genome walking. The complete cDNA sequence was obtained by SMARTer rapid amplification of cDNA ends. The gene was cloned and sequenced, and the putative protein was characterized *in silico*. Results: In the halophilic black yeast *Hortaea werneckii*, we have identified a gene encoding a putative protein with a considerable degree of similarity to a number of uncharacterized sugar transporters, and also to Stl1p, a well-characterized member of the sugar transporter family, the glycerol/H^+ symporter of the plasma membrane in *Saccharomyces cerevisiae*. We have named it HwSTL1. HwSTL1 consists of 1631 bp, encodes a protein of 543 aa with a calculated MW of 59417. Conclusions: We have identified, cloned and characterized the gene HwSTL1 encoding a glycerol-transporter-like protein of *H. werneckii*. In our further studies we will conclude whether the protein encoded by the HwSTL1 indeed functions as a glycerol transporter protein in *H. werneckii*.

glycerol transport, Hortaea werneckii, MFS superfamily, osmoadaptation, STL1-like transporter,

PR4.15

Enzymatic characterization of six recombinant serine-type carboxypeptidases of *Aspergillus oryzae* RIB40

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Serine-type carboxypeptidase (SCP) is an exopeptidase that has Ser, His, and Asp residues as a catalytic triad construct and can sequentially release C-terminal amino acid residues of peptides and proteins. In the genome of *Aspergillus oryzae* RIB40, 12 genes have been predicted to encode SCPs. However, the carboxypeptidase activities of the gene products have not yet been confirmed experimentally. Therefore, we have constructed those gene overexpressing strains using *Aspergillus nidulans* and characterized their overproduced recombinant proteins. Here, we report enzymatic character of six of the gene products. The recombinant proteins were able to release amino acid residues from the C terminus of peptides, and the activity of the enzymes was inhibited by phenylmethylsulfonyl fluoride, indicating the enzymes to be SCPs. The enzymes were stable at lower than 40-55°C, at low and neutral pH. The optimum pHs of the enzymes except for AOCP16 were around pH 4. That of AOCP16 was pH5.5. The substrate specificities of each enzyme for *N*-acyl-peptides were different. Result of transcriptional analysis of these genes suggested differences in transcriptional regulation between these genes. The enzymatic properties of AOCP6 and AOCP9 were different from those of any reported SCP. AOCP4 and AOCP13 had similar enzymatic properties to carboxypeptidases O1 and O2 and carboxypeptidase O from *A. oryzae* IAM2640. Results of sequence analysis of DNA and N-terminal amino acid sequences showed AOCP4 correspond to carboxypeptidases O1 and O2, and AOCP13 correspond to carboxypeptidase O.

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PR4.16

Characterization of newly found intracellular metallo-carboxypeptidases by genome sequencing of *A. oryzae*

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Aspergillus oryzae is one of industrial microorganism as using for the Japanese traditional fermented food. The genome project of *A. oryzae* clarified that there were 134 genes coding proteolytic enzymes. We have been trying to characterize all proteolytic enzymes.

We found that *A. oryzae* had twelve genes for metallo-carboxypeptidases. Nine of them do not have the signal peptide, and it is presumed that these enzymes would be localized intracellularly. The enzymes were all classified in M20 super-family. As four of the enzyme genes were translated under the liquid culture condition, the genes were cloned and expressed in *E. coli*. The two purified enzymes (AOEXE305 and AOEXE306) showed maximum activity at alkaline pH in spite of those are intracellular enzymes. It was shown that acidic amino acid was favorable in P1' site for AOEXE305. AOEXE306 showed wider substrate specificity than AOEXE305. The enzyme could cleavage between even Xaa-Pro bonds but did not favor acidic amino aicd in S1' in substarates. The results might indicate that fungal intracellular metallo-carboxypeptidases had different roll in fungal cells.

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PR4.17

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

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Aspergillus fumigatus is an opportunistic pathogen predominantly affecting immunocompromised individuals, resulting in pulmonary illnesses such as Invasive Aspergillosis. Sequencing of the genome has led to an increased understanding of the organism; however the functions of many genes remain unknown. A putative translation elongation factor 1Bγ (EF1Bγ, termed *elf*A; 750 bp) is expressed, and exhibits glutathione s-transferase activity, in *A. fumigatus* [1]. Normally, EF1Bγ plays a key role in the elongation step of protein synthesis. Our hypothesis is that *elfA* may also play a role in regulating the cellular redox state adjacent to the ribosome during protein synthesis. Consequently, *elfA* was disrupted in *A. fumigatus* ATCC46645 (wild-type) using a bipartite construct containing overlapping fragments of a pyrithiamine resistance gene (*ptrA*). The *elfA* mutant (Δ*elfA)* was complemented using a hygromycin resistance marker *(hph).* Southern Blot analysis was used to confirm the generation of Δ*elfA* and the complemented strain. RT-PCR confirmed the expression of *elfA* in wild-type and complemented strains, and absence of expression in Δ*elfA*. The availability of the mutant has facilitated phenotypic analysis of *elfA* functionality. *A. fumigatus* wild-type and Δ*elfA* were grown on AMM plates with the oxidant H_2O_2 (1 - 5 mM), voriconazole (0.25 - 1 µg/ml), and the thiol-reactive reagent, 4,4'-dithiodipyridine (3 - 7.5 µM). At 37°C, the *elfA* mutant was significantly more sensitive (p=0.0003) to H_2O_2 than wild-type. However, Δ*elfA* was significantly less sensitive (p=0.0251) to voriconazole than wild-type. At 37°C, the Δ*elfA* was significantly more sensitive (p=0.0056) to 4,4'-dithiodipyridine than wild-type. These results implicate *elfA* in the oxidative stress response in *A. fumigatus* and also strongly indicate that *elfA* may play a role in the sensitisation of *A. fumigatus* to voriconazole. Global proteomic studies are currently underway using 2D-PAGE and MALDI-MS to explore alterations in the proteome consequential to *elfA* disruption with a view to gaining further insight into the function of *elfA* in *A. fumigatus*.

1. Carberry, S, et al. (2006), Biochem Biophys Res Commun, 341, 1096-1104.

PR4.18

The monomeric gtpase rheb: putative link between the amino acid metabolism regulation and the development control of phytopathogenic fungi

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How phytopathogenic fungi fulfill their nutritional needs during the interaction with their hosts remains poorly documented. They may first mobilize their storage compounds during the early stages of the infection process. Then they may use the simple compounds, released by the subsequent host constituent enzymatic degradation, to complete their development *in planta*. The metabolism of amino acids may thus be redirected and adapted to the different stages of the plant infection strategy. Our studies focus on the amino-acid triggered regulation mechanisms required to successfully achieve plant infections. The monomeric GTPase Rheb (Ras Homologue Enriched in Brain) was known to activate the kinase TOR and to be involved in the regulation of arginine and lysine uptake in yeasts. *Botrytis cinerea Rheb* orthologue was inactivated using different approaches (RNA interference, promoter replacement). The assay of the amino acid content, using reversed chromatography (HPLC), highlighted the implication of Rheb in the control of amino acid metabolism. The putative involvement of Rheb in the amino acid uptake control was suggested with complementation experiments of a *S. cerevisiae* mutant strain. Rheb importance for *B. cinerea* development was analyzed using different model host systems and the microscopic observations of the early differentiation stages. A comparative study of Rheb functions in other phytopathogenic models is being performed by expressing Rheb hyper- and hypo-active mutant forms in *Magnaporthe grisea*. The results obtained with both fungi will give insights on the molecular mechanisms controlling amino acid uptake/metabolism and their requirement for the parasitic development of fungal plant pathogens.

PR4.19

Analysis of the histone deacetylases disruptants of *Aspergillus oryzae*

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Aspergillus oryzae is ubiquitous filamentous fungi in nature and has been used in a number of industries such as Japanese traditional fermented foods and pharmaceutical products. In nature and industries, *A. oryzae* adapt to various environmental conditions by global change of transcriptional regulation. In the previous study, we revealed that the expression of histone acetylation related genes were affected by the growth phase and alteration of growing environment, such as culture conditions and stress exposing. In general, histone acetylation plays the fundamental roles for the genes expression, and closely relates to growth, morphology, differentiation and stress responses. Recently, several reports indicate that histone acetylation also plays important roles in filamentous fungi. In this context, we focused on histone acetylation related genes, particularly histone deacetylases (HDACs). We attempted to disrupt 11 HDACs homologue of *A. oryzae* and 10 of them were disrupted. However, in the deletion of RPD3 homologue, only the heterokaryon transformants had been isolated. This result suggests that RPD3 homologue is essential in *A. oryzae*. The phenotypes of 10 HDACs disruptants were observed in submerged and plate culture with/without several stress conditions. As the results, four disruptants showed considerable growth and developmental defects in these conditions, especially, HOS2 homologue null mutant showed significant decline of growth in submerged culture.

In microscopic analysis, two disruptants showed abnormal hyphal branches and aberrant distribution of hyphae on the interface of medium and air. This study has indicated that HDACs play important roles in the growth and stress adaptation of *A. oryzae.*

PR4.20

Clitocypin and macrocypins cover different mushroom defences

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Mycocypins, clitocypin and macrocypins, are cysteine protease inhibitors isolated from basidiomycetes clouded agaric (*Clitocybe nebularis*) and parasol mushroom (*Macrolepiota procera*). Two new families of protease inhibitors in the MEROPS classification have been established, I48 for clitocypin and I85 for macrocypins, based on their unique primary sequences and biochemical characteristics. Mycocypins are exceptionally stable proteins, exhibiting high thermal and broad pH stability. The physiological function of the two mycocypin families is proposed to be defence against pathogen infection and/or predation by insects or other pests, analogously to the phytocystatins that are involved in plant defence by inhibiting exogenous cysteine proteases during herbivory or infection. Sequence diversity of clitocypin genes is limited to 18 discreet positions that have no influence on its inhibitory activity. On the other hand, the sequence diversity of macrocypin genes is higher and includes amino acid sites of positive selection. The variations in inhibitory profile between different members of the macrocypin family reveal different specificities and strengths of inhibition of cysteine proteases of different evolutionary families, and even a serine protease. These findings together suggest an adaptation process and the selection of appropriate inhibitor isoforms providing effective defence. Analysis of expression regulation of mycocypins using different mycocypin promoters and green fluorescent protein as the reporter gene showed different patterns of expression during fruiting body development for clitocypin and macrocypins. In view of their proposed defensive roles different expression profiles suggest different target organisms as both families of mycocypins are present in each mushroom.

PR4.21

Identification of gene *carD*, encoding the aldehyde dehydrogenase responsible for neurosporaxanthin biosynthesis in *Fusarium fujikuroi*

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The gibberellin producing fungus *Fusarium fujikuroi*, is also used as a model for genetic and biochemical analysis of carotenoid biosynthesis. Its major carotenoid product is neurosporaxanthin (NX), an acidic apocarotenoid formerly discovered in *N. crassa*. The NX precursor beta-apo-4'-carotenal is produced by *F. fujikuroi* through the activity of the enzymes encoded by genes *carRA* (cyclase and phytoene synthase), *carB* (phytoene desaturase), and *carT* (torulene cleaving oxygenase). The enzyme responsible for the oxidation of the aldehyde group of beta-apo-4'-carotenal to yield NX, has not been described in this fungus. Based on our former results with *ylo-1* in *N. crassa*, we have cloned the *F. fujikuroi* gene *carD*, coding for an aldehyde dehydrogenase putatively responsible of this enzymatic reaction. Crude protein extracts from an *E. coli* strain expressing a *carD* cDNA version were able to convert beta-apo-4'-carotenal into the corresponding apocarotenoic acid, confirming the expected enzymatic activity. CarD was also active on shorter carotenoids, including acyclic ones, such as 8'-lycopenal, indicating the irrelevance of the cycled end of the molecule for substrate recognition. Also, we expressed the enzyme in a beta-apo-4'-carotenal producing *E. coli* strain and we got NX production *in vivo*. In contrast to other *car* genes, real-time RT-PCR analyses of *carD* mRNA levels showed a light-independent expression. However, the mRNA levels were increased in a carotenoid overproducing mutant, indicating common regulatory mechanisms for all the *car* genes in this fungus. Phenotypic effect of targeted *carD* disruption, currently in progress, will be reported.

PR4.22

Development and applications of smart screening platforms

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Increased resistance to currently used antifungal compounds and the fact that these agents are often harmful to man and environment have resulted in a growing demand for new antifungals, which selectively act on cellular processes that are unique to fungi. To meet this demand, we have established a luciferin/luciferase based reporter system for high-throughput screening of extracts obtained from natural sources. This system allows us to identify compounds that specifically target fungal cell wall biosynthesis. It has been well established in the yeast *Saccharomyces cerevisiae* that cell wall synthesis is a highly dynamic process, which is orchestrated by the cell wall integrity (CWI) pathway. Such CWI pathway is also present in the filamentous fungus *Aspergillus niger* and probably widespread among fungi. We have previously shown that transcription of the *A. niger agsA* gene, encoding an alpha-1,3-glucan synthase, is strongly and specifically up-regulated in response to cell wall stress (Damveld et.al., 2005b; Meyer et.al., 2007). We have also shown that the induced expression of the *agsA* gene is mediated via the RlmA transcription factor and its putative RlmA binding site within the *agsA* promoter (Damveld et.al., 2005a). We are here presenting results for a new cell wall stress responsive *A. niger* reporter strain obtained by cloning the *agsA* promoter in front of a codon optimized luciferase gene (Morgan et.al., 2003). The performance of the system was verified by screening several antifungal compounds with a known mode of action and isolation of a antifungal compound from teakwood sawdust. The specific response dynamics of the new reporter system will allow us to identify new putative antifungal compounds from extracts of the whole biodiversity.

PR4.23

Utilization of exogenous heme by *Aspergillus niger*

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

PR4.24

A glutathione S-Transferase, GliG, may mediate thiol incorporation in gliotoxin biosynthesis and is not involved in auto-protection against gliotoxin.

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

PR4.25

Studying the interaction of DON-producers *Fusarium graminearum* with tolerant or susceptible varieties of *Triticum aestivum*: a biochemical and molecular approach

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Under suitable conditions, some fungal species, such as *Fusarium*, growing on many food commodities, can produce secondary metabolites dangerous for humans and animals.

It has been assessed that one of the main virulence factor involved in the *Fusarium* head blight (FHB) disease of wheat (leading to a severe reduction of grain yield and quality) is the *Fusarium graminearum* production of toxins, predominantly deoxynivalenol (DON) that delays germination and growth of wheat plants, inducing hydrogen peroxide (H_2O_2) production, inhibiting protein synthesis and stimulating cell death in *planta*.

Mycotoxigenic fungi contamination is a real issue, especially for cereal industry. Therefore, in order to reduce the diffusion of plant disease and health risks, there is a real need to develop analytical methods able to identify DON-producing fungal variety and to quantify mycotoxins.

In this work, the interaction between two *Triticum aestivum* varieties, BLASCO (tolerant) and SAGITTARIO (susceptible), inoculated with two *F. graminearum* strains (Fg126 and Fg8308), was studied.

Recent advances in DNA-based techniques confer to Real Time-PCR (RT-PCR) assays an important role because of the accelerated diagnostic outcome, so that, this method is providing new tools for fungal detection and quantification in complex matrix. Thus, two primer pairs, designed by other authors, on the gene sequences belonging to the thricothecene gene cluster were used to identify high DON producing *Fusarium* strains through PCR method. Furthermore, a SYBR green Real Time-PCR assay was developed to quantify *F. graminearum* strains in artificially contaminated soft wheat. These results were correlated with the quantification of ergosterol by HPLC. Moreover, the expression of different genes activated in the interaction environment, was analysed by a relative RT-PCR approach. In the pathogen these genes encode for Fg*ap1* that is a transcription factor active in the cell defence against oxidative stress, *ePG* a poligalatturonase involved in cell degradation and *tri6*, one of the thricotecenes biosynthesis regulator. In *T. aestivum,* the expression analysis of one glucosyl transferases (*gt*), one of biochemical mechanisms of resistance to DON is the plant ability to convert DON in a less toxic glucosylated form, and of the pathogenesis-related protein PR1 (*PR1*) were carried out.

It is known that, among the broad range of defence responses, occurring in *planta* when *Fusarium* invasion occurs, the generation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), is one of the earliest events. The activities of three antioxidant enzymes (catalase, superoxide dismutase and glutathione peroxidase) correlated to ROS and of one more enzyme related to the defensive response (lipoxygenase), were monitored.

Finally, the application of HPLC method for the quantitative detection of DON and 15-acetyl DON produced from *Fusarium* species present on samples, confirmed, also, through ELISA analysis, was described.

In conclusion, as far as fungal diseases are wide diffused, the control of contaminated matrices it's a priority. Thus, it's very important to deepen plant-pathogen interaction study, in order to develop control strategies (i.e. quantitative, specie-specific methods) to be applied in diagnostics (i.e. advanced analytical method for mycotoxin detection).

PR4.26

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

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The objective of this research was to investigate whether *A. carbonarius* contamination induces resveratrol production in grape berry. A possible correlation between OTA production and resveratrol biosynthesis has also been considered. *Aspergillus carbonarius* is an important ochratoxin A (OTA) producing fungus which is responsible for toxin contamination of grapes and wine. OTA is a secondary metabolite which has been shown to be nephrotoxic, nephrocarcinogenic, teratogenic and immunosuppressive. Resveratrol (3,5,4-trihydroxy-trans-stilbene), a natural polyphenolic antioxidant found in red wine and grapes has been described as stress metabolite produced by *Vitis vinifera* in response to biotic and abiotic stress as well as to fungal infection and it has been demonstrated to have a particularly wide spectrum of mycotoxin control. In this study, *Vitis vinifera* berries were infected, during ripening, by a conidial suspension of *A. carbonarius* and incubated for 6, 12, 24, 48, 120 hours at 30°C. After incubation, each berry was analyzed, at each time interval considered, for quantifying *A. carbonarius*, OTA and resveratrol in grapes. Real Time PCR method with specie-specific primers (Ac*pks*), designed on the basis of the OTA-related polyketide synthase sequences, was carried out quantifying the fungal development in grapes. Our results show a correlation between the growth of the fungus and biosynthesis of OTA and resveratrol content into grape berries, leading to hypothesize that some grapevine cultivars are more capable of self-protection against fungal contamination.

PR4.27

Identification of cell wall factor(s) adsorbing Taka-amylase in submerged culture of *Aspergillus oryzae*

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We have observed that Taka-amylase (TAA) activity disappeared in submerged culture of *Aspergillus oryzae* at the later-stage of cultivation. This disappearance was revealed to be caused by adsorption of TAA on fungal mycelia, but not by degradation by own extracellular proteolytic enzymes. We have also showed that cell wall of *A. oryzae* prepared from mycelia at the later-stage of cultivation has an adsorption ability for TAA. This suggested that a certain cell wall factor(s) can adsorb TAA, resulting in the disappearance of TAA in liquid medium during cultivation. To identify the adsorption factor(s) in fungal cell wall, we carried out stepwise fractionation of cell wall prepared from mycelia at the later cultivation stage by alkali extraction and cell wall lytic enzymes. The alkali-insoluble fraction of cell wall, CW4, showed high adsorption ability for TAA, but digestion of CW4 with chitinase resulted in a significant decrease in the adsorption ability. These results indicated that the adsorption factor for TAA is chitin, which is one of major polysaccharides in fungal cell wall. However, the cell wall prepared from mycelia at the earlier cultivation stage barely adsorbed TAA, although it contained equivalent amount of chitin to that of later-stage mycelia. Taken together, it is suggested that there exists unidentified factor(s) that could prevent from adsorption of TAA onto the cell wall at the earlier-stage of cultivation and the factor(s) would be removed from or decreased in the cell wall with longer cultivation periods.

PR4.28

Non-ribosomal peptides play an important role in the virulence of the opportunistic pathogen *Aspergillus fumigatus*

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Aspergillus fumigatus is a ubiquitous filamentous fungus, and a serious opportunistic human pathogen. Availability of the complete genome sequence for *A. fumigatus* has revealed that there are at least eighteen genes coding for non-ribosomal peptide synthetases (NRPS). NRPS's are usually large, multi-modular enzymes, comprised of discrete domains, which synthesise bioactive peptides via a thiotemplate mechanism. To date, a wide range of virulence factors have been reported for *A. fumigatus*, including adhesions, conidial pigments and proteases. Some of the best documented virulence factors for *A. fumigatus* include Gliotoxin and the iron-chelating Siderophores, which are of NRPS origin. Despite these important findings, there have been few studies relating the majority of *A. fumigatus* NRPS encoding genes to specific peptide products. This work aims to elucidate the peptide product encoded by a mono-modular NRPS, *pesL* (Afu6g12050/NRPS11), and to determine a possible role in virulence. A *pesL* deletion strain was generated, termed *ΔpesL*. *ΔpesL* displays severely reduced virulence in the *Galleria mellonella* model ($p < 0.0001$). Phenotypic analysis has confirmed increased sensitivity of *ΔpesL* to H_2O_2 (> 1 mM) compared to the wild-type ($p = 0.05$), and severely increased susceptibility towards the antifungal voriconazole (> 0.25 µg/ml) compared to wild-type ($p < 0.01$). These results indicate a role for *pesL* in protection against oxidative and antifungal stress within *A. fumigatus*. Comparative RP-HPLC analysis identified conidial specific material (Rt = 15.9 min; λmax at 220 nm) synthesised by *A. fumigatus* wild-type. This metabolite was absent from *ΔpesL* conidia. Increased production of this metabolite was observed in conidial extracts cultured in 2 mM H_2O_2, indicating up-regulation in response to oxidative stress. This material is currently undergoing further analysis. Furthermore, a recombinant PesL enzyme has been purified for use in an assay to determine the specific PesL amino acid substrate. This will contribute to the currently limited information on fungal NRPS substrate selectivity. Interestingly, another NRPS mutant generated previously, termed *Δpes3* (*Δ*Afu5g12030/*Δ*NRPS8) displays increased virulence in the *Galleria mellonella* model ($p < 0.0001$). Furthermore, *Δpes3* exhibited severely increased susceptibility towards the antifungal voriconazole (> 0.5 µg/ml) compared to wild-type ($p < 0.001$). RP-HPLC has not yet revealed a candidate *pes3* peptide. However, the search is on-going. This data further highlights the importance that NRPS plays in this serious human pathogen, and may reveal novel drug targets in the future.

PR4.29

Endobacteria affect the metabolic profile of their host *Gigaspora margarita*, an arbuscular mycorrhizal fungus

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AM fungi are obligate biotrophs of a large spectrum of plants with which they establish a mutualistic symbiosis. The presence of endobacteria living inside the cytoplasm of some AM fungi has long been documented, but the impact of these prokaryotes on fungal biology is still unknown.

The aim of this work was to understand whether the endobacterium *Candidatus* Glomeribacter gigasporarum has an impact on the biology of its fungal host *Gigaspora margarita* through the study of the modifications induced on the fungal proteome and lipid profile. The availability of *G. margarita* cured spores (i.e. spores that do not contain bacteria), represented a crucial tool to enable the comparison between two fungal homogeneous populations in the presence and the absence of the bacterial component. A differential protein expression was detected between wild type and cured spores under different physiological conditions (quiescent, germinating and strigolactone-elicited spores). The results obtained indicate that the fungal primary metabolism does not seem to be affected by the absence of the endosymbiont. By contrast, heat shock proteins are unambiguously upregulated, suggesting that the fungus has to face a stress situation when endobacteria are lacking. Furthermore, the fungal fatty acid profile resulted to be modified both quantitatively and qualitatively in the absence of endobacteria, being fatty acids more abundant in the presence of the endobacterium.

The results not only revealed that endobacteria have important impacts on the host fungal biology, but also offered one of the first contributions to the knowledge of the metabolic features of *G. margarita*.

PR4.30

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

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The function of genes is usually inferred from mutants in which the desired gene has been deleted or strongly overexpressed. However, studies at these extreme discrete points give only limited information about the gene functions. Moreover, many overexpression studies make use of metabolism-dependent promoters which often cause pleiotropic effects and thus impose further limitations on their use and significance.

Here we report a promoter system for *Aspergilus niger* that can be fine-tuned to user-specified expression levels, that is independent from carbon and nitrogen metabolism, that can be induced within minutes and that shows remarkable reproducibility. The system is based on the tetracycline-dependent promoter and the bacterial rtTA transcriptional activator protein and has been validated under various cultivation conditions. From the data obtained we conclude that the tetracycline-dependent promoter provides rapid and tunable gene control in *A. niger*. The system should be applicable to other filamentous fungi with only minor modifications.

PR4.31

Purification and cloning of trans-3- and trans-4-proline hydroxylase from the fungus *Glarea lozoyensis*

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Glarea lozoyensis is an anamorph fungus, which was initially assigned *Zalerion arboricola* based on morphological traits. *G. lozoyensis* is of pharmaceutical interest since it is producing the antifungal secondary metabolite pneumocandin B0. This cyclic lipopeptide is chemically converted into a water-soluble derivative (caspofungin acetate) that is used against clinically relevant fungi pathogens.

In Pneumocandin B0 trans-4- and trans-3-hydroxyprolines are incorporated which are derived from hydroxylation of L-Proline by proline trans-3-hydroxylase (P3H) and proline trans-4-hydroxylase (P4H),respectively. The P3H activity discovered in G. lozoyensis is unique and specific for pneumocandin B0 biosynthesis.

We are interested in this new selectivity for biocatalysis, but also on the molecular and genetic level. To understand the physiological parameters that influence pneumocandin B0 and C0 production better, we proceed by purification of potential P3H and P4H proteins using gel filtration. The activity is checked in each step by HPLC and LC-MS. In parallel, we are trying to clone these genes by use of some conserved domains known from bacterial proline hydroxylases for designing degenerative primers. In future experiments, we want to express the genes heterologously in *E. coli*, knock them out in the native strain and study their expression which will allow a deeper understanding of the mechanism of pneumocandin biosynthesis.

PR4.32

Transcriptomic insights into the physiology of *Aspergillus niger* approaching zero specific growth rate

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The physiology of filamentous fungi at growth rates approaching zero has been subject to limited study and exploitation. With the aim of uncoupling product formation from growth, we have revisited and improved the retentostat cultivation method for *Aspergillus niger*. A new retention device was designed allowing reliable and near complete cell retention even at high flow rates. Transcriptomic analysis was used to explore the potential for product formation at very low specific growth rates. The carbon- and energy-limited retentostat cultures were highly reproducible. While the specific growth rate approached zero (<0.005 h^{-1}), the growth yield stabilized at a minimum (0.20 g_{DW} g^{-1} maltose). The severe limitation led to asexual differentiation and the supplied substrate was used for spore formation and secondary metabolism. Three physiologically distinct phases of the retentostat cultures were subjected to genome-wide transcriptomic analysis. The severe substrate limitation and sporulation were clearly reflected in the transcriptome. The transition from vegetative to reproductive growth was characterized by down-regulation of genes encoding secreted substrate hydrolases and cell cycle genes, and up-regulation of many genes encoding secreted small cysteine-rich proteins and secondary metabolism genes. Transcription of known secretory pathway genes suggests that *A. niger* becomes adapted to secretion of small cysteine-rich proteins. The perspectives are that *A. niger* in approach of zero growth can be used as a cell factory for production of secondary metabolites and cysteine-rich proteins. We propose that the new retentostat method can be used in fundamental studies of differentiation and is applicable to filamentous fungi in general.

PR4.33

The main cell cycle genes in the pathogenic yeast *Cryptococcus neoformans*

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We have been involved in studies towards molecular understanding of cell cycle regulation in the pathogenic yeast *Cryptococcus neoformans*. Our group has reported the unique cell cycle pattern of *C. neoformans*, different from that of the model yeast *Saccharomyces cerevisiae*. In contrast to *S. cerevisiae*, very little is known about the molecular regulation of *C. neoformans* cell cycle. To clarify cell regulation at the molecular level, cell cycle control genes in *C. neoformans* were cloned and analyzed, and further studies are currently being done to confirm their function in *C. neoformans* cell cycle. The homologues of CDC28/Cdc2 (CnCdk1), the main cell cycle gene which regulates the major processes in eukaryotic cell cycle, and its cyclin counterparts, known to interact with CDC28/Cdc2 and activate it to carry out specific controls throughout different stages of the cell cycle, were isolated and identified from *C. neoformans*. In addition to CnCdk1, at least three cell-cycle related cyclin homologues were identified in *C. neoformans*. Analysis of putative amino acid sequences of these cyclin homologues showed that one is a G1 cyclin homologue, named CnCln1. The molecular characterization of the two main cell cycle genes, CnCdk1 and CnCln1, in the pathogenic yeast *C. neoformans*, will be reported and discussed.

Poster Category 5:

Fungal Way of Living; Sex and Other Encounters

PR5.1

Identification of natural αAAα diploid hybrids in the *Cryptococcus neoformans* population

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Cryptococcus neoformans is an opportunistic encapsulated pathogenic basidiomycetous yeast that causes meningoencephalitis in immunocompromised (e.g. AIDS) patients world-wide. The fungus is typically haploid, and sexual reproduction occurs normally between two individuals with opposite mating types, α and **a**. *C. neoformans* occurs in two serotypes (A and D), which correspond to *C. neoformans* var. *grubii* (molecular type: VNI & VNII) and *C. n.* var. *neoformans* (molecular type: VNIV). In addition, rare isolates with AD serotype have been reported and suggested to be diploid or aneuploid. In this study, several *C. neoformans* isolates were collected globally and their molecular type was assigned by *URA*5-RFLP analysis with *Hha*I and *Sau*96I in a double digest. The results showed unusual combination patterns of VNI and VNII for 8 isolates. These isolates were tested for ploidy by fluorescence flow cytometry analysis and were found to be diploid. PCR analysis with primers specific for serotype A or D genes and serotype- and mating-type genes revealed that the isolates were serotype A and mating type α. Also, the serotype of the isolates was further confirmed by *CAP59*-RFLP analysis with *Bsm*FI and *Hpa*II. This study confirmed the presence of same sex αAAα hybrids in nature, indicating that diploids can also occur naturally as a result of intra-varietal crossings. The hybrids identified, were isolated from different geographic locations indicating independent non-clonal origins. The fact that the majority of those hybrids are clinical strains possibly indicates that hybridization events may have an impact on *C. neoformans* virulence and subsequent infection. Moreover, this finding provides insights on the origin and impact of unisexual hybridization on the *Cryptococcus neoformans* population.

PR5.2

A mutation in *Coprinopsis cinerea* Ubc2 affects multiple cellular processes required for dikaryosis and sexual development

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We isolated and characterized a REMI mutant (B28) defective in sexual development (fruiting initiation) from a *Coprinopsis cinerea* homokaryotic fruiting strain 326 (*Amut Bmut pab1-1*). Genetic analysis strongly suggested that the defect in fruiting directly results from insertion of plasmid pPHT1. A plasmid rescue followed by complementation experiments revealed that the gene disrupted and responsible for the phenotype in strain B28 encodes a homologue of Ustilago maydis Ubc2. We designated this gene *Cc.ubc2*.

The *U. maydis ubc2* gene has been shown to be required for pheromone responses as well as for pathogenicity. Also, Ubc2 has been proposed to act as an adaptor for a pheromone responsive MAPK cascade. On the basis of these findings in *U. maydis*, we performed further analyses, which revealed that strain B28 is defective in two pheromone responses in *C. cinerea*, clamp cell fusion and nuclear migration for dikaryosis. We then carried out immunoblot analyses, which demonstrated that the *Cc.ubc2-1* mutation inhibits phosphorylation of a MAP kinase similar to Ubc3/Kpp2 and that the phosphorylation is not under the control of the B mating type genes encoding pheromone and their conjugate receptors.

PR5.3

The novel 9.5-kDa protein SIP2 specifically interacts with STE12 in *Sordaria macrospora*

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In fungi, the homoeodomain protein STE12 controls diverse developmental processes and derive its regulatory specificity from a wide range of different protein interactions. We have recently shown that in the homothallic ascomycete *Sordaria macrospora* STE12 is essential for ascospore development and able to interact with the alpha-domain mating-type protein SMTA-1 and the MADS box protein MCM1. To further evaluate the functional roles of STE12, we used the yeast two-hybrid approach to identify new STE12-interacting partners. Using *ste12* as bait, a small, serine-threonine rich protein (designated STE12 interacting protein 2, SIP2) was identified. SIP2 is conserved among members of the fungal class *Sordariomycetes*. *In vivo* localization studies revealed that SIP2 was targeted to nuclei and the cytoplasm. The STE12/SIP2 interaction was further confirmed in vivo by biomolecular fluorescence complementation (BiFC). Nuclear localization of SIP2 was apparently mediated by STE12. Unlike to the deletion (D) of *ste12*, deletion of *sip2* in *S. macrospora* led only to a slight decrease in ascospore germination, but no other obvious morphological phenotype. In comparison to the Dste12 single knock-out strain, ascospore germination was significantly increased in a Dsip2/Dste12 double knock-out strain. Our data provide evidence for a regulatory role of the novel fungal protein SIP2 in ascospore germination.

PR5.4

***Penicillium chrysogenum* – is it another shy sexual ascomycete?**

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Penicillium chrysogenum is the most ubiquitous filamentous fungus worldwide; however, it is best-known as the major industrial producer of penicillin. It is classified as an asexual ascomycete because there has never been any direct observation of mating or meiosis in the fungus. Significantly, despite its apparent asexuality there is increasing evidence for a latent sexual cycle in *P. chrysogenum*, similar to what was previously observed for the cryptically-sexual human pathogen *Aspergillus fumigatus*. Isolates of opposite mating type are found in near-equal proportion in nature and transcriptionally express pheromone and pheromone receptor genes. Furthermore, analysis of the genome sequence has revealed the presence of other key genes required for completion of the sexual cycle. Experiments are in progress to both characterise the functions of the 'master regulator' mating-type genes and induce a sexual cycle under laboratory conditions. As a first step, a *MAT1-1* knockout strain was generated by gene replacement with a phleomycin resistance cassette in a ΔPcku70 background. Asexual conidiophore development in the mutant strain was affected; it produces significantly higher quantities of conidia relative to the recipient strain P2niaD18, although the ratio of light-to-dark conidial production remains the same. The *MAT1-1* gene was also found to play a role in hyphal morphogenesis, with implications for pellet formation in industrial fermentation systems. These results highlight the diverse regulatory roles of the *MAT1-1* gene in developmental processes other than sexual reproduction.

PR5.5

Live-cell imaging of conidial fusion in the bean pathogen, *Colletotrichum lindemuthianum*

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Fusion of conidia and conidial germlings by means of conidial anastomosis tubes (CATs) is a common phenomenon in filamentous fungi, including many plant pathogens. It has a number of different roles, and has been speculated to facilitate parasexual recombination and horizontal gene transfer between species. The bean pathogen *Colletotrichum lindemuthianum* naturally undergoes CAT fusion on the host surface and within asexual fruiting bodies in anthracnose lesions on its host. It has not been previously possible to analyze the whole process of CAT fusion in this or any other pathogen using live-cell imaging techniques. Here we report the development of a robust protocol for doing this with *C. lindemuthianum in vitro.* The percentage of conidial germination and CAT fusion was found to be dependent on culture age, media and the fungal strain used. Increased CAT fusion was correlated with reduced germ tube formation. We show time-lapse imaging of the whole process of CAT fusion in *C. lindemuthianum* for the first time and monitored nuclear migration through fused CATs using nuclei labelled with GFP. CAT fusion in this pathogen was found to exhibit significant differences to that in the model system *Neurospora crassa*. In contrast to *N. crassa*, CAT fusion in *C. lindemuthianum* is inhibited by nutrients (it only occurs in water) and the process takes considerably longer.

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PR5.6

Mating type loci of *Botrytis cinerea*

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Botrytis cinerea is a heterothallic ascomycete with two mating types, MAT1-1 and MAT1-2. *B. cinerea MAT* loci have novel features. Fragments of the *MAT1-2-1* and *MAT1-1-1* genes were detected bordering idiomorphs of the MAT1-1 and MAT1-2 isolates, respectively. Both of these fragments encode truncated, non-functional proteins. *B. cinerea* has probably evolved from a homothallic ancestor containing complete *MAT1-1-1* and *MAT1-2-1* genes at the same locus, with MAT1-1 and MAT1-2 arising from the loss of HMG and alpha-domain sequences, leaving the disabled gene fragments seen in current *MAT* loci. Two ORFs, designated *MAT1-1*-5 and *MAT1-2-3*, have not previously been reported from other fungi. Homologs of *MAT1-1-5* are present in other leotiomycetes, whereas the *MAT1-2-3* gene is exclusively present within the genus *Botrytis.* Knockout mutants in *MAT1-1-5* are sterile, due to the inability of the dikaryon to develop a cap structure.

B. cinerea is unusual in that some isolates are capable of 'dual mating'. This refers to the observation that most isolates act in a standard heterothallic fashion (MAT1-1 or MAT-1-2), but some isolates can mate with both MAT1-1 ánd MAT1-2 isolates. Some dual mater isolates can self-fertilize and are truly homothallic. The *MAT* locus of five homothallic *B. cinerea* isolates was analysed. Four of those contain a MAT1-2 locus, without any sequence of the MAT1-1 locus being detected. Remarkably, one homothallic isolate contains a MAT1-1 locus, without any sequence of the MAT1-2 locus being detected. We conclude that dual mating and homothallism is controlled by sequences outside the *MAT* locus.

PR5.7

A novel effector protein in the defense of mushrooms against predatory nematodes

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We identified a novel lectin, termed RedA, from the saprobic mushroom *Coprinopsis cinerea* by virtue of its binding to the plant glycoprotein horseradish peroxidase (HRP). Like many fungal lectins, RedA is highly soluble, lacks a signal sequence for classical secretion and is specifically produced in the fruiting body. Homologous proteins are encoded in the genomes of *C. cinerea* and the symbiotic mushroom *Laccaria bicolor*. Glycan array analysis of recombinant RedA revealed a pronounced carbohydrate-specificity for the disaccharide Fucα1,3GlcNAc. Structural studies by NMR showed that the lectin adopts a beta-trefoil (R-type) fold and coordinates one ligand molecule per monomer at a site different from the canonical carbohydrate-binding site of R-type lectins. The glycoepitope recognized by RedA is found at the non-reducing ends of animal glycans and at the core of N-linked glycans of plants, insects and nematodes but not of fungi. Toxicity bioassays with model organisms including *Aedes aegypti* (insect), *Caenorhabditis elegans* (nematode) and *Acanthamoeba castellanii* (amoeba) showed an exclusive toxicity of RedA towards nematodes. Resistance of *C. elegans* mutants defective in the biosynthesis of the α1,3-core fucoside revealed that this nematotoxicity is dependent on the binding of this specific glycoepitope. Feeding *C. elegans* with the dTomato-RedA fusion protein showed that this binding occurs at the intestinal epithelium of the nematode. Our results are in agreement with the existence of a protein-mediated defense system of fungi against predators and parasites and suggest that the fruiting body lectin RedA is an effector protein against predatory nematodes.

PR5.8

Determinants of *Aspergillus fumigatus* mating

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Sexual reproduction of the human pathogen *Aspergillus fumigatus* (teleomorph: *Neosartorya fumigata*) was assumed to be absent or cryptic until recently, when fertile crosses among geographically restricted environmental isolates were described. Here, we provide evidence for mating, fruiting body development, and ascosporogenesis accompanied by genetic recombination between unrelated, clinical isolates of *A. fumigatus*, which demonstrates the generality and reproducibility of this long undisclosed phase in the life cycle of this heterothallic fungus. Successful mating requires the presence of both mating type idiomorphs *MAT1-1* and *MAT1-2*, as does expression of genes encoding putative factors involved in this process. Moreover, analysis of an *A. fumigatus* mutant deleted for the *nsdD* gene suggests a role of this conserved regulator of cleistothecia development in hyphal fusion and hence heterokaryon formation.

PR5.9

Sex, virulence, stress, and histidine kinase response regulator proteins

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Two-component histidine kinase (HK) phosphorelay signaling systems are a major mechanism by which fungi sense and adapt to their environment. In response to a signal, the HK autophosphorylates a conserved histidine residue, then the phosphate is transferred to a conserved aspartic acid residue in a response regulator (RR) protein, resulting in an output. Nearly all eukaryotic HKs are hybrid with the HK and the RR domains in a single polypeptide; most characterized hybrids require an additional phosphorelay step through a histidine phosphotransfer (HPt) domain protein and a second RR protein. This additional phosphorelay step may allow the organism to integrate multiple input signals into a single output. The maize pathogen, *Cochliobolus heterostrophus* has 21 HKs, 4 RRs (*SSK1, SKN7, RIM15, REC1*) and one HPt gene. Because all HKs signal through the 4 RRs, we chose the latter to make gene deletion mutants and then screened for altered phenotypes (virulence, asexual and sexual development, stress responses, drug resistance). No altered phenotypes were detected for *rim15* and *rec1* mutants. *C. heterostrophus* and *Gibberella zeae* Ssk1p are required for virulence to maize and wheat, respectively. Lack of Ssk1p affects fertility of heterothallic *C. heterostrophus*, self-fertility and ascospore maturation in homothallic *G. zeae,* and proper timing of sexual development in both fungi. Pseudothecia from crosses involving *C. heterostrophus ssk1* mutants ooze masses of single ascospores unlike those from WT crosses, which do not ooze, and tetrads cannot be found. Ssk1p also controls asexual spore proliferation, and represses asexual spore production in the sexual reproductive phase of both fungi. Double *C. heterostrophus ssk1skn7*-deletion mutants are more sensitive to oxidative and osmotic stress, and more exaggerated in their spore-type balance phenotype than single *ssk1* and *skn7*-deletion mutants. *ssk1*-deletion mutant phenotypes largely overlap with *hog1*-deletion mutant phenotypes, allowing us to place *SSK1* upstream of the *HOG1* MAPK pathway.

PR5.10

Localization and protein-protein interaction studies of pro22, a protein involved in hyphal fusion and sexual development in *Sordaria macrospora*

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The formation of fruiting bodies in filamentous fungi is a multicellular differentiation process and controlled by many developmentally regulated genes [1]. The filamentous ascomycete *Sordaria macrospora* represents an excellent model system for cell differentiation during fruiting body development, because this homothallic fungus is self-fertile.

For *Sordaria macrospora*, several developmental mutants are described. Though these mutants can build young protoperithecia, they are not able to form mature perithecia. These mutants, named “pro”-mutants, include the mutant pro22, which shows a point mutation in the *pro22* ORF, leading to undifferentiated protoperithecia consisting of a loose hyphal coil. By means of forced-heterokaryon tests using auxotrophic strains and fluorescence microscopic investigations with nuclear labeled strains it could be demonstrated that pro22 is restricted in hyphal anastomosis in homozygous crosses [2].

The PRO22 protein possesses two highly conserved domains A and B and homologs can be found in many other eukaryotes from yeast to humans. Fluorescence microscopy of PRO22 tagged with GFP and mRFP1 provides evidence for a localization in vacuoles. Our aim is to extend the functional analysis of the pro22 mutant via fluorescence microscopy and to identify interaction partners of PRO22 *in vitro* via yeast two-hybrid and *in vivo* via tandem-affinity purification.

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PR5.11

Applications of sexual development of *Trichoderma reesei* in research and industry: Perspectives and challenges

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Sexual development is one of the most important achievements in evolution. The case of sexuality in the industrial workhorse *Trichoderma reesei* (anamorph of *Hypocrea jecorina*) is special in so far as both research as well as industrial strain improvement is solely based on the single isolate QM6a and its (asexual) progeny. As with all other filamentous fungi applied in industry, the possibility of crossing was not available for *T. reesei*, although in silico data had identified *H. jecorina* as its teleomorph more than a decade ago. Recently, we described the two mating type loci of *T. reesei* (MAT1-1 and MAT1-2) and we were for the first time able to induce sexual reproduction of *T. reesei* QM6a (MAT1-2) in crossings with a MAT1-1 wild-type isolate of *H. jecorina*, and we obtained fertilized stromata and mature ascospores . However, a serious issue with our findings was, that while male fertile, *T. reesei* QM6a is female sterile. We now investigated the role of the peptide pheromone precursor genes, the pheromone receptors as well as of the known photoreceptors in sexual development in T. reesei. Additionally we provide first insights into the relevance of RIP for crossing in this organism.

PR5.12

Mushroom forming basidiomycetes: Mating and more

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The recent genome sequence of the wood-rotting homobasidiomycete *Schizophyllum commune*, in comparison to genomes of other Agaricomycotina like *Coprinopsis cinerea, Laccaria bicolor, Phanerochaete chrysosporium , Postia placenta* and the heterobasidiomycetes *Ustilago maydis* and *Cryptococcus neoformans* now allow comparative "omics" studies on mating interactions, pheromone response pathways. Linking the identified genes to phenotypes in sexual development and mushroom formation can provide insight into development of structures typical for the kingdom of the mycota. In mushroom forming homobasidiomycetes, the two mating type loci *A*, coding for homeodomain (HD) type transcription factors, and *B* encoding a pheromone/receptor system, regulate mating and sexual development. We could localize the pheromone receptor solving the old question of whether pheromone signalling is involved in cell-cell recognition or rather, after mycelial fusion, recognition of nuclei in a dikaryon. In addition to the mating type loci, *in silico* analyses now permit the identification of putative components of the signaling pathways leading to dikaryotic growth, mushroom formation and meiotic spore production. The involvement of G proteins like Ras could be shown for nuclear migration, nuclear positioning, clamp formation and fruitbody morphogenesis, involving cAMP-dependent protein kinase A signaling. Nuclear migration has been investigated looking into dynein function. The heavy chain of dynein in S. commune, like in other basidiomycetes, is encoded by two separate genes which are investigated by performing knock-out analyses. The resulting strain is viable, with phenotypes which, again, can be linked to sexual development, fruitbody formation and spore production as well as to apical hyphal growth. With availability of the fully sequenced genome, thus, many of the long-established pathways of signalling and morphogenesis can be revisited and surprises from the genome sequence provide insight into long-standing questions.

PR5.13

Analysis of proteins involved in sexual development in zygomycetes

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Sexual development within the class *Zygomycetes* is regulated by retinoid-like beta-carotene derivatives, the trisporoids. Trisporoids serve as pheromones in partner recognition and as internal transcription regulators. Numerous studies deal with their complex and co-operative synthesis, some of the biosynthesis enzymes have been studied into molecular detail, but their mode of action, molecular reaction mechanisms and signal perception events are completely unknown. We are researching the putative reaction partners involved in recognition and signal perception events. Our main goal is to identify putative trisporoid receptors and binding proteins, but we also include the search for proteins undergoing regulatory interactions with the trisporoid biosynthesis enzymes. 2D-PAGE reveals only a small number of deviating proteins between the (+) and the (-) mating type in cell surface-attached proteins. Several receptor candidates have been selected and are currently under analysis. Using antibodies raised against animal retinoid signaling and developmental regulation proteins, we identified a putative retinoid binding protein, cross-reacting with an antibody against cellular retinaldehyde-binding protein CRALBP, and a putative homeobox protein. Direct transcriptional modulation of effector genes may occur via trisporoid binding to specific transcription factors and trisporoid synthesis enzymes themselves might act as transcription factors during sexual development. Based on the observation that both the trisporoid biosynthesis enzymes 4-dihydrotrisporin dehydrogenase and 4-dihydromethyltrisporate dehydrogenase are inactivated at certain developmental stages by binding to other proteins forming multimeric complexes, we are using heterologously expressed 4-dihydrotrisporin dehydrogenase as bait for the purification of such binding proteins from cytoplasmatic protein fractions from *Mucor mucedo*.

PR5.14

Development of a tool for genetic manipulation of the zygomycete *Mucor mucedo*

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Genetic analysis of *Mucor mucedo*, a zygomycete model organism for studying sexual communication and action of the trisporoid system, is hampered by a strong tendency towards autonomous replication of introduced plasmids instead of stable integration. We are working at developing a tool for genetic manipulation based on interference with the DNA double strand break-repair system.

Targeted gene disruption via homologous recombination is routinely used for analysis of gene function. Although integration of exogenous DNA at homologous sites in the genome occurs easily in *Saccharomyces cerevisiae*, it is rare in many filamentous fungi where DNA integration occurs predominantly through non-homologous end joining (NHEJ) and exogenous DNA can be integrated at ectopic sites in the genome. Direct ligation of DNA strands in NHEJ is mediated by a DNA-dependent protein kinase, a DNA ligase complex, and the Ku70-Ku80 heterodimer. Homologues of Ku70 and Ku80 have been identified in many organisms. Recent studies in filamentous fungi have shown increased gene targeting frequencies in *KU* deficient mutants, indicating that *KU* disruption strains are efficient recipients for gene targeting. We identified and cloned a *M. mucedo* homologoue to the human *KU*70 gene using PCR, inverse PCR, and Southern hybridization. Genetic manipulation will be done using an RNA silencing approach with Morpholino antisense constructs directed against *KU*70 mRNA, thus resulting in the functional knock-down of the *KU*70 gene product. The suitability of this approach is shown by morpholino-mediated down-regulation of crgA, a protein involved in the regulation of carotene synthesis in zygomycete fungi.

PR5.15

The diaphanous-related formin Drf1 is an effector of the rho-GTPase Cdc42 in the basidiomycetous fungus *Ustilago maydis*

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The basidiomycetes *Ustilago maydis* is a tumour-causing agent on corn plants. Tumour development can occur in all green parts of the plant and is initiated by the invasion of dicaryotic hyphae from the plant surface. These hyphae are formed after fusion of two compatible haploid sporidia. Hyphal growth is unipolar and the cytoplasmic part of elongating filaments is restricted by the sequential formation of retraction septa leaving empty sections behind.

We have identified a regulatory network essential for the formation of the secondary septum in budding cells composed of the guanine-nucleotide exchange factor Don1, the corresponding small GTPase Cdc42 and the germinal-centre kinase Don3.

We will demonstrate our recent data that the diaphanous related formin Drf1 is an effector of the Cdc42-module for secondary septum formation. Formins are proteins that polymerize and bundle actin cables.

Furthermore we will show that among these proteins only Cdc42 is essential for the morphological switch from yeast-like to hyphal growth. We will present data how Cdc42 regulates the MAP kinase cascade. The complete Don1-Cdc42-Drf1 module organizes the retraction septa formation in filaments. In addition, this cascade is also necessary for appressoria formation. This also indicates that actin nucleation is important for these processes.

Currently we are investigating the role of the Don3 kinase during filamentous growth to elucidate its interplay with the Cdc42 module.

PR5.16

Genetic and epigenetic control of a nonself recognition complex in *N. crassa*

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Heterokaryon incompatibility (HI) in filamentous fungi is a form of nonself recognition that operates during the vegetative phase of the life cycle. One HI complex in *Neurospora crassa*, the *het-6* locus, comprises two tightly linked incompatibility genes, *het-6* and *un-24*, each having two allelic variants, Oak Ridge (OR) and Panama (PA). The *un-24* gene also encodes the large subunit of ribonuclotide reductase while *het-6* appears to be a member of a repetitive gene family with no other known function aside from HI. These two genes are in severe linkage disequilibrium such that only *un-24*^{OR} *het-6*^{OR} and *un-24*^{PA} *het-6*^{PA} haplotypes occur in nature. In this study we constructed novel *un-24*^{PA} *het-6*^{OR} strains and *het-6* deletion strains. The *un-24*^{PA} *het-6*^{OR} strains are initially self-incompatible, but escape from incompatibility in a predictable manner that is associated with loss of *het-6*^{OR} incompatibility function. HI tests using *het-6* deletion strains reveals an allelic incompatibility interaction between OR and PA forms of *un-24*, which is asymmetrically enhanced by the presence of *het-6*^{OR} or *het-6*^{PA}. Mutations in *vib-1* suppress HI associated with allelic differences at *un-24* or at *het-6*, but only partially suppress incompatibility reactions that occur when both *un-24* and *het-6* differ. We have also identified a second genetic suppressor that acts synergistically with *vib-1* and will present evidence that this suppressor compromises epigenetic silencing of HI.

PR5.17

The fungal immune response: from detection of the pathogen to the cellular response

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Living organisms have developed complexe processes to fight off pathogens. Plants and animals initiate an immune response upon detection of Pathogen Associated Molecular Patterns (PAMPS) by dedicated Pattern Recognition Receptors (PRRs) that belong to the STAND class of proteins involved in signal transduction. Pathogen driven evolution of the PRR encoding genes can lead to auto-immune diseases. No such fungal immune systems have been described so far.

In *Podospora anserina* vegetative incompatibility (VI) is under the control the *NWD* gene family that interacts with incompatible alleles of the *het-c* locus encoding for a glycolipid transfer protein (GLTP). The *NWD* gene family evolves extremely rapidly and new variants in the WD repeat domain involved in protein protein interactions are permanently being generated. NWD proteins also belong to the STAND class of proteins. We have hypothesized that these proteins function as PRRs and that the VI reaction corresponds to a pathological manifestation of an auto-immune disease. We have discovered a fungal species that initiates a strong reaction in *P. anserina* that is dependant on *het-c* and all known VI suppressors. We have undertaken the task of describing the fungal immune response from pathogen recognition to the cellular response.

PR5.18

Genetic relatedness of multi-azole resistant *Aspergillus fumigatus* isolates

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Objectives

In the last few years multi-azole resistant *Aspergillus fumigatus* seem to emerge in the Netherlands. The dominant resistance mechanism is a point mutation in the *cyp51A* gene resulting in the substitution of leucine to histidine at codon 98 (L98H) combined with a 34 bp tandem repeat (TR) in the promoter region of this gene. The emergence of this single resistance mechanism suggests that the resistant isolates have a common evolutionary origin. We analyzed the CSP gene, microsatellites and the mating type locus to study azole susceptible and resistant *A. fumigatus* isolates of clinical and environmental origin to investigate the genetic relatedness of TR/L98H isolates.

Methods

Resistant isolates containing the TR/L98H resistance mechanism (n=57) were subjected to CSP, microsatellite and mating type analysis. As a control, both susceptible (n=57) and resistant isolates without the TR/L98H resistance mechanism (n=17) were analyzed.

Results

The TR/L98H isolates clustered together in microsatellite analysis and were grouped into three CSP types (2, 4B and 11). Susceptible and non-TR/L98H resistant control isolates were classified to other CSP types than the TR/L98H isolates, except for several isolates belonging to CSP type 2. Mating type genes *MAT1-1* and *MAT1-2* were almost equally represented in each group.

Conclusions

1. Clinical and environmental TR/L98H isolates were grouped to the same CSP types and clustered together using microsatellite typing, indicating that the TR/L98H resistance mechanism has only recently developed and might not be induced in the patient but might be acquired from the environment.
2. As the TR/L98H isolates grouped to different CSP types and clustered apart from the control isolates, the TR/L98H isolates might belong to a separate genetic *A. fumigatus* lineage.
3. Although microsatellite and CSP analysis indicate that the TR/L98H isolates are rather homogeneous, segregation of the mating type locus is equal to the control isolates. This might suggest that sexual reproduction played a role in resistance development.

PR5.19

The fungal immune response: from detection of the pathogen to the cellular response

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Poster Category 6:

Fungal Way of Living; Cell Biology

PR6.1

The antifungal activity of the *Penicillium chrysogenum* antifungal protein PAF disrupts calcium signalling and homeostasis in *Neurospora crassa*

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The antifungal protein PAF from *Penicillium chrysogenum* exhibits growth inhibitory activity against a broad range of filamentous fungi. Recent evidence has suggested that calcium (Ca^{2+}) signalling may play an important role in the mechanistic basis of PAF as a growth inhibitor. Supplementation of the growth medium with high Ca^{2+} concentrations counteracted PAF toxicity towards sensitive moulds. By using a transgenic *Neurospora crassa* strain expressing codon optimized aequorin, PAF was found to cause a significant increase in the $[Ca^{2+}]_c$ resting level. The Ca^{2+} signatures in response to stimulation by mechanical perturbation or hypo-osmotic shock were significantly changed in the presence of PAF. BAPTA, a Ca^{2+} selective chelator, ameliorated the PAF toxicity in growth inhibition assays and counteracted the PAF induced perturbation of Ca^{2+} homeostasis. These results indicate that extracellular Ca^{2+} was the major source of these PAF-induced effects. The L-type Ca^{2+} channel blocker diltiazem disrupted Ca^{2+} homeostasis in a similar manner to PAF. Diltiazem in combination with PAF acted additively in enhancing growth inhibition and aggravating the change in Ca^{2+} signatures. Notably, both substances, PAF and diltiazem increased the $[Ca^{2+}]_c$ resting level possibly by blocking Ca^{2+} channel activity. However, examination of a *N. crassa Δcch1* deletion strain excluded the L-type Ca^{2+} channel *cch1* to be the major target of PAF.

PR6.2

Multivesicular body-ESCRT components and pH response regulation in *Aspergillus nidulans*

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Involvement of MVB class E components in pH signalling is well characterised in yeasts. In *S. cerevisiae*, all components, of ESCRT-I, -II and Vps32p-Vps20p of ESCRT-III are required for pH signalling. Regulation of gene expression by ambient pH in *Aspergillus nidulans* is mediated by the transcription factor PacC. The ESCRT –III components Vps32 and Vps24 interact with the pH signalling components PalA and PalB respectively, thus participating in pH signalling.

We report that the deletion of genes encoding Vps20, Vps32 and Vps36 in *A. nidulans* is nearly lethal and nearly always accompanied by selection of suppressor mutations greatly improving growth. These (partial) suppressors occur in two genes, *supA* and *supB*. SupA is a transcription factor and SupB is a putative protein kinase. The suppressor mutations do not affect pH regulation or trafficking but they do alter the volume and quantity of vacuoles, even in *vps*$^+$ strains. *vps20, -32* and *-36* deletions prevent pH signalling, consistent with results in yeast. These deletions also impair trafficking of FM4-64 and of the dicarboxylic amino acid transporter to the vacuolar membrane. We conclude that at least ESCRT-II and -III components are required for pH signalling.

PR6.3

Control of organelle differentiation and copy number by positive feedback to import competence

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The differentiation of organelles is fundamental to the growth and development of eukaryotic cells. Woronin bodies are low -copy fungal organelles produced from a high-copy number organelle – the peroxisome. Here, we show that Woronin body producing peroxisomes differentiate *de novo* and are hyper-competent for matrix protein import. This produces a few dominant organelles that receive the majority of nascent matrix protein import. Differentiation depends on a key oligomeric contact in the Woronin body core protein HEX. Mutational disruption of this oligomer abolishes the differentiation of peroxisomes and interaction with two proteins that are enriched in the membrane of WB producing peroxisomes. Our results are consistent with a model where the HEX oligomer promotes peroxisome differentiation through positive feedback to the targeting of key membrane proteins.

PR6.4

***Penicillium chrysogenum* Pex14/17p: a novel component of the peroxisomal membrane that is important for penicillin production**

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By genome analysis, we identified Pex14/17p as a putative novel peroxin of *Penicillium chrysogenum*. Here we show that Pex14/17p is a component of the peroxisomal membrane that is essential for efficient PTS1 and PTS2 matrix protein import, implying that the protein is indeed a *bona fide* peroxin. Additionally, a *PEX14/17* deletion strain is affected in conidiospore formation. Pex14/17p has properties of both Pex14p and Pex17p in that the N-terminus of this protein is similar to the highly conserved Pex5p binding region present in the N-termini of Pex14p's, whereas its C-terminus shows weak similarity to yeast Pex17p's. We have identified a novel motif in both Pex17p and Pex14/17p that is absent in Pex14p.

We show that an N-terminally truncated, but not a C-terminally truncated Pex14/17 protein is able to complement both the matrix protein import and sporulation defects of a *Δpex14/17* strain, implying that it is the Pex17p-related portion of the protein that is crucial for its functioning as a peroxin. Possibly, this compensates for the fact that *P. chrysogenum* lacks a Pex17 protein. Finally, we show that in P. chrysogenum Pex14/17p plays a role in the efficiency of the penicillin biosynthesis process.

PR6.5

Comparative gene expression analyses reveal genes with functional roles during fruiting body development of filamentous ascomycetes

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Many filamentous ascomycetes form fruiting bodies during a highly complex differentiation process. Four major morphological types (apothecia, perithecia, pseudothecia and cleistothecia) are differentiated that derive from an ancestral fruiting body. Thus, fruiting body differentiation most likely is controlled by a set of common core genes. One way to identify such genes is to search for genes with evolutionary conserved expression patterns, which is a powerful criterion for functional importance.

Using "Suppression Subtractive Hybridization", we selected differentially expressed transcripts during fruiting body development in *Pyronema confluens* (Pezizales). By real time PCR, expression patterns were shown to be conserved in members of the Sordariales (*Sordaria macrospora* and *Neurospora crassa*), a derived group of ascomycetes. Knockout studies with correlated *N. crassa* orthologues, revealed a functional role during fruiting body development for NCU05079, a putative MFS peptide transporter.

Additionally we verified a conserved expression pattern for the homologues of yeast *asf1* (anti-silencing function protein 1) during fruiting body development of four distantly related ascomycetes (*N. crassa*, *S. macrospora*, *Fusarium graminearum* and *P. confluens*) by microarray and real time PCR analysis. Asf1 is a highly conserved histone chaperone involved in the balance of nucleosome assembly and disassembly. Knockout and complementation analysis with *S. macrospora* Asf1 indicate a functional role of the protein during sexual development of the fungus.

These data indicate conserved gene expression patterns and a functional role of the corresponding genes during fruiting body development, which are candidates of choice for further analysis.

PR6.6

The *Aspergillus nidulans* cortical marker teaa mediates microtubule-cortex interactions through an interaction with the XMAP215 family protein AlpA

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In eukaryotic cells microtubule (MT) length is determined by polymerization and depolymerization phases. One important parameter for length determination is the contact with the cell cortex. Here, we show in the model organism *Aspergillus nidulans* that the contact of MT plus ends with the cortex is mediated through interaction between a putative MT polymerase (XMAP215, *A. nidulans* homologue AlpA), and a cortical cell end marker protein, TeaA. Although both proteins localized to MT plus ends during MT growth, AlpA-TeaA interaction was observed in a bimolecular fluorescence complementation assay only after MT plus ends contacted the cortex. In the absence of TeaA, MT plus ends contacted random places along the tip cortex and occasionally continued to grow after reaching the cortex. In the absence of AlpA, the microtubule array was laregely affected and MTs grew very slowly. In an *in vitro* MT polymerase assay we show that fast MT polymerization depends on the presence of AlpA and is inhibited by TeaA. Further investigation with truncated variants of TeaA suggests that the interaction of TeaA with AlpA is necessary not only for the proper behavior of MTs at tips but also proper polarity establishment and maintenance.

PR6.7

Signaling through the *Aspergillus nidulans* orthologue of PKC mediates septum formation

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We have shown that the *Aspergillus nidulans* orthologue of protein kinase C (PkcA) participates in regulating cell wall integrity (CWI) and localizes at sites of cell wall synthesis, including growing hyphal tips and septa. PkcA's role in CWI is regulated independently of its capacity to target to sites of wall growth, as shown by the ability of the *calC*2 mutation to inhibit resistance to wall-damaging compounds without affecting growth or cytokinesis. To better understand the mechanisms by which PkcA localizes to tips and septa, we have observed the formation of cortical rings at sites of septation by fluorescently tagged PkcA in hyphae defective in expression of other proteins necessary for septum formation, using either temperature-sensitive mutants or regulation under the *AlcA* promoter. In addition, we have co-imaged PkcA and other septation proteins bearing complementary tags. Here we report that localization of PkcA to septa lies "downstream" of the functions performed by MobA (Mob1p orthologue), TpmA (tropomyosin), SepA (formin), SepD, SepG, and proteins encoded by two other not-yet-cloned *Sep* loci. In the absence of function of these proteins, PkcA cortical rings were not observed. PkcA localization lies "upstream" of MyoB (myosin II orthologue), the *A. nidulans* orthologue of Bud4p (in yeast, a bud site selection marker), and a protein encoded by a third uncloned *Sep* locus. PkcA cortical rings still form in the absence of function of these proteins, though septa do not develop. SepA, TpmA, MyoB, and MobA all appear to colocalize with PkcA during normal septum formation. While PkcA localizes to the very apex of hyphal tips and to the leading edge of growing septa, the protein phosphatase BimG localizes to sites lateral to the most active sites of growth. Studies with other septation-related proteins are ongoing.

PR6.8

Calcium and pH homeostasis in *Aspergillus*: small molecules under control

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The well tuned orchestra of enzymes is precondition for any cell to function. A coordination of enzymes' activities is manly a responsibility of wide variety of secondary messengers. Some of them like cAMP, PIPs are locally synthesized. Others like free calcium ions are constantly present, but stored in organelles and the concentration is rigorously controlled by homeostatic machinery. And then there is a pH, which might serve as a mechanism by which cells co-ordinate the regulation of various processes that lack any other common regulating factors and may provide a link between metabolic state and physiological responses. However, not lot is known how calcium and pH homeostatic machinery are working which is most likely due to lack of easy methods to monitor changes in intracellular free calcium ions concentration and pH *in vivo*. The methods for tracking changes in intracellular calcium concentration and pH will be explained and evidence for calcium and pH homeostasis will be given. An examination of cytoplasmic pH in growing cells of Aspergillus niger was performed by simultaneous, dual excitation confocal ratio imaging of the ratiometric pH probe RaVC. For calcium homeostasis studies a genetically encoded calcium probe aequorin was used. The role of PMRA, Golgi P-type ATPase and PMCA, plasma membrane P-type ATPase in maintaining calcium homeostasis of filamentous fungus *Aspergillus niger* will be demonstrated.

PR6.9

***Fusarium graminearum* as a model for human Niemann-Pick Type C disease**

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Niemann-Pick Type C (NPC) disease is a fatal autosomal recessive lipid storage disorder in humans. An accumulation of endocytosed cholesterol in lysosomes/late endosomes of spleen, liver and brain cells causes progressive dementia, usually resulting in death by the age of twenty. NPC is caused by defects in two genes which function in cholesterol transport, *npc1* and *npc2*, with mutations in the former being responsible for 95% of documented cases. Filamentous fungi make attractive models to study the endomembrane system, growing rapidly in a highly polarized manor. Here, we explore the potential of the ascomycete *Fusarium graminearum* as a tool to study NPC. A BLAST search revealed the presence of a putative *F. graminearum* homolog of *npc1*, possessing 34% sequence identity (51% positives) at the amino acid level. Deletion of *F. graminearum npc1* produced a viable mutant which displayed defects in ergosterol localization. A combination of fluorescence and differential interference contrast microscopy revealed an accumulation of ergosterol in vacuoles of mutant cells. Furthermore, a GFP tagged version of NPC1 was found to localize to the vacuolar membrane, analogous to lysosomal membrane anchored human NPC1p. Our results suggest that filamentous fungi may provide good model systems to study NPC.

PR6.10

Functional analyses of α-1,3-glucan synthase genes, *agsA* and *agsB*, in *Aspergillus nidulans*

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The cell wall of filamentous fungi is a complex structure that is essential for the maintenance of cell's shapes and integrity, for the prevention of cell lysis, and for protection against adverse environmental conditions. We previously reported that the transcriptional regulation of a MAP kinase gene *mpkA* and of cell wall-related genes (CWGs) in *Aspergillus nidulans* differs significantly from that in *Saccharomyces cerevisiae*. The transcription of two α-1,3-glucan syntase genes, *agsA* and *agsB*, were regulated by MpkA pathway, but most CWGs were not. Recently, the importance of α-1,3-glucan in host-parasite interactions has been studied in both mammalian and plant pathogenic fungi. In this study, to understand the role of α-1,3-glucan in *A. nidulans*, functional analyses of the *agsA* and *agsB* genes were performed. The deletion mutants of *agsA* gene did not show any significant phenotypes under normal growth conditions. In contrast, the disruptants of *agsB* gene could not be obtained, suggesting that AgsB seems to play a crucial role in α-1,3-glucan synthesis of *A. nidulans*. To assess this issue, we constructed the conditional *agsB* strain whose *agsB* expression is conditionally regulated under the control of *alcA* promoter. The transcription of many CWGs coding for β-1,3-glucan synthase and chitin synthase were induced under *agsB* repressed conditions. This suggests that the decrease of α-1,3-glucan content was counterbalanced by an increase in other cell wall components. The results of the sensitivities to cell wall stress compounds such as micafungin, CFW and Congo Red and the susceptibility test for cell wall degrading enzymes will be presented.

PR6.11

Reduced expression of SccA increases sensitivity to wall stress

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Decreased expression of the extragenic suppressor, designated *SccA*, affects cell wall integrity in the filamentous fungus *Aspergillus nidulans*. Overexpression of *SccA* suppresses the phenotype of the *calC*2 mutation in the *A. nidulans* orthologue of protein kinase C (PkcA), which results in hypersensitivity to the chitin-binding agent Calcofluor White (CFW). In addition, we have shown that *SccA* rescues 6 wall-sensitive strains. In filamentous fungi, as in yeasts, hypersensitivity to CFW correlates with defects in cell wall integrity. *SccA* is predicted to have a single transmembrane domain with 42% of its amino acids residues being serine or threonine, which indicates it is bound to carbohydrates in the cell wall. Homologues exist in the genomes of other filamentous fungi, but not in yeasts or other organisms. A SccA-GFP hybrid localizes to the plasma membrane and septa of vegetative hyphae. When *SccA* is placed under the control of the regulatable *AlcA* promoter and grown under low expression conditions (glucose), we observed a sensitivity to CFW, indicating it plays an important role in cell wall integrity. Taking into consideration the protein's cell surface location and its influence on the function of PkcA, we hypothesize that SccA plays a role in signal transduction as part of a cell wall integrity pathway.

PR6.12

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

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The exosome is a multi-subunit 3'→5' exonucleolytic complex that is conserved in eukaryotes. The ring-shaped core structure of the exosome is constituted of nine subunits. In yeast, all of nine exosome subunits are essential for viability. On the other hand, in plant, Csl4 is dispensable for growth and development, whereas Rrp41 and Rrp4 are essential for the development of female gametophytes and embryogenesis. These results suggest that the function of individual subunit of the exosome is different in each eukaryotic cell. Since there has been no report on the exosome itself in filamentous fungi, we attempted to construct the disruptants of genes encoding exosome subunits in *Aspergillus oryzae*.

We have chosen two orthologous genes for *csl4* and *rrp4* as targets for disruption, and successfully obtained a *csl4* disruptant but not an *rrp4* disruptant. The disruption of *csl4* gene had no apparent defect on growth in *A. oryzae*. Since the *rrp4* disruptant could not been obtained, this gene would be essential for cell viability. Thus, we generated the conditional *rrp4* expression mutant strain by using the promoter of *nmtA*, expression level of which is regulated by riboswitch existed within its 5'UTR and is repressed considerably in the presence of thiamine. The resultant conditional *rrp4* expression strain displayed a remarkable growth defect when thiamine was added to the medium. These results suggested that Rrp4 is essential but Csl4 is not for cell growth in *A. oryzae* and that function of individual exosome components in *A. oryzae* is similar to that in plant.

PR6.13

Directed growth during germling fusion in *Neurospora crassa* requires bem-1, a protein which is dispensable for general polar growth

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We are using *Neurospora crassa* as a model system to study the molecular mechanisms of chemotropic growth and cell fusion. When spores of *N. crassa* germinate, they attract each other to undergo cell fusion and to form the mycelial colony. Earlier studies have shown that the MAP kinase MAK-2 is an essential part of a signaling cascade involved in chemotropic growth during germling fusion (Pandey *et al.* 2004; Fleissner *et al.* 2009). To further characterize this signaling pathway we analyzed the role of BEM-1. The homologous protein in *Saccharomyces cerevisiae* is interacting with several upstream factors of the Fus3 cascade, which is homologous to the MAK-2 pathway in *N. crassa*. In contrast to yeast, where BEM-1 is involved in polarity establishment, a *N. crassa* delta *bem*-1 knock out mutant is not significantly impaired in spore germination and polar hyphal growth. However, delta *bem-1* germlings do not interact chemotropically. Complementation with a *bem* delta PB-1 construct did not complement the knock out mutant phenotype, suggesting that the PB-1 domain is essential for BEM-1 function. Subcellular localization of BEM-1 using GFP fusion constructs showed that BEM-1-GFP accumulates at every growing hyphal tip. In germling fusion pairs, BEM-1-GFP concentrates at the fusion point and localizes around the opening fusion pore. We also detected BEM-1-GFP at septa of germ tubes and mature hyphae. Taken together, our data suggest novel functions of BEM-1 in chemotropic growth, fusion pore formation and at the septa. In our further studies we will try to unravel and identify the distinct molecular functions of BEM-1 during *Neurospora* development.

PR6.14

Heterogeneity in micro-colonies of *Aspergillus niger* in liquid shaken cultures

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Colonies of the filamentous fungus *Aspergillus niger* secrete large amounts of proteins. Previously, it has been shown that on solid media only part of the colony participates in secretion. We assessed whether heterogeneity can also be found between and within micro-colonies of *A. niger* from submerged cultures.

Strains expressing *GFP* from the glucoamylase (*glaA*) or ferulic acid esterase (*faeA*) promoters were grown. The Complex Object Parametric Analyzer and Sorter (COPAS) was used to analyze the diameter and fluorescence of micro-colonies. At least two populations were found in these cultures, one population of small (25%) and one of large micro-colonies (75%). Fluorescence correlated with volume. 27% and 73% of the micro-colonies expressing *GFP* from the *faeA* promoter were lowly and highly fluorescent, respectively. This implies that heterogeneity in this strain depends on the volume only. In contrast, lowly fluorescent micro-colonies of the *glaA::GFP* strain comprised about 79% of the culture; this implies an additional factor besides volume.

To assess heterogeneity within a micro-colony, central and peripheral parts were isolated by laser microdissection and pressure catapulting (LMPC). QPCR showed that *glaA* and *faeA* expression is similar in both zones. However, RNA content per hypha was 50 times higher in the periphery.

Here, COPAS and LMPC were used for the first time to assess gene expression in a microbial system. Our results show heterogeneity between micro-colonies from a submerged culture. Moreover, it is shown that zones within a pellet are also heterogenic. Peripheral hyphae have about 50 times more RNA than central hyphae.

PR6.15

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

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

PR6.16

PBS (Phenotype Based Screening) System: an effective strategy for bidirectional genetic approach in the rice blast fungus, *Magnaporthe oryzae*

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The most straightforward strategy of reverse genetic approach includes targeted gene deletion process. It requires homologous recombination (HR) events and efficiency of gene deletion is subject to frequency of HR. In many filamentous fungi, however, HR occurs at low frequency due to the dominance of non-homologous end joining (NHEJ) and in case of the rice blast fungus *Magnaporthe oryzae*, frequency of HR is locus-dependent and very low that has been reported less than 10%. It causes inefficiency of experimental progress and generates lots of undesirable ectopic transformants. For the improved efficiency of genetic study, we designed an add-on system named PBS (Phenotype Based Screening). This system is added to the targeted gene deletion process and makes efficient use of ectopic transformants previously abandoned. As a forward genetic approach, ectopic transformants showing gene disruptions probably in random manner are screened for various defectives in several phenotypes such as growth rate, pigmentation, colony morphology, and conidiation. Location of ectopic integration is achieved by inverse-PCR and sequencing. With aid of PBS system, two growth-retarded ectopic transformants were confirmed as related to disruption of two loci MGG_00839.6 and MGG_04395.6. Consequently, coupling of PBS system and targeted gene deletion process can be regarded as the union of forward and reverse genetic approaches. This bidirectional approach will advance the functional genomic studies of filamentous fungi with high efficiency.

PR6.17

Comparative Proteomic Analysis of *Colletotrichum higginsianum* Infection Structures

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The hemibiotrophic ascomycete *Colletotrichum higginsianum* causes anthracnose disease of crucifers, including the model plant *Arabidopsis*. Following conidial germination, the pathogen differentiates a specialised infection structure called an appressorium that is essential for initial host invasion. We used a comparative proteomics approach to uncover changes in the total and secreted proteome during germination and appressorium formation, taking advantage of the ability to mass-produce *Colletotrichum* appressoria *in vitro* on polystyrene substrata. Proteins harvested at 5h, corresponding to early appressorium formation, and 22h, representing mature melanized appressoria, were compared to the proteome of undifferentiated mycelia. A total of 677 protein spots were identified using MALDI-TOF-MS and MS/MS by reference to the *C. higginsianum* genome sequence. 27 secreted proteins were identified in the germination liquid surrounding mature appressoria. Of the proteins that were co-regulated during appressorium formation, enzymes involved in fungal wall modification and melanin biosynthesis were represented during early morphogenesis, while enzymes potentially involved in plant cell wall degradation and toxin biosynthesis were represented in mature appressoria. The identified peptides provide experimental support for annotation of the *C. higginsianum* genome sequence.

PR6.18

Mutations in two Golgi Apparatus COG proteins affect growth and glycosylation in *Aspergillus nidulans*

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

PR6.19

How coupled oscillators shape daily timing

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The circadian clock is a complex trait, involving a network of interlocked molecular loops that depend on oscillatory feedback. There are many indications that also in the fungus *Neurospora crassa* the clock consists of multiple oscillators. One molecular oscillator system that has been described in great detail is the frequency-white collar (FRQ-WC) transcription-translation feedback loop (FWC-TTFL). In the absence of a functional FWC loop, strains are still able to entrain systematically to temperature, they show oscillations in nitrate reductase activity and, under particular conditions, rhythmic conidiation is within the circadian range. There thus is ample evidence for one or more FRQ-less oscillators (FLOs). Microarrays have revealed several candidate components of these FLOs. We have studied the circadian behaviour of one of these: *cpc-1* (for cross pathway control), an orthologue of the *gcn4* gene from yeast. *Cpc-1* encodes a transcription factor involved in the coordination of many amino acid biosynthetic pathways. It is therefore a candidate node in several metabolic feedback loops. We show that coupling oscillations of FWC and CPC-1-dependent feedback loops control fundamental clock attributes like amplitude, precision and robustness.

PR6.20

Regulation of the SNARE complex formation in *Trichoderma reesei*

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Eukaryotic cells contain membrane-bound compartments that are connected by trafficking of vesicular intermediates. Merging of the donor and acceptor membranes accomplishes three tasks: 1) Surface of the plasma membrane increases (by the surface of the fused vesicle), which is important for cell growth. 2) The substances within the vesicle are released into the exterior. 3) Proteins embedded in the vesicle membrane become part of the plasma membrane.

To maintain compartmental organization, proper targeting of transport vesicles is required. In the process of exocytosis, where Golgi derived secretory vesicles fuse to the plasma membrane, three major classes of proteins are needed SNARE, Sec1 and Rab proteins. Although t- and v-SNAREs are sufficient to drive the fusion of membranes *in vitro*, it is clear that other proteins are important regulators of this event in the cell.

It has been shown that the formation of SNARE complex is very strictly regulated in mammalian and yeast cells. The NH_2-terminal domain from isolated mammalian t-SNARE, syntaxin, has been shown to fold back onto the SNARE motif, and this closed conformation is stabilized by the binding of members of the Sec1-munc (SM) family, apparently preventing SNARE complex formation. Two reports have demonstrated that key neuronal mammalian SM protein, munc18-1, can interact with the assembled SNARE complex, via an N-terminal peptide in syntaxin. This motif is conserved in some fungal SSO homologs and in mammalian syntaxin1. The function of the SM family of proteins in membrane fusion is still controversial, but it has been shown to be essential regulator of the membrane fusion and in yeast has been shown to concentrate on the sites of secretion.

To address the question of the control of membrane fusion, we have cloned the Sec1 homologue of *T. reesei* and expressed it as a fusion with a yellow fluorescent marker protein (Venus). The localisation of the fusion protein is cytoplasmic. Interactions of the SECI protein with two plasma membrane t-SNARE proteins SSOI and SSOII has been studied using multi-dimensional time-correlated single photon counting (TCSPC) fluorescence lifetime imaging microscopy (FLIM) to detect FRET. It was shown that there is FRET occurring between mCer::SSOI and the SECI::Venus fusion proteins indicating that there's a protein-protein interaction between these two proteins. The major site of interaction is at the plasma membrane. The finding was further verified by immunoprecipitation (IP) studies showing that SSOI and SECI can be isolated as a complex. Both SSOI and SECI proteins seem to be essential for growth as deletion of either is lethal to the cells. The FLIM/FRET results from the mCer::SSOII and SECI::Venus interaction were inconclusive and in IP studies, no interaction has been seen between the SECI and SSOII proteins.

PR6.21

Characterisation of functional domains of the iron-dependent transcription factor HapX

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Recently, a putative fourth CCAAT-binding complex (CBC) subunit with an unknown function was identified in *A. nidulans* and designated HapX. *hapX* expression is repressed by iron via the GATA-factor SreA. Various iron-dependent pathways (e.g., heme biosynthesis) are repressed during iron starvation by the interaction of HapX with the CBC. These data suggest a model, in which HapX/CBC interaction is regulated at both transcriptional and post-translational levels. Iron starvation causes expression of *hapX*. Subsequent binding of HapX to the CBC results in transcriptional repression of iron-dependent pathways. During iron-replete conditions, *hapX* is repressed and, therefore, iron-dependent pathways are derepressed. Moreover, HapX/CBC interaction is inhibited by increased iron concentrations. This post-translational mechanism allows rapid adjustment to iron availability by disruption of the HapX/CBC complex. Mutual transcriptional control of *hapX* and *sreA* coordinates iron acquisition and iron-dependent pathways, thereby serving for both iron supply and prevention of iron toxicity. These data indicate that the CBC has a general role and that HapX function is confined to iron depleted conditions.

Here, we describe the domain architecture of the HapX protein. Phylogenetic analysis revealed the conservation of certain domains, which were characterised further with the help of surface-plasmon-resonance and complementation experiments.

PR6.22

Regulated silencing of the spindle assembly checkpoint without mitotic spindles

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The spindle assembly checkpoint (SAC) is a universal mechanism which arrests mitosis if a bipolar spindle cannot be formed. Although higher eukaryotic cells that are unable to satisfy the SAC eventually die, such a mechanism would not confer an obvious advantage to filamentous fungi encountering environmental conditions that interfere with spindle formation. Following the status of nuclear pore complex and SAC proteins we show that *Aspergillus nidulans* cells treated with the spindle poison benomyl activate the SAC and arrest in mitosis. However after a defined period of time, the SAC is actively turned off and cells exit mitosis without chromosomal segregation. Most remarkably we find that cells which have undergone one such failed mitosis surprisingly transit interphase and enter a second mitosis in which the SAC is re-activated before again being inactivated. This cyclic activation then inactivation of the SAC can occur for at least three cell cycles in which the nucleus completes all aspects of mitosis except those depending on spindle function. Further, following one or more cell cycles without spindle function, if cells are allowed to reform microtubules they assemble spindles upon mitotic entry and can undergo successful mitosis. Therefore, we propose that inactivation of the SAC allows filamentous fungi to continue growth under environmental conditions which prevent spindle formation and then periodically test the environment for conditions which are compatible with mitosis. We conclude that the SAC can be silenced in a cyclic regulated manner independent of spindle formation.

PR6.23

Tip-focused Rho GTPase activity and the actin cytoskeleton regulate directional growth of *Neurospora crassa* germlings

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The ability to establish and maintain cell polarity is a prerequisite for spore germination and cell fusion in *Neurospora crassa*. Without it spores are limited to isotropic growth and unable to protrude germ tubes and conidial anstomosis tubes (CATs). Germ tubes avoid each other, whilst CATs attract each other. During CAT homing, tip orientation follows a chemoattractant gradient to establish cell-cell contact and fusion. Using strains co-expressing Lifeact-TagRFP and β-tubulin-GFP we recently analyzed the interrelated localization pattern and dynamic rearrangement of F-actin and microtubules during conidial germination and cell fusion. We found that recruitment of both cytoskeletal elements occurs in a distinct but coordinated manner that might influence which protrusion is being formed and maintained at any point in time. Molecular mechanisms involving cortical markers whichlocally and specifically recruit the cytoskeleton in order to achieve re-orientation during germ tube avoidance and CAT homing, are virtually unknown in *Neurospora*. Focused activation of Rho GTPases, such as CDC42 and RAC-1, at the cell cortex is a key determinant of polarized F-actin organization, and thought to occur in sterol-rich microdomains within the plasma membrane. Displacement of these microdomains in response to external stimuli might regulate directional growth. Interestingly, Δ*rac-1* mutants of *Neurospora* still show germ tube avoidance but are unable to undergo CAT-mediated cell fusion. To study the spatial and temporal relationship between GTPase activity and F-actin rearrangement during tip orientation in more detail, we engineered a green fluorescent Cdc42-Rac-interactive binding (CRIB) biosensor for activated GTPases and co-expressed it with Lifeact-TagRFP-T.

PR6.24

Novel peroxin, Fam1p of *Colletotrichum orbiculare* is essential for pathogenesis and associates with Woronin bodies

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The cucumber anthracnose fungus *Colletotrichum orbiculare* forms an infection structure called an appressorium. Melanization of appressoria is essential for host penetration and requires the β-oxidation of fatty acids in peroxisomes. Here, we identified and characterized a protein specific to filamentous ascomycete fungi, Fam1, that is essential for peroxisome function and which associates with Woronin bodies. The *FAM1* gene was isolated by screening random insertional mutants for deficiency in fatty acid metabolism. The *fam1* disrupted mutants were unable to grow on medium containing oleic acids as the sole carbon source. Green fluorescent protein carrying the peroxisomal targeting signal 1 (PTS1) or PTS2 were not imported into peroxisomes of *fam1* mutants, suggesting that *FAM1* is a novel peroxisomal biogenesis gene (peroxin). Accordingly, *fam1* mutants were defective in both appressorium melanization and host penetration. Microscopy showed that the Fam1p-GFP fusion protein localized to small punctate structures in the apical region of hyphae, near septa and adjacent to peroxisomes. This resembled the distribution of Woronin bodies, which are peroxisome-derived organelles involved in septal pore plugging. After cloning *CoHEX1*, a homolog of the *HEX1* Woronin body structural gene, we generated a Cohex1p-mRFP1 fusion protein, which co-localised with Fam1p-GFP in Woronin bodies. Furthermore, the apical and septum localization of Fam1p was impaired in *cohex1* mutants. Our results indicate that Fam1p is a novel Woronin body-associated protein and raise the possibility that filamentous ascomycete fungi coordinate peroxisome function *via* Woronin bodies.

PR6.25

NOX genes of *Cochliobolus heterostrophus*: role in ROS production, development and virulence

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Reactive oxygen species (ROS) are synthesized by specific NADPH oxidases (NOX), enzymes inserted in the plasma membrane. NOX enzymes use cytoplasmic NADPH to produce superoxide. ROS can provide both defense and differentiation signaling roles in animals and plants. Fungal NADPH oxidases have a structure very similar to the human gp91phox. Specific isoforms of fungal NOX have been reported to be required for various physiological processes and cellular differentiation events, including development of sexual fruiting bodies, ascospore germination, and hyphal growth in both mutualistic and antagonistic plant–fungal interactions. We identified three NADPH oxidase homologues in the necrotrophic filamentous fungus *Cochliobolus heterostrophus*, and we set out to investigate their function and importance in the fungal life cycle through study of loss-of-function mutants in genes encoding catalytic and regulatory subunits of NADPH oxidases. Mutants in noxA, noxC and the predicted regulatory subunit gene noxR have decreased pigmentation and delayed conidiation. noxA and noxR show decreased virulence on the host plant, maize. All nox mutants produce superoxide as detected by nitro blue tetrazolium (NBT) staining. We are investigating ROS production in single and multiple *nox* mutants under different conditions.

PR6.26

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

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Many cells and organisms go through polarized growth phases during their life. Cell polarization is achieved by local accumulation of signaling molecules which guide the cytoskeleton and vesicular trafficking to specific parts of the cell and thus ensure polarity establishment and maintenance. Polarization of signaling molecules is also fundamental for the lifestyle of filamentous fungi such as *Aspergillus niger* and essential for their morphogenesis, development and survival under environmental stress conditions. Considerable advances in our understanding on the protagonists and processes mediating polarized growth in filamentous fungi has been made over the past years. However, how the interplay of different signaling pathways is coordinated has yet to be determined. We found recently that the *A. niger* RmsA protein is central for the polarization of actin at the hyphal tip (1). However, we show here that RmsA is also of vital importance for the metabolism, viability and stress resistance of *A. niger*. This suggests that RmsA could occupy an important position in the global network of pathways that balance growth, morphogenesis and survival of *A. niger*.

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PR6.27

Subcellular localisation of AreA and MeaB is driven by different nitrogen sources.

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In *A. nidulans* the utilisation of nitrogen sources other than ammonium or glutamine is dependent on the transcriptional factor AreA, which enables transcription of a broad range of genes specifically under nitrogen limitation. A second transcription factor, MeaB, also plays a role in the cellular response to nitrogen availability. Although the function of MeaB is less well characterised it appears, at least in part, to act in opposition to AreA, being most active under nitrogen sufficiency. Both AreA and MeaB have been tagged with GFP and their intracellular localisation monitored in response to changes in nitrogen regime using confocal microscopy. As controls histone H1:RFP was used to identify the nuclei and mitochondria were stained with MitoTracker. The subcellular localisation of MeaB and AreA both respond to changes in nitrogen regime. In the case of AreA, under conditions of nitrogen repression (eg Gln or NH_4^+) the protein appears to be distributed throughout the cell. Surprisingly under nitrogen derepression conditions (eg NO_3^- or –N) AreA is primarily localised to the mitochondria and not the nuclei. In the case of MeaB, growth on Gln or NH_4^+ results in localisation in and around the nuclei and within the mitochondria. In the presence of NO_3^- or during nitrogen starvation MeaB accumulates exclusively in mitochondria, although this is not the case with all deprepressing nitrogen sources (eg proline) where MeaB is evenly distributed throughout the cell. In *Fusarium fujikuori* subcellular localisation of MeaB is also subject to nitrogen regulation and, as in *A. nidulans*, it is located in the nucleus under nitrogen repressing conditions. However, in contrast to the *A. nidulans*, there was no obvious accumulation of MeaB within the mitochondria under the regimes tested. In summary, as expected AreA and MeaB both show very distinct response to nitrogen availability. Unexpectedly, in *A. nidulans* both transcription factors localise to the mitochondria under nitrogen regimes.

PR6.28

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

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A characteristic hallmark of *Aspergillus niger* is the formation of black conidiospores. In this study, we report the characterization of a color mutant, *colA*, which was isolated because of a complete loss of pigmentation resulting in white conidia. Pigmentation of the *colA* mutant was restored by a gene that encodes the *A. niger* ortholog of the 4'-phosphopantetheinyl transferase protein (PptA). 4'phosphopantetheinyl transferase activity is required for the activation of Polyketide synthases (PKSs) and Non-Ribosomal Peptide Synthases (NRPSs) (1). Complementation analysis showed that the *colA* mutant is allelic to a previously isolated color mutant, *gryA.* Sequencing of the *colA* and *gryA* loci and the targeted deletion of the *pptA* gene further confirmed that the *colA /gryA* mutants are mutated in the *pptA* gene. Spores from the *ΔpptA* deletion are paler in color that spores of an *A. niger* strain disrupted in the *pksA* gene. PksA encodes the polyketide synthase required for melanin biosynthesis (2) and spores from the *pksA* disruption strain become fawn colored. Spores from both the *ΔpksA* and the *ΔpptA* were hypersensitive to UV-radiation indicating that melanin is required for resistance against UV-radiation. The *ΔpksA* strain was equally sensitive to hydrogen peroxide as the parental strain, but spores from the *ΔpptA* strain showed increased sensitivity. The results suggest the involvement of PKS or NRPS-derived metabolites that confer resistance towards oxidative stress conditions.

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Poster Category 7:

Fungal and Oomycete Effectors

PR7.1

GEMO: Evolutionary Genomics of *Magnaporthe oryzae*

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Developing integrated control methods against pests of cultivated plants can significantly contribute to increasing food production while reducing inputs threatening the environment. The durability of a control method can be improved by a better knowledge of the pathogen's genetic determinants that are responsible for this adaptation. We were granted by the French National Research Agency for a project that aims at sequencing the genomes of several strains of the phytopathogenic model species *Magnaporthe oryzae* and at exploiting these complete sequences to characterize the repertoire of genes involved in pathogenicity and host specificity, and study their evolution. We will sequence 7 strains of the species *M. oryzae* representing different genetic groups pathogenic of different species of Poacees and one strain of the sister species *M. grisea*. ESTs produced during the infection by two strains pathogenic of rice and wheat on their respective host will also be sequenced. Different available annotation pipelines will permit to list and do comparative analyses of different gene families known or speculated to be involved in pathogenicity. Transcriptomic data of the two strains with different host specificities will be compared to identify key genes in specialization to the host. Genome fluidity will be characterized by synteny analyses and by the identification and localization of repeated elements. The impact of these rearrangements on pathogenicity genes and host specificity genes will be tested. Molecular signatures of positive or purifying selection in coding and regulatory sequences will be searched for by different methods. The whole set of data will be integrated in a database that will be designed to be accessible publicly.

PR7.2

Molecular characterisation of effector proteins from the fish pathogenic oomycete *Saprolegia parasitica*

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Water molds (oomycetes) are destructive pathogens of aquatic animals and terrestrial plants. *Saprolegnia* species cause Saprolegniosis, a disease that is characterized by visible white or grey patches of filamentous mycelium on the body or fins of freshwater fish. *Saprolegnia parasitica* is economically one of the most important fish pathogens, especially on catfish, salmon and trout species, causing millions of dollar losses to the aquaculture business worldwide. Several *Saprolegnia* species have also been linked to declining wild fish stocks and amphibian populations around the world. Currently, the genome of *S. parasitica* (isolate CBS223.65) is being sequenced by combined Sanger, 454 and Illumina data. The annotation is supported by paired-end EST sequences. Analysis of the preliminary genome sequence and EST libraries resulted in the identification of putative effector proteins. Electron microscope analysis showed that *Saprolegnia* interacts with fish cells by forming haustoria-like structures. Detailed expression studies of the genes encoding the putative effectors were performed during the biotrophic and necrotrophic infection stages of *S. parasitica*. Also localization and uptake studies were performed to show a role of the effector proteins in the interaction of *S. parasitica* with a rainbow trout cell-line.

PR7.3

Genome sequence of anamorphic basidiomycetous yeast *Pseudozyma antarctica*

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Pseudozyma antarctica (renamed from *Candida antarctica*) is an anamorphic basidiomycetous yeast, which is closely related to the corn smut fungus *Ustilago maydis*. *P. antarctica* is known to produce the two different lipases, lipase A and B, which are useful biocatalysts with a broad range of industrial applications. *P. antarctica* was also found to produce itaconic acid, which is used in the manufacture of synthetic resins, coating, and other industrial products. Interestingly, *P. antarctica* is able to produce a large amount of the extracellular glycolipid biosurfactants, mannosylerythritol lipids (MELs) from a variety of biomass such as vegetable oils, glycerol, and some sugars. MELs show not only excellent surface-active properties but also versatile biochemical actions, and thus have been received increasing attentions as new bio-based materials [1]. Previously, in order to apply genetic engineering to the large-scale production of MELs, we carried out the expressed sequence tags (EST) analysis of *P. antarctica* T-34, and described the genes expressing under the MEL production conditions [2]. Consequently, we believe that the genomic information of the basidiomycetous yeast should allow us to develop the novel processes for producing many practical bio-products such as proteins, organic acids, sugars, and glycolipids. Furthermore, the comparative analysis of the genome sequences between *P. antarctica* and *U. maydis* would give the important knowledge for plant infection mechanism. We thus focused our attention on the genome sequence of *P. antarctica*. Here, we present the draft genome sequence of *P. antarctica*, the putative genes derived from the previous result of EST analysis. This study was supported by the Industrial Technology Research Grant Program in 06A17501c from the New Energy and Industrial Technology Development Organization (NEDO) of Japan. [1] Morita et al., *Biotechnol Appl Biochem*, 53, 39-49, 2009. [2] Morita et al., *Yeast*, 23, 661-671, 2006.

PR7.4

The whole genome sequence of *Venturia inaequalis*, the causal agent of apple scab, using next generation sequencing technology: implications for identifying candidate effector genes

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The hemi-biotrophic fungus *Venturia inaequalis* infects members of the Maloideae, causing the economically important apple disease, scab. The genetics of the interaction between *Malus* and *V. inaequalis* follow the gene-for-gene model; effectors (pathogen proteins required for infection) are presumably secreted to the plant/pathogen interface early during the infection cycle where a subset can be recognised by plant resistance gene products to induce a hypersensitive response. Previously, expressed sequence tag and proteomic approaches were adopted to identify candidate effector genes but were limited in their success by the extent of sequence coverage. Therefore, sequencing the whole genome of an isolate of *V. inaequalis* theoretically carrying a full complement of effectors was performed using Illumina technology. A total scaffold length of 36Mb was assembled at an estimated 96% coverage of genes calculated by mapping 131 SSR markers and ESTs. Orthologues of several fungal effector genes were identified including *Ecp6*, *AvrLm6* and *Avr-Pita*. Previously, using reverse genetics, we had obtained amino acid sequence information from a candidate AvrRvi4,5 effector that elicited a hypersensitive response on hosts 4 and 5. This sequence exactly matched part of a predicted novel 154-amino acid protein in the whole genome sequence which has a leader sequence and eight cysteines. Functional analysis of this and other candidate genes is currently underway by silencing/disruption. Further sequencing, including transcriptome analysis and mate-end paired reads, will be carried out to reduce the number of scaffolds in the assembly and to aid annotation.

PR7.5

Functional analysis of Six proteins: effectors of *Fusarium oxysporum*

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The plant xylem-colonizing fungus *Fusarium oxysporum* f.sp. *lycopersici* (Fol) secretes small proteins into xylem sap during colonization of its host, tomato. We call these small proteins 'Six' proteins for 'Secreted in xylem'. Through gene knock-out and complementation we established that several Six proteins are required for full virulence. In addition, three Six proteins trigger R gene-dependent immunity and are therefore called Avr1, Avr2 and Avr3.

Some *SIX* genes are activated specifically upon entry into roots, and expression of all *SIX* genes investigated is fully dependent on the transcription factor Sge1 ('Six gene expression 1'). In accordance with this, strains deleted for *SGE1* are non-pathogenic and are inhibited in growth inside tomato roots.

Results of transient expression assays in leaves of *Nicotiana benthamiana* suggest that some Six proteins can suppress disease resistance reactions (hypersensitive response) while others enhance these reactions. Our aim is to uncover the molecular mechanisms underlying these effects.

PR7.6

Chasing effectors in the secretome of *Melampsora larici-populina*, the causal agent of poplar leaf rust

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The foliar rust caused by *Melampsora larici-populina* is the main disease affecting poplar plantations in Northern Europe with severe economic losses. In the wake of the Populus genome sequencing, the ~100 Mb genome of M. larici-populina have been sequenced (7X depth) by the Joint Genome Institute (JGI, Department of Energy, USA). The analysis of this genome is a great opportunity to identify loci coding for small-secreted proteins (SSP) produced by the rust fungus to penetrate and exploit its host. The genome sequence of M. larici-populina has revealed a large arsenal of approximately two thousand secreted proteins, half corresponding to SSP (≤ 300 aa). Similarities with effectors previously described in Pucciniales were also uncovered, such as homologs of avirulence factors from *Melampsora lini* or of the Rust Transferred Protein RTP1 from *Uromyces fabae*. Transcriptome analyses based on 454-pyrosequencing and NimbleGen systems oligonucleotide arrays allowed to identify transcripts encoding SSP specifically and highly expressed during parasitic growth. Expression profiles of these candidates were monitored by RT-qPCR in *M. larici-populina* during infection of poplar leaves, showing a preferential expression after haustorium formation. Immunolocalization of selected SSP containing RxLR-like motif using confocal laser scanning microscopy indicated accumulation around haustorial structures, likely in the extra-haustorial matrix. Such approaches led to the identification of candidate rust effectors, for which functional characterisation is ongoing.

PR7.7

Identification and characterization of candidate effector proteins secreted by the crucifer anthracnose fungus *Colletotrichum higginsianum*

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The hemibiotrophic ascomycete *Colletotrichum higginsianum* causes anthracnose disease on brassica crops and the model plant *Arabidopsis thaliana*. Successful plant infection requires the development of a specialized infection structure called an appressorium and the establishment of biotrophic hyphae inside living epidermal cells. We hypothesize that appressoria and biotrophic hyphae secrete effector proteins that permit the fungus to evade or disarm host defence responses and to reprogram host cells. As a first step towards the discovery of secreted effectors in *C. higginsianum*, we have generated expressed sequence tags (ESTs) from appressoria grown *in vitro*, isolated biotrophic hyphae and appressoria piercing the epidermal cell wall. Biocomputational prediction tools were used to identify small, soluble secreted proteins showing no homology to known proteins – hallmarks of many microbial effectors. *Colletotrichum higginsianum* effector candidates (ChECs) that were upregulated at developmental stages relevant to the establishment of biotrophy were selected for functional analysis. This included targeted gene disruption in *C. higginsianum*, localization in infected plants by over-expression of tagged proteins, and transient *in planta* expression assays to assess the ability of selected effectors to suppress elicitor-induced cell death. ChEC3 and its paralogue ChEC3a, both displaying weak similarity to *C. gloeosporioides* CgDN3, were found to suppress necrosis induced by *C. higginsianum* Nep1-like protein 1 (ChNLP1). Effector ChEC4, containing a predicted nuclear localization signal, was found to target the plant nucleus when expressed *in planta* as a GFP fusion protein suggesting that this effector has the potential to interfere with host gene expression.

biotrophy, Colletotrichum higginsianum, Effectors, expressed sequence tags ,

PR7.8

Genome mining and functional genomics of small secreted proteins (SSPs) in *Cladosporium fulvum, Mycosphaerella graminicola* and *M. fijiensis*

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Fungal pathogens secrete effector proteins to target and manipulate host plants for successful infection. They primarily function as virulence factors but during co-evolution plants have evolved resistance proteins to recognize them resulting in effector-triggered immunity (ETI). Successful pathogens, in turn, evolved additional effectors to evade recognition or to suppress ETI resulting in host susceptibility. In this study we mined the genomes of *Cladosporium fulvum, Mycosphaerella graminicola* and *M. fijiensis* and identified 289, 266 and 180 SSPs, respectively, that represent a common feature of effector proteins (<300 aa residues and containing ≥4 cysteine residues). We used a combined proteomics and expression profile approach and selected over 100 SSPs for further functional analysis. The SSPs are cloned into an *Agrobacterium tumefaciens* overexpression vector with or without the PR-1A signal peptide sequence for transient expression *in planta*. We are testing the effect(s) of SSPs in suppression of HR or necrosis induced by Inf1, CfHNNI1, BAX or Cfs/Avr gene pairs in tobacco plants. The role of some promising candidates in virulence will be assayed by gene knock-out studies. In addition, we will produce *M. graminicola* SSPs in an *Escherichia coli* heterologous expression system and purify them using FLAG affinity purification. Subsequently, *M. graminicola* SSPs will be infiltrated in a set of differential cultivars to identify SSPs that are able to cause HR or necrosis. Systemic overexpression of *M. graminicola* SSPs will be performed using the BSMV-mediated expression system in wheat differentials. Preliminary data and comparison of SSPs from the different fungal pathogens will be discussed.

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PR7.9

Characterisation of two necrosis and ethylene inducing protein-like (Nlp) genes from *Sclerotinia sclerotiorum*

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The necrotrophic phytopathogen, *Sclerotinia sclerotiorum,* induces plant cell death in order to colonise host plants and release nutrients. This pathogen is known to secrete various compounds, including oxalic acid and lytic enzymes during infection. Infection is often facilitated in other necrotrophs (such as *Fusarium oxysporum* and *Botrytis cinerea*) by the secretion of small, phytotoxic effector molecules (including necrosis and ethylene inducing peptides, NEPs). We have cloned two genes from *S. sclerotiorum* with significant similarity to *NEP*s called *NEP-like1* (*Nlp1*) and *Nlp2*. Both NLPs appear to induce necrosis when transiently expressed in *Nicotiana tobaccum* and *N. benthamiana*. Multiple 35S fusion constructs, designed to investigate the effect of *in planta* NLP protein expression (with or without signal peptides) have also been completed and transformations are currently underway. Both genes have also been expressed in *Pichia pastoris* resulting in purified NLP1 and NLP2 that will be used for further transient assays to assess the specific response induced in host plants when presented with one or both proteins. Multiple Nlp1RNAi mutant lines have been generated which display inhibited growth rates both *in vitro* and *in planta*; further characterisation of these will be presented. Expression localisation studies are also underway using *NLP* promoter:GFP fusion constructs in transformed *S. sclerotiorum*.

PR7.10

Identification of a *Sporisorium reilianum* effector involved in host specificity

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Sporisorium reilianum and *Ustilago maydis* are smut fungi with a narrow host range. *U. maydis* and the *S. reilianum* variety SRM produce smut symptoms only on maize, while the *S. reilianum* sorghum variety SRS produces spores on sorghum. Microscopic analysis showed that after host plant penetration *S. reilianum* reaches the inflorescence via growth along the vascular bundles. SRS hyphae can also ramify in maize plants and reach the inflorescence but do not differentiate into spores. We wanted to identify genes that support virulence on maize but hamper virulence on sorghum. Using genome comparison and heterologous PCR/Southern analysis we identified three putative candidate genes that are present in the maize pathogens *U. maydis* and SRM but absent in the sorghum pathogen SRS. One of these genes, *c1*, encodes a secreted protein that is highly conserved between *U. maydis* and SRM. Deletion of *c1* in *U. maydis* drastically reduced virulence. Expression of *c1* of SRM in SRS led to strains that more efficiently reached the inflorescence as deduced by an increased incidence of phyllody – a typical symptom associated with *S. reilianum* infection. This shows that the identified effector functions as a virulence factor for maize.

Expression of *c1* in SRS led to the appearance of colored spots on veins of infected sorghum plants indicating a host defense response. In addition, virulence on sorghum was reduced, suggesting that *c1* functions as a virulence-attenuating factor for sorghum. Thus, the identified effector contributes to host specificity of *S. reilianum*.

PR7.11

Hunting for effectors-elicitors in the fungal wheat pathogen *Mycosphaerella graminicola* with a quantitative proteomic approach using comparative (label-free) LC-MSE

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Septoria tritici blotch caused by the haploid ascomycete *Mycosphaerella graminicola* (Fuckel) J. Schröt. in Cohn, is the most important wheat disease in Europe. Despite the recent identification of 15 resistance genes and their potential application in breeding, disease control is currently achieved mainly by fungicides. The genome of *M. graminicola* has been sequenced and finished by the US. Department of Energy- Joint Genome Institute and together with the high quality of the genome annotation, the high-density genetic linkage maps, including 11 quantitative trait loci involved in species specificity as well as in cultivar-specificity (Ware et al. 2006; Wittenberg et al. 2007) this provides an excellent foundation for proteomic studies that focus on the pathogen side of the interaction. The lifestyle of *M. graminicola* is significantly different from other cereal pathogens. It is a hemibiotroph with an initial symptomless biotrophic and intercellular phase that is followed by a necrotrophic phase resulting in disease symptoms starting from approximately 14 days after infection. The switch from biotrophy to necrotrophy is poorly understood and we therefore have started experiments using established protocols for apoplast analysis from the *Cladosporium*-tomato pathosystem. Extracellular liquid (apoplast) was collected from (in)compatible interactions at several time points after inoculation. Proteins were extracted and digested with trypsin. The complex peptide digests were separated and detected with nano-UPLC-QTOF operating in an alternating mode of low and high collision energy. With this approach peptide abundances can be quantitatively compared between multiple complex protein samples. In the initial experiment 18 LCMS traces (6 samples in triplicate) were compared providing highly detailed quantification and identification. Using the identification algorithm 3150 peaks were identified, resulting in 1926 unique peptide sequences that were identified and quantified. We identified several proteins of *M. graminicola* that appear to be secreted in specific stages of the infection to the apoplast and currently study these candidate effectors of the *M.graminicola*-wheat interaction.

PR7.12

Trehalose synthesis in *Aspergillus niger* and other fungi

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The disaccharide trehalose has several important roles in fungi, e.g. in stress protection, spore germination and virulence. It also serves as a major component in asexual and sexual spores. In order to reveal the exact function of this extraordinary sugar, it is essential to identify and reveal the functions of the enzymes involved and how these are regulated. In filamentous fungi some components have been identified, mostly in *Aspergillus nidulans*, but no overall characterization has been performed. In *Saccharomyces cerevisiae* the four enzymes behind the pathway from glucose to trehalose are characterized. Using bioinformatics we found that in other yeasts and in filamentous fungi the protein composition differs substantially. In some sequenced Aspergilli, including *A. niger*, *A. flavus* and *A. fumigatus* six homologues proteins were identified. We call the enzymes with proposed trehalose phosphate synthase activities TpsA, TpsB and TpsC. Three proteins with believed phosphatase activities are called TppA, TppB and TppC. We have started a comprehensive characterization of all enzymes using *A. niger* as target organism. In most deletion mutants, the content of trehalose was initially reduced but after maturation there were no significant differences compared to wildtype. Two notable exceptions: In the *tppA* deletion mutant only abnormal conidiophores were formed and in the very few conidia we could detect only traces of trehalose. The other exception, the *tppB* mutant, had wildtype-like conidiephores but with a severely reduced conidial trehalose content. This mutant is a usable tool to reveal the role of trehalose in spore germination and survival.

PR7.13

The transcriptome of an intracellular hemibiotroph, *Colletotrichum higginsianum*

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Colletotrichum higginsianum causes anthracnose disease on *Arabidopsis thaliana*, providing a model pathosystem in which pathogen and host genomes are available and both partners can be genetically manipulated. After initial penetration by appressoria, the fungus grows biotrophically in living epidermal cells, producing bulbous hyphae that invaginate the host plasma membrane, before entering a destructive necrotrophic phase. To survey fungal gene expression during biotrophy, we made a stage-specific cDNA library from hyphae isolated from infected leaves by fluorescence-activated cell sorting. The high purity of the isolated hyphae eliminates contamination by transcripts from host cells or other fungal cell types. EST sequencing showed that genes related to redox homeostasis, biosynthesis of amino acids and vitamins and the uptake of amino acids, mono- and disaccharides were well-represented. To search for fungal effectors, we used computational prediction tools to identify genes encoding small, soluble secreted proteins. Expression profiling showed that many candidate effector genes are plant-induced and highly stage-specific. One such gene, CIH1, encodes a protein with two LysM chitin-binding domains that accumulates at the biotrophic interface and may function in PAMP concealment. Targeted gene disruption suggested that CIH1 is required for the establishment of intracellular biotrophic hyphae. In *Nicotiana benthamiana* transient expression assays, one candidate effector induced plant cell death, while six others suppressed necrosis induced by a *C. higginsianum* Nep1-like protein 1. Overall, our results suggest that *Colletotrichum* biotrophic hyphae are organs for both nutrient acquisition and effector delivery.

PR7.14

Secreted oomycete proteins in *Phytophthora* rot of garden pea

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Host-pathogen interactions rely on secreted proteins. Studying the secretome is therefore a suitable way to identify genes implicated in pathogenicity. Here, we present a project to investigate the interaction between pea and a root-rot pathogen, a mostly uncharacterized *Phytophthora sp.*, by identifying proteins secreted by pea and oomycete during infection. To this end, *Phytophthora sp.*-infected pea roots are used to produce dual organism cDNA libraries. These libraries are screened for cDNA sequences encoding relevant proteins that are then further characterized. Of interest are both enzymes to breakdown tissue into nutrients and other proteins that function directly in virulence. Through the secretome studies we will deepen the understanding of the novel *Phytophthora* species and its function as causal agent of an economically important pea disease.

PR7.15

Functional analysis of homologues of the *Cladosporium fulvum* Avr4 and Ecp2 effectors present in other (pathogenic) fungal species

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Cladosporium fulvum is a non-obligate biotrophic fungus of the Dothideomycetes class that causes leaf mould of tomato. During infection, *C. fulvum* secretes effectors that function as virulence factors in the absence of cognate Cf resistance proteins and induce effector-triggered immunity in their presence. Recently, homologues of the *C. fulvum* Avr4 and Ecp2 effectors were identified in species of Dothideomycetes, including *Mycosphaerella fijiensis* the causal agent of the black Sigatoka disease of banana. We have demonstrated that the *M. fijiensis* Avr4 is a functional orthologue of the *C. fulvum* Avr4 that binds to chitin and triggers a Cf-4-mediated hypersensitive response (HR) in tomato, suggesting that a common recognition site in the two effectors is recognized by the Cf-4. Using a targeted mutational approach, we are examining whether the chitin-binding domain present in these two effectors represents this recognition site. Three homologues of the *C. fulvum* Ecp2 are found in *M. fijiensis,* two of which induce different levels of necrosis or HR in tomato lines that lack or contain a cognate Cf-Ecp2 protein. Therefore, Ecp2 is suggested to promote virulence by interacting with a putative host target, causing host cell necrosis. Using a yeast-two-hybrid assay we will try to isolate Ecp2-interactors from tomato to further unravel the role of this effector in virulence. Finally, we are expanding our searches for Avr4 and Ecp2 homologues in fungal species outside the class of Dothideomycetes. Preliminary data suggest that Ecp2 is widely distributed among fungal species but has significantly diverged after speciation of these fungi.

PR7.16

Dissecting the role of *Cladosporium fulvum* (secreted) proteases and protease inhibitors

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In order to facilitate infection, fungal pathogens produce various types of secreted proteases likely to target and perturb important plant proteins that are involved in controlling basal defense. In addition, they secrete several protease inhibitors such as Avr9 of *Cladosporium fulvum* that, based on its structure, is predicted to be a carboxy peptidase inhibitor. Protease inhibitors are potentially able to deactivate or detoxify host target proteases and, therefore, might play an important role during infection. In this study we are investigating the role of *C. fulvum* protease and protease inhibitors in disease establishment by using both functional genomics and biochemical approaches. We mined the genome of *C. fulvum* and found numerous proteases and protease inhibitors of which many are secreted. Expression analyses of these genes were performed using RNA extracted from fungal mycelium grown *in vitro* on liquid media under different conditions as well as from inoculated susceptible tomato plants. Interestingly, many of these genes are highly expressed only *in vitro* and/or *in planta* and based on their expression profiles we selected a number of candidates for further functional analyses. We will generate knock-out mutants of the selected proteases and protease inhibitors to identify their role in virulence. In addition, biochemical approaches will be used to pull-down the host target proteins of some presumably important candidate proteins such as for example the Avr9 protein.

PR7.17

Identification and functional characterization of *Cladosporium fulvum* effectors by genomics, transcriptomics and proteomics approaches

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Cladosporium fulvum is a biotrophic fungal pathogen that causes leaf mold of tomato. During infection *C. fulvum* secretes a number of small proteins into the apoplast of tomato leaves, which are collectively called effectors. So far, ten effector proteins have been characterized that in general show no or limited sequence similarity to other proteins present in public databases. In this study we try to identify and functionally characterize additional *C. fulvum* effector proteins that are involved in fungal pathogenesis. Recently, the genome of *C. fulvum* has been sequenced using the 454 technology. This genome sequence enables the identification of all secreted proteins from the fungus, collectively called the secretome. However, for accurate mining and annotation of the effector secretome, gene calling programs need to be first optimized by analyzing high quality expressed sequence tags (ESTs). Therefore, several cDNA libraries of *C. fulvum* grown under various *in vitro* and *in planta* conditions were constructed and are sequenced to support genome annotation. Initial automated annotation of the genome revealed that the fungus contains approximately 13.000 genes, of which approximately 1200 encode putatively secreted proteins. Bioinformatic analyses identified a subset of 300 putative effectors within the predicted secretome, while additional proteomics analysis from apoplastic fluids of tomato leaves infected by *C. fulvum*, revealed 30 proteins that are specifically produced in the compatible interaction. At this moment we are performing functional profiling of these novel effector proteins by examining their ability to inhibit PAMP triggered Immunity and/or effector-triggered immunity in custom made assays.

PR7.18

Identification and analysis of secreted proteins from the maize pathogen *Colletotrichum graminicola*

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The filamentous Ascomycete *Colletotrichum graminicola* is the causal agent of stem rot and leaf anthracnose on *Zea mays*. After germination and penetration of epidermal cells, this hemibiotrophic fungus enters a short biotrophic phase that is followed by a destructive necrotrophic phase resulting in the production of conidia. Secreted fungal proteins are believed to play important roles in the progress of both phases of pathogenesis. The Yeast Secretion Signal Trap (YSST) was used to identify cDNAs encoding peptides containing signal sequences, starting from mRNA from *in vitro*-grown mycelium, induced with a corn leaf-extract. Of the 94 obtained sequences, 45 showed significant similarities to genes with a reported function, 24 were similar to genes annotated in fungal genome projects and 27 showed no similarity to database entries. Macroarray hybridisation showed that most of these genes are expressed *in planta*. Transcript abundance of most genes peaks at specific periods during pathogenesis, while some are expressed constantly. A minor set exhibits two peaks and a minimum during the biotrophy-necrotrophy transition phase. Expression patterns of several genes from each set were confirmed by qRT-PCR.

To test possible roles of secreted proteins as pathogenicity or virulence factors, knock-out strains were generated for 18 genes identified in the screen, encoding proteins of various classes: enzymes, small cystein-rich proteins and proteins of unknown function. Results from infection assays will be presented.

PR7.19

Crystal structure of the avirulence gene AvrLm4-7 Of *Leptosphaeria* maculans illuminates its evolutionary and functional characteristics

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Leptosphaeria maculans, a phytopathogenic ascomycete causing stem canker of oilseed rape, develops “gene-for-gene” interactions with its host plant where fungal avirulence (*AvrLm*) genes are the counterpart of plant resistance (*Rlm*) genes. *AvrLm4-7* encodes a 143 amino-acid cysteine-rich protein, secreted outside of the fungus cells and strongly induced during the early stages of plant infection. AvrLm4-7 can bind to phosphoinositides and is translocated within plant cells. AvrLm4-7 crystal structure was determined following heterologous production in *Pichia pastoris*. The protein shows the presence of 4 disulfide bridges, and is strongly positively charged, suggesting interaction with minus charged molecules such as DNA or phospholipids. *AvrLm4-7* confers a dual specificity of recognition by *Rlm7* or *Rlm4* resistance genes and occurs as three alleles only: the double avirulent (A4A7), the avirulent towards *Rlm7* only (a4A7), or the double virulent (a4a7). Sequencing of diverse alleles coupled with targeted point mutagenesis strongly suggested that one single base mutation, leading to the change of a glycine residue to an arginine, was responsible for the A4A7 to a4A7 phenotype change. This amino acid being located on an external loop of the protein, its change is unlikely to alter the 3-D structure of the protein, but rather must correspond to a specific recognition target for the plant cell. In contrast, multiple mechanisms are responsible for the complete loss of avirulence (a4a7 phenotype), mostly drastic events leading to inactivation or complete deletion of the gene. The few single point mutations found targeted amino acids essential for the 3-D structure of the protein.

PR7.20

Functional characterization of LaeA

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Here we present the initial findings of a biochemical and genetic investigation into the mechanism of LaeA, a putative methyltransferase that functions as a global regulator of secondary metabolism in Aspergillus nidulans. LaeA has been found to be part of a large nuclear velvet complex that is required for secondary metabolite production as well as light regulated morphological development. Preliminary data suggests LaeA may control secondary metabolite gene clusters through chromatin remodeling. However, there is no direct evidence linking the velvet complex to chromatin remodeling. We have initiated a study to functionally characterize LaeA. Several LaeA orthologs were recombinantly expressed in E. coli and assayed for solubility. The full length LaeA protein from A. nidulans is only soluble as a MBP fusion protein, which has proved to be uninformative for in vitro activity assays. A partial proteolysis study was performed to identify soluble domains that could be amenable to in vitro analysis. Soluble truncation mutants were identified and have proved useful for in vitro methyltransferase activity assays. Validation of the truncated LaeA proteins was carried out through successful in vivo complementation of a ΔlaeA mutant. These truncation mutants are functionally equivalent to the full-length protein by restoring sterigmatocystin (ST) biosynthesis to wild type levels. Using the truncated LaeA protein, we have confirmed binding of S-adenosyl-L-methionine (methyl group donor) and have identified a methyltransferase activity. Each candidate protein substrate's site of methylation is being mapped by trypsin digestion coupled with LC/MS. The in vivo role of LaeA methylation will be evaluated with point mutants for effects on ST biosynthesis. Our findings confirm LaeA has methyltransferase activity and provide the first functional insights into the mechanism of LaeA regulation of secondary metabolism.

PR7.21

Cloning and characterization of SnTox1, a novel virulence effector gene important in the wheat-*Stagonospora nodorum* interaction

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Stagonospora nodorum blotch caused by *S. nodorum* (teleomorph *Phaeosphaeria nodorum*) is a destructive disease of wheat that causes significant yield and quality losses worldwide. Although this disease is a major concern for breeders, it has been difficult to characterize the interaction due to its quantitative nature. We have shown that the quantitative nature of the S. nodorum-wheat pathosystem is due at least in part to a complex of proteinaceous host-selective toxins (virulence effectors) that interact either directly or indirectly with dominant host sensitivity/susceptibility gene products in an inverse gene-for-gene manner. SnToxA, which was originally identified in Pyrenophora tritici-repentis as well as SnTox1, SnTox2, SnTox3, and SnTox4 have each been shown to be highly important in disease development in the presence of the corresponding dominant wheat sensitivity genes, Tsn1, Snn1, Snn2, Snn3 and Snn4, respectively. The SnTox1-Snn1 interaction was the first to be identified and this interaction was shown to account for as much as 58% of the disease development. Recently, we have cloned the gene responsible for the production of the SnTox1 protein. The mature SnTox1 is highly cysteine rich and predicted to be approximately 10 kDa. As expected, the immature protein contains a 20 amino acid predicted signal sequence. The cloning of SnTox1 adds another piece of information to this already fascinating system of virulence effectors produced by *S. nodorum*.

Poster Category 8:

Fungal Biotechnology

PR8.1

Recombinant expression in *Trichoderma reesei* for improved hydrolysis of pretreated corn stover

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The majority of commercial enzyme products for biomass hydrolysis are produced by the saprophytic mesophilic fungus *Trichoderma reesei*. *Trichoderma* produces two cellobiohydrolases (CBHI and CBHII), five endoglucanases (EGs), and two β-glucosidases (BGs). This mix of enzymes is relatively efficient at cellulose degradation and large quantities of these proteins are secreted from the fungus. However, improvements in the total enzyme specific activity and secretion yield may improve this enzyme mix. A primary factor in the high cost of enzymes for biomass hydrolysis is the amount of enzyme that must be applied for efficient cellulose conversion to glucose. Compared with starch hydrolysis, 15-100fold more enzyme is required to produce an equivalent amount of ethanol, depending on specific process conditions. It is well known that efficient cellulose hydrolysis requires a complex, interacting mix of cellulose degrading proteins. To significantly reduce the enzyme loading required, one may replace *Trichoderma* components with more efficient candidates, or augment the enzyme system with additional components to improve the overall enzyme performance. In this study, identification of new genes that improve specific performance in hydrolysis of pretreated corn stover, and their expression in *Trichoderma* will be discussed.

PR8.2

Highly efficient gene replacements in *ku70* disruption strain of filamentous fungus *Penicillium decumbens*

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Penicillium decumbens is an important industrial filamentous fungus and has been widely used in biorefinery for its high production of cellulase and hemicellulase. However, genetic engineering strategies are still rarely applied for strain improvement in *P. decumbens*. It has been proven that the frequency of targeted gene replacement in fungi can be increased by inactivation of the main components required for non-homologous end joining (NHEJ) pathway.

To improve gene targeting efficiency in *P. decumbens*, the putative *pku70* encoding KU70 homolog involved in the NHEJ process was identified and deleted. The Δ*pku70* strain displayed wild-type phenotypes regarding vegetative growth, conidiation and cellulase production, and appeared the similar sensitivity to hygromycin, EMS and H_2O_2 at different concentrations, compared with that of wild-type strain. The effect of the deletion of the *pku70* on gene targeting was tested by disruption of the *creA* gene encoding carbon catabolite repressor and the *xlnR* gene encoding general xylanolytic activator. Efficiency of gene targeting for both genes was 100% in the Δ*pku70* strain, compared with low efficiency (30%) in the wild-type strain.

Establishment of highly efficient gene targeting system opens the way to large-scale functional genomics analysis in *P. decumbens* and contributes to the study of the mechanism of lignocellulose degradation by *P. decumbens*.

PR8.3

Mutations in a beta-carotene oxygenase gene result in beta-carotene overaccumulation in the fungus *Phycomyces blakesleeanus*

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The biosynthesis of beta-carotene and its regulation by environmental factors, including blue light and sexual interaction, has been investigated in detail in the Zygomycete fungus *Phycomyces blakesleeanus*. Mutations in the regulatory genes *carS*, *carF*, or *carD* result in mycelia that accumulate beta-carotene.

The gene *carS* is genetically linked to the structural genes *carB* and *carRA*. From the *Phycomyces* genome sequence we have identified several candidate genes in the vicinity of the *carRA*/*carB* cluster. One of them encodes a putative protein similar to beta-carotene oxygenases, and we found that a *carS* strain had a single mutation that replaced a Ser at position 433 by Leu. We sequenced the gene strains carrying different carS mutations. We have sequenced 7 carS alleles and in all cases we have found single point mutations in the putative beta-carotene oxygenase gene. For this reason we now consider this putative beta-carotene oxygenase gene as the *Phycomyces carS* gene.

The gene *carS* encodes a 628 aminoacid protein. It contains two introns and the cDNA has been expressed in *E. coli*. The oxygenase CARS breaks beta-carotene in two molecules of 25 and 15 atoms of carbon. The *carS* gene is repressed by light and it is activated by sexual interaction.

The discovery that mutations in the gene of a beta-carotene oxygenase causes the acumulation of beta-carotene suggests that a derivate of beta-carotene acts as repressor of the biosynthesis or that the CARS enzyme itself could act as a repressor of the pathway.

PR8.4

BrlA and StuA are essential for conidiation in *Penicillium chrysogenum*

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Penicillium chrysogenum is the main industrial producer of the β-lactam antibiotic penicillin. Asexual conidiation is a key developmental process in this filamentous fungus. Evidence in filamentous fungi suggests that secondary metabolism might be linked with developmental processes such as conidiation.

We therefore generated deletion mutants of the transcription factors *brlA* and *stuA* using a *Pcku70* deletion strain with improved gene targeting efficiency. *brlA* is known as a central regulator of asexual sporulation and plays a crucial role in the development of conidiophores. The APSES domain transcription factor *stuA* influences conidiation by modulating the central developmental pathway that directs the differentiation of conidiophores and conidia from vegetative hyphae.

Both deletion mutants, *ΔbrlA* and *ΔstuA*, showed drastic impairment in conidiation and morphology under light and electron microscope. Besides, micro array analysis disclosed altered regulation of various genes involved in differentiation in both mutants compared to the parental strain *Pcku70*. While conidiation was completely blocked in *ΔbrlA*, it was severely decreased in *ΔstuA.* Furthermore, HPLC-, qPCR- and array analysis revealed a dramatic regulation of penicillin biosynthesis in the *ΔstuA* mutant.

PR8.5

Development of a low-cellulase background *Chrysosporium lucknowense* C1 strain.

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Filamentous fungi, particularly species such as *Aspergillus niger*, *A. oryzae*, *Trichoderma reesei* and recently also *Chrysosporium lucknowense* C1, have shown to produce large quantities of extracellular enzymes. As a result these fungi are used in industry as work horses for enzyme production. Usually, a mixture of enzymes is produced, which may be beneficial with regard to the degradation or modification of complex substrates such as plant cell wall polysaccharides. However, with respect to the production of a desired single target enzyme, the presence of large amounts of background proteins with non-relevant or even contra-productive enzyme activities is not desirable. A C1-strain almost devoid of background protein was obtained after random mutagenesis and extensive screening. The new C1 strain was shown no longer to produce extracellular cellulases. Furthermore, remaining undesired extracellular proteins were removed by targeted disruption of the corresponding genes. The new C1 strains proved in particular to be useful for the targeted production of specific single enzymes at high yields.

PR8.6

Development of a *Chrysosporium lucknowense* enzyme library

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Plant cell wall polysaccharides (PCWP) are large interconnected biopolymers that together form complex rigid structures. For efficient breakdown of PCWP, e.g. in second generation biofuel production or in feed and food applications, a variety of enzymes is needed to efficiently hydrolyze the cellulosic and hemi-cellulosic fibers. The *Chrysosporium lucknowense* C1-genome contains over 115 genes that encode PCWP-degrading enzymes and each of these is being over-expressed in specially designed low protein background C1 strains. This has now resulted in an enzyme library of over 60 specific carbohydrate active enzymes. This growing C1-enzyme library is used for mixing and matching experiments, e.g. to establish limitations in current biofuel enzymes, as well as for specific enzyme characterization and application studies in the area of feed, food and pulp & paper. We continue to expand the enzyme library in C1, which now contains mainly cellulases, hemi-cellulases and other corresponding accessory enzymes.

PR8.7

Development of protease deficient *Chrysosporium lucknowense* strains

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Filamentous fungi have proven to produce and secrete large quantities of extracellular enzymes. Species such as *Aspergillus niger*, *A. oryzae*, *Trichoderma reesei* and recently also *Chrysosporium lucknowense* C1 are used in industry as work horses for enzyme production. High yields of homologous enzymes or enzyme mixtures are readily obtained. The production of heterologous proteins, on the contrary, is often hampered by the presence of host proteases that partially or fully degrade the heterologous protein. In order to significantly improve the production of heterologous proteins, C1 strains are developed that have an as low as possible protease level without having their vital functions disturbed. To this end C1 was subjected to random mutagenesis and subsequent screening approaches. Strains with greatly reduced overall protease activity levels were obtained . Additionally, the presence of specific proteases in C1 culture samples was shown by MS analyses. Some of these showed homology to notoriously harmful proteases known from other fungal systems. A protease gene disruption approach was used to knock-out the corresponding genes. This work was greatly facilitated by the use of strains defective in the non-homologous-end-joining of DNA. Thus a number of versatile low protease C1 hosts were developed for high level expression of heterologous proteins.

PR8.8

Vector development to simplify multiple gene expression in *Aspergillus oryzae*

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Vector pTAex3 was modified for use in characterising fungal polyketide synthases (PKS) and hybrid polyketide synthase-non-ribosomal peptide synthases (PKS-NRPS) in an arginine auxotroph of *Aspergillus oryzae*. In pTAex3GS the strong, starch-inducible *amy*B expression cassette was modified for GATEWAY LR recombination, and in further derivatives the *arg*B selectable marker was replaced with basta- (*bar*) or bleomycin- (*ble*) resistance genes to allow co- or sequential transformation. Tailoring enzymes convert PKS/PKS-NRPS products to final secondary metabolites; tenellin production in *Beauveria bassiana* requires an enoyl reductase and two cytochrome P450s in addition to a PKS-NRPS encoded by *tenS*. We produced tenellin in *A. oryzae* by introducing all four genes in *amy*B expression cassettes on three separate plasmids. To simplify plasmid construction for whole-pathway expression pTAex3GS was converted to a yeast-*E. coli* shuttle vector. The *A. nidulans arg*B gene functions well in *A. oryzae*, so promoters from three other arginine biosynthesis genes (carbomylphosphate synthase (P1), arginosuccinate synthase (P2) and arginosuccinate lyase (P3) were inserted by homologous recombination in yeast. The resultant pTAYAGSarg3P was further modified, replacing *arg*B with *bar* and *ble* markers. Yeast recombination simultaneously placed the three tenellin tailoring genes downstream of the *arg* promoters, creating pTAYAGSarg3genes, and introduction of *tenS* by GATEWAY recombination reconstructed the whole tenellin synthesis pathway in pTAYAargTENELLIN, which was introduced into *A. oryzae*. While the easy construction principle has been proven, tenellin synthesis is not yet a likely outcome because in tests of the *arg* promoters only P2 gave an acceptable level of eGFP expression.

PR8.9

The role of the bZIP transcription factor MeaB in the nitrogen metabolite repression of *Fusarium fujikuroi*

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The rice pathogen *Fusarium fujikuroi* produces a broad spectrum of secondary metabolites. The synthesis of two of them, gibberellin and bikaverin, is subject to the nitrogen metabolite repression. One of the key elements of this complex regulatory network is the GATA transcription factor AreA which activates the expression of the gibberellin biosynthesis genes. In *A. nidulans* the main antagonist of AreA is NMR. The expression of NMR is positively controlled by the bZIP transcription factor MeaB which is therefore an indirect antagonist of AreA. We examined the role of MeaB in the nitrogen metabolite repression of *Fusarium fujikuroi*.

Knockout and overexpression mutants of *meaB* were created, and the impact on the expression of nitrogen repressed genes was studied by Northern analysis. It was shown that MeaB has also a mainly negative effect on the expression of these genes in *F. fujikuroi*, but that this effect is not as strong as in *A. nidulans*. Interestingly the repressing effect of MeaB is not mediated via the expression control of *nmr* as proved by promoter studies in the Δ*meaB*-background. Additionally, we identified two transcript sizes of *meaB* that are part of a complex regulation system. Western analysis indicated that only the large transcript is translated. This regulation system allows MeaB to migrate into the nucleus only under high nitrogen concentrations as shown by MeaB-GFP-Fusion. The results indicate the role of MeaB as a fine tuning regulator in the nitrogen regulation network and highlight differences between *F. fujikuroi* and *A. nidulans* concerning this role.

PR8.10

Formation of beta-fructofuranosidase by *Aspergillus niger* in submerged cultivations

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Filamentous fungi like *Aspergillus niger* are used as versatile and efficient cell-factories for the production of lipases and proteases, exopolysaccharides, nutrition additives, and therapeutic agents in many industrial bioprocesses. Although *A. niger* shows a high production capacity and secretion efficiency, and is capable of carrying out post-translational modifications, obtainable yields of recombinant proteins are considerably lower than those of homologous proteins. Besides improvement of productivity due to genetically modified strains, current research is focussed on the optimisation of cultivation processes resulting in an increased, controlled and tailored formation of desired products while avoiding by-products.

The contribution displays the influence of pH-value, volumetric power input and inoculum concentration on the observed morphology and the formation of homologous recombinant β-fructofuranosidase under a constitutive promoter as model product. Batch cultivations are monitored starting with the germination of *A. niger* spores and ending with the stationary phase of growth. Every step of the protein formation path – transcription, translation and secretion – is shown: The expression of the β-fructofuranosidase gene as well as of genes, which show significant expression levels within the bioprocess, are quantified via real-time PCR. Intra- and extracellular enzyme activities are measured and related to gene expression levels and observed morphology, pellet size and concentration. Undesired by-products are analysed by HPLC.

In conclusion, the protein formation in batch processes is linked to defined cultivation conditions to reveal bottlenecks within the complex production path from gene to product. Therefore the shown results indicate targets for improving, optimising and controlling industrial bioprocesses.

PR8.11

A marker recycling system for filamentous fungi

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To overcome the limited availability of antibiotic resistance markers in filamentous fungi we have established a system for marker recycling. For this purpose we have chosen the FLP/*FRT* recombinase system from the yeast *Saccharomyces cerevisiae* for application in the penicillin producer *Penicillium chrysogenum*. In a first step, we generated a nourseothricin resistance cassette flanked by the *FRT* sequences in direct repeat orientation (*FRTnat1* cassette) and ectopically integrated this construct into a *P. chrysogenum* recipient strain. In a second step the gene for the native yeast recombinase and in parallel a codon optimized *Pcflp* recombinase gene were transferred into the *P. chrysogenum* strain, carrying the *FRTnat1* cassette. The corresponding transformants were analyzed by PCR, growth tests and sequencing to verify successful recombination events. Our analysis of several single and multicopy transformants showed that only with the codon-optimized recombinase a fully functional recombination system was achievable in *P. chrysogenum*. To further extend the application of the FLP/*FRT* recombinase system, we produced a ΔPcFRTku70PcFLP strain to establish a highly efficient homologous recombination system for the construction of marker free knockout strains. The applicability of the developed FLP/*FRT* recombinase system was further demonstrated by marker recycling in the ascomycete *Sordaria macrospora*. In summary we have developed a optimized FLP/*FRT* recombinase system as a molecular tool for the genetic manipulation of filamentous fungi.

PR8.12

The Velvet-like complex In *Penicillium chrysogenum* – a novel target for industrial strain improvement

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The recent discovery of a *velvet* complex containing several regulators of secondary metabolism in the model fungus *Aspergillus nidulans* raises the question whether similar type complexes direct fungal development in genera other than *Aspergillus*. *Penicillium chrysogenum* is the industrial producer of the beta-lactam antibiotic penicillin, whose biosynthetic regulation is barely understood. Here we provide a functional analysis of major homologues of the *velvet* complex in *P. chrysogenum*. Data from northern hybridizations, HPLC quantifications of penicillin titres as well as detailed microscopic investigations clearly show that all regulators play not only a major role in penicillin biosynthesis but are also involved in different and distinct developmental processes. While for example deletion of the *velvet* homologue PcvelA leads to light-independent conidial formation, dichotomous branching of hyphae and pellet formation in shaking cultures, a ΔPclaeA strain shows a severe impairment in conidiophore formation in both the light and dark. Furthermore, detailed bimolecular fluorescence complementation assays together with yeast two-hybrid screens led not only to the identification for a *velvet*-like complex in *P. chrysogenum* but also to the detection of new components of this complex. Our results extend the current picture of regulatory networks controlling both fungal secondary metabolism and morphogenesis which is significant for the genetic manipulation of fungal metabolism as part of industrial strain improvement programs.

PR8.13

From chitin to a highly valuable, pharmaceutically relevant fine chemical: inserting a multi-step enzyme cascade into *Trichoderma*

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Second to cellulose, chitin is a most abundant, renewable organic source in nature with an estimated annual biosynthesis of 10^9 to 10^{11} tons. The polymer is composed of ß-(1,4)-linked units of the amino sugar N-acetyl glucosamine (GlcNAc). For industrial applications this renewable resource is mainly extracted from crustacean shells. However, thitherto only a minute amount of this renewable resource is used in industrial and agricultural applications.

N-acetyl neuraminic acid (NeuNAc), a C9 mono-saccharide, is the most prevalent exponent of sialic acids. Currently, more than 50 derivatives of sialic acids are known to exist in nature. NeuNAc is believed to serve as a precursor of all these derivatives as all biochemical pathways precede via this substance.

In biological systems sialic acids are mostly terminal components of glycoproteins presented at the respective cell surface. Because of its exposed position in cellular systems they play an important role in infection cycles of viral diseases, e.g. influenza viruses A and B. Therefore, sialic acid derivatives are successfully applied in the therapy of such virus-born diseases. Preparations on the market are e.g. „Relenza“ (GlaxoSmithKline), functioning as neuraminidase inhibitor and produced from the precursor NeuNAc.

We will present an alternative strategy of NeuNAc production based on a genetically engineered *Trichoderma* strain producing and excreting NeuNAc. For the first time, this poses the introduction of a multi-step enzyme cascade into a filamentous fungus as heterologous host. This strategy involves the application of *Trichoderma* as a whole-cell catalyst for direct synthesis of NeuNAc from the cheap, renewable biopolymer chitin.

PR8.14

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

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For industrial applications and (medical) scientific research efficient (heterologous) production of compounds like, enzymes, antibodies, viral epitopes, chemical compounds and antibiotics is of great importance. For these purposes *Aspergillus* functions as a suitable production host. Over the years all kinds of commercially available expression systems have been developed. A well established expression system is the one based on the protein Glucoamylase (GlaA). However, in the case of heterologous protein production the efficiency of these systems is still very depending on the protein to be produced. Recently, we identified the *inuE* gene, encoding for the exo-inulinase protein in *A. niger*, as the most strongly induced gene in the presence of inulin and sucrose (Yuan et al., 2006; Yuan et al., 2007).

Characteristics of the system were studied by placing *gfp* behind the *inuE* promoter. This reporter strain showed that the *inuE* gene is highly expressed when grown on inulin and sucrose. No expression was observed when grown on glucose, fructose and xylose indicating a tight control on different carbon sources. This tight control can be a benefit if the heterologous protein to be produced can be a disadvantage for fungal growth. Finally, different peroxidases and a laccase were successfully produced in *Aspergillus* using this novel inducible expression system.

PR8.15

Engineering an N-acetylneuraminic acid synthesis pathway into *Trichoderma*

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N-acetylneuraminic acid (NeuNAc) belongs to the structural class of sialic acids and is an acidic α-keto sugar with a C9 backbone. Derivatives of NeuNAc are used as neuraminidase inhibitors to treat viral infections like influenza. Therefore, the pharmaceutical industry is interested in a cheap source for NeuNac, but its synthesis is costly (current market price: 3000 to 4000 US $/kg). Currently, NeuNac can be produced either by *E. coli* biosynthesis or enzymatic conversion. In both cases the substrate used is N-acetylglucosamine, which itself is costly.

Instead of N-acetylglucosamine, we use the renewable source chitin as a substrate. We developed a whole-cell-bio-catalysis process based on the fungus *Trichoderma*. *Trichoderma* is known for its high secretory capacity for hydrolytic enzymes. This filamentous fungus is able to utilize polysaccharides like cellulose or chitin which commonly occur in nature. We use this ability of the fungus to degrade chitin into its monomer N-acetylglucosamine. From N-acetylglucosamine we designed an intracellular enzyme-catalyzed pathway for the synthesis of NeuNac. Therefore, we heterologously expressed two bacterial enzymes, N-acetylglucosamine-2-epimerase and NeuNAc synthase, in *Trichoderma.* This is necessary because the wild-type of *Trichoderma* itself is not able to produce NeuNac. We will illustrate the properties of a recombinant *Trichoderma* strain, which expresses the two bacterial enzymes, and we will show the ability of this strain to form NeuNac. This work demonstrates the potential of using *Trichoderma* as a whole-cell catalytic system.

PR8.16

Transposon mutagenesis using a resident DNA transposon Crawler in *Aspergillus oryzae* industrial strains

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An active DNA transposon *Crawler* isolated from the genome of industrially important fungus *Aspergillus oryzae* transposes under extreme stress conditions. The DNA sequencing surveys revealed that the *Crawler* element is widely distributed among *A. oryzae* and *A. sojae* strains, which are commonly used in Japanese traditional fermentation manufacturing. In the present study, we analyzed the relationship between various stress stimuli and inhibition of cryptic splicing of the *Crawler* mRNA by qRT-PCR to enhance the frequency of *Crawler*-mediated mutagenesis in an *A. oryzae* industrial strain, AOK139. Under the optimized stress conditions, in which conidiospores were treated for 6 hr in 20 mM $CuSO_4$ or at 52□, various phenotypic mutants different from the parent strain were isolated. These mutants exhibited white color in conidiospores, less number of spores formed, shortened aerial hyphae, thin colony mat and so on. DNA sequencing analysis of a white conidia mutant revealed that *Crawler* was newly inserted within a coding region of *wA* gene encoding polyketide synthase, which resulted in *wA* deficiency. The insertion occurred also at a TA site with duplication according to the manner of *Crawler* transposition. These results suggested that transposon mutagenesis using active *Crawler* is potentially valuable to improve characteristics of *A. oryzae* industrial strains.

PR8.17

Bikaverin biosynthesis in *Fusarium fujikuroi*: Genes, their function and regulation

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Fusarium secondary metabolites are structurally diverse, have a variety of activities, and their biosynthesis and regulation are generally poorly understood. The *F. fujikuroi* polyketide synthase gene *bik1* was previously shown to be responsible for formation of the mycelial pigment bikaverin. Here we present the characterization of five genes adjacent to *bik1* as encoding a putative FAD-dependent monooxygenase (*bik2*), an *O*-methyltransferase (*bik3*), an NmrA-like protein (*bik4*), a $Zn(II)_2Cys_6$ transcription factor (*bik5*), and an MFS transporter (*bik6*). Deletion of each gene resulted in total loss or significant reduction of bikaverin synthesis. Over-expression of genes involved in biosynthesis enabled us to identify at least one intermediate of the bikaverin pathway. Expression studies revealed that all *bik* genes are repressed by high amounts of nitrogen in an AreA-independent manner and are subject to a time- and pH-dependent regulation. Deletion of the pH regulatory gene *pacC* resulted in partial de-repression while complementation with a dominant active allele resulted in repression of *bik* genes at acidic ambient pH. Furthermore, the global regulator Velvet represses *bik* gene expression and product formation most likely by interconnection with the nitrogen regulation machinery. Thus, bikaverin synthesis is regulated by a complex regulatory network. Understanding how different factors influence the synthesis of this model secondary metabolite will aid understanding secondary metabolism in general.

PR8.18

The role of the early genes of the mevalonate pathway, HMGR and FPPS, for gibberellin biosynthesis in *Fusarium fujikuroi*

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Each year about ten tons of gibberellins (GAs) are consumed by the agricultural industry as plant growth regulators. GAs function as phytohormones in higher plants and application of GAs induces flower bud formation and shoot elongation as well as an increased fruit size and yield of crops such as seedless grapes and corn.
The rice pathogen *Fusarium fujikuroi* is known to produce high amounts of this secundary metabolite and constitutes a capable species for GA production by microorganisms. Therefore, we developed different strategies for strain improvement. By providing a higher amount of the GA precursor molecule geranyl-geranyl-pyrophosphate (GGPP) the yield of GAs could be enhanced and costs of the production would be reduced. For that reason expression of the key enzymes of the mevalonate pathway, 3-hydroxy-3-methyl-glutaryl-CoA-reductase (HMGR) and farnesyl-pyrophosphate-synthase (FPPS), should be increased.

In former studies truncation and overexpression of HMGR in *Saccharomyces cerevisiae* led to a higher accumulation of terpenoids. This is referred to the deletion of the transmembrane domains of HMGR where the enzyme is regulated by feedback inhibition.

Additionally, the ergosterol biosynthesis pathway, a branch of the mevalonate pathway, shall be downregulated as it competes with the GGPP synthase for FPP. Furthermore it would avoid formation of abnormal structures in the fungal membrane.

Finally, localization studies with GFP fusion proteins of HMGR and FPPS shall clearify subcellular organization of the mevalonate pathway and the role of the two key enzymes in *F. fujikuroi*.

PR8.19

Comparison of the polysaccharide degrading ability of 8 *Aspergilli*

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Plant polysaccharides are among the most prominent carbon sources for fungi, which degrade these substrates through the production of diverse sets of extracellular enzymes. This topic has been best studied in the Aspergilli, in particular in *A. niger* and *A. oryzae* due to the many industrial application of plant polysaccharide degrading enzymes and the good fermentative properties of these fungi. The availability of genome sequences for several Aspergilli has allowed detailed comparisons between these species and enables us to identify differences in the strategies that they employ to release their carbon source from crude plant biomass.

CAZy-annotation of the genomes revealed significant differences in the sets of hydrolytic enzymes and growth profiling of these fungi demonstrated strong correlations between genome content and ability to degrade specific polysaccharides. In addition, analysis of the secreted enzymes demonstrated further differences that are likely caused by differences in transcriptional or post-transcriptional regulation. Highlights from these results will be presented.

These data will help with the further development of improved enzyme cocktails as they enable a link between the composition of the enzyme set and the efficiency with which different polysaccharides are degraded.

PR8.20

Targeted morphology engineering of *Aspergillus niger* for improved enzyme production

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Among filamentous fungi, *Aspergillus niger* is important as the major world source of citric acid and higher-value enzyme products including pectinases, proteases, amyloglucosidases, cellulases, hemicellulases and lipases. One of the outstanding and, unfortunately often problematic, characteristics of filamentous fungi is their complex morphology in submerged culture. Hereby, the productivity in biotechnological processes is often correlated with the morphological form.

In the present work, supplementation with microparticles was used as novel approach to control the morphological development of *A. niger* [1]. With careful variation of size and concentration of the micro material added, a number of distinct morphological forms including pellets of different size, free dispersed mycelium and short hyphal fragments could be reproducibly created. Exemplified for different recombinant *A. niger* strains enzyme production could be strongly enhanced by the addition of microparticles. Titres for glucoamylase and fructofuranosidase, were up to four fold higher. Moreover, accumulation of the undesired by-product oxalate was suppressed by up to 90 %. Using co-expression of glucoamylase with green fluorescent protein, enzyme production was localized in the cellular aggregates of *A. niger*. For pelleted growth, production was maximal only within a thin layer at the pellet surface and markedly decreased in the pellet interior, whereas the interaction with the microparticles created a highly active biocatalyst with the dominant fraction of cells contributing to production.

The use of microparticles, allowing targeted engineering of cellular morphology opens novel possibilities for future design and optimization of recombinant enzyme production in *A. niger* and probably also other fungi.

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PR8.21

Determination of viable spores in seeding cultures using fluorescent dyes

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In many biotechnological processes filamentous fungi like *Aspergilli* are used for the production of organic acids, enzymes and antibiotics. Furthermore, there is an increasing application of fungi as catalysts in biotransformations. Therefore spores are used in inoculum development or directly as starting culture in submerged cultivation processes. While the influence of process conditions during cultivation is well-known, the influence of the spore quality in seeding cultures has not been investigated in depth. However the spore viability in seeding culture is an important criterion for product quantity.

The contribution displays the investigation of the transferability of fluoresence based rapid screening assays, developed for bacterial cells, to study spore quality. The results of this method show the applicability to determine the proportion of viable and defect spores in seeding cultures of filamentous fungi. Additionally, good correlations to germination characteristics in submerged cultivation are demonstrated and allow fast, reliable and quantitative distinction between viable and dead spores. Furthermore a biochemical method, based on enzyme activity, has been verified to characterize the spore viability in seeding cultures. The assay allows the characterization of the growth stadium of the filamentous fungi during the sporulation process. Furthermore the enzyme based assay enables the determination of biomass activity in the early phase of submerged cultivation and reveals first information about the feasibility of the cultivation.

In conclusion the presented investigations display the possibility of assessing the ratio of viable to defect spores in seeding cultures, whereby a routine method prior to large scale cultivations could be applied.

PR8.22

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

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

PR8.23

Secrelection: a novel fungal expression system for selection of secreted enzymes

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

PR8.24

Towards science-based process design in filamentous fungi

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Meeting QbD (Quality by Design) and PAT (Process Analytical Technology) guidelines launched by the U.S. Food and Drug Administration, biopharmaceutical manufacturers need to move away from traditional empirically based process development towards optimized and science-based process design and control. Within recent years, numerous PAT compliant tools allowing effective, non-invasive in-situ monitoring of microbial production processes have been successfully introduced. Moreover, several approaches for online process characterization in bacterial and yeast systems have been suggested. Promising procedures include applying metabolic modeling and online evaluation of stoichiometric balances enabling to distinguish between different metabolic states of cellular production systems.

For cultivation processes of filamentous fungi however, implementation of such methods has been hampered by increased complexity of filamentous fungal systems and additional factors affecting process performance, e.g. growth morphology and extracellular proteolytic activity.

The aim of this work is to establish a methodology allowing generically characterizing fungal filamentous systems by accurately quantifying fungal response behavior to physiological stress under transient culturing conditions, linking metabolic regulations to morphological changes. Dynamic experiments are designed using DoE (Design of experiment) strategies, and carried out in fully instrumented bioreactors coupled to a process management system performing online data evaluation and reconciliation.

Combining process technology, morphological, and physiological analysis, allows accurately quantifying filamentous fungal process behavior. Our approach will thus facilitate process development and allow understanding biological variability and, in turn, control those complex processes.

PR8.25

Novel low-temperature performing cellulase for textile industry

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Cellulases are traditionally used in textile, detergent, pulp and paper, feed and food industries, and are currently also extensively studied for cellulosic biomass conversion to ethanol. Endoglucanases have been widely used in textile industry for biostoning and biofinishing of cellulosic fibers. There has been trend in the market, especially in Asia, to use lower washing temperatures for energy savings and environmental reasons. Here we describe molecular cloning of glycosyl hydrolase (GH) family 45 cellulase from the *Geomyces pannorum* as well as its heterologous expression in *Trichoderma reesei*. *Geomyces pannorum* Cel45A contains bimodular structure composed of a catalytic module linked to C-terminal carbohydrate-binding module (CBM), and the protein has low homology to the known Cel45 cellulases. *Geomyces pannorum* Cel45A enzyme shows exceptional broad application range from 20 ºC to 50 ºC with high biostoning performance and excellent depilling properties.

PR8.26

Secretome analysis of concentric zones of *Aspergillus niger* colonies

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In nature fungi degrade organic matter such as wood. To this end, they secrete enzymes that convert the polymeric substrates into small molecules that can be taken up to serve as nutrients. Hyphae at the periphery of the colony are exposed to unexplored organic material, whereas the center of the colony experiences a (partly) utilized substrate. This suggests that the enzymes that are secreted by distinct zones in the colony are different. This hypothesis was tested using the industrial important fungus *Aspergillus niger*. Spatial protein secretion was monitored by growing *A. niger* colonies between two porous polycarbonate membranes that have been placed on top of agar medium. This mode of growth inhibits sporulation and forces the colony to grow two-dimensionally. After 6 days of growth, the colony was transferred to a ring plate. This plate contains 5 concentric wells that are filled with liquid medium. After growing the colony for 24 h on the ring plate the medium of the wells was collected. Proteins in each of the wells were identified by mass spectrometry. 70 and 50 different proteins were found to be secreted at the periphery and the centre of xylose-grown colonies, respectively. Of these, only one is predicted not to have a signal sequence for secretion. This indicates that lysis in the colony is minor, if present at all, even within the colony centre. Interestingly, six proteins were secreted in the central zone that were not found at the periphery of the colony. Thus heterogeneity of the colony is reflected in the spatial secretion pattern.

PR8.27

Electrotransformation of the obligate biotrophic cucurbit pathogen *Podosphaera fusca*

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Powdery mildew fungi (*Erysiphales*) are probably the largest group of plant pathogens that remain uncharacterized from the genetic and molecular points of view. Their lifestyle as obligate biotrophic parasites and consequent inability to grow on culture media has significantly hampered research. Appropriate disease management requires a good understanding of the biology of the responsible pathogen. Regarding powdery mildews research, a technologically important unsolved issue is their genetic manipulation. Since the first reports of DNA-mediated transformation in model filamentous fungi such as *Neurospora crassa* and *Emericella nidulans*, many commercially and agriculturally important fungal species have been successfully transformed. However, the obligate biotrophs have, hitherto, proved recalcitrant to this process.Most common methods such as protoplast fusion or *Agrobacterium*-mediated transformation are not suitable for such organisms like the first, or show important methodological limitations like the second. In fact, to date particle bombardment has been reported as the only valid method to transform this type of parasites, although this technique has shown certain lack of reproducibility.

We report here on progress in developing a reliable and stable transformation protocol for *Podosphaera fusca*, the causal agent of cucurbit powdery mildew, by the highly versatile electrotransformation method. Two selectable markers have been used:the hygromycin B resistance gene (*hph*) and the enhanced green fluorescent protein gene (*egfp*). These markers were tested under the control of different promoter and terminator sequences such as those of the *gpd* and *trpC* genes of *E. nidulans*, the *isl* gene of *N. crassa*, and the promoters of the b-tubulin and CYP51 genes of *P. fusca*. Transformants were obtained with most of the constructs used. Under selective conditions the hygromycin B resistant transformants grew very slow. Similarly, weak GFP signals were associated to fluorescent hyphae of transformants. In all cases we detected sequences of marker genes by PCR and found copies of the transforming plasmids integrated in the fungal genome. To our knowledge this is the first case of genetic transformation of a powdery mildew fungus by electroporation of conidia. An update of the results obtained so far will be presented.

PR8.28

The regulation of ergot alkaloid biosynthesis in *Claviceps purpurea*

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Ergot alkaloids (EA) are secondary metabolites synthesized by the phytopathogenic ascomycete *Claviceps purpurea.* The altogether 14 genes encoding the specific enzymes for the biosynthesis of EA are clustered. To date the molecular mechanisms of cluster regulation in *C. purpurea* are unknown. No transcription factor gene has been found within the cluster region involved in the synthesis of EA. It is only known to date that the EA in *C. purpurea* wild-type are produced during the ripening of the sclerotium and not in axenic cultures. Mutant strains producing alkaloids in submersed cultures require under specific conditions: (a) tryptophan as inducer and precursor, (b) a high osmotic value, (c) a low phosphate level. The alkaloid biosynthesis was speculated to be regulated by changes in the chromatin organization, a hypothesis checked by the cultivation of *C. purpurea* in the presence of either inhibitors of histone deacetylases (HDACis) or histone acetyltransferases (HATis). The production strain P1 of *C. purpurea* was cultivated under both alkaloid producing (T25N medium) and non-producing (BII medium) conditions. Unexpectedly, the alkaloid biosynthesis is repressed instead of induced by the addition of HDACis (euchromatin formation) and vice versa, indicating a complex regulation system (1). Another global regulatory system of secondary metabolism and development was discovered in *Aspergillus nidulans, velvet* (2). We are investigating if in *C. purpurea* the biosynthesis of ergot alkaloids could be regulated through velvet. We have identified and sequenced a velvet homologue in *C. purpurea* (*cpvel1)* and started a functional analysis. (1) Lorenz et al. (2009) *Phytochemistry.* 70:1822-1832. (2) Kim et al. (2002) *Fungal Genet Biol.* 37:72-80.

PR8.29

Disruption of a vacuolar protein sorting receptor gene, Aovps10, enhances production level of heterologous protein by the filamentous fungus *Aspergillus oryzae*

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Filamentous fungi have received attention as a host for heterologous protein production because of their high secretion capability. However, a bottleneck in post-transcriptional processing in the secretory pathway limits protein production yields. The vacuolar protein sorting gene *VPS10* encodes a sorting receptor for the recognition and delivery of several yeast vacuolar proteins. Although it can also target several recombinant and aberrant proteins for the vacuolar degradation, there is limited knowledge of the effect of its disruption on heterologous protein production. In this study, cDNA encoding AoVps10 from the filamentous fungus *Aspergillus oryzae* was isolated. Microscopic observation of the transformant expressing AoVps10 fused with enhanced green fluorescent protein showed that the fusion protein localized at the Golgi and prevacuolar compartments. Moreover, disruption of the *Aovps10* gene resulted in missorting and secretion of vacuolar carboxypeptidase AoCpyA into the medium, indicating that AoVps10 is required for sorting of AoCpyA to vacuoles in *A. oryzae.* To investigate the extracellular production level of heterologous protein, we constructed an Δ*Aovps10* mutant expressing bovine chymosin (CHY). Interestingly, Δ*Aovps10* increased the maximum extracellular production by three fold. Western blot analysis of extracellular CHY also demonstrated an improvement in productivity. These results suggest that the AoVps10 plays a role in the regulation of heterologous protein secretion in *A. oryzae*, and may be involved in vacuolar protein degradation through the Golgi apparatus. This is the first report demonstrating that disruption of a vacuolar protein sorting gene in filamentous fungi leads to enhanced production levels of heterologous protein.

PR8.30

Cloning, expression, and characterization of beta-glucosidases from a black *Aspergillus* spp.

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Through a broad cellulolytic activity screening of fungi isolated from various locations, a very promising fungus, identified as a black *Aspergillus*, was selected for further studies. An enzyme extract of this fungus was obtained through solid state fermentation and tested to profile its beta-glucosidase activity. Four beta-glucosidase genes from this fungus have been cloned through pcr with degenerate primers designed from conserved motif regions of known beta-glucosidase genes from *Aspergilli* followed by genome walking strategies. The goal is to have these genes expressed in *Trichoderma reesei* QM6a ΔpyrG. An expression system was constructed with the constitutive ribosomal promoter RP27 for expression of the his-tagged beta-glucosidase genes and with the pyrG gene to complement the mutation. Analysis of the different transformants is currently on-going and subsequently the different beta-glucosidase proteins will be purified for detailed analysis of specific activity, Km, sugar tolerance, thermostability as well as their ability to break down shorter chained oligosaccharides.

PR8.31

Laccases from the biopulping fungus *Physisporinus rivulosus*: molecular characterization of thermotolerant oxidoreductases and reactions with mediator compounds

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The white rot, softwood biopulping basidiomycetous fungus *Physisporinus rivulosus* isolate T241i produces two laccase isozymes with novel catalytic properties such as thermotolerance and atypically low pH optima (pH 3.0-3.5) for the methoxylated phenolic laccase substrate compounds syringaldazine, 2,6-dimethoxyphenol and guaiacol (1). The fungus was previously distinguished in a large screening study as the most selective and efficient lignin decomposer of spruce (*Picea abies*) wood (2, 3), and is shown to produce several manganese peroxidase and laccase isozymes as well as oxalic acid when growing on spruce wood chips (4). Recently, we have cloned and characterised at molecular level two *P. rivulosus* laccase genes. The translated mature Priv-lac1 and Priv-lac2 enzymes demonstrate structural similarity with *Trametes versicolor* and *Lentinus tigrinus* laccases, respectively, as deduced by protein 3-D homology modelling. The *Priv-lac1* and *Priv-lac2* coding sequences are interrupted by 11 and 12 introns and they host the four conserved His-containing Cu-binding regions typical for laccases. The protein architectures are viewed at the modelled substrate-binding pocket to elucidate the atypical pH optima for the phenolic compounds as electron donors. When *P. rivulosus* T241i is cultivated in spruce sawdust-charcoal containing liquid medium, higher levels of laccase activity is produced. The activity is separated within over five isoforms, and purified *P. rivulosus* laccases display catalytic efficiency for oxidation of the higher-redox potential substrates Reactive Black 5 and veratryl alcohol when the reactions are supplied with natural-type of oxidation-mediator compounds. These properties well support the *P. rivulosus* laccases to be applied as robust biocatalysts for e.g. bleaching of dye compounds.

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PR8.32

Improving *A. niger* asparaginase via directed evolution

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

PR8.33

The secreted yields of heterologous proteins from *Saccharomyces cerevisiae* and *Pichia pastoris* are dependent on protein stability and the UPR

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S. cerevisiae and *P. pastoris* are hosts for the production of heterologous proteins. The secreted yield of a heterologous protein varies according to the protein being expressed and we aim to provide predictability based on the stability of protein folded states. Evidence was gained from the secretion of human lysozyme variants that have single amino acid changes. A direct relationship between decreasing protein stability and secreted yield was demonstrated in *P. pastoris* (Kumita et al. 2006 FEBS J 273, 711-720) and in *S. cerevisiae* (unpublished). The reasons underlying the impact of stability on secreted yields were investigated and the unfolded protein response (UPR) was the focus in terms of its kinetics of induction and its severity. We showed that genes encoding both BiP (chaperone) and PDI (foldase) were transcriptionally up-regulated more quickly and to higher levels in response to secretion of less-stable lysozymes. The transcript level of *HAC1* mRNA is not altered substantially in *S. cerevisiae* by ER stress but the transcript level of *HAC1* from *P. pastoris* was enhanced markedly and increasingly so with the more unstable variant lysozymes. We conclude that the folded state of a protein is detected and responded to in the ER lumen by the quality control system and the response is a measured one and proportionate to the folded state stability of a secreted protein. This analysis is being extended to other proteins, e.g. antibody fragments, and the differences in the UPR between *S. cerevisiae* and *P. pastoris* are being explored further.

PR8.34

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

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

PR8.35

Overproduction and characterization of prolyl aminopeptidase from *Aspergillus oryzae*

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Overproduction and characterization of prolyl aminopeptidase from *Aspergillus oryzae* Ken-Ichi Kusumoto[1], Mayumi Matsushita-Morita[1], Ikuyo Furukawa[1], Youhei Yamagata[2], Yoshinao Koide[3], Hiroki Ishida[4], Michio Takeuchi[2], Yutaka Kashiwagi[1], Satoshi Suzuki[1] [1]National Food Research Institute, Tsukuba, Ibaraki, Japan; [2]Department of Agriscience and Bioscience, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan; [3]Gifu R & D Center, Amano Enzyme Inc., Kagamihara, Gifu, Japan; [4]Research Institute, Gekkeikan Sake Company Ltd., Fushimi-ku, Kyoto, Japan

Introduction: Prolyl aminopeptidase (PAP) degrades only amino-terminal proline from peptides. The food-grade fungus *Aspergillus oryzae* produces this enzyme only in small amounts. Therefore, we present here an efficient production of recombinant PAP with an overexpression system of *A. oryzae* and characterization of its biochemical properties.
Methods and Results: The gene encoding PAP was overexpressed as a His-tag fusion protein under a *amyB* promoter with a limited expressing condition in *A. oryzae*. The PAP activity in the mycelia grown in rich media containing glucose (repressing condition) was twice that in starch (inducing condition). The enzyme prepared as cell-free extract was partially purified through two-step column chromatography. *A. oryzae* PAP was purified 1800-folds. The PAP was estimated to be a homo hexameric protein and exhibited salt tolerance against NaCl of up to 4 mol l^{-1}.
Discussion: Overproduction of PAP under promoter inducing conditions led to an increase of inactive PAP, possibly because of irregular folding. PAP with a high specific activity and salt tolerance may be used effectively in the manufacturing processes of fermented foods.

PR8.36

The *Trichoderma harzianum* pH regulation factor Pac1 is involved in osmotic and saline stress resistance

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Trichoderma spp. is a filamentous fungus used in biocontrol. Many attemps were carried out to improve its antagonistic ability. However, *Trichoderma* adaptability to different soils might be affected under environmental stress conditions, and finding resistance to adverse conditions would be a desirable feature for the use of *Trichoderma* in agriculture. Recently, implication of Pac1, the homologue of the pH transcriptional regulator PacC, in *Trichoderma harzianum* parasitic relationships has been demonstrated. As its homologous PacC proteins in other filamentous fungi, Pac1 is expected to be directly involved in fungal stress response. Two mutants of *T. harzianum* CECT2413 have been obtained in Pac1: P2.32 expresses a constitutively active Pac1, and R13 contains a null allele by gene silencing. Strain P2.32 shows better growth than wild type in NaCl-, KCl- or sorbitol-containing media, while strain R13 shows higher sensitivity in the same conditions. Phenotypes of P2.32 and R13 mutants in the presence of Lithium are opposed but contrary to those described for PacC/Rim mutants in yeasts and other filamentous fungi. Transcriptional regulation of *pac1* is altered under salt/osmotic stress. Pac1 mRNA levels are higher and the protein produced able to bind DNA. However, Pac1 DNA binding ability did not correlate to those levels of *pac1* expression in non stressing media. These results indicated that Pac1 is a key factor for saline and osmotic stress resistance and might be subjected to a complex combination of traductional and posttranslational regulation in *T. harzianum*.

PR8.37

Applications of genetic transformation of *Aspergillus oryzae* with bleomycin resistant selection

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Introduction: Since *Aspergillus oryzae* produces large quantity of enzymes, it is significant to establish a host-vector system to use this microorganism as a host for heterologous protein expression. In various filamentous fungi, the genes, which confer resistance to hygromycin B, aureobasidin, and G418, have been used as genetic markers for the gene manipulation. However, *A. oryzae* is resistant to these antibiotics. We have recently developed a transformation system for *A. oryzae* RIB40 by using bleomycin-resistance gene as a selective marker. In the present study, we generated *ligD* knock out strain, by using bleomycin resistance selection. And we generated exogenous or endogenous enzyme over-expresser by transformation with expression vector carrying bleomycin resistant gene. Methods: The *ligD* disruption cassette consisted of bleomycin-resistance expression cassette and 2kb of 5' and 3' franking sequence of *ligD*, the *β*-glucuronidase encoding gene *uidA* from *E. coli* over-expression vector and a polygalacturonase encoding gene *pgaB* from *A. oryzae* over-expression vector were introduced into the protoplasts from *A. oryzae* RIB40 by the polyethylene glycol method, respectively. Results: The disruption of the *ligD* locus in genomic DNA of transformants was confirmed by colony PCR and the Southern blot analysis. Activity of each enzyme was detected from cell free extract or culture filtrate of the each transformant selected by bleomycin resistance. Discussion: In the present study, we successfully generated a gene disruptant and over-expresser of two kinds of enzymes by using bleomycin resistance transformant selection system of *A. oryzae*.

PR8.38

Genetic chemotyping in *Fusarium* species predicts toxin content in wheat

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Fusariotoxins present in wheat and barley are a major cause of economic loss, especially for alimentary industries and breweries. Many predictive models based on climatic and agronomic practices have been implemented in order to estimate toxin content in grains. Here we show that genetic chemotyping of the Fusarium population within a field may improve significantly the prediction of toxin content on wheat. We analysed nivalenol (NIV, a trichothecene with higher toxicity when compared to deoxynivalenol) content in wheat by LC/MS-MS analysis from 17 different wheat fields sampled for two consecutive years. We determined species and chemotype by using multiplex PCRs on a total of 1084 isolates. A significant correlation ($R= 0.75$, $p<0.00001$) was found between the quantity of *Fusarium culmorum* isolates per 100 seeds with the NIV chemotype and the amount of NIV in grains. A threshold, based on the amount of NIV isolates per 100 seeds for detecting NIV in grains has been tested successfully on 9 independent additional fields ($P<0.001$). Moreover we showed that the development of a real time PCR method for the detection of *F. culmorum* with NIV chemotype from grains further improved the accuracy and speed of the test. Here we demonstrated the reliability of Fusarium genetic chemotyping for toxin prediction in the field. Furthermore, we verified the importance of targeting genes linked to function (in this case trichothecene synthetic genes) in PCR based screening for mycological application.

PR8.39

Development of a dual marker selection system for repeated gene deletions in *Fusarium venenatum* and the resulting strain harboring five gene deletions

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The industrially important Quorn™ fungus *Fusarium venenatum* has previously been used as a host for heterologous protein expression. Since the wild-type organimsm can grow on the toxic analogue 5-fluoroorotic acid (FOA) despite having an intact *pyrG* gene, an alternative couter-selectable marker was needed for sequential gene deletions to improve the host. A system was developed using two separate gene cassettes, each controlled by a different heterologous promoter and terminator, flanked by direct repeats. The *E. coli* hygromycin phosphotransferase gene *(hph*), conferring resistance to the herbicide hygromycin, was used for positive selection and deletion of the gene of interest. The *herpes simplex virus* thymidine kinase (*tk*) gene was used for counterselection on the uridine analogue 5-flourodeoxyuridine (FdU), allowing selection for spontaneous excision of the region containing both markers. Deletion constructs incorporating the two markers listed above, flanking direct repeats, as well as flanking sequence for the gene of interest were generated for two mycotoxin genes (trichodiene synthase gene (*tri5*) and enniatin synthase gene (*dps1*)), the orotidine- 5- phosphate decarboxylase gene (*pyrG*), an alpha amylase gene (*amyA*) and an alkaline protease (*alpA*) gene.

Using deletion constructs above genes were sequentially deleted in *Fusarium venenatum* strain A3/5 in the following order: *tri5, pyrG, amyA, alpA, dps1*. The uridine auxotrophy caused by the *pyrG* deletion could be rescued by transformation with a *pyrG* fragment, allowing its use as a marker for heterologous protein expression. The resulting strain also showed desired host characteristics as a result of the gene deletions.

PR8.40

Enzymatic hydrolysis of lignocelluloses: identification of novel cellulases genes from filamentous fungi

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Lignocellulosic materials form a huge part of the plant biomass from agricultural and forestry wastes. They consist of three major components: cellulose, hemicellulose and lignin. Cellulose, the main constituent of plant cell wall, is a polymer of D-glucopyranose units linked by β-1,4-glucosidic bonds. Cellulose can be degraded to simple sugar components by means of enzymatic hydrolysis. However, due to its complex, crystalline structure it is difficult to break it down and the cooperative action of a variety of cellulolytic enzymes is necessary. Fungi are known to have potential in production of a variety of cellulolytic enzymes. The aim of this work is to discover new thermostable and robust cellulolytic enzymes for improved enzymatic hydrolysis of biomass. For this purpose two screening methods are applied in different fungal strains with high cellulolytic activities: an expression-based method using suppression subtractive hybridization and a targeted genomic screening approach using degenerate PCR. Suppression subtractive hybridization facilitates identification of genes encoding cellulolytic enzymes that are expressed when cultivating a fungal strain in medium with cellulose as the carbon source. By means of degenerate PCR, specific genes, homologous to the genes of previously classified glycoside hydrolases from CAZY database, are searched for in selected strains of *Aspergillus sp*., *Trichoderma sp*. and *Penicillium sp*. Both methods are anticipated to facilitate identification of target genes which subsequently will be cloned and expressed in a relevant fungal host for further characterization of the expressed enzymes. The goal is to introduce new enzymes to industrial processes.

PR8.41

Colony picker as screening-tool in fungal biotechnology

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The aim of this project is to establish the QPix2 colony picker (Genetix) to pick colonies from different filamentous fungi (e.g. *Aspergillus*, *Trichoderma*) in a fast and efficient way. This is important because classical mutagenesis and screening projects require huge amounts of mutants to be picked and analysed which is highly time consuming if done manually. One additional benefit is that all the work is carried out in an enclosed chamber which is easily kept sterile by exposure to UV-light.
Since the robot was originally designed for picking bacteria or yeast we had to overcome different obstacles regarding e.g. the composition of the culture media, the design of the picking-pins or the speed of movement of the picking head. Other critical parameters are the density of plated spores, age of cultures and size of the colonies. Several experiments will be shown in which we try to define the optimal values for each of these parameters.
Additionally, the colony picker will be used to carry out simple plate assays to measure e.g. enzymatic activities of generated mutants semi-automatically.
All of these improvements will substantially enhance the speed of classical strain development by mutagenesis and screening approaches.

PR8.42

Identification and regulation of gene *fusA* responsible for fusarin production in *Fusarium fujikuroi*

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Fusarium fujikuroi is a rice pathogen which produces different secondary metabolites. Some of them are mycotoxins, i.e. bikaverins and fusarins, and others have biotechnological applications, i.e., gibberellins and carotenoids. Fusarins are near UV-absorbing polyketides that, accumulated at high concentration, provide a yellowish pigmentation to *F. fujikuroi* mycelia. Little information is available on the regulation of fusarin biosynthesis by this fungus. As formerly described, the synthesis was enhanced at high temperature (30°C *vs* 22°C). However, in contrast to the nitrogen repression described for the synthesis of other metabolites, such as gibberellins and bikaverins, we found that fusarin production is repressed by nitrogen starvation. As other fungal polyketides, the synthesis of fusarins starts through the activity of a multidomain enzyme, termed type I polyketide synthase (PKS). The PKS gene responsible for fusarin biosynthesis was formerly indentified in *Fusarium graminearum* and other *Fusarium* species. High sequence conservation between *Fusarium* genomes has allowed us to clone the orthologous gene of *F. fujikuroi,* which we named *fusA*. Deletion mutants for this gene showed a total absence of fusarin production, confirming the role of PKS FusA in the biosynthesis of this metabolite. Real-time RT-PCR analyses of *fusA* mRNA were achieved using a 3' gene segment, absent in the other known *Fusarium* PKS genes. The results showed that the two regulatory conditions tested, temperature and nitrogen availability, act at least partially on *fusA* gene expression.

PR8.43

Effects of CBM insertion on the properties of *Trichoderma reesei* α-Amylase

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The carbohydrate binding modules (CBMs) are non-catalytic domains of carbohydrate-acting enzymes, and are known to promote the association of enzyme with its insoluble substrates. We studied the effects of CBM attachment on the properties of *Trichoderma reesei* α-amylase (ID 105956 in http://genome.jgi-psf.org/Trire2/Trire2.home.html). This amylase does not, in its native form, contain a CBM. A sequence encoding a family 20 CBM from another fungal α-amylase gene was fused 3' to the *T. reesei* amylase gene. The *T. reesei* amylase gene and amylase-CBM fusion construction were overexpressed in *T. reesei* using the strong *cbh1* (*cel7A*) promoter. The two enzymes were purified from the culture supernatants, and their physicochemical properties and performance in stain removal were determined. The amylase-CBM fusion protein was stable in *T. reesei* culture supernatant. Its temperature and pH profiles were similar to those of the native *T. reesei* amylase. However, its specific activity on Azurine-crosslinked amylase (AZCL-Amylose) was over three times higher when compared to the native amylase. Addition of the CBM20 was shown to have a positive effect on starch hydrolysis rate but the hydrolysis product profiles were similar with the two enzymes. The hydrolysis products consisted mainly of glucose and maltose during the whole time-course of the reaction, and only low amounts of maltotriose, -tetraose and –pentaose were detected. Insertion of the CBM had no positive effect on stain removal performance on standard starch stain, possibly due to attachment of the amylase-CBM form to the stain and/or on fabric surface.

PR8.44

Using spore autofluorescence to measure viability of fungal inocula

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Filamentous fungi such as Aspergilli are widely used in numerous biotechnological applications. Their use ranges from the large scale production of citric acid to the expression of highly complex catalytic and therapeutic proteins. Furthermore, they are used in biotransformations both, inside and outside the bioreactor. The widespread industrial usage of filamentous fungi has consequently triggered tremendous interest in the research and optimization of the cultivation process which is now well understood. However, what has been left out so far is the influence of spore quality. This is partially due to the lack of adequate methods for spore quality assessments and hinders the interpretation of cultivation outcomes.

The contribution shows the applicability of spore autofluorescence as an indicator for spore quality. Autofluorescence allows high throughput, non-invasive investigations of important cell compounds and can be correlated to key metabolic substrates also present in spores. The results of the autofluorescence assay show a good correlation to different standard viability assays.

The findings of these investigations show the possibility of assessing spore quality as a routine measure prior to large scale cultivations, thereby improving the cultivation outcome and minimizing bioprocesses with filamentous fungi with inferior yields. The presented results also offer a new and fast tool for the optimization of sporulation media.

PR8.45

Biochemical producing fungi

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

PR8.46

2-DE analysis of the *Penicillium chrysogenum* extracellular proteome: An impressive number of isoforms

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Penicillium chrysogenum is well-known by its ability to synthesize penicillin and related b-lactam antibiotics as well as several other secondary metabolites. In addition, *P. chrysogenum* might be consideered, like other filamentous fungi, as an excellent host for secretion of extracellular proteins due to the high capacity of their protein secretion machinery. In this work, we have analysed by 2-DE the extracellular proteome reference map for *P. chrysogenum* Wisconsin 54-1255. IPG strips with a pH range from 4 to 7 were used and visualization of spots was achieved by "Blue Silver" staining. This method allowed the correct identification of 279 spots by peptide mass fingerprinting (PMF) and tandem MS. The 279 spots represented 328 correctly identified proteins (with an average error of 6.47 ppm), since some spots contained more than one protein. Almost all the significant MASCOT searches provided an exact match with proteins inferred from the *P. chrysogenum* Wisconsin 54-1255 genome, which indicates high accuracy of the genome annotation process. From the 328 proteins identified in the reference map, 62 proteins showed a total of 259 isoforms. Proteins with higher representation in the extracellular proteome were those involved in plant cell wall degradation (polygalacturonase, pectate lyase, glucan 1,3-beta-glucosidase), extracellular acid phosphatases, 6-hydroxy-D-nicotine oxidase and catalase R. These two proteins showed a high number of isoforms (20 and 18, respectively). The deep analysis of the *P. chrysogenum* extracellular proteome may be of relevant interest for white biotechnology.

PR8.47

Transcriptional analysis of *Trichoderma reesei* cultivated in the presence of different lignocellulose substrates

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Transcriptional analysis of *Trichoderma reesei* cultivated in the presence of different lignocellulose substrates Mari Häkkinen , Markku Saloheimo, Merja Penttilä and Tiina Pakula VTT Technical Research Centre, P.O.Box 1000 (Tietotie 2, Espoo), Fin-02044 VTT, Finland; mari.hakkinen@vtt.fi, tel. +358 20 722 111, fax. +358 20 722 7071 *Trichoderma reesei* is a soft rot Ascomycete fungus able to secrete enzymes extremely efficiently and which is therefore used for industrial production of cellulolytic and hemicellulolytic enzymes and heterologous proteins for applications in pulp and paper, detergent, food, textile and feed industries and in biorefinery applications. Production strains have been traditionally improved by classical mutagenesis as well as by specific genetic modifications. The availability of the complete genome sequence of *T. reesei* has made it possible to utilise genome wide analysis methods to study physiology and protein production by the fungus at different conditions and to utilise the information obtained to develop new strains with better enzyme production qualities, such as capability for enhanced protein production or production of modified enzyme mixtures for degradation of specific types of lignocellulose materials. In this study *Trichoderma reesei* Rut-C30 was cultivated in the presence of different lignocellulose substrates in order to study the hydrolytic system of *T. reesei* in the presence of the substrates. The substrates included e.g. sophorose, cellulose, pretreated wheat straw, pretreated spruce, xylan and bagasse. For bagasse, different pre-treatments were used in order to get different combinations of the inducing substances. The cultures were subjected to transcriptional profiling using oligonucleotide microarrays. Differentially expressed genes were indentified from the data by comparing the signal intensities between the induced samples and un-induced controls, and expression profiles of glycoside hydrolase genes and other genes encoding lignocellulase degrading enzymes were compared in the presence of the different substrates to identify co-regulated groups of genes

PR8.48

Production of a CBH1-EG1 fusion enzyme in *Trichoderma reesei* and evaluation of its activity on lignocellulosic substrates

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The enzymatic cocktail of *Trichoderma reesei* is widely used for the hydrolysis of lignocellulosic substrates to fermentable sugars in the bioethanol production processes. Its efficiency has still to be increased, however, to lower the cost of the hydrolysis step. In order to increase the specific activity of the enzyme mixture, a fusion protein comprising the entire CBH1 enzyme linked to the catalytic domain of EG1 was constructed. The construct was transformed into a *T. reesei* strain lacking the *cbh1* gene (CL847D*cbh1*) where it was expressed under the control of the *cbh1* promoter. Two stable clones secreting the fusion protein were isolated and one of them was cultivated in a larger scale for purification of the recombinant protein by FPLC. A partially pure protein could be obtained and its activity on cellulosic substrates determined. The initial hydrolysis rate and final sugar yield of a *T. reesei* enzyme cocktail supplemented with the fusion enzyme were measured on pretreated wheat straw and softwood and compared to the hydrolytic activity of the native mixture. In addition, the degree of synergy was evaluated by comparing the activity of the fusion protein with an equimolar mixture of CBH1 and EG1.

PR8.49

Real time qPCR-based assay for the early detection of aflatoxigenic fungi on maize kernels

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

PR8.50

Applications of *Trichoderma harzianum* in postharvest fungicidal biocontrol

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Although chemicals are usually used to preserve postharvest fruit quality, the acceptability of these treatments by consumers is becoming increasingly limited. Alternatively, biocontrol of postharvest fruit decay using antagonistic microorganisms has been considered a desirable method. *Trichoderma* is a filamentous fungus widely used as a biocontrol agent against phytopathogenic fungi. One of the mechanisms employed by *Trichoderma* during antagonism is the production of cell wall degrading enzymes (CWDEs) and antibiotics (e.g. pyrones). In this study, treatments with conidia of *Trichoderma* in sterile distilled water were conducted to assess the effect on *Penicillium expansum* inoculated on apple and pear fruits. Application of *T. harzianum* CECT 2413 on apples was able to reduce to 60% the number of infected fruits. However, treatment of pears with *T. harzianum* PF1 –a derived mutant overproducing CWDEs and secondary metabolites- was more effective in disease reduction compared to the treatment with the wild type. According to this, effect of enzymes and antibiotics excreted by *Trichoderma* was tested. Combinations of supernatants of PF1 growing either in hydrolase-inducing (fungal mycelia) or pyrone-inducing media significantly inhibited the growth *in vitro* of *P. expansum*. Moreover, the treatment with these combinations of supernatants drastically reduced the number of apple fruits infected with *P. expansum*. These results indicated the importance of *T. harzianum* enzymes and antibiotics in protecting apple fruits from blue mold infection and the suitability of these components in postharvest biocontrol.

PR8.51

Production of meso-galactarate (mucic acid) in fungi

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Mucic acid is a dicarboxylic 6-carbons-containing organic acid, which has applications in food and pharmaceutical industries and can also be used as a platform chemical. Nowadays, mucic acid is produced either by oxidation of D-galactose with nitric acid or by electrolytic oxidation of D-galacturonate. These industrial processes are energy demanding and produce a significant amount of toxic waste. Here we present an alternative route of synthesis of mucic acid by means of biotechnology in *Aspergillus niger* and *Hypocrea jecorina*.

In nature, mucic acid is the first intermediate in the D-galacturonate catabolic pathway in some bacteria, such as Agrobacterium or Pseudomonas. The reaction is catalyzed by D-galacturonate dehydrogenase (udh), which is an NAD-dependent enzyme. D-galacturonate is a main component of pectin, abundant and cheap raw material derived from agricultural production. The bacterial udh gene was introduced into the fungal strains with disrupted intrinsic D-galacturonate metabolism in order to direct metabolic flux towards mucic acid. Both modified strains converted D-galacturonate to mucic acid. *H. jecorina* produced mucic acid in high yield. *A. niger* regained the ability to grow on D-galacturonate, which implies the existence of yet undiscovered pathway for mucic acid degradation in this organism.

PR8.52

The master regulator of the Unfolded Protein Response revisited

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The Unfolded Protein Response (UPR) regulates gene expression in response to stress in the endoplasmic reticulum (ER). In *Aspergillus niger* the beta-zip transcription factor HacA plays a central role in the activation of genes involved in this pathway. Activation of HacA is mediated via an unconventional splicing event of 20 nucleotides in its mRNA. In the present study we engineered an *A. niger* strain that expresses only the activated form of HacA and we show that the removal of the 20 nucleotides results in a constitutive activation of the well established UPR target genes. The wild type strain ($HacA^{WT}$) and the strain expressing the constitutive active form of HacA ($HacA^{CA}$) were cultured in glucose-limited batch cultures using bioreactors. Initially, HacA grew with the same specific growth rate (≈0.22 h^{-1}) as the wild type. However, halfway into the growth phase HacA shifted to linear growth. RNA from the batch cultures was isolated for transcriptomic analysis of the effect of expressing a constitutive activated HacA. Statistical analysis defined 1119 genes as differentially expressed (significance $p<0.005$) relative to the wt. Not surprisingly, genes described as UPR direct targets - *pdiA*, *bipA*, *clxA, prpA* - are found among the most differentially expressed ones. GO enrichment analysis revealed that the expression of several secretion and protein modification related genes was up-regulated in the $HacA^{CA}$ mutant. Biological processes overrepresented in the down-regulated genes include several metabolic pathways, and terms related to transcription and translation. A comprehensive overview of the transcriptional response in the $HacA^{CA}$ mutant will be presented.

PR8.53

Genome mining of secondary metabolites in *Aspergillus*

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Aspergillus species are known to produce high value secondary metabolites such as lovastain. Recently the genomes of several *Aspergillus* species have been sequenced. One of the most important findings from these genome efforts is the realization that these organisms have the potential to produce far more secondary metabolites than have ever been isolated and identified. I will present our recent efforts in identifying new secondary metabolites and their corresponding biosynthesis pathways from several different *Aspergillus* species. Finally we will present approaches to use natural products we have isolated from *Aspergillus* as starting point for further drug discovery.

PR8.54

Characterization of the *Aspergillus nidulans* biotin biosynthesis gene cluster: the bifunctional biDA gene as a new transformation marker in *Aspergilli*

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Biotin (vitamin H) is an essential cofactor for some (trans)carboxylation reactions in carbon metabolism. It also plays roles in cell signaling, epigenetic regulation, and chromatin structure. In fungi, biotin biosynthesis genes are clustered. In *Aspergilli*, the central gene we termed *biDA*, encodes a protein that bares similarity to both *Escherichia coli* dethiobiotin synthetase (BioD) and 7,8-diaminopelargonic acid (DAPA) aminotransferase (BioA). The divergently transcribed *biF* gene codes for a protein similar to 7-keto-8-aminopelargonic acid synthase (BioF) while the *biB* gene, situated downstream of *biDA*, is similar to biotin synthase (BioB). *E. coli* mutants deleted for either of the structural genes *bioF*, *bioA*, *bioD* or *bioB*, could be complemented by the expression of *A. nidulans biF*, *biDA* or *biB* cDNAs. This confirmed that while seperate genes encode DAPA aminotransferase and dethiobiotin synthetase in bacteria and yeast, both these activities are performed by a single, bifunctional protein in *A. nidulans*. Three classical *A. nidulans* (*biA1-3*) biotin-auxotroph mutants were found to have distinct mutations in the part of the *biDA* gene that specifies the DAPA aminotransferase domain. Such mutants could be complemented by transformation with the functional *biDA* gene from either *A. nidulans* or *Aspergillus fumigatus*. Approximately ten biotin autotroph colonies per 10^6 protoplasts per microgram of plasmid, were routinely obtained. Co-transformation of *biDA* with a plasmid carrying sGFP under *A. nidulans* transcriptional control yielded co-transformants stably expressing sGFP over several generations. These results showed that *biDA* orthologs can serve as the basis for a robust and convenient transformation system in *Aspergilli*.

PR8.55

Transposon Vader: From a mobile element towards a molecular tool

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Transposons are mobile DNA sequences, which are found in all eukaryotic genomes (1-3). We have analyzed the transposon content in two fungal genomes, *Aspergillus niger* and *Penicillium chryosogenum*. One non-autonomous element, the *A. niger* transposon *Vader*, was shown to be active during strain development (4). *Vader* mobility could also be shown in a transposon trap experiment. Due to its obvious activity and to its ability to insert into genes *Vader* appears to be suitable as a gene tagging tool. A vector was constructed which carries a *Vader* element between the promoter and open reading frame of the hygromycin resistance gene. The *Vader* element was modified by adding a unique oligonucleotide binding sequence. Conidiospores were plated on hygromycin B containing media which allows for selection of excision of transposon *Vader*. Reintegration sites were determined using TAIL-PCR. *Vader* transposition shows a high insertion rate into genes which appears very promising. At current we analyse a large number of integration sites to determine any target site preferences. In addition we will introduce Vader into *A. nidulans* and other heterologous hosts.

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PR8.56

Array comparative genomic hybridization analysis of *Trichoderma reesei* high cellulase producing strains

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Background: *Trichoderma reesei* is the main industrial producer of cellulases and hemicellulases used to depolymerize biomass in many biotechnical applications. Many production strains in use have been generated by classical mutagenesis. In this study we characterized genomic alterations in hyperproducing mutants of *T. reesei* by high-resolution comparative genomic hybridisation tiling array (aCGH). Our aim was to obtain genome-wide information which could be utilized for better understanding of the mechanisms that underlie the efficient cellulase production and enable targeted genetic engineering for improved production of proteins in general. Results: We carried out aCGH analysis of four hyperproducing strains (QM9123, QM9414, NG14 and RutC-30) using QM6a genome as a reference. In QM9123 and QM9414 we detected altogether 44 previously undocumented mutation sites including deletions, chromosomal breakpoints and single nucleotide mutations. In NG14 and RutC-30 we detected 126 mutation sites of which 17 were new mutation sites not documented previously. Among these new sites there are first chromosomal breakpoints found in NG14 and RutC-30. We studied the effect of two deletions identified in RutC-30 (a deletion of 85 Kb in the scaffold 15 and a deletion in the gene 72076 encoding a fungal transcription factor) on cellulase production by constructing knock-out constructs in the QM6a background. Neither of the deletions affected cellulase production. Conclusions: ArrayCGH analysis identified dozens of mutation sites in each strain analyzed. The resolution was at the level of single nucleotide mutation. High-density aCGH is a useful tool for genome-wide analysis of organisms with small genomes e.g. fungi, especially if large set of interesting strains is to be analyzed.

PR8.57

Comparative analysis of koji mold's genomes

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Koji mold is the traditional name of *Aspergillus* species that are used for Japanese fermentation industries. *Aspergillus oryzae* has been widely used in Japanese fermentation industries, Japanese alcohol beverage, soy sauce and so on for longer than a thousand of years. Comparison of *A. oryzae* genome with those of other

Aspergillus species of smaller genome size revealed existence of non-syntenic blocks (NSBs) specific to the *A. oryzae* genome. *Aspergillus awamori* is another industrial filamentous fungus, widely used for brewing Japanese traditional spirits, Awamori, in Okinawa prefecture. We have sequenced *A. awamori* NBRC 4314 (RIB2604). The total length of non-redundant sequences reached 34.7 Mb consisting of contigs fallen into 44 major linkage groups. High potential of secretory production of proteins has led *A. Oryzae* and *A. awamori* to extensive use also in modern biotechnology. *A. awamori* is genetically very close to *Aspergillus niger* and close to *A. oryzae*. Like *A. niger*, *A. Awamori* vigorously produces citric acid, lowering pH of the product. *A. awamori* is genetically very close to *Aspergillus niger*. However, mapping of short reads from *A. awamori* by SOLiD revealed that the species have remarkable difference. Comparison between the genomes of *A. awamori* and *A. oryzae* showed higher diversity of genes located on the non-syntenic blocks of the *A. oryzae* genome. We are currently sequencing various strains of *A. awamori* by SOLiD. Analysis of the relationship between genetical and phenotypical differences among the strains should provide important information for gene function.

PR8.58

Single-use bioreactors for the heterologous production of a peroxidase by *Aspergillus niger*. An alternative for stirred tank reactors?

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The application of single-use equipment becomes more and more common in the biopharmaceutical industry. Disposable filters, bags for medium and product storage are more and more used and the application of single-use process equipment becomes more common nowadays. Compared to the traditional glass or stainless steel stirred tanks, the single-use bioreactor offers clear advantages: a shorter turnaround time (no SIP or CIP required), resulting in a cost reduction; minimal utilities required; greatly reduced potential of cross contamination (safety); greater operational flexibility; reduced validation requirements.

Due to its two-dimensional movement profile, the CELL-tainer® single-use bioreactor creates a superior gas-liquid mass-transfer compared to other single-use bioreactors presently available. This offers the opportunity to reach higher cell-densities and therefore higher volumetric productivities. With the CELL-tainer k_la values of 300 h^{-1} and above are measured, making the system suitable for high density mammalian cell cultures, but especially also for microbial, yeast, and mycelia cultures. Measurement of the heat exchange capacity (cooling capacity) indicates that accurate temperature control of such exothermic type of cultures is possible as well.

A comparison is made between the CELL-tainer® and the standard stirred bioreactor during the heterologous production of a peroxidase in *Aspergillus niger* under the control of the *inuE* expression signals.

PR8.59

Heterologous protein production and purification of a His-tagged peroxidase in *Aspergillus niger*

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Filamentous fungi have a very efficient protein-production capacity which make them suitable host organisms for the overproduction of commercially interesting homologous and heterologous proteins. The overall efficiency of an enzyme production process is influenced by the production yield (fermentation) and purification yield (Down Stream Processing). Unfortunately, since every protein is different, in many cases production and purification protocols and strategies must be developed for each individual protein. In *E. coli* the fusion of proteins to oligohistidine tags followed by affinity chromatography is a very common protein purification strategy. As far as we know, the use of His-tags in the extracellular production and purification of heterologous proteins in *Aspergillus* has not yet been demonstrated.

Recently, we successfully produced *Arthromyces ramososus* peroxidase (ARP) in *Aspergillus niger* under control of the *inuE* (exo-inulinase) expression signals. To allow fast and easy purification we introduced N- and C-terminal His_6-tags, respectively. Extracellular peroxidase activity could be measured and was obtained with both, N- and C-terminal His_6-tagged ARP. The ability to purify the different His_6-tagged proteins by affinity chromatography is under investigation.

PR8.60

Transcriptome and proteome analysis of an antibody Fab fragment producing *Trichoderma reesei* strain and its parental strain at different cultivation temperatures

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The work is part of a EUROSCOPE programme project Genophys, in which the aim is to compare the physiology of heterologous protein production in different microbial production hosts using a common model protein and applying genome-wide analysis methods to study the cellular events at a selected set of cultivation conditions.
We have carried out transcriptome and proteome analysis of a *Trichoderma reesei* strain producing antibody 3H6 Fab-fragment and a control strain expressing only the selection marker gene *amdS*, and analysed the effects of production of the hetereologous protein as well as cultivation temperature on the cellular responses. For the analyses, the strains were cultivated in lactose-limited chemostats at 21.5°C, 24°C and 28°C. The dilution rate used in the cultivations, 0.03/h, has been previously shown to be optimal for protein production in similar type of chemostat cultures of *T. reesei*. The lower temperatures used in the study, favoured protein production by both strains. In the Fab producing strain, both the total protein production into the culture medium as well as Fab production were the highest at 21.5°C, the values obtained at 24°C being close to the ones obtained at 21.5°C. In the control strain the highest protein production was at 24°C. Comparison of differentially expressed genes between the strains showed relatively few genes affected by the heterologous gene expression, whereas comparison of the cultures at different temperatures revealed a large number of genes with altered expression level. However, the proteome analysis of the cultures using the 2D DIGE method indicated also stress responses to the heterologous protein production. The analysis showed differences especially in the intensity of protein spots corresponding to different type of heat shock proteins (ER, mitochondrial, cytoplasmic, ribosome-associated HSPs), actin and tubulin assembly factors, and proteins related to ER-associated protein degradation.

PR8.61

Transient disruption of the *kusA* gene in a citric acid producing strain of *Aspergillus niger* facilitates high throughput gene deletion

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Mutation of the kusA gene in several fungi, including *Aspergillus niger*, has proven to greatly increase the rate of homologous integration. The kusA gene is required for non homologous end joining and is required for repair of double strand breaks in genomic DNA. A transient kusA- mutant was created in *A. niger* strain ATCC 11414 by inserting pyrG, a counter-selectable marker, at the kusA locus. Screening of targeted gene deletion transformants in this background reveals the rate of homologous recombination was significantly higher (95%) than in the non mutant parent (20%). Restoration of KusA activity is important for maintaining strain stability in *A. niger*, an ascomycete with no established sexual crossing procedures. Spores from the wildtype, kusA, and reverted kusA+ strains were exposed to gamma radiation ranging from 50 to 400 Gy. Our analysis confirms that the ability to recover from radiation-induced mutagenesis was restored in the kusA revertant strains. High throughput genesis of gene deletion constructs can be achieved by utilizing yeast GAP repair and has been used to great effect in other large scale genomic analyses. We have adapted these tools to *A. niger.* Large numbers of gene deletions are underway in order to analyze the metabolic and morphological changes associated with highly efficient citric acid production. This capability will rapidly advance the analysis of organic acid production and morphology in this organism.

PR8.62

Functional characterization of the CPCR1 ortholog (regulatory factor PcRFX) in *Penicillium chrysogenum*

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Beta-lactam biosynthesis is subjected to complex regulatory processes. One of the transcriptional factors recently identified in *Acremonium chrysogenum* is CPCR1, which acts as a regulator of the cephalosporin C biosynthesis and shows a high similarity to transcriptional factors from the human family of "winged helix" regulatory X factors (RFX).

The CPCR1 ortholog was characterized in *Penicillium chrysogenum*. The gene encoding this transcription factor (*Pcrfx*) was identified in the *P. chrysogenum* genome (Pc20g01690). The protein encoded by this gene (PcRFX) shared a 47% homology and 30% identity with the *A. chrysogenum* CPCR1 factor. Analysis of the promoter region of the penicillin biosynthetic genes (*pcbAB*, *pcbC* and *penDE*), revealed the presence of one binding site (X-box) for this factor in each promoter region. Gene silencing of *Pcrfx* gave rise to a decrease in the production of isopenicillin N and penicillin G in the knock-down mutant after 48 and 72 h of culture. In this mutant, the steady-state levels of the penicillin biosynthetic genes transcripts were diminished, thus confirming the regulatory role of PcRFX in penicillin biosynthesis.

PR8.63

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

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Homologous recombination frequencies in filamentous fungi, including *Aspergillus niger*, are low. Several reports over the last years have shown that mutants defective in the Non-Homologous-End Joining (NHEJ) pathway display increased homologous integration efficiencies up to 80 to 100%. Recently, we described that deletion of the *A. niger kusA* gene, encoding the orthologue of the Ku70 protein in other eukaryotes, dramatically improved homologous integration efficiency. However, deletion of *kusA* also causes increased sensitivity of *A. niger* towards UV and X-ray and the consequences of loss of *kusA* in relation to DNA repair and genome stability are currently unknown.

To avoid any potential side effects of a *kusA* loss-of-function mutation on growth and viability of *A. niger*, we transiently disrupted *kusA*. We made use of the counter selectable *amdS* marker, flanked by 300 bp direct repeats of the *kusA* gene. Disruption of the *kusA* gene resulted in similar homologous recombination frequencies compared to the *ΔkusA* strain. After completion of the gene targeting approach, we re-established an intact *kusA* copy using counter selection on fluoroacetamide, proven by sequencing of the *kusA* locus. In *A. niger*, which lacks an sexual reproduction cycle, the transient disruption system is especially important as the NHEJ pathway cannot be restored by performing a sexual cross.

PR8.64

Improved tools for fungal strain development

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Already from the very start of the molecular era for filamentous fungi, TNO has been involved in developing molecular tools for gene expression and protein secretion for filamentous fungi. In this poster we present a number of improved tools for fungal strain development, like novel promoters and versatile selection markers.
From the development of fungal gene transfer systems, the isolation of efficient expression signals is one of the research lines to obtain efficient protein production. As a result of gene expression studies we have discovered a new and very strong promoter from *A. niger*. Furthermore, we have optimized the existing very strong *A. niger gpdA* promoter by modification of its promoter sequence.

Versatile genetic modification of filamentous fungi requires the possibility to modify, disrupt and express a number of different genes in a single fungal strain. Therefore, the availability of a (series) of different selection markers is essential. However, the possibility of repeated use of the same marker in subsequent experiments can circumvent the requirement for multiple selection markers. The usage of counter selectable markers like *pyrG* and *amdS* is very suitable for this purpose, because these allow selection of the mutant and the transformant. Also a synthetic *amdS* selection marker was constructed which is devoid of most restriction sites that are present in the native *amdS* gene.

PR8.65

Single cell analysis as a way to study hyphal heterogeneity

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Mycelial fungi use the hyphae growing at their apices to colonize a substrate. These hyphae secrete enzymes that convert complex polymers into small molecules that can be taken up by the fungus to serve as nutrients. Using GFP as a reporter it has been shown that exploring hyphae of *Aspergillus niger* are heterogenic with respect to enzyme secretion; some hyphae strongly express the glucoamylase gene *glaA*, while others express this gene lowly. This was a surprising finding considering the fact that all hyphae were exposed to the same nutritional conditions. Apparently, a vegetative mycelium is more complex than generally assumed.

We here demonstrate that the expression of other genes encoding secretion enzymes in *A. niger* is also heterogenic. Co-expression studies, using GFP and dTomato as reporters, showed that hyphae that highly express one of these genes also highly express other genes encoding secreted proteins

To unravel the mechanisms underlying heterogeneity we aim to perform a genome-wide expression analysis of highly and lowly expressing hyphae. To this end, protocols have been set up to collect individual hyphae using laser micro-dissection and pressure catapulting (LMPC) and to isolate RNA from these samples. QPCR has shown that we are able to extract RNA from a single hypha only.

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PR8.66

Oligosaccharide transport by *Neurospora crassa* during cellulolytic growth

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The filamentous fungus, *Neurospora crassa,* is capable of depolymerizing and metabolizing plant cell walls, but this process is not understood in great detail. Our group recently reported a systematic study of plant cell wall degradation by *N. crassa,* utilizing transcriptomics, secretome analysis, and the phenotypic characterization of gene knockouts (1). Whole genome microarrays identified 114 genes that show overlapping expression differences when *N. crassa* is grown on either ground *Miscanthus* stems, or pure cellulose as carbon sources. Within this set were 10 predicted major facilitator superfamily transporters. We hypothesized that at least one of these proteins may transport oligosaccharides, an activity that has been reported in *H. jecorina* (2), but never attributed to a particular gene. Mutations in three of these transporters significantly affected growth of *N. crassa* on crystalline cellulose. We developed a method by which to rapidly assay the substrates of these transporters, and report the identity of three oligosaccharide transporters in *N. crassa*. We believe these transporters may be useful in downstream processing of depolymerized plant cell walls.

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PR8.67

Genome-wide analysis of the transportome of the industrial filamentous fungal strain *Penicillium chrysogenum*

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Industrial production of β-lactam antibiotics using the filamentous fungus *Penicillium chrysogenum* is based on successive microbial strain improvement cycles. Analysis of these strains has led to the identification of several important mutations in high-producing strains. These are amongst others the amplification of the penicillin biosynthesis genes, the elevated transcription of genes involved in biosynthesis of the amino acid precursors, and genes encoding microbody proteins. However, many of the key (intra)cellular transport processes have remained obscure.

To identify ABC-transporters involved in β-lactam production, *P. chrysogenum* was grown in the presence and the absence of phenylacetic acid (PAA), a side chain precursor of penicillin G. Expression of all ABC transporters was determined by Quantitative Real Time PCR. This revealed a significantly increased expression level of ABC40 when grown with PAA. In another experiment, expression of all ABC transporters was determined upon a penicillin G challenge, resulting in elevated levels of the expression of ABC30, ABC42 & ABC48. Independent knock-outs strains of ABC30, ABC40, ABC42 and ABC48 in a high producing strain were constructed, but none of these strains showed a detectable phenotype. In another approach, the same genes were inactivated in an early (low) production strain. The further analysis of these strains is in progress.

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