11th European Conference on Fungal Genetics
Philipps- Universitat Marburg, Germany
March 30- April 2, 2012

Committees
Welcome
Sponsors

Scientific Programme

Keynote & Plenary Lecture Abstracts

Parallel Symposia Abstracts

1. Fungal Cell Biology
2. Sex and Sexual Development
3. Genomes and Genome Evolution
4. Organismic Interactions
5. Mitochondria
6. ROS, Autophagy and Apoptosis
7. Sensing and responding
8. Biotechnology
9. The Fungal Cell Wall

Poster Abstracts

1. Fungal Cell Biology
2. Sex and Sexual Development
3. Genomes and Genome Evolution
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7. Sensing and responding

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9. The Fungal Cell Wall
10. Other Fungal Features and Oddities

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Participants List

Committees ECFG11

Local Organising Committee

Michael Bölker

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Regine Kahmann

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Paul Tudzynski

Gillian Turgeon

Dear Colleagues,

It is with great pleasure that I welcome you to Marburg for the 11th European Conference on Fungal Genetics. With this conference ECFG has returned to Germany, where it was held only once before in Münster in 1996. We are proud to host such an exciting meeting that reflects the most recent developments in fungal biology in the areas of genomics, evolution, systems biology and synthetic biology, while witnessing an amazing progress in understanding how fungi live, adapt, develop and interact with different host organisms. I am personally extremely happy that so many of you decided to come and share with us new insights into the intricacies and mechanisms of the unique fungal lifestyle. The local organizing committee has assembled a scientific program with input from the national and the international advisory committee, which we hope will be truly exciting. With two keynote lectures, 18 plenary lectures and 69 oral presentations in parallel sessions we hope to provide a frame for intensive discussions - which you should take as incentive to continue at the more than 400 posters.

Organizing this conference has been a major effort and would not have been possible without Vera Matschiske-Peters who runs my secretarial office and Christian Bengelsdorff, the head of administration at the Max Planck Institute for Terrestrial Microbiology. They were tireless up to the point of sheer collapse when numbers for this congress went up and up and problems steadily increased. I also want to thank the local organizing committee consisting of Michael Bölker, Gunther Doehlemann, Roland Lill, Hans-Ulrich Mösch, Björn Sandrock, Eva Stukenbrock, Christof Taxis and Alga Zuccaro for all their contributions and help behind the scene. Last, but not least I want to thank the SYNMIKRO office for all their organizational help and the many additional helpers from the Max Planck Institute for Terrestrial Microbiology and the Philipps-Universität. Without their input, ECFG11 would not be what it turned out to be. I also want to thank Celia Lloyd and Ray Gibson from Intelligent Events Ltd Edinburgh who helped us organizing this big event. I also wish to thank all our sponsors, in particular FEMS who supports attendance of 27 young scientists and the DFG who generously funds the plenary speakers. ECFG11 is truly an international meeting: more than two thirds of the participants come from outside Germany and represent scientists from 38 different countries. I am also happy about the many industry participants as our field is rooted both in basic science and application. And I am delighted that about one third of the participants are PhD students. I hope that they will make use of this wonderful opportunity to decide on their future areas of research in academia or industry. Most of them will also participate in the conquest for 6 poster prizes that are sponsored by the VAAM.

I very much hope that ECFG11 will be scientifically exciting and thought-provoking. At the same time I hope that you will enjoy the charm of the medieval city of Marburg. We will try our best to make your stay comfortable and hope that you will remember Marburg for its excellent science and unique environment.

Kind regards,
Regine Kahmann
Chair of the local organizing committee
Marburg, March 2012

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Programme

Friday 30 March

14:00-19:30 Registration and poster set up

18:15-18:25 **Welcome** (Hörsaal 215 - Audimax, Hörsaalgebäude Biegenstraße 10)
Regine Kahmann, chair of the organizing committee

18:25-18:35 **Welcome**
Frank Bremmer, Vice President of Philipps-Universität Marburg

18:35-18:45 **"What you are going to miss tonight"**
Michael Bölker, member of the organizing committee

18:45-19:45 **Keynote Lecture**
From genotype to phenotype - an inconvenient truth
Gerald R. Fink, Whitehead Institute for Biomedical Research, USA
Chair: Hans-Ulrich Mösch, Philipps-Universität Marburg, Germany

19:45-22:00 Welcome reception (on the 2nd and 3rd floor of Hörsaalgebäude, Biegenstraße 10)

Saturday 31 March

09:00-10:30 **Plenary session 1: Plant and Human Pathogens** **Audimax**
Chair: Paul Tudzynski, University of Münster, Germany

09:00-09:30 PL1.1 **How do arbuscular mycorrhizal fungi and plants recognize each other?**
Guillaume Bécard, Paul Sabatier University, France

09:30-10:00 PL1.2 **Dissecting phospholipid signalling in *Phytophthora infestans***
Francine Govers, Wageningen University, The Netherlands

10:00-10:30 PL1.3 **Metabolic adaptation and virulence factor expression in *Cryptococcus neoformans***
Jim Kronstad, University of British Columbia, Canada

10:30-11:00 Coffee

11:00-12:30 **Plenary session 2: Epigenetics and RNA Biology** **Audimax**
Chair: Michael Feldbrügge, University of Düsseldorf, Germany

11:00-11:30 PL2.1 **Centromeres of filamentous fungi**
Michael Freitag, Oregon State University, USA

11:30-12:00 PL2.2 **Diverse small RNA biogenesis pathways in *Neurospora***
Yi Liu, University of Texas, USA

12:00-12:30 PL2.3 **Post-transcriptional operons in the rice blast fungus**
Ane Sesma, Technical University of Madrid, Spain

12:30-14:00 Break for lunch

14:00-15:20 **Parallel session 1** **Audimax**
Fungal Cell Biology
Chairs: Gero Steinberg, University of Exeter, UK and Nick Read, University of Edinburgh, UK

14:00-14:20 PS1.1 **In *Candida albicans* the negative regulator of morphogenesis, Nrg1, is itself regulated at multiple post-transcriptional levels by kinase action**
Peter Sudbery, Sheffield University, UK

14:20-14:40 PS1.2 **Investigating the biology of plant infection by the rice blast fungus *Magnaporthe oryzae***
Nicholas Talbot, University of Exeter, UK

14:40-15:00 PS1.3 **Comparative live-cell imaging analyses of SPA-2, BUD-6 and BNI-1 in *Neurospora crassa* reveal novel features of the filamentous fungal polarisome**
Alexander Lichius, CICESE, Mexico

15:00-15:20 PS1.4 **The *Aspergillus nidulans* kinesin-3 tail is necessary and sufficient to recognize modified microtubules**
Constanze Seidel, Karlsruhe Institute of Technology, Germany

14:00-15:20 **Parallel session 2** **Room 113**
Sex and Sexual Development
Chairs: Ursula Kües, University of Göttingen, Germany and Robert Debuchy, University of Paris Sud, France

14:00-14:20 PS2.1 **A mating type loci in *Coprinopsis cinerea* differ in numbers of homeodomain transcription factor genes**
Ursula Kües, University of Göttingen, Germany

14:20-14:40 PS2.2 **Systematic deletion of homeogenes in an ascomycete fungus supports analogy of clamp and crozier in Dikaryomycota**
Robert Debuchy, University of Paris Sud, France

14:40-15:00 PS2.3 **Sexual development and spore pathogenesis of Cryptococcus**
Michael Botts, University of Wisconsin, USA

15:00-15:20 PS2.4 **Living together - genetics and genomics of the dikaryotic lifestyle**
Antonio G. Pisabarro, Public University of Navarre, Spain

14:00-15:20 **Parallel session 3** **Room 114**
Genomes and Genome Evolution
Chairs: Eva Stukenbrock, Max Planck Institute for Terrestrial Microbiology, Germany and Marco Thines, Biodiversity and Climate Research Centre (BiK-F), Germany

14:00-14:20 PS3.1 **Evolution of obligate parasitism in the white rust pathogen of *Arabidopsis thaliana***
Eric Kemen, The Sainsbury Laboratory, UK

14:20-14:40 PS3.2 **Harnessing natural genetic variation to elucidate the relationship between genotype and phenotype in *Saccharomyces paradoxus***
Jeremy Roop, University of California, USA

14:40-15:00 PS3.3 **Evolutionary genomics of accessory chromosomes in *Mycosphaerella graminicola***
Daniel Croll, Institute of Integrative Biology Zurich, Switzerland

15:00-15:20 PS3.4 **Genome sequence of Shiitake mushroom *Lentinula edodes* and comparative mushroom genomic analyses**
Hoi Shan Kwan, Chinese University of Hong Kong, China

15:20-16:00 Coffee and cakes

16:00-17:20 **Parallel session 1 continued** **Audimax**
Fungal Cell Biology
Chairs: Gero Steinberg, University of Exeter, UK and Nick Read, University of Edinburgh, UK

16:00-16:20 PS1.5 **Active plus-end capture of dynein at astral microtubules increases the efficiency of anaphase B**
Yvonne Roger, University of Exeter, UK

16:20-16:40 PS1.6 ***Candida albicans*: sensing the host environment**
Carol Kumamoto, Tufts University School of Medicine, USA

16:40-17:00 PS1.7 **A steep phosphoinositide phosphate gradient is critical for filamentous growth in *Candida albicans***
Robert Arkowitz, University of Nice, France

17:00-17:20 PS1.8 **In vivo nonlinear spectral imaging of fungi**
Helene Knaus, University of Utrecht, The Netherlands

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16:00-17:20 **Parallel session 2 continued** **Room 113**
Sex and Sexual Development
Chairs: Ursula Kües, University of Göttingen, Germany and Robert Debuchy, University of Paris Sud, France

16:00-16:20 PS2.5 **The transcription of the putative mating type genes *sexM* and *sexP* is regulated by trisporic acid in *Mucor muce*do**
Jana Wetzel, Friedrich Schiller University Jena, Germany

16:20-16:40 PS2.6 **Interspecific sex in grass smuts and the genetic diversity of their pheromone-receptor system**
Ronny Kellner, Ruhr University Bochum, Germany

16:40-17:00 PS2.7 **The pheromone system of *Hypocrea jecorina (Trichoderma reesei)* and its regulation by photoreceptors upon sexual development**
Monika Schmoll, Vienna University of Technology, Austria

17:00-17:20 PS2.8 **A developmental protein as scaffold for a MAP kinase pathway controlling fungal fruiting body development?**
Ines Teichert, Ruhr University Bochum, Germany

16:00-17:20 **Parallel session 3 continued** **Room 114**
Genomes and Genome Evolution
Chairs: Eva Stukenbrock, Max Planck Institute for Terrestrial Microbiology, Germany and Marco Thines, Biodiversity and Climate Research Centre (BiK-F), Germany

16:00-16:20 PS3.5 **Comparative genomics of *Fusarium pseudograminearum* and other cereal fungal pathogens**
John Manners, CSIRO Plant Industry, Australia

16:20-16:40 PS3.6 **Comparative genomics of basidiomycetes telomere and subtelomere regions**
Lucía Ramírez, Public University of Navarre, Spain

16:40-17:00 PS3.7 **Comparative genomics of *Cochliobolus* cereal pathogens: the core and pan genome**
Gillian Turgeon, Cornell University, USA

17:00-17:20 PS3.8 **A dynamin-like protein affects both RIP and premeiotic recombination**
Kyle Pomraning, Oregon State University, USA

17:30-20:30 Poster session sponsored by Genencor

Sunday 1 April

09:00-10:30 **Plenary session 3: Secondary Metabolism** **Audimax**
Chair: Barry Scott, Massey University, New Zealand

09:00-09:30 PL3.1 **Secondary metabolism in *Fusarium fujikuroi* – the role of nitrogen availability and histone modifications**
Bettina Tudzynski, University of Münster, Germany

09:30-10:00 PL3.2 **LaeA-directed natural product discoveries**
Nancy Keller, Madison School of Medicine and Public Health, USA

10:00-10:30 PL3.3 **Secondary metabolites of *Leptosphaeria maculans*, the causal agent of blackleg of canola**
Barbara Howlett, University of Melbourne, Australia

10:30-11:00 Coffee

11:00-12:30 **Plenary session 4: Synthetic and Systems Biology** **Audimax**
Chair: Axel Brakhage, Friedrich Schiller University Jena, Germany

11:00-11:30 PL4.1 **Mathematical modeling of yeast stress response and cell cycle regulation**
Edda Klipp, Humboldt University of Berlin, Germany

11:30-12:00 PL4.2 **Aspergillus spp.: ironing out iron problems**
Hubertus Haas, Innsbruck Medical University, Austria

12:00-12:30 PL4.3 **Systems biology of industrially important filamentous fungi**
Jens Nielsen, Chalmers University of Technology, Sweden

12:30-14:00 Break for lunch

14:00-15:20 **Parallel session 4** **Audimax**
Organismic Interactions
Chairs: Alga Zuccaro and Gunther Döhlemann, Max Planck Institute for Terrestrial Microbiology, Germany

14:00-14:20 PS4.1 **ß-1,3-glucan synthase of the maize anthracnose fungus *Colletotrichum graminicola* is essential at specific stages of pathogenesis**
Holger Deising, The Martin Luther University of Halle-Wittenberg, Germany

14:20-14:40 PS4.2 **Effectors and biotrophic invasion by the rice blast fungus, *Magnaporthe oryzae***
Barbara Valent, Kansas State University, USA

14:40-15:00 PS4.3 **Downy mildew effectors and their activity in the host plant**
Guido van den Ackerveken, Utrecht University, The Netherlands

15:00-15:20 PS4.4 **SpHtp1 from the oomycete *Saprolegnia parasitica* shows fish cell-specific entry and tyrosine-O-sulphate-dependent import**
Stephan Wawra, Aberdeen Oomycete Laboratory, UK

14:00-15:20 **Parallel session 5** **Room 113**
Mitochondria
Chairs: Roland Lill, Philipps-Universität Marburg, Germany and Benedikt Westermann, University of Bayreuth, Germany

14:00-14:20 PS5.1 **Functional analysis of ERMES and TOB (SAM) complex components in *Neurospora crassa***
Frank Nargang, University of Alberta, Canada

14:20-14:40 PS5.2 **On mitochondrial genes, genomes and proteomes**
Franz Lang, University of Montreal, Canada

14:40-15:00 PS5.3 **Mitochondrial dynamics and organismal ageing in *Saccharomyces cerevisiae***
Christian Q. Scheckhuber, Groningen Biomolecular Sciences and Biotechnology Institute, The Netherlands

15:00-15:20 PS5.4 **Mitochondrial dynamics in yeast**
Benedikt Westermann, University of Bayreuth, Germany

14:00-15:20 **Parallel session 6** **Room 114**
ROS, Autophagy and Apoptosis
Chairs: Jesus Aguirre, National Autonomous University of Mexico and Heinz Osiewacz, Johann Wolfgang Goethe University, Germany

14:00-14:20 PS6.1 **ROS signal transduction and cell differentiation in filamentous fungi**
Jesus Aguirre, National Autonomous University of Mexico

14:20-14:40 PS6.2 **The NADPH oxidase complexes in *Botrytis cinerea***
Ulrike Siegmund, University of Münster, Germany

14:40-15:00 PS6.3 **Identifying targets of NADPH oxidase-mediated redox signalling in *Fusarium graminearum* using proteomics approaches.**
Christof Rampitsch, Agriculture and Agrifood, Canada

15:00-15:20 PS6.4 **Production and epidemiological importance of photodynamic toxins produced by the necrotrophic fungus *Ramularia collo-cygni***
Michael Hess, Technical University München, Germany

15:20-16:00 Coffee and cakes

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16:00-17:20 **Parallel session 4 continued** **Audimax**
Organismic Interactions
Chairs: Alga Zuccaro and Gunther Döhlemann, Max Planck Institute for Terrestrial Microbiology, Germany

16:00-16:20 PS4.5 **Calnexin complex is involved in the establishment of fungal biotrophy in *Ustilago maydis***
José Ibeas, The Pablo de Olavide University, Spain

16:20-16:40 PS4.6 **Functional analysis of candidate effector proteins by host-induced gene silencing in *Blumeria graminis* f. sp. *hordei***
Clara Pliego, Imperial College London, UK

16:40-17:00 PS4.7 **Elucidating the response of wheat to the exposure of *Stagonospora nodorum* effectors**
Lauren Du Fall, Australian National University, Australia

17:00-17:20 PS4.8 **How do mobile pathogenicity chromosomes collaborate with the core genome?**
Charlotte van der Does, University of Amsterdam, The Netherlands

16:00-17:20 **Parallel session 5 continued** **Room 113**
Mitochondria
Chairs: Roland Lill, Philipps-Universität Marburg, Germany and Benedikt Westermann, University of Bayreuth, Germany

16:00-16:20 PS5.5 **Mitochondrial protein quality control influences lifespan and stress adaptation in *Podospora anserina***
Fabian Fischer, Johann Wolfgang Goethe University, Germany

16:20-16:40 PS5.6 **A mitochondrial molecular marker for estimating arbuscular mycorrhizal fungal biomass in soil and roots**
Cristina Micali, University of Montreal, Canada

16:40-17:00 PS5.7 **The mitochondrial genome of the wood-decaying basidiomycete *Phlebia radiata* is the largest in size (156 kb) among fungi and contains a 6 kb inversion, stretches with repetitive elements and long introns invaded with homing endonucleases**
Taina Lundell, University of Helsinki, Finland

17:00-17:20 PS5.8 **Phylogenetic analysis of the complete mitochondrial genome of *Madurella mycetomatis* confirms its taxonomic position within the order Sordariales**
Wendy van de Sande, Erasmus MC, The Netherlands

16:00-17:20 **Parallel session 6 continued** **Room 114**
ROS, Autophagy and Apoptosis
Chairs: Jesus Aguirre, National Autonomous University of Mexico and Heinz Osiewacz, Johann Wolfgang Goethe University, Germany

16:00-16:20 PS6.5 **ROS damage defence mechanisms in *Podospora anserina***
Andrea Hamann, Johann Wolfgang Goethe University, Germany

16:20-16:40 PS6.6 **Mitophagy is linked to the general stress response pathway in *Saccharomyces cerevisiae***
Andreas Reichert, Frankfurt Institute for Molecular Life Sciences, Germany

16:40-17:00 PS6.7 ***Botrytis*-plant interaction: interplay of cell death**
Amir Sharon, Tel-Aviv University, Israel

17:00-17:20 PS6.8 **Farnesol-induced cell death in the filamentous fungus *Aspergillus nidulans***
Gustavo Goldman, University of São Paulo, Brazil

17:30-20:30 Poster session sponsored by Genencor

Monday 2 April

08:30-10:05 **Plenary session 5**: **Genomes** **Audimax**
Chair: Jörg Kämper, Karlsruhe Institute of Technology (KIT), Germany

08:30-09:00 PL5.1 **Population genomics uncovers the *Verticillium dahliae* effector that is recognized by the tomato Ve1 immune receptor**
Bart Thomma, Wageningen University, The Netherlands

09:00-09:30 PL5.2 **Interacting with plants: lessons from fungal genomes**
Marc-Henri Lebrun, INRA-APT, France

09:30-10:00 PL5.3 **Genomic dynamics and host specificity in *Fusarium oxysporum* species complex**
Li Jun Ma, University of Massachusetts Amherst, USA

10:00-10:05 **Genome sequencing and beyond: A new JGI call for proposals**
Igor Grigoriev, DOE Joint Genome Institute, USA

10:05-10:30 Coffee

10:30-12:00 **Plenary session 6: Regulation and Development** **Audimax**
Chair: Erika Kothe, Friedrich Schiller University Jena, Germany

10:30-11:00 PL6.1 **Coordination of fungal development and secondary metabolism**
Gerhard Braus, University of Göttingen, Germany

11:00-11:30 PL6.2 **Tipping the balance: what turns a fungal mutualist into a pathogen**
Barry Scott, Massey University, New Zealand

11:30-12:00 PL6.3 **Novel intrinsically disordered proteins assemble at septal pores and regulate diverse aspects of hyphal homeostasis**
Greg Jedd, Temasek Life Sciences Laboratory, Singapore

12:00-13:00 International Scientific Committee meeting Room 109

12:40-13:20 Demonstration: Room 113
PhytoPath - a new resource for integrating genomic and phenotypic information from plant pathogens

13:20-14:00 Demonstration: Room 113
PhytoPath - a new resource for integrating genomic and phenotypic information from plant pathogens

12:00-14:30 Extended lunch break for sightseeing and guided tours

14:30-15:50 **Parallel session 7** **Room 114**
Sensing and responding
Chairs: José Pérez-Martin, Centre for Biotechnology Madrid, Spain and Jürgen Wendland, Carlsberg Laboratory, Denmark

14:30-14:50 PS7.1 **The Pals wink at the ESCRT: pH signalling in the plasma membrane**
Miguel Penalva, Centre for Biotechnology Madrid, Spain

14:50-15:10 PS7.2 **pH control of infectious growth in *Fusarium oxysporum* involves reprogramming of MAPK signalling cascades**
Antonio di Pietro, University of Cordoba, Spain

16:20-16:40 PS7.3 **Subcellular localization of the *Neurospora crassa* MAP kinase MAK-2 influences its activity and function during cell-cell signalling**
Julia Illgen, Braunschweig University of Technology, Germany

15:30-15:50 PS7.4 **Functional characterization of G-protein-coupled receptors in the cereal pathogen *Fusarium graminearum***
Van Thuat Nguyen, University of Hamburg, Germany

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14:30-15:50 **Parallel session 8** **Audimax**
Biotechnology
Chairs: Peter Punt, TNO, The Netherlands and Bernhard Seiboth, Vienna University of Technology, Austria

14:30-14:50 PS8.1 **Systems biology approaches to dissecting plant cell wall deconstruction in a model filamentous fungus**
Louise Glass, University of California-Berkeley, USA

14:50-15:10 PS8.2 **Modeling the XlnR regulon of *Aspergillus niger***
Leo H. de Graaff, Wageningen University, The Netherlands

15:10-15:30 PS8.3 **Breaking the silence: protein stabilization uncovers silenced biosynthetic gene clusters in the fungus *Aspergillus nidulans***
Jennifer Gerke, University of Göttingen, Germany

15:30-15:50 PS8.4 **Lactose induces all genes related to plant biomass hydrolysis and corresponding mono- and oligosaccharide transporters in *Trichoderma reesei***
Christa Ivanova, Vienna University of Technology, Austria

14:30-15:50 **Parallel session 9** **Room 113**
The Fungal Cell Wall
Chairs: José Ibeas, The Pablo de Olavide University, Spain and Steffen Rupp, Fraunhofer IGB, Germany

14:30-14:50 PS9.1 **Advances in fungal cell wall proteomics**
Piet de Groot, University of Castilla – La Mancha, Spain

14:50-15:10 PS9.2 **Interaction of cell wall polysaccharides with amyloid forming proteins**
Han Wösten, Utrecht University, The Netherlands

15:10-15:30 PS9.3 **Microarray analysis of antifungal synergy between inhibitors of chitin synthases and beta-(1,3)-glucan synthase**
Emmanuelle Galland, Bayer CropScience, France

15:30-15:50 PS9.4 **Cell wall stress affects chitin synthase delivery and secretion in the pathogen *Ustilago maydis***
Magdalena Martin-Urdiroz, University of Exeter, UK

15:50-16:30 Coffee and cakes

16:30-17:30 **Parallel session 7 continued** **Room 114**
Sensing and Responding
Chairs: José Pérez-Martin, Centre for Biotechnology Madrid, Spain and Jürgen Wendland, Carlsberg Laboratory, Denmark

16:30-16:50 PS7.5 **Specific structural features of sterols affect cell-cell signalling and fusion in *Neurospora crassa***
Martin Weichert, Braunschweig University of Technology, Germany

16:50-17:10 PS7.6 **Structural and functional comparison of pyrrolnitrin- and iprodione-induced modifications in the class III histidine-kinase Bos1 of *Botrytis cinerea***
Sabine Fillinger, INRA Thiverval-Grignon, France

17:10-17:30 PS7.7 **Transcriptomic and molecular analysis of germination and plant infection of *Botrytis cinerea***
Michaela Leroch , University of Kaiserslautern, Germany

16:30-17:30 **Parallel session 8 continued** **Audimax**
Biotechnology
Chairs: Peter Punt, TNO, The Netherlands and Bernhard Seiboth, Vienna University of Technology, Austria

16:30-16:50 PS8.5 **Endophytic fungi; a novel biotechnology tool**
Milan Gagic, Agresearch, New Zealand

16:50-17:10 PS8.6 **Characterisation of constitutive promoters of *Aspergillus niger* exhibiting different expression intensities as a useful tool for metabolic engineering**
Marzena Blumhoff, BOKU University of Natural Resources and Life Sciences, Austria

17:10-17:30 PS8.7 **Control of morphogenesis and pellet architecture by cell surface proteins in *Streptomyces coelicolor***
Dennis Claessen, Leiden University, The Netherlands

16:30-17:30 **Parallel session 9 continued** **Room 113**
The Fungal Cell Wall
Chairs: José Ibeas, The Pablo de Olavide University, Spain and Steffen Rupp, Fraunhofer IGB, Germany

16:30-16:50 PS9.5 **Self-assembly at air/water interfaces and chitin-binding properties of the small cell wall protein EPL1 from *Trichoderma atroviride***
Verena Seidl-Seiboth, Vienna University of Technology, Austria

16:50-17:10 PS9.6 **Analysis of the cell wall integrity (CWI) pathway in *Ashbya gossypii*.**
Klaus Lengeler, Carlsberg Laboratory, Denmark

17:10-17:30 PS9.7 **Efg1 shows a haploinsufficiency phenotype in modulating cell wall architecture and immunogenicity of *Candida albicans***
Steffen Rupp, Fraunhofer IGB, Germany

17:45-18:45 **Keynote Lecture** **Audimax**
***Ustilago maydis*: an experimental organism for 21st century biology**
William Holloman, Weill Cornell Medical College, USA
Chair: Michael Bölker, Philipps-Universität Marburg, Germany

18:45 Poster prize winners announced

19:30-22:30 Conference dinner at Marburg Castle

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Saturday 31 March

Keynote lecture

From Genotype to Phenotype ---An Inconvenient Truth

Gerald R. Fink
Whitehead Institute/MIT Nine Cambridge Center Cambridge, MA 02467

Genome wide comparisons of transcriptional circuits among different fungal species have shown that these regulatory networks are extremely variable. This network plasticity has been most thoroughly documented in the comparison of mating type regulation between the evolutionarily distant species *Candida abicans* and *S. cerevisiae*. Although the group of genes controlling mating and the transcriptional regulators are similar, the circuit that regulates the mating genes uses an activator in Candida and a repressor in Saccharomyces. As this change in circuitry between species involves many mutational changes that are unlikely to have occurred simultaneously, the conversion of one circuit to another raises the question: How much network variation exists within a single species?

To address this question we compared the deletion phenotypes of two closely related strains of *Saccharomyces cerevisiae*, the reference strain S288c and a closely related strain, Σ1278b, by individually deleting every gene in Σ1278b. Despite their overall sequence similarity, these strains show dramatic differences in phenotype even among those genes considered “essential.” This difference in essentiality is polygenic, often depending on more than 5 genes. A second comparison of the two strains for the genes that affect adhesion/filamentation again revealed unexpected differences. Adhesion in Sigma is controlled by the filamentation MAPK pathway (fMAPK), which activates the transcription of a downstream structural gene *FLO11*.

However, in Sigma the fMAPK pathway is not required to activate *FLO11* for adhesion/filamentation despite the fact that the MAPK pathway is still present and active in mating. In S288c the requirement for the fMAPK pathway is bypassed by many different polymorphisms that lead to fMAPK independence. We identified one of these suppressors of the fMAPK pathway SUP1(S288c), a transcription factor, capable of bypassing the fMAPK pathway in both S288c and Sigma. The Sigma allele, SUP1(Sigma) cannot bypass the fMAPK pathway either in S288c or Sigma. Thus, both for essential genes and morphological traits there are fundamental differences in gene control. Our studies show that even within a species there is substantial variation in the networks that control gene expression. These polymorphisms are the obvious grist for speciation.

Monday 2 April

Keynote lecture

***Ustilago maydis*: an experimental organism for 21st century biology**

William Holloman
Cornell University Medical College

The fundamental principles of cell growth, division, differentiation, communication, and development have come from discoveries made in an odd collection of organisms including flies, worms, and fungi. Given that advances in DNA sequencing technologies and gene transfer methods makes exploration of genetic questions possible in almost any organism, a question for students to ponder is why do only a handful of organisms serve as model systems? The answer is that the choice of species as systems for experimentation has often been driven by need and desire for improving agriculture and industry, expedience and amenity for laboratory study, and more often than not, simply the vagaries of the day. Organisms that emerged in the last century as choice models did so because their entire biology was explorable, interesting mutants were obtained, enabling methodologies were developed, and devoted scientific groups coalesced. The same criteria still apply now in the twenty-first century.

Ustilago maydis, a biotrophic fungal plant pathogen that is the causative agent of smut disease of maize, has emerged at the beginning of the twenty-first century, as an outstanding model system for genetic analysis. It has proven to be valuable for understanding genetic mechanisms involved in numerous cellular pathways including parasite virulence and host defense during pathogenesis, homologous recombination, cytoskeleton architecture, intracellular trafficking and movement of macromolecules, secondary metabolite production, evolution of sex determining loci, cell cycle regulation, dikaryosis, and so on. The story of its development as organism for experimentation in genetics is remarkable in that it has came to light as model system more lately, but originating, nonetheless, from the currents of intellectual and scientific fervor from which *Drosophila melanogaster* and *Neurospora crassa* emerged.

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Saturday 31 March

Plenary session 1: Plant and Human Pathogens

PL1.1
How arbuscular mycorrhizal fungi and plants recognize each other?
Guillaume Becard
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Before physical contact between the partners of the arbuscular mycorrhizal symbiosis each partner, the plant and the fungus, secretes diffusible molecules that can trigger in the opposite partner rapid metabolic responses, new gene expression and developmental modifications. Trace molecules such as strigolactones present in root exudates serve as plant host recognition signals for AM fungi.

They induce spore germination and/or hyphal branching at extremely low concentrations by rapidly stimulating the fungus mitochondrial activity. Interestingly strigolactones are also germination stimulant of the obligate parasitic weeds *Orobanche* and *Striga* and they have recently been proposed as a new class of hormones controlling various developmental stages in plants.

Symmetrically, based on genetic, molecular, biochemical and physiological evidence, AM fungi were also expected to produce important early signals perceived by their plant host. The so called Myc factors were expected to trigger the plant mycorrhization programme via the common SYM pathway (CSP), like the Nod factors produced by rhizobia trigger nodulation of legumes. Molecules that respond to the definition of Myc factors have recently been identified. They are simple lipochitooligosaccharides (LCOs) that can stimulate, at extremely low concentration, mycorrhization and lateral root formation of non legumes (dicots and monocots). In *Medicago truncatula*, activity on root development is dependent on DMI1, DMI2, DMI3 and NSP2, four proteins involved in the CSP.

The fact that LCOs, involved in a very ancient plant biotic interaction, are also important signals in “modern” interactions with rhizobia, raises the question of their possible occurrence in other plant biotic interactions, including in other plant- fungus interactions.

PL1.2
Dissecting phospholipid signalling in *Phytophthora infestans*
Francine Govers
Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands

Phytophthora, which literally means plant destroyer, is a genus in the Stramenopile lineage in the class oomycetes with over a hundred species. Well known is *Phytophthora infestans,* the causal agent of potato late blight. Its ~ 240 Mb genome is the largest and most complex in the Stramenopile lineage. Comparative genome analysis revealed features illuminating its success as a pathogen, such as rapid turnover and massive expansion of families encoding secreted proteins and peculiar gene innovations resulting in proteins with oomycete-specific domain combinations. One outstanding class of novel proteins comprises GPCR-PIPKs. The 12 members all have a N-terminal 7-transmembrane domain typical for G-protein coupled receptors (GPCRs) combined with a phosphatidylinositol phosphate kinase (PIPK) domain at the C-terminus. Their differential expression and localization suggest distinct roles in various cellular processes. Another typical class represents secreted proteins with hallmarks of phospholipase D (PLD). We demonstrated PLD activity in medium of mycelium cultures and are currently investigating how these secreted PLDs effect plant cells. Other well-known proteins in the *Phytophthora* secretome are the RXLR effectors that act inside host cells where they function as virulence factors, mostly by suppressing host defence. In certain interactions they act as avirulence factors that are recognized by corresponding resistance proteins thereby triggering host defence. For the RXLR effector IPI-O, we identified a putative host target and demonstrated that this lectin receptor kinase is a *Phytophthora* resistance component in Arabidopsis.

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PL1.3

Metabolic adaptation and virulence factor expression in *Cryptococcus neoformans*

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The Michael Smith Laboratories, University of British Columbia, Vancouver, B.C., Canada, Department of Biotechnology, Chung-Ang University, 72-1 Nae-ri, Daedeok-Myeon, Anseong-Si, Gyeonggi-Do, 456-756, Republic of Korea

The pathogenic basidiomycete fungi *Cryptococcus neoformans* and *Cryptococcus gattii* cause life-threatening meningoencephalitis in immunocompromised and immunocompetent people. We are employing genetic and genomics approaches to identify the factors in the *Cryptococcus* species that contribute to metabolic adaptation and virulence factor expression in the host environment. The emerging view is that these processes are tightly integrated to support colonization and proliferation. One area of investigation focuses on elucidating the mechanisms of iron acquisition during mammalian infection by *C. neoformans.* We previously found that the reductive, high-affinity uptake system encoded by the *CFT1* (iron permease) and *CFO1* (ferroxidase) genes is required for iron use from transferrin, for full virulence and for dissemination to the CNS. The fact that the mutants still caused some disease in mice suggested that other iron sources were important during infection. Heme is a likely candidate because of its abundance in mammals and because *C. neoformans* grows particularly well on heme as the sole iron source. We found that the extracellular mannoprotein Cig1 is important for iron utilization from heme. In addition, transcriptional profiling and an insertional mutagenesis screen via Agrobacterium-mediated transformation identified a number of additional functions involved in heme utilization. One of these functions is endocytosis and mutants with defects in this process are debilitated for growth on heme.

Saturday 31 March

Plenary session 2: Epigenetics and RNA Biology

PL2.1

Centromeres of filamentous fungi

Kristina Smith, Pallavi Phatale, Lanelle Connolly, Sarah Ferrer, Alec Peters, Jonathan Galazka Michael Freitag
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Centromeric DNA, the centromere-specific histone H3 variant (CenH3), and centromeric DNA binding proteins form the foundation for attachment of kinetochore protein complex assembly. Correct assembly and proper maintenance of these large and cell cycle-regulated complexes is essential for attachment of spindle microtubules, which transport chromosomes into daughter nuclei during nuclear division. Over the past decade little information has emerged on centromere and kinetochore organization in filamentous fungi, even though these protein complexes are essential, and to date, only centromeres of *Neurospora crassa* have been studied in any detail. This was enabled by early groundbreaking studies on the underlying centromeric DNA structure [1, 2] and the availability of an arsenal of genetic, biochemical and cytological tools to study centromere proteins and centromere DNA composition. We analyzed the genomes of *Neurospora*, *Fusarium* and *Mycosphaerella* species for the presence of satellite or other near-repetitive sequences, confirming earlier studies that predicted centromeric DNA to be composed of active or silent transposable elements. To learn more about centromere assembly and maintenance, we subjected *Neurospora crassa* and *Fusarium graminearum* to ChIP-sequencing with tagged CenH3 and antibodies against histone modifications thought to be required for centromere function [3]. Our findings suggest that centromere maintenance in Neurospora is qualitatively different from that in fission yeast, where expression of small RNA and subsequent heterochromatin formation is required for the assembly but not maintenance of centromeres. To better understand centromere assembly we are dissecting protein interactions between different centromere foundation proteins, namely CenH3, CEN-B, CEN-C and CEN-T, by genetic and biochemical means.

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PL2.2

Diverse Small RNA Biogenesis Pathways in *Neurospora*

Yi Liu

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A variety of small RNAs, including miRNAs and Piwi-interacting RNAs (piRNAs), associate with Argonaute family proteins to regulate gene expression in diverse cellular processes. In *Neurospora crassa*, we identified several types of Argonaute-associated small RNAs. The analyses of these sRNAs uncovered the existence of diverse sRNA production mechanisms. qiRNAs are a type of Argonaute QDE-2 associated sRNAs that are induced after DNA damage. qiRNAs originate from the highly repetitive rDNA locus and their biogenesis the RDRP QDE-1, the recQ DNA helicase QDE-3 and the Dicers. Our genetic and biochemical results suggest that, after DNA damage, QDE-1 is recruited to ssDNA by RPA and QDE-3. QDE-1 first acts as a DNA-dependent RNA polymerase to produce ssRNA and then as an RDRP to converts the ssRNA into dsRNA, a process that is strongly by RPA. miRNA-like small RNAs (milRNAs) and Dicer-independent sRNAs (disiRNAs) are two additional types of QDE-2 associated sRNAs. Despite their similarities to miRNAs in higher eukaryotes, there are at least four different pathways for milRNA production, including Dicer-dependent and Dicer-independent pathways. On the other hand, although disiRNAs may originate from dsRNA, they are generated independent of all known RNAi components. The mechanistic diversity observed for sRNA biogenesis in *Neurospora* shed lights on the diversity, evolutional origins, and biogenesis of eukaryotic small RNAs.

PL2.3

Post-Transcriptional Operons in the Rice Blast Fungus

Ane Sesma

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Transcriptional (*de novo* transcription) and post-transcriptional mechanisms (mRNA stability/degradation, export/localisation, translation, silencing...) coordinate the expression of related genes that drive a biological process, i.e. invasive growth during host colonisation. RNA-binding proteins orchestrate the expression of these gene networks at post-transcriptional level. They normally form RNA-protein complexes and regulate the translation of functionally related subpopulations of mRNAs. Very little is known about the post-transcriptional mechanisms that control fungal infection of plants and animals.

Formation of pre-mRNA 3' ends occurs in two steps: cleavage of the pre-mRNA (at a ***canonical*** polyA site) followed by synthesis of the adenosine tail. Isoforms of mRNAs with different exons or 3' untranslated region (UTR) lengths are generated by ***alternative*** polyadenylation (APA), a mechanism that regulates the presence of cis elements in the mRNA. Proteins involved in APA include the Cleavage Factor I complex (CFI_m) in metazoans and Hrp1 in yeast.

A virulence-deficient mutant in *Magnaporthe oryzae* led us to the identification of RBP35, a novel RNA-binding protein component of *M. oryzae* polyadenylation machinery that is missing in yeasts, plants or metazoans. RBP35 interacts *in vivo* with the orthologue of the metazoan cleavage factor I 25kDa (CFI_m25), indicating that RBP35 is the functional equivalent of CFI_m68 in filamentous fungi. Intriguingly, *M. oryzae* genome contains a clear orthologue of Hrp1, suggesting that combined mechanisms regulate the 3' end processing of *M. oryzae* pre-mRNAs. In *M. oryzae*, RBP35 is not essential for fungal viability but acts as a gene-specific polyadenylation factor regulating alternative 3' UTR processing of infection-related mRNAs.

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Sunday 1 April

Plenary session 3: Secondary Metabolism

PL3.1

Secondary metabolism in Fusarium fujikuroi – the role of nitrogen availability and histone modifications

Bettina Tudzynski, Philipp Wiemann, Caroline Michielse, Lena Studt, Eva-Maria Niehaus, Sabine Albermann, Dominik Wagner
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The species *F. fujikuroi* was first *discovered* more than 100 years ago as the causative agent of the "Bakanae" (foolish seedling) disease of rice leading to hyperelongation of etiolated seedlings due to the secretion of gibberellins (GAs) by the fungus. Today, GAs are biotechnologically produced worldwide as plant growth regulators in large scale. Beside GAs, the fungus produces a broad range of other secondary metabolites such as bikaverin, fusarubin, fusarins, and fusaric acid. This spectrum of secondary metabolites and the distinct response of biosynthetic genes to nitrogen quantity and quality encouraged us to sequence the genome of *F. fujikuroi*, the first member of the Asian clade of the *Gibberella fujikuroi* species complex. The genome sequence enabled us not only to identify all genes coding for key-enzymes of secondary metabolites (PKS, NRPS, terpene cyclases), but also to link these genes and entire gene clusters to natural products. Furthermore, the discovery that the biosynthesis of many secondary metabolites in *F. fujikuroi* depends on nitrogen in different ways intensified our attempts to shed light on the nitrogen regulation network and the cross-link to secondary metabolism. We have applied microarray- and ChIP-seq analyses under different nitrogen conditions (no nitrogen, glutamine, nitrate) to study in detail, how secondary metabolite gene clusters are regulated by nitrogen, and which histone modifications are characteristic for active/silenced expression. These genome-wide analyses of gene clusters and their nitrogen-dependent regulation deepened our understanding on the biological role of secondary metabolites in the fungus' life style and the potential link with growth, differentiation and virulence.

PL3.2

LaeA-directed natural product discoveries

Nancy Keller, Saori Amaike, JinWoo Bok, Yiming Chiang, Ry Forseth, Dirk Hoffmeister, Fang Yun Lim, Berl Oakley, Daniel Schenk, Frank Schroeder, Ali Soukup, Clay Wang, Wenbing Yin
Medical Microbiology and Immunology-The University of Wisconsin

Several years ago the methyltransferase LaeA was identified in a mutagenesis screen for genes important in secondary metabolite (also termed natural products) synthesis in the model ascomycete *Aspergillus nidulans*. Since its discovery, LaeA has been characterized as a conserved member of the fungal specific nuclear Velvet Complex required for orchestration of fungal secondary metabolism with morphological and physiological competence in many Ascomycete genera. The power of LaeA led the discovery of a number of fungal natural products and their associated biosynthetic pathways to understand hows and whys of fungal natural product formation. This has proven unrivaled by any other single fungal protein as demonstrated by pertinent examples of our newest understanding of LaeA directed fungal biology in *Aspergillus* species.

PL3.3

Secondary metabolites of *Leptosphaeria maculans*, the causal agent of blackleg of canola

Barbara Howlett
School of Botany, the University of Melbourne, Vic 3010 Australia

Like many filamentous fungi, *Leptosphaeria maculans*, the blackleg pathogen of canola, secretes a diverse range of secondary metabolites. Such metabolites are synthesized by gene clusters that include key genes such as non-ribosomal peptide synthases (NRPSs) or polyketide synthases (PKSs). The *L. maculans* genome has 13 NRPSs and 12 NRPS and we are identifying the end products of several of these genes. An NRPS designated as *sirP* is involved in biosynthesis of sirodesmin, which is the major secreted metabolite. Sirodesmin is toxic due to its disulphide bridge, which can inactivate proteins via reaction with thiol groups, or via generation of reactive oxygen species by redox cycling. Sirodesmin is produced during infection of canola by *L.maculans* and contributes to virulence during colonization of the stem. A candidate NRPS involved in the biosynthesis of the depsipeptide, phomalide, which causes necrosis on Indian mustard, has also been identified. The most abundantly secreted polyketide is phomenoic acid, which like sirodesmin, has antifungal activity. Domain modelling and comparative genomics with closely related fungi has been used to predict a candidate PKS for phomenoic acid biosynthesis. Silencing of this gene resulted in significantly reduced levels of phomenoic acid in culture, indicating that this PKS is responsible for phomenoic acid production. It is likely that the toxicity of these secondary metabolites allow *L. maculans* to outcompete other micro-organisms including fungi *in planta* and also during its saprophytic growth phase on canola stubble in the soil.

Sunday 1 April

Plenary session 4: Synthetic and Systems Biology

PL4.1

Mathematical modeling of yeast stress response and cell cycle regulation

Edda Klipp
Humboldt-Universität zu Berlin, Theoretical Biophysics

Cells have to grow and to divide. This is a well-organized, highly regulated process. Since cells also have to react to changes in the environment, cell cycle must be both robust against and sensitive to changes. The ability to perceive and respond to information from their environment is one of the most ubiquitous properties of cellular organisms. It is crucial for a cell to react appropriately to changes or signals in its environment. This becomes apparent in many situations such as the search for nutrients, the detection of potentially harmful external conditions and in cell-cell communication as it is required for any multi-cellular organism. Even though there is a huge selection of perceivable signals the underlying mechanisms are surprisingly alike, which suggests that they are highly conserved in the course of evolution.

Here, we apply different modeling techniques to understand cell cycle progression and cell cycle regulation in changing environments, with specific focus on mechanisms and experimental data for the model organism *Saccharomyces cerevisiae*. Specifically, new aspects in cell cycle regulation and the interaction of stress-activated signaling pathways with cell cycle progression will be discussed. The results indicate that yeast cells have developed different mechanisms for coping with external stress during different periods of their life time.

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PL4.2

***Aspergillus* spp.: ironing out iron problems**

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Iron is an essential but in excess toxic nutrient. Therefore, fungi evolved fine-tuned mechanisms for uptake and storage of iron, such as the production of siderophores (low-molecular mass iron-specific chelators). In *Aspergillus fumigatus,* iron starvation causes extensive transcriptional remodeling involving two central transcription factors, which are interconnected in a negative transcriptional feed-back loop: the GATA-factor SreA and the bZip-factor HapX. During iron sufficiency SreA represses iron uptake, including reductive iron assimilation and siderophore-mediated iron uptake, to avoid toxic effects. During iron starvation HapX represses iron-consuming pathways, including heme biosynthesis and respiration, to spare iron and activates synthesis of ribotoxin AspF1 and siderophores, the latter partly by ensuring supply of the precursor ornithine. In agreement with the expression pattern and mode of action, detrimental effects of inactivation of SreA and HapX are confined to growth during iron sufficiency and iron starvation, respectively. Deficiency in HapX, but not SreA, attenuates virulence of *A. fumigatus* in a murine model of aspergillosis, which underlines the crucial role of adaptation to iron limitation in virulence. Consistently, production of both extra- and intracellular siderophores is crucial for virulence of *A. fumigatus*. Recently, the sterol-regulatory element-binding protein SrbA was found to be essential for adaptation to iron starvation, thereby linking regulation of iron metabolism, ergosterol biosynthesis, azole drug resistance and hypoxia adaptation.

The studies were supported by Austrian ScienceFoundation Grants FWF P-21643-B11 and I-282-B09.

PL4.3

Systems Biology of Industrially Important Filamentous Fungi

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Aspergilli are used extensively in the fermentation industry for the production of a range of different industrial enzymes, organic acids and high-value secondary metabolites. In connection with further development of bioprocesses for the production of fuels and chemicals these fungi are interesting versatile cell factories as they tolerates low pH, can utilize a wide range of carbon sources and has relatively high conversion rates. Furthermore, their metabolism extends to cover many different secondary metabolites. Due to these factors the metabolism of *Aspergilli* is quite complex and involves a very large number of enzyme catalyzed reactions, probably among the microorganisms containing the largest number of metabolic capabilities. With the availability of the genome sequence for several different *Aspergilli* it has become possible to query the metabolic capabilities of these microorganisms at the genome-level. Using a bottom-up approach using genomic information together with information from databases, research papers and books, we have reconstructed the metabolic networks of *A. nidulans*, *A. niger*, *A. oryzae* and *P. chrysogenum*. We have used these metabolic networks for gaining novel insight into the metabolic functions of these organism through integrative analysis of different kinds of data, e.g. data from genome-wide transcription analysis. Among the processes studied are carbohydrate metabolism (use of different carbon sources like glucose, xylose and glycerol), enzyme production and penicillin production.

Monday 2 April

Plenary session 5: Genomes

PL5.1

Population genomics uncovers the *Verticillium dahliae* effector that is recognized by the tomato Ve1 immune receptor

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Cell surface receptors, generally referred to as pattern recognition receptors (PRR), detect conserved microbial molecules, generally referred to as microbe-associated molecular patterns (MAMPs), to activate MAMP-triggered immunity (MTI). Successful plant pathogens overcome MTI by the use of secreted effectors which perturb host immunity in a pro-active manner. An example is provided by LysM effectors that are secreted by various fungal plant pathogens during infection to sequester fungal cell wall-derived MAMPs to prevent detection by the host and activation of MAMP-triggered immunity. To overcome effector-triggered susceptibility, plants in turn evolved immune receptors that monitor the presence or activity of particular effectors to re-install immunity.

The tomato immune receptor Ve1 governs resistance to race 1 strains of the soil-borne vascular wilt fungus *Verticillium*, while race 2 strains are not recognized. Thus far, the *Verticillium* effector that is monitored by Ve1 remained unknown. By high-throughput population genome sequencing, we identified a sequence stretch that only occurs in race 1 strains, and that is absent from race 2 strains. Within this region the *Ave1* (for *Avirulence on Ve1 tomato*) gene was identified. Functional analyses confirmed that Ave1 activates Ve1-mediated resistance and demonstrated that Ave1 contributes to fungal virulence. Interestingly, Ave1 is homologous to a widespread family of plant proteins. Besides plants, homologous proteins were also found in few plant pathogenic fungi, including the fungal pathogens *Colletotrichum higginsianum* and *Fusarium oxysporum* f. sp. *lycopersici*. Remarkably, some of these Ave1 homologs can activate Ve1-mediated resistance in tomato as well.

PL5.2

Interacting with plants: lessons from fungal genomes

Marc-Henri Lebrun
Bioger,INRA, Thiverval-Grignon, URGI, INRA, Versailles, France. With the help of many fungal genome consortia and the Cazy database

Fungi display contrasting interactions with plants (symbiotic *vs* pathogenic; biotrophic *vs* hemi-biotrophic/necrotrophic; large *vs* restricted host range). Analysis of the increasing number of fungal genomes may help understanding how fungi have evolved towards such diverse infection strategies. Comparison of related species that differ in their infection strategies is a powerful approach to uncover such trends. This is illustrated by the related Leotiomycetes *Blumeria graminis (Bg),* and *Botrytis cinerea* (*Bc*), which are either necrotrophic (*Bc*) or biotrophic (*Bg*). *Bg* has a large genome (120 Mb) extensively invaded by transposons (70% genome) with few protein-coding genes (6000). These genomic features differ significantly from those of *Bc* (38 Mb, 4% transposons, 14270 genes), but are shared by other Erysiphales, suggesting that this type of genome evolution is a hallmark of this family. This evolutionary trend may help define minimal gene sets required for infection. Indeed, Bg has only one functional secondary metabolism key gene encoding a polyketide synthase likely involved in pigment biosynthesis. Likewise, the ascomycete symbiont *Tuber melanosporum* also has a very restricted set of secondary metabolism genes. This contrasts with the large number of such genes found in other plant pathogens (38 in *Bc*, 49 in *Magnaporthe grisea*). This suggests that biotrophic pathogens and symbionts have lost most secondary metabolism pathways except those needed for survival. Similarly, these biotrophic and symbiotic species have reduced numbers of genes encoding plant cell wall-degrading enzymes, suggesting that functions harmful to the host plant have been counter-selected during evolution towards biotrophy and symbiosis. In contrast, biotrophic fungi have similar numbers of transporters and effectors as other plant pathogenic fungi. These selective gene losses in biotrophs and symbionts suggest that repertoires of genes encoding secondary metabolism enzymes, plant cell wall-degrading enzymes and effectors, as well as their *in planta* expression patterns, can provide useful signatures of fungal infection strategies. The analysis of these signatures in genomes of other plant pathogenic fungi will be presented.

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PL5.2 continued…

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PL5.3

Genome dynamics of the *Fusarium oxysporum* species complex

Li-Jun Ma [1,2] Shiguo Zhou [3] Liane R. Gale [4] Terry Shea [2] Sarah Young [2] H Corby Kistler [3]
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The *Fusarium* comparative genomes of *F. graminearum*, *F. verticillioides* and *F. oxysporum* revealed greatly expanded lineage-specific (LS) chromosomes in *F. oxysporum f*. sp. *lycopersici*. These LS chromosomes contribute to the organism pathogenicity and host–specificity, providing explanation for the polyphyletic origin of host specificity and the emergence of new pathogenic lineages in the *F. oxysporum* species complex (FOSC). In this presentation, I will describe the comparative study including one human isolate and 11 plant pathogenic isolates that represent 8 forma specialis, selected from *F. oxysporum* species complex. The optical maps confirmed that LS chromosomes exist in all the strains we have examined. Different races of the same forma specialis share the determinant pathogenicity chromosomes and effectors encoded in these chromosomes. Our preliminary analysis of the genome assemblies using only the illumine data indicates these *de novo* assemblies capture the effectors that determine the host-specificity. The genomic data also reveals the power in detecting genome-wide mutation pattern in short evolutionary divergent time, while RNA-seq data shows great promise in detecting novel genes encoded in the LS chromosomes and studying the gene expression under different conditions.

Monday 2 April

Plenary session 6: Regulation and Development

PL6.1
Coordination of fungal development and secondary metabolism
Gerhard H. Braus
Molekulare Mikrobiologie und Genetik, Georg-August-Universität Göttingen, D-37077 Göttingen, Germany

Differentiation and secondary metabolism are correlated processes in fungi that respond to various abiotic or biotic external triggers. The velvet family of regulatory proteins plays a key role in coordinating secondary metabolism and differentiation processes as asexual or sexual sporulation and sclerotia or fruiting body formation. The velvet family shares a protein domain that is present in most parts of the fungal kingdom from chytrids to basidiomycetes. The structure of this domain will be discussed. The heterotrimeric *velvet* complex VelB/VeA/LaeA which includes the velvet domain proteins VelB and VeA as well as the conserved eight subunit COP9 signalosome complex are required for the link between secondary metabolism and developmental programs. The current state of the work in the laboratory will be presented.

PL6.2
Tipping the balance. What turns a mutualist into a pathogen?
Barry Scott, Daigo Takemoto, Aiko Tanaka, Yvonne Becker, Matthias Becker, Carla Eaton, Gemma Cartwright
Massey University,NZ 2. Nagoya University, Japan

Epichloë festucae is a biotrophic fungus of the family Clavicipitaceae that forms a mutualistic symbiotic interaction with *Lolium* and *Festuca* temperate grass species. This fungal symbiont forms an interconnected hyphal network that extends throughout the aerial tissues of the grass including the leaf surface. Hyphal apical and intercalary growth within the host is highly regulated and coordinated with leaf growth. Fungal synthesis of reactive oxygen species, by a specific NADPH oxidase (Nox) complex, is a crucial signalling mechanism for maintaining a stable symbiotic association. Additional components of the Nox complex include a regulatory subunit NoxR, the small GTPase, RacA and homologues of the yeast polarity proteins Bem1, a scaffold protein, and Cdc24, a known guanine nucleotide exchange factor (GEF) for Rac. Disruption of any one of these genes leads to a breakdown in the symbiotic interaction resulting in dramatic changes in hyphal morphology and growth as well as plant development. Surprisingly, disruption of the *E. festucae* guanine nucleotide dissociation inhibitor (RhoGDI) or the p21-activated kinase, PakA/Cla4 had no obvious effect on the symbiotic interaction even though both mutants had severe culture growth phenotypes. We have also been examining the role of oxidative stress signalling systems in culture and in the symbiotic interaction through a functional analysis of the *E. festucae* homologues of the *S. cerevisiae* GPX3/YAP1 and *S. pombe* TPX1/PAP1 redox relay systems. Insights into our current understanding of what controls the balance between mutualism and pathogenicity will be presented.

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PL6.3

Novel Intrinsically disordered proteins assemble at septal pores and regulate diverse aspects of hyphal homeostasis

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Like animals and plants, multicellular fungal hyphae possess cell-to-cell channels that allow intercellular cooperation, and communication. Using a combination of mass spectrometry of Neurospora Woronin body associated proteins, and a bioinformatics approach that identifies related proteins based on composition and character, we have identified 17 septal pore associated (SPA) proteins that localize in rings around the pore, and in pore-centered foci. SPA proteins are not homologous at the primary sequence level, but share overall physical properties with intrinsically disordered proteins. Some SPA proteins form aggregates at the septal pore, and in vitro assembly assays suggest self-assembly through a novel non-amyloidal mechanism involving mainly random coil structural moieties. SPA loss-of-function phenotypes include excessive septation, septal pore degeneration, and uncontrolled Woronin body activation. These data identify a new family of disordered proteins that control cell-to-cell communication, and diverse aspects of septal homeostasis.

Saturday 31 March

Parallel session 1: Fungal Cell Biology

PS1.1

In Candida albicans the negative regulator of morphogenenis, Nrg1, is itself regulated at multiple post-transcriptional levels by kinase action

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[1.] Sheffield University [2.] Aberdeen University [3.] Universidad de Extremadura

The human fungal pathogen *C. albicans* is responsible for common mucosal disease with significant morbidity and among immunocompromised patients it can cause life-threatening hematogenously disseminated infections. A key virulence factor is its ability to switch from yeast to hyphal growth forms. This switch is positively regulated by a network of signal transduction pathways that target a panel of transcription factors that program the expression of hyphal-specific genes. Negative regulated is applied by the transcriptional repressor Tup1, targeted to the promoters of hyphal specific genes by the co-repressor Nrg1. While the signal transduction pathways targeting transcription factors have been extensively studied, less attention has been paid to the control of Nrg1 action. It has long supposed main mechanism of Nrg1 regulation is exerted through control of Nrg1 transcription which is repressed upon hyphal induction. However, Nrg1 transcript levels decline over a time scale of at least 100 minutes, whereas hyphal specific transcription can be detected much more rapidly than this. Here we show that upon hyphal induction, the repressive action of Nrg1 is relieved by a combination of alterations to protein stability, exclusion from the nucleus and alteration of binding to promoters of hyphal-specific genes. These changes are mediated by the action of a panel of kinases that target Nrg1.

PS1.2

Investigating the biology of plant infection by the rice blast fungus *Magnaporthe oryzae*

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Plant diseases are major causes of crop losses throughout the world and a significant constraint on worldwide agricultural production. Developing new means to control plant diseases is an important component of any strategy to ensure global food security. *Magnaporthe oryzae* is the causal agent of rice blast, one of the most devastating diseases of cultivated rice. Each year rice blast disease destroys enough rice to feed 60 million people. The availability of complete genome sequences for *M. oryzae* and its host rice, *Oryza sativa*, has provided the means to investigate this fungal-plant interaction in great detail. 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 plant infection process by the rice blast fungus. Recently, we have shown that development of a functional appressorium is linked to cell cycle progression and programmed autophagic cell death of the fungal spore. New information on the role of a family of five septin GTPases in the function of appressoria will be presented, indicating that they serve an important role in translating turgor into mechanical force necessary for plant infection. The role of reactive oxygen species generation in the control of appressorium-mediated plant infection, will also be discussed.

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PS1.3

Comparative Live-Cell Imaging Analyses of SPA-2, BUD-6 and BNI-1 in *Neurospora crassa* Reveal Novel Features of the Filamentous Fungal Polarisome

Alexander Lichius[1,2] Mario E. Yanez-Guiterrez[1] Cynthia Araujo-Palomares[1] Nick Read[2] Ernestina Castro-Longoria[1]

[1.] CICESE [2.] Edinburgh University

A key multiprotein complex involved in regulating the actin cytoskeleton and secretory machinery required for polarized growth in fungi, is the polarisome. Recognized core constituents in budding yeast are Spa2, Pea2, Aip3/Bud6, and the key effector Bni1. Multicellular fungi display a more complex polarized morphogenesis than yeasts, suggesting that the filamentous fungal polarisome might fulfill additional functions. In this study we analyzed the subcellular organization and dynamics of SPA-2, BUD-6 and BNI-1 in a wide range of developmental stages of *Neurospora crassa*, in order to characterize the filamentous fungal polarisome more comprehensively, and identify potential differences to other fungal species. Our analyses showed that during early, unicellular developmental stages the filamentous fungal polarisome closely resembles the apical cap configuration known from yeasts, but during later, multicellular developmental stages all three polarisome components become spatiotemporally separated within the apical dome, and thus adopt a so far unknown configuration. Most notably, in vegetative hyphal tips BUD-6 accumulated as a subapical cloud excluded from the Spitzenkörper (Spk), whereas BNI-1 and SPA-2 partially colocalized with the Spk and the tip apex. Phenotypic analyses of gene deletion mutants revealed additional functions for BUD-6 and BNI-1 in septum formation, septal plug consolidation, tip re-polarization, cytokinesis, cell fusion regulation, and the maintenance of Spk integrity. Considered together, our findings reveal novel polarisome-dependent and -independent functions of BUD-6 and BNI-1, and their complex arrangement with SPA-2 in the apical dome of mature hypha represents a novel aspect of filamentous fungal polarisome architecture.

PS1.4

The *Aspergillus nidulans* Kinesin-3 Tail Is Necessary And Sufficient To Recognize Modified Microtubules

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Posttranslational microtubule modifications are numerous; however, the biochemical and cell biological roles of those modifications remain mostly an enigma. The *Aspergillus nidulans* kinesin-3 UncA uses preferably modified MTs as tracks for vesicle transportation. Here, we show that a positively charged region in the tail of UncA (amino acids 1316 to 1402) is necessary for the recognition of modified MTs. Chimeric proteins composed of the kinesin-1 motor domain and the UncA tail displayed the same specificity as UncA, suggesting that the UncA tail is sufficient to establish specificity. Interaction between the UncA tail and alpha-tubulin was shown using a yeast two-hybrid assay and in *A. nidulans* by bimolecular fluorescence complementation (BiFC). Our data show that specificity determination depends on the tail rather than the motor domain, as has been demonstrated for kinesin 1 in neuronal cells.

In a non-targeted Y2H approach interaction partners of this region were identified, because they are most likely involved in the recognition of MT subpopulations. Several candidates were confirmed using BiFC. Two are associated with vesicles; one is a predicted siderophore uptake transmembrane transporter and the other one was previously shown to be involved in ER to Golgi vesicle-mediated transport. The deletion of another fished interactor with similarity to Phosphatidylinositol 3- & 4-kinase family showed strongly reduced growth. Further characterization of a potentinal role in regulating the activity and specificity of UncA is in progress.

PS1.5

Active plus-end capture of dynein at astral microtubules increases the efficiency of anaphase B

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Dynein is involved in several cellular processes, including early endosomes motility. To do so, dynein concentrates at microtubule plus-ends from where it is released to mediate motility towards minus-ends. We recently have shown that in hyphae of *Ustilago maydis* an interaction between EB1 and dynactin capture ~25 dynein motors at apical microtubule plus-ends and that additional ~25 motors are stochastically clustering due to local crowding effects. In mitosis, Dynein concentrates at astral microtubule plus-ends, from where it is released to the cortex to exert force on the spindle, thereby powering rapid spindle elongation. Here, we investigate the dynein anchorage mechanism at these astral microtubule plus-ends in mitotic cells. In contrast to hyphal cells, only ~3 dynein motors concentrate at astral microtubule ends, and dynein is not anchored via EB1-dynactin. Instead, deletion of the plus-end binding protein Clip1 and the dynein activator Lis1 reduces the amount by one dynein each, respectively, which led to spindle position defects and 50% reduction in spindle elongation rate. Dynein numbers and spindle elongation rates were further reduced in a ΔClip1/Lis1↓ double mutant. However, in the absence of anchorage mechanisms ~1.7 dynein motors were still concentrated at astral microtubule plus-ends. Consequently, the spindle apparatus is still able to segregate chromosomes, albeit with much reduced efficiency. Mathematical modelling suggests that this can be a consequence of stochastic clustering effects at microtubule ends. These results suggest that mitosis can function without active anchorage. In order to increase the efficiency of chromosome segragation, Clip1 and Lis1-dependent processes support dynein concentration at astral microtubule ends.

PS1.6

Candida albicans: sensing the host environment

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Candida albicans, an opportunistic pathogen and a human commensal, is found almost exclusively in association with a host. Intestinal tract colonization by this organism is common in humans and disease is thought to arise due to overgrowth or escape of organisms from the gut. In the host, the organism's ability to sense cues from the environment would be expected to enhance its growth and survival. C. albicans responds to numerous environmental cues that could be encountered in the host such as temperature, pH, presence of O2, CO2, nutrients or antimicrobial compounds, and stress conditions. Our studies focus on the ability of C. albicans to sense contact with a surface and to sense the environment within the intestinal tract. These types of sensing mechanisms promote the organism's ability to sense its location within the host, the nature of the tissue that it is encountering and the status of the host's immune response. As a result of these sensing mechanisms, the organism controls its physiology so that it maintains benign colonization in a healthy host but becomes a destructive pathogen that invades host tissue in a compromised host.

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PS1.7

A steep phosphoinositide phosphate gradient is critical for filamentous growth in *Candida albicans*

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Membrane phospholipids, such as phosphoinositide phosphates, despite being minor membrane components, have been shown to be required for cytoskeleton organization, G-protein signaling, cell polarity and morphogenesis in a range of organisms. In *Candida albicans*, neither PI(3,4,5)P_3 nor PI-3-kinase homologs have been found, raising the possibility that the PI(4,5)P_2 fulfills some functions of PIP_3. In this organism there is a single PI(4)P-5-kinase (encoded by *MSS4*) and three PI-4-kinases (encoded by *LSB6*, *STT4* and *PIK1*). In the yeast *S. cerevisiae*, both Mss4 and Stt4 are required for viability, organization of the actin cytoskeleton and are localized to the plasma membrane.

We examined whether PI(4,5)P_2 is required for *C. albicans* filamentous growth. We have generated strains in which the level of the Stt4 or Mss4 PI-kinases can be manipulated using the Tetracycline repressible promoter system. In repressive conditions, the *stt4* and *mss4* mutants are viable, yet defective in filamentous growth. Using a fluorescent lipid associated reporter, we have observed a striking PI(4,5)P_2 asymmetry in budding cells and a steep gradient which occurs concomitant with germ tube emergence. Both sufficient PI(4)P synthesis and an intact actin cytoskeleton are necessary for this steep PI(4,5)P_2 gradient. In contrast, neither microtubules nor asymmetrically localized mRNAs are critical for this gradient. Furthermore, the Mss4 protein is localized to the tip of the bud and hyphal filament. Our results indicate that a gradient of PI(4,5)P_2, generated in part by filament tip-localized Mss4 and the slow diffusion of plasma membrane PI(4,5)P_2, is crucial for the yeast to filamentous growth transition.

PS1.8

In Vivo Nonlinear Spectral Imaging Of Fungi

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Non-linear microscopy combined with fluorescence spectroscopy is known as non-linear spectral imaging (NLSI), providing simultaneously the specimen morphology and (auto)fluorescence spectra. Hence, it allows deducing the biochemical composition, while distinguishing different parts of the tissue.

We introduce NLSI to in vivo monitor the metabolism of fungi. Fungi are consumables (food) and are utilized to produce industrial and pharmaceutical compounds, requiring quality control. With NLSI, we present a fast method to determine the metabolic state and relate it to protein production. Moreover, we introduce NLSI and NLSI-data processing tools as an easy to use method, capable of addressing a broad range of microbiological questions.

Saturday 31 March

Parallel session 2: Sex and Sexual Development

PS2.1

_A_ mating loci in _Coprinopsis cinerea_ differ in numbers of homeodomain transcription factor genes

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The 25 kb-long *A* mating type locus in the mushroom *Coprinopsis cinerea* controls defined steps in the formation of a dikaryotic mycelium after mating of two compatible monokaryons as well as the formation of the fruiting bodies on the established dikaryon. Usually, three paralogous pairs of divergently transcribed genes for two distinct types of homeodomain transcription factors (termed HD1 and HD2 after) are found in the *A* locus. For dikaryon formation and regulation of sexual development, heterodimerization of HD1 and HD2 proteins from allelic pairs from different *A* loci is required. In some A loci found in the wild, alleles of gene pairs are not complete or one of two genes have been made in-active. Functional redundancy allows the system still to work as long as an *HD1* gene in one and an *HD2* gene in the other allelic gene pair are operative. In this study, we present for the first time two completely sequenced *A* mating loci of *C. cinerea*. Evidences for gene duplications, deletions and inactivations are found. The loci differ in the number of potential gene pairs (five versus three), in genes that have been lost in evolution and in genes that are still present but have been made inactive.

PS2.2

Systematic deletion of homeogenes in an ascomycete fungus supports analogy of clamp and crozier in Dikaryomycota

Evelyne Coppin[1] Veronique Berteaux-Lecellier[2] Frédérique Bidart[2] Sylvain Brun[3] Gwenaël Ruprich-Robert[4] Eric Espagne[2] Jinane Aït Benkhali[2] Robert Debuchy[2] Philippe Silar[3] students master 2 Mycologie Fondamentale[3]
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The dikaryotic state, a unique characteristic shared by superior fungi, is maintained by similar structures, the clamp in Basidiomycetes and the crozier in Ascomycetes. A debate is going on regarding whether these structures are analogous or homologous. The essential role of basidiomycetes homeodomain transcription factors (HDTF) in clamp formation led us to examine their possible involvement in crozier formation in Ascomycetes. We identified the seven homeobox genes of the ascomycete filamentous fungus *Podospora anserina* and constructed mutants inactivated for either one or several homeogenes. Croziers developped normally in these mutants, including those inactivated for up to six homeogenes. However, two mutants are defective in the beak formation, an essential differentiation for efficient ascospore ejection. This defect can be rescued by wild-type maternal hyphae, thus demonstrating its maternal origin. Multiple mutant analyses revealed interactions between homeobox genes, suggesting that they operate as a complex network. Like in animals and plants, HDTF are involved in shaping multicellular structures in Ascomycetes. In contrast, HDTF are not required for the dikaryotic tissue formation and development. These data demonstrate that the crozier of Ascomycetes and the clamp of Basidiomycetes are under different genetic control, suggesting that they are analogous and not homologous structures.

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PS2.3

Sexual Development and Spore Pathogenesis of *Cryptococcus*

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Cryptococcus neoformans is a human pathogen that causes fungal meningoencephalitis, primarily in immunocompromised individuals, resulting in over one million cases of cryptococcosis and ~600,000 deaths annually worldwide. Infection appears to occur via a respiratory route from environmental sources such as disrupted soil and bird droppings. Although the vegetatively growing yeast form of *C. neoformans* has been shown to cause disease in animal models, the infectious particles in human disease are not known. Given their small size and resistance properties, spores are a likely cause of natural infections.

Spores are the products of sexual development of *C. neoformans*, which appears to occur in the environment and can also be induced in the laboratory. To understand molecular events governing sexual development, we carried out an analysis of gene expression over time during development using microarray analysis. We are using these data to identify genes and regulatory elements involved in spore formation.

To investigate whether/how spores may function as infectious particles, we purified spores to homogeneity and evaluated them. Using a variety of methods, we determined that spores are covered by a thick coat that is both morphologically and compositionally distinct from yeast cells that appears to provide spore resistance to environmental stress. We are continuing to explore the basic physical and biochemical properties of spores with a focus on the spore surface.

To understand how spores interact with the host immune response, we carried out virulence assays using a mouse intranasal infection model of cryptococcosis. We discovered that spores are disease-causing particles; mice infected with spores developed disseminated cryptococcosis. Strikingly, however, we found that spore-mediated disease is distinct from yeast-mediated disease. Spores led to higher burdens of *C. neoformans* in the brain, and the mice were more likely to show signs of central nervous system (CNS) disease than yeast-infected mice. Furthermore, yeast strains that are avirulent in mice can cause fatal CNS disease when infected as spores, revealing that disease outcomes can differ depending on the infectious particle to which the host is exposed.

These data represent the first glimpses into the process of spore biogenesis, the basic properties spores, and the host response to these novel particles. Continuing studies of spores promise to reveal how *C. neoformans* infects the host and ultimately causes disease.

PS2.4

Living together. Genetics and genomics of the dikaryotic lifestyle

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In basidiomycetes, karyogamy occurs immediately before the meiosis and long after plasmogamy. Because of this delay, these fungi spend most of their life cycle as dikaryotic mycelia containing two separate nuclei per cell. We are studying the genetic and genomic mechanisms of dikaryosis as a model for internuclear communication and its impact on gene expression. To tackle it, we have sequenced the genomes of the two nuclei present in the strictly dikaryotic basidiomycete *Pleurotus ostreatus* in collaboration with the JGI, and we have used RNAseq to monitor the differences in gene expression in monokaryotic and dikaryotic mycelia. The questions dealt with the genome synteny, the genetic control of the monokaryotic/dikaryotic lifestyle, the differential expression of genes and alleles in the two conditions, and the identification of genes specifically expressed in monokaryons and dikaryons.

The synteny analysis revealed multiple single and nested chromosomal inversions that suppress chromosomal crossover and produce supergenes that can be associated to quantitative trait loci in some cases. Moreover, synteny studies of different fungi suggested that inversions can be a main evolutive mechanism in basidiomycetes.

The sequence analysis revealed the presence of genome-specific genes and of several helitron families involved the promoting structural heterozygosis and the amplification of genes involved in degradation processes.

The RNAseq experiments identified a number of genes whose expression is tightly associated to the monokaryotic/dikaryotic condition. Some of them were known to be involved in the maintenance of the cellular conditions, but others could be responsible for the differences between the two lifestyles.

PS2.5

The transcription of the putative mating type genes sexM and sexP is regulated by trisporic acid in Mucor mucedo

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Friedrich-Schiller University

The putative mating type locus of mucoralean fungi consists of a single HMG-domain transcription factor gene, sexM or sexP, flanked by genes for a RNA helicase and a triose phosphate transporter. Sequencing of the sex locus of Mucor mucedo follows this general scheme as previously found in Phycomyces blakesleeanus, Rhizopus oryzae and Mucor circinelloides. Only the TP transporter is deleted in both mating types of Mucor mucedo. The participation of transcription factors in the regulation of sexual development in mucoralean fungi has not been proven yet. We performed transcriptional analysis of sexM and sexP by quantitative real-time PCR and found that under sexual stimulation, i.e. either in cross cultures or after addition of the mating pheromone trisporic acid, both transcripts are upregulated. Interestingly, the promoter sequences controlling sexM and sexP show no similarities to each other. Comparison of the promoter sequences of sexP from different zygomycetes show a conserved transcription factor binding side, whereas the promoter sequences of sexM of different zygomycetes do not show any conserved regions. At the protein level, both transcription factors are completely unrelated with each other, and only sexM harbors a putative nuclear localization sequence. Heterologous expression of sexM coupled to GFP in Saccharomyces cerevisiae shows that sexM is indeed accumulated in the nucleus of yeast.

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PS2.6

Interspecific sex in grass smuts and the genetic diversity of their pheromone-receptor system

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The grass smuts comprise a speciose group of biotrophic plant parasites, so-called Ustilaginaceae, which are specifically adapted to hosts of sweet grasses, the Poaceae family. Mating takes a central role in their life cycle as it initiates parasitism by a morphological and physiological transition from saprobic yeast cells to pathogenic filaments. As in other fungi sexual identity is determined by specific genomic regions encoding allelic variants of a pheromone-receptor (PR) system and heterodimerising transcription factors. Both operate in a biphasic mating process that starts with PR-triggered recognition, directed growth of conjugation hyphae and plasmogamy of compatible mating partners. So far, studies on the PR system of grass smuts revealed diverse interspecific compatibility and mating type determination. However, many questions concerning the specificity and evolutionary origin of the PR system remain unanswered. Combining comparative genetics and biological approaches we report on the specificity of the PR system and its genetic diversity in 10 species spanning about 100 million years of mating type evolution. We show that three highly syntenic PR alleles are prevalent among members of the Ustilaginaceae favouring a triallelic determination as the plesiomorphic characteristic of this group. Furthermore, interspecies sex tests detected a high potential for hybridisation that is directly linked to pheromone signalling as known from intraspecies sex. Although the PR system seems to be optimised for intraspecific compatibility, the observed functional plasticity of the PR system increases the potential for interspecific sex, which might allow the hybrid-based genesis of newly combined host specificities.

PS2.7

The pheromone system of *Hypocrea jecorina (Trichoderma reesei)* and its regulation by photoreceptors upon sexual development

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[1] Vienna University of Technology [2] Vienna University of Technology, Institute of Chemical Engineering, Research Area Molecular Biotechnology

Discovery of sexual development in the biotechnologically important ascomycete *Trichoderma reesei* (*Hypocrea jecorina*) as well as detection of a novel class of peptide pheromone precursors in this fungus indicates promising insights into its physiology and lifestyle. We investigated the *H. jecorina* pheromone-system and its regulation by the light response pathway.

The pheromone receptors HPR1 and HPR2 confer female fertility in their cognate mating types. Neither pheromone precursor genes nor pheromone receptor genes of *H. jecorina* were transcribed in a strictly mating type dependent manner, but showed enhanced expression levels in the cognate mating type. Transcripts of the pheromone precursor genes respond to the presence of a mating partner. In the female sterile *T. reesei* strain QM6a, these responses were altered. Inappropriate reaction to the mating partner may hence cause female sterility in QM6a.

In agreement with the dependence of sexual development on light, the photoreceptors BLR1 and BLR2 were found to impact fruiting body formation, although they are not essential for mating. In contrast, ENV1 was needed for female fertility. BLR1, BLR2 and ENV1 negatively regulate transcription of both pheromone receptors as well as peptide pheromone precursors in a mating type dependent manner. Thereby, the effect of ENV1 is most intense and at least partially mediated via regulation of *mat1-2-1*. ENV1 is concluded to be crucial for balanced regulation of genes regulated in a mating type dependent manner by the MAT-locus, and consequently for determination of sexual identity and fruiting body formation.

PS2.8

A developmental protein as scaffold for a MAP kinase pathway controlling fungal fruiting body development?

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The formation of fruiting bodies during sexual development in filamentous fungi is very complex and involves the generation of new cell types. Using the homothallic ascomycete *Sordaria macrospora* as a model system, we have identified a number of developmental proteins essential for this differentiation process. One of these, PRO40, the homolog of *Neurospora crassa* Soft, was employed for protein-protein interaction studies. In a yeast two-hybrid screen, the MAP kinase kinase (MAPKK) MEK1 was identified as a putative PRO40 interaction partner, and further analysis revealed an additional interaction between PRO40 and the MAPKKK MIK1. Both kinases can be assigned to the cell wall integrity pathway by their homology to the corresponding yeast kinases. Interestingly, while a *pro40* knockout strain is impaired in sexual development and hyphal fusion, a *mek1* knockout strain shows additional defects in vegetative growth and resistance to cell wall stress. We hypothesize that PRO40 scaffolds the MAPKKK and the MAPKK of the designated cell wall integrity pathway of *S. macrospora* in a development-specific manner leading to the formation of fruiting bodies.

Saturday 31 March
Parallel session 3: Genomes and Genome Evolution

PS3.1

Evolution of Obligate Parasitism in the White Rust Pathogen of *Arabidopsis thaliana*

Eric Kemen[1] Ariane Kemen[1] Morten Jørgensen[1] Anastasia Gardiner[1] Alexi Balmuth[1] Jan Sklenar[1] Kim Findlay[2] Jones Alexandra[1] Jonathan Jones[1]
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Biotroph evolution and molecular mechanisms of biotrophy in eukaryotic plant pathogens are poorly understood. To address evolutionary and mechanistic aspects of biotrophy we sequenced the genome of the obligate biotroph oomycete *Albugo laibachii*. Comparisons with other oomycete plant pathogens revealed independent loss of biosynthetic pathways while other proteins like HSP90 show an unexpected expansion. Biotrophic organisms require “effectors” to suppress host defence; we revealed RXLR and Crinkler effectors shared with other oomycetes, and furthermore discovered a novel class of effectors, the “CHXCs”.

A requirement for a biotrophic life style is an intimate complex haustorial interface with parasitized cells. Beside its role in nutrient uptake, little is known about structural features, protein content and protein trafficking. We used proteomics and cytological approaches to dissect the haustorial interface and revealed the accumulation of a HSP90 subclass within the extrahaustorial matrix.

We hypothesize that evolution of biotrophy involves a series of steps: step 1, involving progressively more effectors to suppress defence that might evolve from conserved proteins step 2, attenuated activation of defence by reduction in the inventory of cell wall hydrolyzing enzymes, resulting in, step 3, weak selection to maintain certain biosynthetic pathways if the products of the pathways can be obtained from the host. This results in progressively more comprehensive auxotrophy and culminates in irreversible biotrophy.

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PS3.1

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Eric Kemen[1] Ariane Kemen[1] Morten Jørgensen[1] Anastasia Gardiner[1] Alexi Balmuth[1] Jan Sklenar[1] Kim Findlay[2] Jones Alexandra[1] Jonathan Jones[1]
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Biotroph evolution and molecular mechanisms of biotrophy in eukaryotic plant pathogens are poorly understood. To address evolutionary and mechanistic aspects of biotrophy we sequenced the genome of the obligate biotroph oomycete *Albugo laibachii*. Comparisons with other oomycete plant pathogens revealed independent loss of biosynthetic pathways while other proteins like HSP90 show an unexpected expansion. Biotrophic organisms require “effectors” to suppress host defence; we revealed RXLR and Crinkler effectors shared with other oomycetes, and furthermore discovered a novel class of effectors, the “CHXCs”.

A requirement for a biotrophic life style is an intimate complex haustorial interface with parasitized cells. Beside its role in nutrient uptake, little is known about structural features, protein content and protein trafficking. We used proteomics and cytological approaches to dissect the haustorial interface and revealed the accumulation of a HSP90 subclass within the extrahaustorial matrix.

We hypothesize that evolution of biotrophy involves a series of steps: step 1, involving progressively more effectors to suppress defence that might evolve from conserved proteins step 2, attenuated activation of defence by reduction in the inventory of cell wall hydrolyzing enzymes, resulting in, step 3, weak selection to maintain certain biosynthetic pathways if the products of the pathways can be obtained from the host. This results in progressively more comprehensive auxotrophy and culminates in irreversible biotrophy.

PS3.2

Harnessing Natural Genetic Variation to Elucidate the Relationship Between Genotype and Phenotype in *Saccharomyces paradoxus*

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A more thorough understanding of the causative relationship between genotype and phenotype is of great interest to many fields of biology. In the interests of improving our understanding of this relationship, we have pursuing several lines of research that have revealed specific phenotypic consequences resulting from natural genetic variation and divergence in the wild yeast *Saccharomyces paradoxus*. In one study, we use gene expression and population genetics tests to investigate the effects of natural selection on a group of co-regulated membrane protein genes in a European population of *S. paradoxus.* We provide evidence for expression level divergence of these membrane protein genes relative to other *Saccharomyces* species, and suggest that this divergence has been partially driven by the heightened selective pressures that we find to be acting on the promoters of these genes. In a second study, we investigate the genetic variants that underlie the invasive growth and flocculant phenotypes that are displayed by several members of this same *S. paradoxus* population. We find that these phenotypes are driven by distinct genetic variants that have arisen independently in several isolates from the population and represent a case of convergent evolution. These findings illustrate the potential for harnessing natural variation within and between wild populations to elucidate the relationship between genotype and phenotype.

PS3.3

Evolutionary Genomics Of Accessory Chromosomes In *Mycosphaerella graminicola*

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Fungal genomes evolve very rapidly through the acquisition of foreign genes, hybridization events and ectopic recombination during meiosis. One of the most striking aspects of genomic diversity in fungi is the presence of accessory chromosomes (also termed supernumerary or dispensable). Accessory chromosomes are defined as chromosomes that are specific to a subset of isolates from one species. Accessory chromosomes were found to follow separate evolutionary trajectories due to horizontal transfer and extensive rearrangements during meiosis. A growing number of pathogenic fungi are recognized to carry accessory chromosomes and harbor genes involved in virulence on these chromosomes. The epidemic threat of an emerging pathogen may, hence, depend on the evolutionary dynamics of its accessory chromosomes. To better understand these processes, we studied *Mycosphaerella graminicola*, a major leaf pathogen of wheat harboring the highest known number of accessory chromosomes. We performed whole-genome resequencing of a *M. graminicola* population to identify polymorphisms among the eight known accessory chromosomes. All accessory chromosomes showed significant length variation due to large segmental deletions, including complete absence of certain chromosomes. Furthermore, we performed PCR assays at <100kb intervals along the chromosomes to assess presence-absence of chromosomal segments in a global collection. We found population-specific patterns in segmental deletions and differences in frequencies of accessory chromosomes among populations. We extended the genome re-sequencing and PCR assays to progeny from several controlled crosses and found that meiosis generated a substantial proportion of population-level variation. High degrees of sexual reproduction likely maintain the enormous plasticity in accessory chromosomes of *M. graminicola*.

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PS3.4

Genome sequence of Shiitake mushroom *Lentinula edodes* and comparative mushroom genomic analyses

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School of Life Sciences, The Chinese University of Hong Kong, China

Lentinula edodes, Shiitake mushroom, is one of the most important cultivated mushrooms and wood-degrading fungi. We have been performing genomic analyses of the *L. edodes* monokaryon L54A. The L54 genome was sequenced using Roche 454 and ABI SOLiD sequencing platforms. There are 13,382 predicted protein-coding genes. We constructed a high-density genetic linkage map using the high-quality genetic variations among a mapping population of haploid basidiospores of dikaryon L54. For computational analysis of mushroom genomes, we compiled the genome sequences of *L. edodes* and other fungi into a mushroom genome analysis platform. Comparative mushroom genomic analyses revealed conserved genes in mushroom genomes, including putative regulators, protein-binding proteins and transcription factors. BTB, Fbox, paracaspase and RING domain proteins expanded in mushroom-forming basidiomcyetes. The unique composition of plant biomass-degrading enzymes in *L. edodes* genome was revealed. There are both laccases and peroxidases for lignin degradation. Multiple polysaccharide-degradation enzyme families also expanded, including glycoside hydrolase families which target beta-glucans and pectin. Our works provide insights into the molecular mechanism of mushroom development. The *L. edodes* genome sequence, genetic map and comparative genome analysis platform are key resources for the mushroom research community.

PS3.5

Comparative genomics of *Fusarium pseudograminearum* and other cereal fungal pathogens

Donald Gardiner[1] Megan McDonald[2] Peter Solomon[3] Mhairi Marshall[4] Kemal Kazan[1] Sukumar Chakraborty[1] Bruce McDonald[2] John Manners[1]

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Fusarium pseudograminearum is a pathogen widely associated with crown rot in wheat and barley. We have sequenced the genome of an *F. pseudograminearum* isolate using paired-end short read technology at 180 fold coverage. Comparison of the predicted proteins to those of the genomes of a range of other cereal pathogens, dicot pathogens and saprophytes was undertaken using a reciprocal BLASTp analysis pipeline. This revealed genes that have strong orthologues only in specific cereal pathogens, and suggests multiple horizontal transfer events may have occurred in the evolution of cereal pathogens. One example gene encodes a putative amidohydrolase enzyme with orthologous sequences only detected in *F. pseudograminearum* and *Phaeosphaeria nodorum,* cause of glume blotch disease, but not in any other fungal genome, with the next closest matches all from bacteria. Deletion of this gene from *F. pseudograminearum* resulted in a reduction in virulence on barley and virulence could be restored by expression of the wild type coding sequence, indicating this is a novel virulence gene. Population surveys suggest the gene has been present in both *F. pseudograminearum* and *Phaeosphaeria* lineages for a long time and most probably was independently acquired by both species, possibly from bacteria. Its presence in these two otherwise unrelated pathogens suggests a role for this gene in a common pathogenesis mechanism that targets an important defence pathway in cereals. This study demonstrates that a comparative genomics approach can identify novel events that have influenced the evolution of fungal pathogenesis on cereal crops

PS3.6

Comparative genomics of basidiomycetes telomere and subtelomere regions.

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Telomeres are complex nucleoprotein structures found at chromosome ends and essential for their physical integrity. Telomeres are formed by a species-specific number of repetitions of a conserved sequence unit, a distal domain containing tandem repeated motifs and a proximal domain containing less repeated sequences and clusters of related genes. Because of their structural characteristics, telomeres are underrepresented in most assembled genomes and they must be characterized using dedicated molecular and bioinformatics strategies.

We are interested in determining which genes are preferentially placed at the subtelomeric regions, to study the effect of this position on their expression and to correlate it with the physiological and ecological characteristics of the organism. For this purpose, we have analyzed the genomes of several basidiomycetes involved in environmental and other biotechnological processes sequenced by the JGI.

We found that the basic telomere repetitive unit as well as its copy number varied among species. Gene density at the subtelomeric regions ranged from less than 0.20 to 0.49 genes per Kbp. Synteny analysis of these regions using *Pleurotus ostreatus* as a reference, revealed that seven of the 12 *P. ostreatus* chromosomes harboured gene models also found in the subtelomeric regions of other basidiomycetes, the subtelomeric chromosome regions were statistically enriched in specific gene sets, and that a mosaic of modules of subtelomeric genes described in other basidiomycetes was identified at *P. ostreatus* chromosome 4. These facts suggest that the ecologic niche of a species could be the responsible for the movement of subtelomeric chromosome specific genes to core chromosome locations.

PS3.7

Comparative genomics of Cochliobolus cereal pathogens: the core and pan genome

Bradford Condon, Gillian Turgeon
Cornell. Univ.

Cochliobolus is a species-rich genus of taxa that have caused devastating losses to US agriculture. The superpathogens, *Cochliobolus heterostrophus* (host/corn), *Cochliobolus carbonum* (corn), *Cochliobolus victoriae* (oats), *Cochliobolus sativus* (cereals), and *Cochliobolus miyabeanus* (rice), form a tight phylogenetic group, suggesting a progenitor gave rise, over a short period, to this series of distinct biotypes, each distinguished by unique pathogenic capability to particular plants. Working with the Joint Genome Institute and its Fungal Genomics Program, we have sequenced, assembled, and compared genomes of all of these species. The close relationship has allowed characterization of a core and pan-genome, consisting of species-unique and conserved regions. We have inventoried genes for secondary metabolism and secreted proteins, in particular, as these are candidate virulence effector molecules. Included in these categories are secondary metabolite and protein host selective toxins (HSTs), the calling-card of Dothideomycete necrotrophs, known to confer hypervirulence on susceptible hosts. This project provides insights into how unique genomic regions contribute to virulence, and thus how new pathogens emerge.

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PS3.8

A Dynamin-like Protein Affects Both RIP and Premeiotic Recombination

Kyle Pomraning [1] Ann Kobsa [2] Eric Selker [2] Michael Freitag [1]
[1.] Oregon State University [2.] University of Oregon

Repeat-induced point mutation (RIP) and premeiotic recombination affect gene-sized duplications in many filamentous fungi. RIP causes G:C to T:A transition mutations while premeiotic recombination can result in loss of repeated DNA segments (J. Galagan and E. Selker, 2004). Both processes occur after fertilization but prior to meiosis and can be very efficient, in some cases mutating and/or deleting the duplication in essentially every nucleus. At least in *Neurospora crassa*, RIP has countered the expansion of gene and transposon families (E. Selker, 1990), suggesting that genome streamlining and protection from transposition events may yield long-term benefits to Neurospora populations. We employ genetic approaches to elucidate the mechanism of premeiotic recombination and RIP. Here we report the successful identification of semi-dominant mutations that affect both of these processes by using UV mutagenesis, followed by a screen for reduced RIP of linked duplications of *hph* and *pan-2*. Classical genetic mapping and complementation tests revealed that a mutation in the histone H3 gene,*hH3*$^{dim-4}$, is responsible for greatly reduced RIP of one mutant. We identified two additional mutations by bulk segregant analysis and high-throughput Illumina sequencing. Single point mutations were found in the same gene, encoding a novel dynamin-like long GTPase, albeit in different conserved domains. Both premeiotic recombination and RIP frequencies are affected, supporting the idea that these processes are mechanistically linked. To investigate this further, we are screening the Neurospora single gene deletion collection for mutants that show RIP defects, starting with deletion mutants that are known or expected to affect recombination pathways.

Sunday 1 April

Parallel session 4: Organismic Interactions

PS4.1

ß-1,3-glucan synthase of the maize anthracnose fungus *Colletotrichum graminicola* is essential at specific stages of pathogenesis

Ely Oliveira-Garcia, Holger B. Deising
Martin-Luther-University Halle-Wittenberg, Interdisciplinary Center for Crop Plant Research, Betty-Heimann-Str. 3, D-06120 Halle (Saale), Germany.

Covalently cross-linked β-1,3-glucan and chitin are the most prominent and morphogenetically relevant carbohydrate polymers of fungal cell walls. In most filamentous fungi several chitin synthases, but only a single β-1,3-glucan synthase contribute to forming the glucan-chitin core. While the role of individual chitin synthase genes in pathogenic development has been analyzed in several plant pathogenic fungi, functional analyses of β-1,3-glucan synthase genes, encoding the catalytic subunit of the β-1,3-glucan synthase complex, is lacking.

We investigated the role of the β-1,3-glucan synthase gene (*GLS1*) in infection structures of the maize pathogen *Colletotrichum graminicola*. Infection assays with a *GLS1*:*eGFP* replacement strain, in combination with aniline blue fluorochrome-staining, showed that massive β-1,3-glucan synthesis occurs in conidia, appressoria and necrotrophic hyphae, but, surprisingly, not in biotrophic hyphae. As targeted deletion of *GLS1* was lethal, we employed RNA interference (RNAi) to generate transformants gradually differing in *GLS1* transcript abundance. Appressoria of RNAi strains had reduced turgor pressure, elastic, inefficiently melanized cell walls, and many of these infection cells exploded spontaneously. Due to loss of appressorial adhesion, penetration of intact maize leaves did not occur and normally shaped biotrophic primary hyphae formed on the maize cuticle. In wounded leaves, only necrotrophic hyphae were found, as indicated by strains carrying biotrophy- and necrotrophy-specific promoters controlling expression of *eGFP*. Necrotrophic hyphae formed by RNAi strains in the host tissue were severely distorted, hyper-melanized, and unable to cause spreading disease. Our studies suggest that *GLS1* is essential in appressoria and fast growing necrotrophic, but not in biotrophic hyphae of *C. graminicola*.

PS4.1

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PS4.2

Effectors and Biotrophic Invasion by the Rice Blast Fungus, *Magnaporthe oryzae*

Barbara Valent [1] Mihwa Yi [1] Martha C. Giraldo [1] Chang Hyun Khang [1,2] Melinda Dalby [1] Kirk Czymmek [3] Mark Farman [4]

[1]Department of Plant Pathology, Kansas State University, Manhattan, Kansas, U.S.A.; [2]Department of Plant Biology, University of Georgia, Athens, Georgia, U.S.A.; [3]Department of Biological Sciences and Delaware Biotechnology Institute, Newark, Delaware, U.S.A.; [4]Department of Plant Pathology, University of Kentucky, Lexington, Kentucky, U.S.A.

Blast disease, caused by the haploid ascomyceteous fungus *Magnaporthe oryzae*, remains a major disease of rice, and wheat blast has emerged as a threat to global wheat production since it was first identified in Brazil in 1985. To cause disease, *M. oryzae* sequentially invades living rice cells using intracellular invasive hyphae (IH) that are enclosed in host-derived extrainvasive-hyphal membrane. In each subsequently colonized host cell, IH initially grow as filamentous hyphae and then switch into pseudohyphal-like bulbous hyphae that proliferate throughout the host cell. Fluorescently-labeled avirulence effectors are secreted from the fungus and accumulate in the biotrophic interfacial complex (BIC), which forms at the filamentous IH tip and remains beside the first differentiated bulbous IH cell as the IH continues to grow. Effectors that show preferential BIC localization are translocated into the rice cytoplasm, suggesting that BICs are a staging center for effector delivery into the host cell. In addition to known effectors, 80 biotrophy-associated-secreted (BAS) proteins localize to BICs. Twenty-six of these BAS proteins are translocated into the cytoplasm of invaded rice cells. Additionally, 25 translocated effector or BAS proteins move ahead into neighboring rice cells before invasion by the fungus, presumably to prepare these host cells before invasion. Two translocated proteins naturally accumulate in rice nuclei and six others accumulate where the IH crossed the rice cell wall into neighboring cells. This talk will focus on current understanding of effector secretion into BICs and translocation into rice cells, and on effector sequences that mediate these processes.

PS4.3

Downy mildew effectors and their activity in the host plant

Stan Oome [1,2] Joost Stassen [1] Adriana Cabral [1] Ruslan Yatusevich [3] Jane Parker [3] Guido van den Ackerveken [1,2]

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Downy mildews are obligate biotrophic pathogens belonging to the oomycetes. Each downy mildew species is highly specialized on a particular host plant with which it has strongly co-evolved. This is evident from the secretomes of these oomycetes that contain numerous species-specific proteins, many of which are candidate effector proteins that could interfere with plant life in order to create a favourable environment for pathogen infection. We are studying effectors of the Arabidopsis downy mildew *Hyaloperonospora arabidopsidis*, in particular apoplastic effectors that act extracellularly in plant tissue and RXLR effectors that are host-translocated and therefore have their presumed activity inside plant cells. We will report on the progress of the analysis of apoplastic effectors belonging to the group of necrosis- and ethylene-inducing proteins and glucose-6 phosphate epimerases, and on the immune-suppressive activity of RXLR effectors. The mode-of-action of several RXLR effectors is being uncovered by identification of interacting host-target proteins and their immunity-related activity. These studies are revealing the mechanisms by which downy mildews interfere with host cell processes. In addition, knowledge on effectors will aid breeding for resistance to oomycetes in crops. We have made a first step towards application by identifying RXLR effectors in the lettuce downy mildew *Bremia lactuca*e that are now being used to select lettuce lines with new resistance specificities.

PS4.4

SpHtp1 from the oomycete *Saprolegnia parasitica* shows fish cell-specific entry and tyrosine-O-sulphate-dependent import

Stephan Wawra[1] Judith Bain[2] Elaine Durward[1] Irene de Bruijn[1] Kirsty L. Minor[1] Stephen C. Whisson[3] Andy J. Porter[2] Paul R.J. Birch[3] Chris J. Secombes[4] Pieter van West[1]
[1.] Aberdeen Oomycete Laboratory, UK [2.] University of Aberdeen, UK [3.] The James Hutton Institute, Dundee, UK [4.] Scottish Fish Immunology Research Centre, University of Aberdeen, UK

The eukaryotic oomycetes, or water moulds, contain several species that are devastating pathogens of plants and animals. During infection, oomycetes translocate effector proteins into host cells, where they interfere with host defence responses. For several oomycete effectors (e.g. the RxLR-effectors) it has been shown that their N-terminal polypeptides are important for delivery into the host. We found that the N-terminus of the putative RxLR-like effector SpHtp1 from the fish pathogen *Saprolegnia parasitica* shows host cell specific translocation. The translocation process can be blocked by enzymatic desulfation of cell surface proteins, by sulfotransferase inhibitors and antibodies specifically recognising tyrosine-O-sulphate. The quantitative analysis of these inhibitory effects suggests that the uptake process for SpHtp1 requires binding to tyrosine-O-sulfate modified cell surface molecules. No evidence was found that the SpHtp1 translocation involves phospholipid binding, as has been reported for RxLR-effectors from plant pathogenic oomycetes. Here we show a novel effector translocation route based on tyrosine-O-sulfate binding, which could be highly relevant for a wide range of host-microbe interactions.

PS4.5

Calnexin complex is involved in the establishment of fungal biotrophy in *Ustilago maydis*

Alfonso Fernandez-Alvarez, Alberto Jimenez-Martin, Miriam Marin-Menguiano, Alberto Elias-Villalobos, Jose I Ibeas
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Protein N-glycosylation consists in the addition of an oligosaccharide core of acetilglucosemines, glucoses and mannoses to the nascent N-glycoproteins in the Endoplasmic Reticulum (ER). Later, N-glycoproteins undergo maturation processes catalysed by two glucosidases and three mannosidases, which remove specific sugar residues. The α-glucosidase II has been previously described as required for virulence in the corn smut fungus *Ustilago maydis* (Schirawsky, et al., 2005). Here, we characterize the role of the α-glucosidase I, β-glucosidase II and α-mannosidase during the pathogenic development of *U. maydis* by using genetic and biochemical approaches. Interestingly, we have observed that the maturation of glucoses but not mannoses is crucial for virulence. Using a 2D-DIGE proteomic screening to identify putative substrates of α-glucosidase I, we have found that the protein disulfide isomerase Pdi1, a calnexin partner at the ER quality control system, has a different electrophoretic mobility in N-glycosylation mutant cells. This system is highly conserved in eukaryotic cells, although poorly known. We show now that the ER quality control system for N-glycoproteins is functional in the basiodiomycete *U. maydis* and that it is specifically required for the establishment of the biotrophic state between this fungus and its host.

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PS4.6

Functional analysis of candidate effector proteins by Host-Induced Gene Silencing in *Blumeria graminis* sp. *hordei*

Clara Pliego[1] Daniela Nowara[2] Giulia Bonciani[1]Dana Gheorghe[1] Patrick Schweizer[3] Laurence Veronique Bindschedler[4] Rainer Cramer[4] Pietro D. Spanu[1]

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The powdery mildew fungus *Blumeria graminis* is one of the most significant pathogens of cereal crops. In this obligate biotroph haustoria are responsible for nutrient uptake and are hypothesized to deliver effectors which modulate disease development. We selected a panel of fifty *Blumeria* Candidate Effector proteins (BECs) focussing on small proteins detected specifically in haustoria and predicted to be secreted. We then screened them to detect effector action by Host-Induced Gene Silencing on barley. This uses a transient assay system based on bombardment of epidermal cells with RNAi constructs. Seven out of the fifty BECs caused a significant effect in the reduction in the percentage of conidia capable of forming haustorium. The strongest effects were obtained for BEC1011 and BEC1054, two related genes that are part of a small gene family. Complementation analysis of these two effectors by transient over-expression of synthetic homologs resistant to RNAi demonstrated no cross-silencing. BEC1011 appeared to interfere with host cell death. Three BECs, BEC1005, BEC1054 and BEC1019 had domains reminiscent of a glucosidase, an RNAse and a protease, respectively. We are currently testing whether these proteins display the actual enzymatic activity. The outcome of this research will lay the foundation for a detailed understanding of the molecular mechanisms underlying disease in the cereal mildews.

PS4.7

Elucidating the response of wheat to the exposure of Stagonospora nodorum effectors

Lauren Du Fall, Peter Solomon

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The dothideomycete Stagonospora nodorum is a necrotrophic fungal pathogen of wheat and is the causal agent of Stagonospora nodorum blotch (SNB)1. This disease is responsible for over $100 million of yield losses in Australia annually. Recent studies have shown that this fungus produces a number of effector proteins that are internalised into host cells of susceptible wheat cultivars. The mechanism by which these effectors induce tissue necrosis in susceptible hosts is yet to be fully elucidated. We have applied a metabolomics approach to elucidate the cellular processes leading to disease and provide insight into the mode-of-action of these effectors. Gas chromatography-mass spectrometry analysis of primary polar metabolites has been undertaken on tissue extracts and apoplastic fluid from SnToxA infiltrated wheat. Results illustrate widespread perturbations in primary metabolism and reveal the first direct evidence of an increase in energy production in response to a pathogen effector. To further understand the host response to SnToxA at the secondary metabolism level, samples were also analysed using liquid chromatography-mass spectrometry. Our data indicate SnToxA causes an increase in defence-related secondary metabolites. The effect of these metabolites on Stagonospora nodorum growth and sporulation in vitro and in planta is currently under investigation. These complementary approaches have provided a novel insight into the contribution of the SnToxA effector protein to SNB in wheat.

1 Oliver, R. P. & Solomon, P. S. New developments in pathogenicity and virulence of necrotrophs. Current Opinion in Plant Biology (2010).

PS4.8
How do mobile pathogenicity chromosomes collaborate with the core genome?
Charlotte van der Does, Martijn Rep
University of Amsterdam

In the tomato pathogen *F. oxysporum* f. sp. *lycopersici*, most known effector genes reside on a pathogenicity chromosome that can be exchanged between strains through horizontal transfer. As a result, this fungus has sub-genomes with different evolutionary histories: the conserved core genome and the mobile 'extra' genome. Interestingly, expression of the effectors on the mobile genome requires Sge1, a conserved transcription factor encoded in the core genome. Also, a transcription factor on the mobile chromosome, Ftf1, is associated with pathogenicity (de Vega-Bartol et al. 2011). Random insertion of an effector-promoter GFP reporter construct revealed that the position in the genome has a strong influence on the level of expression. Furthermore, in all transformants tested, expression could no longer be induced, but was constitutive. To discover how Sge1 and Ftf1 control gene expression from the core genome and the mobile chromosome(s), their targets will be identified using ChIPseq: sequencing of DNA fragments to which Sge1 binds and RNAseq: in depth-sequencing of transcripts, comparing standard culture conditions to *in planta*-mimicing conditions. Additional components necessary to express effector genes on mobile chromosomes will be identified *via* insertional mutagenesis.

Sunday 1 April

Parallel session 5: Mitochondria

PS5.1
Functional analysis of ERMES and TOB (SAM) complex components in *Neurospora crassa*.
Frank E. Nargang, Sebastian W.K. Lackey, Jeremy G. Wideman
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The *Neurospora crassa* TOB complex (topogenesis of beta-barrel proteins), also known as the SAM complex (sorting and assembly machinery), inserts a subset of mitochondrial outer membrane proteins into the membrane—including all beta-barrel proteins. The TOB core complex contains Tob55, Tob38, and Tob37. Each of these proteins is essential for viability of *N. crassa*. The TOB holo complex contains an additional protein called Mdm10. Deficiency of any of these proteins results in impaired assembly of beta-barrel proteins. Lack of Mdm10 also results in a phenotype of enlarged mitochondria. The Mdm10 protein has also been shown to be a member of the ERMES complex (endoplasmic reticulum-mitochondria encounter structure) in *Saccharomyces cerevisiae*. The complex is thought to function in lipid and calcium exchange between the two organelles. Other members of the ERMES include the Mdm12, Mmm1, and Gem1 proteins. We have shown that deficiency of Mdm12 or Mmm1 also results in the presence of enlarged mitochondria and impaired beta-barrel protein assembly into the mitochondrial outer membrane while lack of Gem1 does not. The possibility that different domains of the proteins of the TOB and ERMES complexes are responsible for specific interactions and functions is currently being explored.

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PS5.1

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The *Neurospora crassa* TOB complex (topogenesis of beta-barrel proteins), also known as the SAM complex (sorting and assembly machinery), inserts a subset of mitochondrial outer membrane proteins into the membrane—including all beta-barrel proteins. The TOB core complex contains Tob55, Tob38, and Tob37. Each of these proteins is essential for viability of *N. crassa*. The TOB holo complex contains an additional protein called Mdm10. Deficiency of any of these proteins results in impaired assembly of beta-barrel proteins. Lack of Mdm10 also results in a phenotype of enlarged mitochondria. The Mdm10 protein has also been shown to be a member of the ERMES complex (endoplasmic reticulum-mitochondria encounter structure) in *Saccharomyces cerevisiae*. The complex is thought to function in lipid and calcium exchange between the two organelles. Other members of the ERMES include the Mdm12, Mmm1, and Gem1 proteins. We have shown that deficiency of Mdm12 or Mmm1 also results in the presence of enlarged mitochondria and impaired beta-barrel protein assembly into the mitochondrial outer membrane while lack of Gem1 does not. The possibility that different domains of the proteins of the TOB and ERMES complexes are responsible for specific interactions and functions is currently being explored.

PS5.2

On mitochondrial genes, genomes and proteomes

Rachid Daoud, Iona Minoiu, B. Franz Lang
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A key event in eukaryotic evolution, the symbiotic introduction of mitochondria (mt), occurred a billion or more years ago. This symbiosis contributed roughly 1/10th the genetic material to extant eukaryotes, which apparently all have or once had a mitochondrion. Most genes of mt origin are no longer encoded in the mitochondrial but in the nuclear genome. Therefore massive protein targeting has to occur, as well as a structural organization specific to mitochondria, although an overall conservation of bacterial features can be expected. Questions that we have been interested in are (i) identification of the complete mitochondrial proteome in yeast and other fungal species, (ii) organization of mitochondrial function in higher-order structures (complexes and super-complexes; in comparison to *E. coli*), and (iii) analysis of the ribonucleo-protein complex RNase P, which in some instances has an mtDNA-encoded catalytic RNA subunit, whereas in others the RNA is imported from the cytoplasm.

We have further followed up on the evolutionary question of mitochondrial origins using both mt genome data and nuclear genes of mt origin - by rooting of the eukaryotic tree and identification of the closest extant bacterial relatives of mitochondria. Our results support the view that eukaryotes are ancient, that the mitochondrial endosymbiosis is a relatively recent event, and that the root of the eukaryotic tree is between the monophyletic 'unikonts' and 'bikonts'. Whether or not *Rickettsia*-like bacteria are the sistergroup of mitochondria as commonly assumed remains an open question.

PS5.3

Mitochondrial dynamics and organismal ageing in *Saccharomyces cerevisiae*

Christian Scheckhuber, Heinz Osiewacz
Goethe University

Mitochondria are essential organelles of energy conversion and other vital pathways in most eukaryotic organisms. The dynamic behaviour of mitochondria, which includes movements of the organelles within the cell as well as opposing fusion and fission processes, is tightly controlled by a set of proteins. Among these, large Dynamin-related GTPases and several proteases in addition to other factors play key roles in this control. Previously, it was shown that reduced mitochondrial fission leads to a network-like morphology, decreased sensitivity for the induction of apoptosis and a remarkable extension of both replicative and chronological lifespan in one of the most important model systems for biological ageing, *Saccharomyces cerevisiae*. On the other hand, promoting mitochondrial fission by deleting the fusion gene *Mgm1* leads to a striking reduction of both replicative and chronological lifespan with a substantial increase of sensitivity to apoptosis elicitation via the reactive oxygen species hydrogen peroxide. It has been shown that Mgm1p is proteolytically cleaved by the rhomboid protease Pcp1p to yield a large and a small isoform of Mgm1p which are both required for efficient fusion of mitochondria. To investigate the importance of this process on mitochondrial functionality and ageing we analysed *Pcp1* deletion mutants containing respiratory competent (rho^{+}) and respiratory incompetent (rho^{0}) mitochondria. Senescent Δ*pcp1* rho^{+} but not senescent Δ*pcp1* rho^{0} cells display a striking mitochondrial morphotype reminiscent of stress-induced mitochondrial hyperfusion (SIMH) in mammalian cells. This correlates with a robust increase of replicative and chronological lifespan of Δ*pcp1* rho^{+} cells compared to the Δ*pcp1* rho^{0} cells. These findings suggest that the SIMH-like process in Δ*pcp1* rho^{+} positively influence ageing by prolonging mitochondrial functionality. Our results bear important clues for translational research to intervene into age-related degenerative processes in multicellular organisms including humans.

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PS5.4
Mitochondrial dynamics in yeast
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Mitochondria are amazingly dynamic organelles. They continuously move along cytoskeletal tracks and frequently fuse and divide. These processes are important for maintenance of mitochondrial functions, for inheritance of the organelles upon cell division, for cellular differentiation, and for apoptosis. As the machinery of mitochondrial biogenesis and inheritance has been highly conserved during evolution, it can be studied in simple model organisms such as yeast. By systematic screening of comprehensive yeast mutant collections and functional analyses we have identified novel components and cellular pathways required for mitochondrial fusion, division, motility, and maintenance of respiratory activity. These data provide a comprehensive picture of the molecular processes required for mitochondrial biogenesis in a simple eukaryotic cell. Our recent studies focus on the roles of mitochondrial cell cortex attachment and myosin-dependent movements in mitochondrial inheritance.

PS5.5
Mitochondrial Protein Quality Control Influences Lifespan and Stress Adaptation in *Podospora anserina*
Fabian Fischer, Andrea Weil, Andrea Hamann, Heinz Dieter Osiewacz
Johann Wolfgang Goethe University

Mitochondria are essential organelles of eukaryotic organisms. Maintaining their integrity is of key relevance, as mitochondrial dysfunction has been linked to a number of adverse phenomena such as aging or the development of degenerative diseases, e.g., Parkinson's and Alzheimer's disease. A complex network of different quality control (QC) pathways has evolved to meet this necessity. Protein QC in mitochondria is accomplished by several proteases located in the intermembrane space, the inner membrane (IM) and the mitochondrial matrix (MM).

Here, we present recent findings regarding the biological role of the *i*-AAA protease, located in the IM, and the ClpXP complex, located in the MM, in the fungal aging model *Podospora anserina*. Deletion of the gene *PaIap*, coding for the *i*-AAA protease, leads to mutant strains that display a pronounced increase in their healthy lifespan under standard growth conditions but are sensitive to heat stress. Consistently, growth at elevated temperatures leads to higher PaIAP abundance in wild type strains. These observations suggest that *P. anserina*'s *i*-AAA protease is part of an inducible QC system that has evolved to allow survival under fluctuating environmental conditions.

The phenotype of *PaClpP* deletion strains strongly resembles that of the *PaIap* deletion strains. Significantly, it was possible to complement the Δ*PaClpP* mutants by heterologous over-expression of the human *ClpP*-cDNA in the fungal deletion background. Although further experiments are required, it now seems feasible to use *P. anserina* for the functional characterization of the as of yet poorly studied eukaryotic Clp proteases.

PS5.6

A Mitochondrial Molecular Marker for estimating Arbuscular Mycorrhizal Fungal Biomass in Soil and Roots

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[1] University of Montreal [2.] Agriculture and Agrifood Canada

Arbuscular Mycorrhyzal Fungi (AMF) are significant contributors to the growth and health of a majority of plant species worldwide. AMF enhance nutrient assimilation from the soil (phosphorous, nitrogen) and enhance plant resistance to drought and pathogen infections. Several qualitative, quantitative and semi-quantitative tools are currently in use to estimate biomass and species diversity of AMF associated with soil and roots. Among them, the use of real-time PCR techniques has been validated in several systems, *in vitro*, in the greenhouse and in field samples, with various degrees of success. The recent sequencing of the first mitochondrial genome of an AMF species, *Glomus intraradices,* has provided a new toolbox to be used among others, for AMF identification and quantification purposes. Herein we present the mitochondrial gene *nad1* as a candidate molecular marker for the estimation of AMF biomass in the soil and roots. We analysed the sequence polymorphisms associated with *nad1* in a panel of reference *Glomus* species. We used a *Glomus*-specific region within the *nad1* gene to design a TaqMan tool for the quantification of several species of *Glomus* in environmental soil samples. We present data on the validation of the tool *in vitro*, in the greenhouse and in agricultural soil and root samples against a diverse collection of *Glomus* species.

PS5.7

The mitochondrial genome of the wood-decaying basidiomycete *Phlebia radiata* is the largest in size (156 kb) among fungi and contains a 6 kb inversion, stretches with repetitive elements and long introns invaded with homing endonucleases

Taina Lundell[1] Ilona Oksanen[1] Heikki Salavirta[1] Jaana Kuuskeri[1] Miia Mäkelä[1] Pia Laakso[2] Lars Paulin[1]
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The mitochondrial (mt) genome of the wood-decaying, white rot saprobic basidiomycete *Phlebia radiata* is the largest in fungi, 156 kb in size, sequenced so far. The mt genome of *P. radiata* is a single circular dsDNA molecule with average GC content of 31.1 %, and reveals several novel features such as a 6 kb duplication-inversion region containing several parallel, anticlockwise orientated open reading frames (ORFs). The exceptionally large size of the mt genome is explained by frequent splicing of the conserved genes with long introns (0.5-3 kb in size), the presence of additional unknown ORFs, existence of the large duplication-inversion region, and most of all, due to long stretches carrying repetitive sequence elements with variant motifs. A few of the repetitive elements containing regions indicate transposable, plasmid or viral origin. The mt genome contains the 14 conserved genes coding for essential proteins participating electron transfer and oxidative phosphorylation, the SSU and LSU rRNA genes, a gene for ribosomal protein subunit 3, and 28 tRNA genes, of which 11 are anticlockwise orientated. Phylogeny of the 14 protein coding sequences confirms current fungal taxonomy. Over 50 homing endonucleases of LAGLIDADGD and GYI-YIG types are recognized within the long introns splicing most of the 14 essential mt protein coding genes, except for *atp6* and *nad2* genes. The repetitive stretches, duplication-inversion and high amount of intron-homing endonucleases are features pointing to exceptional genetic flexibility and allowance of DNA recombination, which have not been recognized to this extent – at genome level – in fungal mitochondria previously.

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PS5.8

Phylogenetic analysis of the complete mitochondrial genome of *Madurella mycetomatis* confirms its taxonomic position within the order Sordariales

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Background: *Madurella mycetomatis* is the most common cause of human eumycetoma. The genus *Madurella* has been characterized by overall sterility on mycological media. Due to this sterility and the absence of other reliable morphological and ultrastructural characters, the taxonomic classification of *Madurella* has long been a challenge. Mitochondria are of monophyletic origin and mitochondrial genomes have been proven to be useful in phylogenetic analyses.

Results: the first complete mitochondrial DNA genome of a mycetoma-causative agent was sequenced using 454 sequencing. The mitochondrial genome of *M. mycetomatis* is a circular DNA molecule with a size of 45,590 bp, encoding for the small and the large subunit rRNAs, 27 tRNAs, 11 genes encoding subunits of respiratory chain complexes, 2 ATP synthase subunits, 5 hypothetical proteins, 6 intronic proteins including the ribosomal protein *rps*3. In phylogenetic analyses using amino acid sequences of the proteins involved in respiratory chain complexes and the 2 ATP synthases it appeared that *M. mycetomatis* clustered together with members of the order Sordariales and that it was most closely related to *Chaetomium thermophilum.* Analyses of the gene order showed that within the order Sordariales a similar gene order is found. Furthermore also the tRNA order seemed mostly conserved.

Conclusion: Phylogenetic analyses of fungal mitochondrial genomes confirmed that *M. mycetomatis* belongs to the order of Sordariales and that it was most closely related to *Chaetomium thermophilum*, with which it also shared both a comparable gene and tRNA order.

Sunday 1 April

Parallel session 6: ROS, Autophagy and Apoptosis

PS6.1

ROS signal transduction and cell differentiation in filamentous fungi

Fernando Lara-Rojas, Olivia Sánchez, Jesús Aguirre
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A phosphorelay system coupled to a MAP kinase module is involved in sensing and processing environmental signals in Fungi. In *Aspergillus nidulans*, response regulator (RR) SskA transmits oxidative stress signals to the stress MAPK (SAPK) SakA, which in turns physically interacts with ATF/CREB transcription factor AtfA in the nucleus. This defines a general stress-signalling pathway, which plays differential roles in oxidative stress responses during growth and development. AtfA is needed for the expression of several genes, the conidial accumulation of SakA and the viability of conidia. Furthermore, SakA is active (phosphorylated) in asexual spores, remaining phosphorylated in dormant conidia and becoming dephosphorylated during germination. SakA phosphorylation in spores depends on certain (SskA) but not other (SrrA and NikA) components of the phosphorelay system. Constitutive phosphorylation of SakA prevents both, germ tube formation and nuclear division. Similarly, *Neurospora crassa* SakA orthologue OS-2 is phosphorylated in intact conidia and gets dephosphorylated during germination. We propose that SAPK phosphorylation is a conserved mechanism to regulate transitions between non-growing (spore) and growing (mycelia) states. The *Aspergilli* contain a second SAPK called MpkC. Although *mpkC* mutants are not sensitive to oxidative or osmotic stress, they produce more spores that the wild type strain, suggesting that SakA and MpkC regulate processes related to the production and germination of spores. In addition, to the SakA pathway, RR SrrA and the AP-1 trancription factor NapA are differentially involved in ROS signalling and cell differentiation.

PS6.2

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PS6.1

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PS6.2

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The NADPH Oxidase Complexes in *Botrytis cinerea*

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Reactive oxygen species (ROS) act as messenger molecules for intercellular signaling or play a role during defense mechanisms against pathogens. One good example is the oxidative burst, within which plants rapidly produce large amounts of ROS as the first defense reaction towards pathogen attacks. NADPH oxidases (Nox) are the most common enzymatic system to produce ROS. Nox are enzymes, which transport electrons through biological membranes and therewith reduce oxygen to superoxide. In fungi they are shown to be involved in differentiation processes and pathogenicity and are therewith in our focus to gain insights into plant - fungi interactions.

Two NADPH oxidases (BcNoxA and BcNoxB) as well as their putative regulator (BcNoxR) were previously identified in the phytopathogenic fungus *B. cinerea* [1]. Besides their involvement in pathogenicity and sclerotia production, deletion studies have revealed that BcNoxA and BcNoxR are also involved in hyphal germling fusions[2].

Preliminary analysis indicate a localization of the catalytical subunits BcNoxA and BcNoxB to the ER and partly to the plasma membrane of hyphae.

Nox are multi-enzyme complexes, whose regulatory process and the participating proteins are well described in mammals. Though, in fungi not all components have been identified, yet. For *B. cinerea* interaction studies with potential candidates identified the regulatory subunit BcNoxR, the small GTPase Rac, the GEF BcCdc24, the scaffold protein BcBem1 and the PAK BcCla4 as interacting proteins within the BcNox complex.

[1] Segmueller N. et al., (2008) *Mol Plant Microbe Interact* **21:** 808-808-819.
[2] Roca M.G. et al., (2011) *Fungal Biology* (in press)

PS6.3

Identifying targets of NADPH oxidase-mediated redox signalling in *Fusarium graminearum* using proteomics approaches.

Manisha Joshi, Rajagopal Subramaniam, Christof Rampitsch
Agriculture and Agrifood Canada

Regulated production of reactive oxygen species by NADPH oxidases (NoxA, NoxB) in *Fusarium graminearum* is essential for the establishment of Fusarium head blight in wheat: the knock-out mutant *FgrΔNoxAB* is non-pathogenic, although it produces normal levels of the mycotoxin (and virulence factor) deoxynivalenol. Nox A and B produce O_2^- and thence H_2O_2 during infection, creating a reducing environment in which susceptible cysteine-cysteine disulphide bonds or other Cys-S-R, can be reduced to native Cys-SH. This can profoundly affect the activity of targeted proteins. Two strategies were used to identify targeted proteins: 1) 2-D electrophoresis, using monobromo-bimane to label reduced Cys residues and 2) an affinity-enrichment strategy based upon biotinylation of targeted cys residues, i.e. those where reduction occurs in WT but not in *FgrΔNoxAB*, under mycotoxin-inducing conditions *in vitro*. We identified 13 potentially targeted proteins by 2-DE and 29 by affinity enrichment, with Cys-S-R in *FgrΔNoxAB* and Cys-SH in WT. One of these proteins, FG10089 – homologous to a putative sporulation specific (SPS2) protein – has been knocked out, and *FgrΔ10089* presents the same phenotype as *FgrΔNoxAB*: non-pathogenic on wheat, but with WT levels of deoxynivalenol. FG10089 is therefore a possible downstream target of NoxAB-mediated redox signalling. Quantification of all Cys-peptides of interest by LC-MRM/MS and conversion of targeted Cys to Ser are underway, with the aim of further understanding the role of redox regulation in *F. graminearum* pathogenesis.

PS6.4

Production and Epidemiological Importance of Photodynamic Toxins Produced by the Necrotrophic Fungus *Ramularia collo-cygni*

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Leaf spotting is a common symptom in barley observed in particular since the late 1980s. The phenomenon is frequently associated with a physiologic reaction of the host plant to abiotic stresses like changes in temperature and radiation involving Reactive Oxygen Species (ROS). The frequent occurrence in past years seems related to climate change. At the same time the fungus *Ramularia collo-cygni* was identified as the causal agent of the Ramularia leaf spot disease. A particularity of the fungus is the production of photodynamic toxins, anthraquinone derivates named Rubellins. After activation through light Rubellins produce ROS, which were shown to be able to cause symptom development through chlorophyll bleaching and lipid peroxidation leading to cell wall degradation and the formation of necrotic spots. It was hypothesized that Rubellins play as virulence factor a role in the host parasite interaction. This is further supported by the correlation of symptom development and epidemics with the breakdown of the antioxidative system during plant senescence.

The testing of archive samples with PCR methods have shown that the fungus was present on seed long before becoming a major disease in barley and the detection during the growth season has discovered a high latency and the opportunity of seed transfer.

The current investigations quantify Rubellins through HPLC and compare it to the occurrence of symptoms and DNA content of *Ramularia collo-cygni* in the plant to find out where and when Rubellins are produced and to further elucidate the role of ROS and abiotic stresses in epidemics.

PS6.5

ROS Damage Defence Mechanisms in *Podospora anserina*

Andrea Hamann, Sandra Zintel, Heinz Dieter Osiewacz
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Reactive oxygen species (ROS) like superoxide or hydrogen peroxide are central components of the 'free radical theory of aging'. One major source for ROS in biological systems is their emergence as by-product during electron transport processes. Fortunately, various pathways to deal with ROS-induced damage have evolved. A first protective mechanism is the prevention of damage by effective ROS scavenging. One central ROS scavenging enzyme is the superoxide dismutase (SOD) converting superoxide into hydrogen peroxide. A number of studies demonstrate a clear impact of this enzyme family on lifespan. However, in the fungal aging model *Podospora anserina* the deletion of all three *Sod* genes hardly affects lifespan. This surprising dispensability of SODs in *P. anserina* is even more astonishing if considering that lack of PaSOD1, the cytosolic Cu/ZnSOD as well as of PaSOD3, the mitochondrial MnSOD results in paraquat sensitivity. We provide data suggesting a 'hormesis' effect of low superoxide doses on lifespan.

While low ROS doses seem to be beneficial, long-term or higher stress needs efficient degradation/recycling of damaged components. We therefore recently started to investigate another protective mechanism, selective autophagy, which degrades and recycles damaged components. The selectivity of this process is determined by the cargo receptor interacting with ATG8, an ubiquitin-like protein required for autophagosome formation. In filamentous fungi, there is only very little information available about cargo receptors. Thus, we used a two-hybrid screen with PaATG8 as bait to identify potential cargo receptors. Here, we provide first data on their characterisation.

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PS6.6

Mitophagy is linked to the general stress response pathway in *Saccharomyces cerevisiae*

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Mitochondria form a dynamic reticular network that is maintained by balance fusion and fission events. Impairment of this dynamic behavior is associated with a number of neuropathies such as optic atrophy and Parkinson's disease. In dysfunctional mitochondria the fusion machinery become inactivated and, as fission is maintained, such mitochondria become spatially separated from the intact network. By that mechanism dysfunctional mitochondria have been proposed to be targeted for selective degradation, by mitophagy, providing a quality control system for mitochondria. In yeast conflicting results concerning the role of mitochondrial dynamics in mitophagy were reported. We investigated the effects of altering mitochondrial fission and fusion on mitophagy using biochemical as well as fluorescence based assays. Rapamycin induced mitophagy depended on Atg11, Atg20, and Atg24 confirming that a selective type of autophagy was induced. Fragmentation of mitochondria or inhibition of oxidative phosphorylation was not sufficient to trigger mitophagy. Neither expression of dominant-negative variants of Dnm1, nor deletion of the fission factors Dnm1, Fis1, Mdv1, or Caf4 impaired mitophagy. Instead, we found that reduced mitophagy initially observed in a Δ*fis1* mutant was not due to the absence of Fis1 but rather due to a secondary mutation in *WHI2*, encoding a factor reported to function in the general stress response and the Ras/PKA signaling pathway. We propose that mitochondrial fission is not a prerequisite for the selective degradation of mitochondria in yeast and that mitophagy is linked to the general stress response and the Ras/PKA signaling pathway. Future studies will address how Whi2 is mechanistically linked to mitophagy.

PS6.7

***Botrytis*-plant interaction: interplay of cell death**

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Botrytis cinerea is used as a model system to study the pathogenicity of necrotrophic fungi. As inferred by the term "necrotrophic", such fungi first kill the tissue and then obtain nutrients from the dead plant cells. Hence, the most critical step in necrotrophic development is fast killing of the host cells. Previous studies have shown that *B. cinerea* promotes apoptotic cell death in infected plants, thereby promoting lesion spreading. How the fungus survives the first encounter with living plant tissue remained unclear. Here we report on the characterization of apoptotic cell death in *Botrytis cinerea* during plant infection and on the role of an anti-apoptotic response in disease establishment.

Useing an automatic search protocol we mapped the fungal homologues of mammalian apoptotic proteins and domains. Among all known apoptotic domains, only BIR domain was found in fungi. We isolated the BIR-containing protein from *B. cinerea* and determined its role in apoptosis and pathogenicity. Knockout or over expression strains of *BcBIR1* revealed that BcBir1 has antiapoptotic activity. We found that the fungus undergoes massive programmed cell death during early stages of infection, but then fully recovers upon transition to second phase of infection. Further studies using the fungal mutants in combination with mutant *Arabidopsis* lines showed that virulence was fully correlated with ability of the fungus to cope with plant-induced PCD. Time lapse analysis showed that the reduced pathogenicity of the *Bcbir1* mutant was due to slow development in the initial infection phase, whereas there was no change in development of the mutant during the second infection phase. A model of the infection process is proposed in which the anti-apoptotic machinery is only necessary during the initial infection phase, during which the fungus is in direct contact with living plant cells.

PS6.8

Farnesol-induced cell death in the filamentous fungus Aspergillus nidulans

Gustavo Goldman
Universidade de São Paulo

FOH (farnesol), a non-sterol isoprenoid produced by dephosphorylation of farnesyl pyrophosphate, has been shown to inhibit proliferation and induce apoptosis. We have been using Aspergillus nidulans and FOH as a model system and cell death stimulus, respectively, aiming to understand by which means filamentous fungi are driven towards cell death. Previously, we demonstrated that the A. nidulans calC2 mutation in protein kinase C pkcA was able to confer tolerance to FOH. We demonstrate that pkcA overexpression during FOH exposure causes increased cell death. FOH is also able to activate several markers of endoplasmic reticulum (ER) stress and the unfolded protein response (UPR). Our results suggest an intense cross-talk between PkcA and the UPR during FOH-induced cell death. Furthermore, the overexpression of pkcA increases both mRNA accumulation and metacaspases activity, and there is a genetic interaction between PkcA and the caspase-like protein CasA. Mutant analyses imply that MAP kinases are involved in the signal transduction in response to the effects caused by FOH.

Financial support: FAPESP and CNPq, Brazil.

Monday 2 April

Parallel session 7: Sensing and responding

PS7.1

The Pals wink at the ESCRT: pH signalling in the plasma membrane

Miguel A. Peñalva [1] A.M. Calcagno-Pizarelli [2] Herb N. Arst [2] Jr. ,Antonio Galindo [1]
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The fungal *pal*/*RIM* signalling pathway regulates gene expression in response to environmental pH. In *Aspergillus nidulans* it involves six dedicated proteins, PalA, PalB, PalC, PalF, PalH and PalI, which mediate the proteolytic activation of the transcription factor PacC. In addition, it requires several components of the ESCRT (endosomal sorting complex required for transport) complexes, which mediate multivesicular body biogenesis at endosomes. This fact suggested that pH signalling proteins might assemble on endosomal platforms. Amongst Pal proteins are the plasma membrane receptor PalH and its coupled arrestin, PalF. PalF becomes ubiquitylated in an alkaline pH- and PalH-dependent manner; three other Pal proteins are ESCRT-III associates, and thus they were considered potentially endosomal. These are the Vps32-interactors PalA and PalC and the Vps24-interactor calpain-like PalB. Therefore previous models speculated that intracellular traffic would mediate the connection between plasma membrane- and endosomal membrane-associated complexes.

We studied by *in vivo* microscopy the subcellular localization at which signalling takes place after activating the pathway by shifting ambient pH to alkalinity. Rather than localising to endosomes, Vps32 interactors PalA and PalC oscillate at the plasma membrane, transiently co-localising to alkaline pH-induced cortical structures in a PalH-dependent manner. Notably, the assembly of this cortical structures is Vps23 (ESCRT-I)- and Vps32 (ESCRT-III)-dependent but Vps27 (ESCRT-0)-independent. These cortical structures are dramatically more stable under conditions leading to Vps4 deficiency, indicating that their half-life depends on ESCRT-III disassembly. Pull-down studies demonstrated that Vps23 interacts strongly with the PalF arrestin. Notably Vps23 co-immunoprecipitates exclusively ubiquitylated PalF forms from extracts. Endogenously tagged Vps23-GFP is also recruited to cortical structures, in addition to endosomes, in a PalF- and alkaline pH-dependent manner. These Vps23-GFP structures become particularly obvious in *vps27Δ* cells where the conspicuous endosomal localisation of Vps23 is prevented. Dual-channel time-lapse epifluorescence microscopy showed that PalC arrives to cortical complexes before PalA. As PalC recruitment is PalA-independent and PalA recruitment is PalC-dependent but PalB-independent, these data complete the participation order of Pal proteins in the pathway. Importantly, they strongly support a model in which pH signalling takes place in ESCRT-containing, plasma membrane-associated, rather than endosome-associated, signalling complexes.

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The fungal *pal*/*RIM* signalling pathway regulates gene expression in response to environmental pH. In *Aspergillus nidulans* it involves six dedicated proteins, PalA, PalB, PalC, PalF, PalH and PalI, which mediate the proteolytic activation of the transcription factor PacC. In addition, it requires several components of the ESCRT (endosomal sorting complex required for transport) complexes, which mediate multivesicular body biogenesis at endosomes. This fact suggested that pH signalling proteins might assemble on endosomal platforms. Amongst Pal proteins are the plasma membrane receptor PalH and its coupled arrestin, PalF. PalF becomes ubiquitylated in an alkaline pH- and PalH-dependent manner; three other Pal proteins are ESCRT-III associates, and thus they were considered potentially endosomal. These are the Vps32-interactors PalA and PalC and the Vps24-interactor calpain-like PalB. Therefore previous models speculated that intracellular traffic would mediate the connection between plasma membrane- and endosomal membrane-associated complexes.

We studied by *in vivo* microscopy the subcellular localization at which signalling takes place after activating the pathway by shifting ambient pH to alkalinity. Rather than localising to endosomes, Vps32 interactors PalA and PalC oscillate at the plasma membrane, transiently co-localising to alkaline pH-induced cortical structures in a PalH-dependent manner. Notably, the assembly of this cortical structures is Vps23 (ESCRT-I)- and Vps32 (ESCRT-III)-dependent but Vps27 (ESCRT-0)-independent. These cortical structures are dramatically more stable under conditions leading to Vps4 deficiency, indicating that their half-life depends on ESCRT-III disassembly. Pull-down studies demonstrated that Vps23 interacts strongly with the PalF arrestin. Notably Vps23 co-immunoprecipitates exclusively ubiquitylated PalF forms from extracts. Endogenously tagged Vps23-GFP is also recruited to cortical structures, in addition to endosomes, in a PalF- and alkaline pH-dependent manner. These Vps23-GFP structures become particularly obvious in *vps27Δ* cells where the conspicuous endosomal localisation of Vps23 is prevented. Dual-channel time-lapse epifluorescence microscopy showed that PalC arrives to cortical complexes before PalA. As PalC recruitment is PalA-independent and PalA recruitment is PalC-dependent but PalB-independent, these data complete the participation order of Pal proteins in the pathway. Importantly, they strongly support a model in which pH signalling takes place in ESCRT-containing, plasma membrane-associated, rather than endosome-associated, signalling complexes.

PS7.2

pH control of infectious growth in *Fusarium oxysporum* involves reprogramming of MAPK signalling cascades

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In plant pathogenic fungi, contact with the host triggers a developmental and metabolic transition towards infectious growth. We are interested in the environmental and host-derived stimuli and cellular pathways that regulate infectious growth in *Fusarium oxysporum*, a soilborne pathogen causing vascular wilt disease on a wide range of plant species and opportunistic infections in immunocompromised humans. One of the key players in plant pathogenicity is Fmk1, a conserved mitogen-activated protein kinase (MAPK) that is essential for infection-related processes such as chemotropism, root adhesion, penetration and invasive growth. Most Fmk1-dependent virulence functions require the homeodomain transcription factor Ste12, and are repressed in the presence of the preferred nitrogen source ammonium through a mechanism that requires the transporter MepB and the bZIP factor MeaB. Repression of invasive growth by ammonium also occurs in *Magnaporthe oryzae*and *Fusarium graminearum*, suggesting that this mechanism is conserved in biologically divergent plant pathogens. Recent data suggest that ammonium repression is mediated by a shift in extracellular pH, which results in rapid changes in the phosphorylation pattern of different MAPKs. Thus, ambient pH controls invasive growth of *F. oxysporum* by reprogramming the activation status of cellular MAPK signalling cascades.

PS7.3

Subcellular Localization of the *Neurospora crassa* MAP Kinase MAK-2 Influence its Activity and Function During Cell-Cell Signalling

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Germinating vegetative spores of the filamentous fungus *Neurospora crassa* mutually attract and grow towards each other and eventually fuse. Earlier studies identified the SO protein and the MAP kinase MAK-2 as essential players for this chemotropic interaction. Both proteins show dynamic recruitment to the tips of opposing germlings and concentrate at the fusion point once the two cells get into physical contact (Fleissner et al, 2009). Our analysis of the subcellular localization of the upstream kinases of MAK-2 suggest similar dynamics for the MAPKK STE-7. Colocalization experiments were performed using heterokaryons expressing *ste-7-gfp* and *mak-2-cherry* constructs. Analysis of the subcellular localization of the MAPKKK NRC-1 indicate a concentration of the kinase at the fusion point after the fusion cells established physical contact.

To test if the dynamic localization of MAK-2 is essential for chemotropic growth, we artificially tethered it permanently to the plasma membrane via a farnesyl anchor. Expression of the respective constructs does not rescue the Δ*mak-2* defects and results in a dominant phenotype in the wild-type background. Western-Blot analysis revealed that the membrane bound form of MAK-2-GFP is hyperactivated. Together these results indicate that the recruitment of MAK-2 to the plasma membrane promotes activation of this kinase and that the dynamic localization of the MAP kinase is essential for cell signalling.

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PS7.4

Functional characterization of G-protein-coupled receptors in the cereal pathogen *Fusarium graminearum*

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G-protein-coupled receptors (GPCRs) are a large family of transmembrane proteins that perceive external signals and communicate them to an intracellular heterotrimeric G-protein-signaling cascade. Thus, GPCRs play a pivotal role in the adaptation of entities to environmental stresses. During establishment of a compatible interaction, pathogenic fungi have to deal with numerous, rapid changes in regard to stresses and nutrition. Here, we present a comprehensive functional characterization of nine GPCRs of the cereal pathogen *F. graminearum*, the causal agent of Fusarium head blight (FHB) of small grain cereals. Single deletion mutants of five putative cAMP receptor-like GPCRs (cAMP-GPCR), two putative nitrogen sensors (NS), one pheromone receptor (PR) and one putative carbon sensor (CS) GPCR were generated. Subsequently, the mutants were characterized in regard to vegetative growth on different media (e.g. different C- and N-sources, various stresses), different conditions (temperature, pH), sexual and vegetative reproduction, cAMP production, lipase acitivity, virulence towards wheat, and deoxynivalenol production. To our surprise, deletion of one cAMP-GPCR (FG7716) and both NS (FG8496 and FG5579) does not provoke any obvious phenotype. Deletion mutants of the cAMP-GPCR FG1861 showed a higher stress tolerance towards oxidative, fungicide and temperature stresses and were reduced in virulence compared to wild type. The latter also applies for deletion mutants of the cAMP-GPCRs FG3023 and FG5239 and the PR FG2655. These four mutants were also reduced in DON-production, which might explain the reduced virulence towards wheat. The CS FG5006 was drastically reduced in the intracellular cAMP level indicating that this GPCR acts upstream of the adenylatcyclase.

PS7.5

Specific Structural Features Of Sterols Affect Cell-Cell Signalling And Fusion In *Neurospora crassa*

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In the early stages of colony formation in *Neurospora crassa*, germinating spores mutually attract each other, establish physical contact, and finally fuse. Communication between germlings requires the coordinated dynamic recruitment of the MAP kinase MAK-2 and the cytoplasmic protein SO to the tips of interacting cells. Subsequent plasma membrane fusion is facilitated by the transmembrane protein PRM1.

Here, we report that mutants affected in the biosynthesis of ergosterol, a major constituent of the fungal cell membrane, show distinct defects during germling fusion. Deletion of *erg-2*, which encodes an enzyme mediating the last step in the pathway, strongly impairs both tropic interactions and cell fusion. Interestingly, both MAK-2 and SO mislocalize at the tips of interacting Δ*erg-2* germlings. In contrast, the absence of ERG-10a and ERG-10b, two enzymes with redundant function that act upstream of ERG-2, does not affect cell-to-cell communication. However, Δ*erg-10a* Δ*erg-10b* germling pairs show Δ*Prm1*-like deficiencies in plasma membrane merger.

By relating the sterol composition and fusion competence of several *erg* mutants, we find that not the absence of ergosterol, but the accumulation of sterol intermediates specifically impairs distinct steps of germling fusion. While the presence of two double bonds in the sterol side chain provokes Δ*erg-2*-like deficiencies, the absence of a double bond in the sterol ring system causes Δ*Prm1*-like defects.

These data suggest that specific structural features of sterols differentially affect membrane properties and functions, such as the membrane recruitment of proteins, the assembly of signalling complexes, and plasma membrane fusion.

PS7.6

Structural and Functional Comparison of Pyrrolnitrin- and Iprodione-induced Modifications in the Class III histidine-kinase Bos1 of *Botrytis cinerea*

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Dicarboximides and phenylpyrroles are commonly used fungicides against plant pathogenic ascomycetes. Although their effect on fungal osmosensing systems has been shown in many studies, their modes-of-action still remain unclear. Laboratory- or field-mutants of fungi resistant to either or both fungicide categories generally harbour point mutations in the sensor histidine kinase of the osmotic signal transduction cascade. We compared the mechanisms of resistance to the dicarboximide iprodione and to pyrrolnitrin, a structural analogue of phenylpyrroles, in *Botrytis cinerea*. Pyrrolnitrin-induced mutants and iprodione-induced mutants of *B*. *cinerea* were produced *in vitro*. For the pyrrolnitrin-induced mutants, high level of resistance to pyrrolnitrin was associated with a high level of resistance to iprodione. For the iprodione-induced mutants, the high level of resistance to iprodione generated variable levels of resistance to pyrrolnitrin and phenylpyrroles. All selected mutants showed hypersensitivity to high osmolarity and regardless of their resistance levels to phenylpyrroles, they showed strongly reduced fitness parameters (sporulation, mycelial growth, aggressiveness on plants) compared to the parental phenotypes. The sequences of the osmosensing class III histidine kinase encoding gene *bos1* showed different mutations in both types of mutants. All of them affected the HAMP-domains of the histidine-kinase showing that each of the six HAMP domains is important for signal-transduction. Structure modelling of the HAMP domains revealed that the replacements of hydrophobic residues within the HAMP domains generally affected their helical structure, probably abolishing signal transduction. The mutation of residues E529, T581, or E692 – without consequences on HAMP structure – highlighted their involvement in signal transduction.

PS7.7

Transcriptomic and molecular analysis of germination and plant infection of Botrytis cinerea

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Germination of *Botrytis cinerea* conidia can be induced by several stimuli. Carbon source-induced germination is dependent on cAMP-dependent signaling, whereas for germination of conidia on hydrophobic surfaces without nutrients, an intact Ste11-Ste7-Bmp1 MAP kinase cascade is required. We have performed transcriptome studies to follow gene expression changes during germination and differentiation of *Botrytis cinerea* wild type conidia and deletion mutants strains. The results showed that in general the greatest changes of gene expression occur between 0 and 1 hour (before germ tube emergence). The genes that were specifically upregulated during germination (1-4 h.p.i.), were found to be enriched in genes encoding secreted proteins, indicating a strong secretory activity during the early stages of development. In contrast in the *bmp1* MAP kinase mutant, which is essential for germination on a hydrophobic surface and host penetration, an upregulation of many of these genes was not observed. As a putative sensor protein Msb2, was identified. Δ*msb2* mutants showed normal germination on hydrophobic surfaces but no appressoria formation and impaired primary lesion formation. Comparison of the transcript profiles of *msb2* and *bmp1* deletion mutants supports a regulatory link of both signal transduction components. Our further research focuses on the contribution of Msb2 in the Bmp1 dependent signaling cascade by phosphorylation studies and yeast-2-hybrid experiments.

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Poster Category 8: Biotechnology

PR8.1

Establishing a novel protein expression system in *Ustilago maydis*

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In industrial biotechnology some heterologous proteins cannot be produce with established expression systems. Thus, there is a great interest to develop novel protein expression platforms. In this project we establish a new expression system using the basidiomycete fungus *Ustilago maydis*. This eukaryotic microorganism is very well suited for genetic, biochemical, genome-wide as well as proteome-wide approaches. During filamentous growth microtubule-dependent mRNA transport plays a crucial role. The RNA-binding protein Rrm4 is responsible for long-distance mRNA transport and essential for the efficient secretion of the endochitinase Cts1. With fusions of β-glucuronidase and Cts1 we demonstrated, that Cts1 can mediate the export of active heterologous proteins. β-glucuronidase is inactivated by N-glycosylation during conventional secretion. Using this enzyme as a reporter our data reveal that Cts1 is secreted via an unconventional secretion process avoiding N-glycosylation. In addition, the lack of a canonical N-terminal secretory signal peptide in Cts1 was confirmed by analysis of truncated Cts1 variants. Circumventing N-glycosylation can be beneficial in various applications, for example to prevent undesirable immune responses in medical applications. As a proof-of-principle we successfully expressed codon optimized lipase CalB in *U. maydis* and confirmed its activity by Tributyrin plate assays. Next, we will express difficult-to produce lipases and characterize them by liquid assays.

PR8.2

Approaches for directed strain improvement targeting enhanced biosynthesis of gibberellic acid in *Fusarium fujikuroi*

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The filamentous fungus *Fusarium fujikuroi* is famous for producing high amounts of gibberellic acids (GAs). These phytohormones exhibit a great biotechnological impact as application of GAs in higher plants induces early flower bud formation and shoot elongation as well as an increased fruit size. Each year about ten tons of gibberellins are used as plant growth regulators by the agricultural and plant breeding industry.

Therefore, we developed strategies to increase GA yields by directed genetic modifications of genes involved in primary and secondary metabolism. Thus, overexpression of the first GA specific gene *Ggs2* (geranylgeranyl-pyrophosphate synthase 2), was performed. In addition, the negative feedback regulation of the key enzyme of the mevalonate pathway, *HmgR* (Hydroxy-methyl-glutaryl-CoA reductase), has been circumvented by deleting the regulatory domains. Overexpression of the truncated gene resulted in higher GA yields. Furthermore, regulation on transcriptional and protein level should be further investigated. This shall be amongst others elucidated by identification of positively or negatively acting transcription factors.

Another approach for enhancing GA-biosynthesis is to knock down a whole set of secondary metabolite pathways competing for the same precursors by deletion of the 4'-phosphopantetheinyl transferase Ppt1. This led to loss of function of all polyketide synthases (PKSs) and non-ribosomal peptide synthases (NRPSs) that are essential for many secondary metabolite syntheses. This modification altered the GA spectrum and resulted in higher GA amounts.

PR8.3

Analysis of the White-Rot Model Pleurotus *ostreatus* Secretome

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The utilization of low-value substrates such as lignocellulosic wastes offers a great potential to reduce the production costs of bioethanol. The biological process of bioethanol production using lignocellulose as feedstock requires its delignification to liberate the cellulose and hemicellulose from their complex with lignin. *P. ostreatus* is a model white rot basidiomycete that produces various ligninolytic enzymes useful in this process.

With the aim to expand knowledge about proteins involved, and through the availability of the whole genome sequence of the two monokaryotic strains PC9 and PC15 that compose the dikaroytic strain N001 obtained in the DOE JGI, we have carried out a study of the secretome of the fungus cultured on three different liquid media using wood, glucose or both as carbon source.

We have used the 2D electrophoresis technique prior to mass spectrometry analyses of the spots in order to make a first approach to the proteins secreted by the fungus. Afterwards, the use of a shot gun technique to deepen in the analysis of the proteins involved, in addition to the analysis of the computationally predicted secretome, have enabled us to compare the enzymatic profile between the monokaryons PC9 and PC15 and the dikaryon N001 and provide valuable insight into how white rot fungi degrade lignocellulosic biomass.

PR8.4

Investigating the yet-unknown biomass degrading and modifying enzymes of Aspergillus *oryzae*

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The associations of plant cell wall components with *A. oryzae*'s extracellular enzyme machinery was investigated from a chemical perspective using an integrated analysis of the transcriptome profile.

*S*train RIB40 of *A. oryzae* was cultured on various carbon sources, namely cellohexaose, mannohexaose, xylopentaose, arabinoheptaose, glucohexaose, glucosyl maltotriosyl maltotriose, galactosyl mannotriose, turanose and sophorose, and the transcribed genes were determined with DNA microarrays. The statistically significant genes were selected and novel hydrolases were identified, which would be further heterologously expressed for subsequent characterization. This work represents a novel way of integrating computational chemical biology and classical enzyme research for improving lignocellulose bioconversion.

More specifically, we aim at the heterologous expression and characterization of seven different hypothetical and non classified proteins of *A. oryzae*, which could prove to be useful tools in the wood biomass separation and modification process.

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PR8.5

Transcriptomics-based genome-scale prediction of secondary metabolite gene cluster members in *Aspergillus niger*

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The biosynthetic pathways for fungal secondary metabolites (SMs) are currently the focus of a large amount of efforts to elucidate the genetic basis of the biosynthesis. Major drivers for this effort are the large potential of fungal metabolites as bioactive compound, as well as an interest in utilizing the enzymes for synthetic biochemistry.

In an effort to alleviate the large amount of work required to identify the biosynthetic genes associated with a given SM synthase, we have previously developed a method for predicting SM clusters size from transcriptomic data, and showed it to be accurate in *A. nidulans*. In this study, we developed a new DNA microarray for *A. niger*, and employed it to build a microarray compendium of 73 samples from a diverse set of growth conditions.

Using the SMURF algorithm (Khaldi et al. 2010), we identified putative NRPS's, PKS's, hybrids and DMATs from the *Aspergillus niger* ATCC 1015 genome. This analysis yielded 81 putative SM synthases. Of those, 75 synthase genes are active in our gene expression catalog. This allowed the assignment of cluster genes for all 75 active synthases. Cluster sizes range from 15 genes (Gene ID: 118581, apparently absent in *A. niger* CBS 513.88) to one gene.

We have further employed the data set to predict cross-chemistry between physically separated gene clusters.

PR8.6

Characterization of the cyp684 gene involved in fenhexamid resistance in the species *Botrytis pseudocinerea*

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The Botrytis species complex responsible for the grey mold disease on multiple crops is composed of several species. The major one is Botrytis cinerea, the second one was called Botrytis pseudocinera. Despite their genetic polymorphism both species cannot be morphologically distinguished. However they differ for their sensitivity to several fungicides, especially to the sterol biosynthesis inhibitor fenhexamid. While B. Cinerea is sensitive to this hydroxyanilide, but can acquire resistance through target site mutations, B. Pseudocinerea is a naturally resistant species. We found a strong synergism between fenhexamid and sterol 14α-demethylation inhibitors (DMIs), especially on B. Pseudocinerea. Since DMIs inhibit Cyp51, a cytochrome P450 protein, we supposed detoxification of fenhexamid by a cytochrome P450 similar to Cyp51 to be involved in B. Pseudocinerea's resistance. The gene with the highest similarity to cyp51, named cyp684, was deleted in a B. Pseudocinerea strain. Cyp684 knock out mutants exhibit increased fenhexamid sensitivity and decreased fenhexamid metabolisation, showing that the Cyp684 cytochrompe P450 is responsible for B. Pseudocinerea's (HydR1) natural resistance to fenhexamid. Although cyp684 is also present in B. Cinerea sensitive to fenhexamid, we observed several polymorphisms: i/ in B. Pseudocinerea the cyp684 promoter shows a deletion of 25 bp, ii/ the peptide sequence varies by 4 amino acid residues between the species. We are currently establishing the cyp684 expression profiles in both species in order to analyze the impact of the promoter deletion on its expression. We will then study which part of the gene is/are responsible for fenhexamid resistance in B. Speudocinerea prior to establish its physiological and enzymatic functions.

PR8.7

Transcriptional and enzymatic profile of *Pleurotus ostreatus* multigene family in submerged and solid state fermentation

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The functional differences among the members of multigene families can be analyzed by studying their transcriptional profiles under different environmental conditions and different genotypes. The white rot fungus *Pleurotus ostreatus* can be a suitable model for genomics and transcriptomics studies in basidiomycetes due to the information available after the sequencing of the two haploid genomes composing the N001 strain. In this work, we have studied the differential regulation of the laccase gene family transcription using a RT-qPCR approach. The study has been made using different monokaryotic and dikaryotic (isogenic and non-isogenic for the growth-rate containing QTL chromosome VIII) strains, cultured in submerged cultures in the presence or in the absence of a laccase inducer, and in solid fermentation. Our results revealed (1) the importance of measuring the amplification efficiency and of carefully selecting the internal standards for the relative quantification of gene expression, (2) that the *Lacc2* and *Lacc10* genes are the responsible of laccase induction in submerged cultures, (3) that these two genes displayed opposite transcriptional response in PC9 type and PC15 type full-sibs strains, suggesting that laccase induction in submerged fermentation is linked to vegetative growth rate, (4) that the expression of these two genes increased in solid fermentation with increased water availability in the culture, (5) that the enzymatic activities and intracellular/extracellular isozyme patterns confirmed the differential behaviour of fast growing and slow growing strains, and characterized the intracellular and extracellular laccase fractions in solid and submerged cultures.

PR8.8

Real-Time Viability Assay for Fungal cells

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Detection of live microorganisms, including fungi, is currently performed by enumeration of colony forming units (CFUs) on agar plates. Although reliable and widely accepted, this method has disadvantages. Determination of CFUs is timely, laborious and limited to readily culturable microorganisms. Cultivation-independent methods are available nowadays, but they mostly involve multiple incubation steps for assessment of the number of viable cells and do not discriminate between dead or live microorganisms. Here a novel method is presented, capable of specifically monitoring living fungal and yeast cells (and bacteria too) in a real-time manner.

The assay is based on a novel viability criterium, the ability of cells to maintain a neutral pH in an acidic environment. Therefore, we searched for probes that show a fluorescent signal in a neutral environment, and not in an acidic environment. Since fluorescence is only produced in cells with a neutral pH (*i.e.* living cells), the fluorescent intensity is a measure for the amount of viable cells.

We have identified a number of probes that allow real-time viability (RTV) assays of fungal samples. These probes have succesfully been tested on *Aspergillus niger* and *A. fumigatus*, *Saccharomyces cerevisiae*, and *Candida albicans*. Based on these results it is expected that the RTV assay will work for other fungal species too. The RTV assay for fungi opens ways to assess in a fast, automated manner the viability in fungal samples such as spore batches, anti-fungal treated fungal cells and all other applications where viability of fungal cells are of interest.

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PR8.9

Hydrophobin fusions for high level intracellular protein production and purification in *Trichoderma reesei*

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Insufficient accumulation levels and the lack of efficient purification methods constitute two major bottlenecks hindering the recombinant protein production. Low yields are often seen in the cases where the host is distantly related to the organism from which the product is derived. Microbial production systems where the product is secreted to the medium are hampered by host proteases, which can destroy the target protein. Secretion in eukaryotic cells is finely tuned, and production is frequently limited by inefficient secretion of exogenous proteins. Microbial intracellular protein production, on the other hand, often results in aggregation of the denatured product in inclusion bodies, from which the active conformation is difficult to recover. Hydrophobins are small amphipathic proteins ubiquitously expressed in filamentous fungi. Hydrophobins are capable of altering the hydrophobicity of their respective fusion partner to enable purification by surfactant-based aqueous two-phase separation (ATPS). We have demonstrated that hydrophobin fusions targeted to endoplasmic reticulum (ER) induces formation of large intracellular protein bodies in *Trichoderma reesei*. The fusion protein remains soluble in the protein bodies surrounded by the ER-membrane and can be easily recovered from the cell lysate by ATPS. It is hypothesized that packing of hydrophobin fusions into these protein bodies may exclude the recombinant protein from the host proteolysis, simultaneously protecting the host cell from toxic effects of massive intracellular accumulation of the target protein. The implications of these results in development of novel strategies for production of recombinant proteins will be discussed.

PR8.10

Application of an optimized FLP/*FRT* recombination system in diverse filamentous fungi

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A molecular tool for marker recycling was established to overcome the limited availability of resistance markers in filamentous fungi. For this purpose the FLP/*FRT* recombination system from *Saccharomyces cerevisiae* was optimized for the penicillin producer *Penicillium chrysogenum*. In a first approach, we used a two-step strategy to test the functionality of the system. Therefore we generated a nourseothricin resistance cassette flanked by *FRT* sequences in direct repeat orientation (*FRTnat1* cassette) and ectopically integrated this construct into a *P. chrysogenum* recipient strain. In a second step a codon-optimized *Pcflp* recombinase gene were transferred into the *P. chrysogenum* strain, carrying the *FRTnat1* cassette. We observed in several tested transformants the successful recombination event due to the use of a codon-optimized recombinase. To further extend the application of the FLP/*FRT* recombination system, we generated a marker-free ΔPcku70FRT2 strain which enables the production of multiple deletion strains by highly efficient homologous recombination. Moreover a *nat1* flipper was generated to establish a one-step marker recycling. Therefore the FLP/*FRT* system and the *nat1* marker gene were combined in a single construct. For induction of the recombinase gene expression we used the *xyl* promoter. In further experiments we will use different flipper cassettes together with the ΔPcku70FRT2 strain to construct marker-free double and triple mutants.

Moreover the applicability of the developed tool was demonstrated by marker recycling in the ascomycetes *Sordaria macrospora* and *Acremonium chrysogenum* indicating, that the optimized FLP/*FRT* recombination system is suitable to a broad range of filamentous fungi.

PR8.11

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

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Fungi secrete enzymes to convert organic matter into small molecules that can serve as nutrients. Hyphae at the periphery of the colony are exposed to unexplored organic material, whereas the center of the colony experiences a utilized substrate. This suggests that the enzymes that are secreted by different zones in the colony are different. *Aspergillus niger* is an important cell factory for the industrial production of enzymes. Here, we determined with stable isotope dimethyl labeling the secretome of 5 concentric zones of 7-day-old xylose-grown colonies of *A. niger* that had either or not been treated with cycloheximide. As expected, cycloheximide blocked secretion of proteins at the periphery of the colony. Unexpectedly, protein release was increased by cycloheximide in the intermediate and central zones of the mycelium when compared to non-treated colonies. Electron microscopy indicated that this is due to partial degradation of the cell wall. A total of 124 and 59 proteins were detected in the medium of xylose grown colonies that had or had not been treated with cycloheximide. Apparently, a major part of the proteins are associated with the cell walls of *A. niger*. Taken together, cycloheximide can be used to obtain a (near) complete secretome of *A. niger*. Moreover, the total amount of protein is increased upon treatment with this antibiotic. The composition of the secretome in each of the 5 concentric zones differed. This study thus describes spatial release of proteins in *A. niger*, which is instrumental in understanding how fungi degrade complex substrates in nature.

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PR8.12

D-xylose Concentration-dependent Hydrolase Expression Profiles and the According Role of CreA and XlnR in *A. niger*

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Aspergillus niger is an industrially important organism for the production of industrial enzymes like hemicellulases and pectinases. The xylan-backbone monomer D-xylose is known as an inducing substance for the coordinate expression of a high number of polysaccharide-degrading enzymes. In this study a total number of 22 genes, which encode enzymes that function as xylan backbone-degrading enzymes, accessory enzymes, cellulose-degrading enzymes, or enzymes involved in the pentose catabolic pathway in *A. niger* have been investigated concerning their response to low (1 mM) and high (50 mM) D-xylose concentrations. Notably, genes encoding enzymes that have similar function (*e.g.* xylan backbone-degradation) respond in a similar way to different amounts of D-xylose. Although low D-xylose concentrations provoke - in particular for hemicellulase-encoding genes - highest transcription response, transcript formation in presence of high amounts of D-xylose was also observed. It even turned out that a high D-xylose concentration is favourable for certain groups of genes. Furthermore, the repressing influence of CreA on the transcription of a selection of these genes was observed on D-xylose, regardless whether low or high amount of D-xylose is used. Interestingly, the decrease in transcription of certain genes on high D-xylose concentrations is not reflected by transcription of their activator XlnR. Regardless of the D-xylose concentration applied and whether CreA was functional or not, *xlnR* was constitutively expressed at a low level.

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PR8.13

The *de novo* designed antifungal hexapeptide PAF26 is internalized by endocytosis prior to killing fungal cells

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Natural and synthetic antimicrobial peptides (AMPs) provide promising alternatives for the control of microbial pathogens. PAF26 is a *de novo*-designed hexapeptide, cationic tryptophan-rich that has been shown to cross the plasma membrane and be fungicidal against pathogenic fungi. In the present work, the mechanism of internalization of PAF26 has been characterized in detail using the model fungus *Neurospora crassa*. PAF26 possesses two well-defined motifs: a N-terminal cationic and a C-terminal hydrophobic regions. We have shown how these motifs are independently responsible during the three steps of the PAF26 action involving its: (a) electrostatic interaction with cells, (b) cellular internalization; and (c) intracellular toxicity. Live-cell imaging of fluorescently labelled PAF26 and organelle probes, and mutant analyses indicate that it is endocytically internalized at low fungicidal concentrations. PAF26 initially accumulated in vacuoles that expanded, and then was actively transported into the cytoplasm, which coincided with cell death. Deletion mutants of the endocytic proteins RVS-161, RVS-167 and RAB-5 exhibited reduced rates of PAF26 internalization and fungicidal activity. Pharmacological experiments with live-cell probes showed that PAF26 internalization and antifungal action were energy-dependent, primarily actin-mediated, disrupted intracellular calcium homeostasis, and also induced rapid plasma membrane depolarization. PAF26 antifungal activity at low concentrations was shown to rely on its endocytic internalization. However at high fungicidal concentrations, PAF26 internalization was energy-independent and involved passive translocation. Our results provide new mechanistic insights into the mode-of-action of cell penetrating AMPs and for the rational design of more effective PAF26-based AMPs.

PR8.14

Molecular and chemical characterization of secondary metabolite gene clusters in *Fusarium fujikuroi*: the fusarin gene cluster

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The filamentous fungus *F. fujikuroi* is known to produce a variety of structurally diverse secondary metabolites such as the plant hormones gibberellins which cause enormous economical losses in trade of crops. In order to reduce the health risk of mycotoxins in food, feed and biotechnologically produced gibberellin preparations, identification of mycotoxin biosynthesis genes is of great importance. The recently sequenced genome of *F. fujikuroi* contains 16 polyketide synthases (PKS). So far only four of them can be linked to specific products: bikaverin, fusarin C, fumonisin and fusarubin. The focus of this work is studying the biosynthesis and regulation of the mutagenic mycotoxin fusarin C.

Since now only the hybrid polyketide synthase/nonribosomal peptide synthetase (PKS/NRPS) gene from the fusarin C cluster in *F. venenatum* is known. Here we present the characterization of the entire fusarin C gene cluster in *F. fujikuroi* by generating deletion mutants of each single cluster gene including the PKS/NRPS-encoding gene. By using these mutants, we are identifying the intermediates to finally unravel the entire biosynthetic pathway. In addition, we have created a deletion mutant missing all cluster genes except for the PKS/NRPS key enzyme gene to identify the first intermediate in the fusarin C pathway.

Besides, we study the regulation of gene expression for fusarin pathway genes by external signals, such as nitrogen availability and pH and the involvement of potential transcription factors and global regulators such as AreA, AreB, PacC and Velvet.

PR8.15

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

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

PR8.16

Analysis of a New Secondary Metabolite Gene Cluster in *Fusarium fujikuroi*

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The filamentous fungus *Fusarium fujikuroi* is best known for its production of gibberellins (GAs), natural phytohormones that lead to hyperelongation and chlorosis in rice plants. Besides the production of GAs, *F. fujikuroi* produces a wide range of other secondary metabolites, such as the polyketide-derived mycotoxin fusarin C or bikaverin.

The recently sequenced genome of *F. fujikuroi* revealed 16 polyketide synthases (PKSs) of which so far only a few can be assigned to their respective product. Here we present the discovery and subsequent investigation of an almost forgotten group of polyketides belonging to the fusarubin (FSR) family in *F. fujikuroi*. The corresponding gene cluster was identified and the regulatory network that governs FSR production has been studied. Our results so far indicate a rather complex regulation, including the importance of the initial pH, the nitrogen availability and the controverse involvement of Velvet and G-Protein mediated signaling. This complex regulatory network leads to an even more complex accumulation of the various FSR derivatives, of which the predominant products were determined using different chemical approaches. In addition, single deletion mutants of all *fsr* cluster genes revealed the biosynthetical pathway leading to the formation of the FSR derivatives. Phylogenetic analyses showed close homologies of the PKS Fsr1 to Pgl1 from *F. graminearum* and *F. verticillioides*, indicating the involvement of these pigments in the coloration of the fruiting bodies. Sexual crosses confirmed that the FSR pigments are the so far unknown perithecial pigments in *F. fujikuroi*.

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PR8.17

ChemoGenomics: Discovery of novel fungicides and their targets in the phytopathogen *Fusarium graminearum*

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Chemical genetics screen is based on the ability of small chemical molecules to bind to biological molecules and alter their function. Screening of pharmaceutical libraries has revealed novel molecules effective against cancer and other diseases. We have adopted similar approach and identify bio-active compounds that will block the growth and development of *F. graminearum*. We have developed a 96-well format to monitor the growth *of F. graminearum* in liquid media. The fungus is tagged with a green fluorescent protein (GFP) and the growth is monitored by the measurement of fluorescence of the GFP. This format facilitates high throughput screening for small molecules that could potentially disrupt the growth of the fungus. As proof of concept, we screened ~560 compounds from theTimTec NDL-3000 natural product collection (TimTec LLC,Newark,DE,USA) and identified several compounds with anti-Fusarium properties.

One compound identified form our screen, “Antofine” was purified from *Vincetoxicum rossicum* and was used in subsequent studies, to identify targets in the fungus. We used the gene deletion library of the budding yeast *Sacchromyces cerevisiae* to identify targets for Antofine. Twenty two potential targets of Antofine were identified and GeneMANIA (http://www.genemania.org), an online multiple association network integration algorithm was used to uncover information pertaining to genetic and physical interactions of these targets. Our efforts to identify targets in Fusarium against Antofine will be discussed

PR8.18

Efficient plant biomass degradation by thermophilic fungus *Myceliophthora heterothallica*

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Rapid and efficient enzymatic degradation of plant biomass into monomeric sugars is currently a major challenge for sustainable production of biochemicals and biofuels. The best studied and most widely used plant-degrading enzymes are produced by *Trichoderma* and *Aspergillus* species, and they are most effective over a temperature range of 40 – 50°C. As a consequence, these moderate temperatures have long reaction times for complete saccharification of plant biomass. It would therefore be desirable to have elevated hydrolysis temperatures using thermostable enzymes. The collection at the CBS Fungal Biodiversity Centre contains several thermophilic fungi, which produce thermostable enzymes up to 70-80°C.

A screening of 32 thermophilic species resulted in several candidates with interesting plant-degrading enzymes. Particularly the genus *Myceliophthora* contains isolates with rapid growth on complex polysaccharides. We elucidated the phylogeny of *Myceliophthora* isolates and distinguished 10 different species, of which four are thermophilic. The isolates with the fastest growth on crude plant material were divided in two species: *M. thermophila* and *M. heterothallica*. The new phylogenetic classification of *M. heterothallica* isolates was further supported by physiological differences between the two species. Also, in contrast to *M. thermophila* isolates, *M. heterothallica* has a functional sexual cycle. *M. heterothallica* isolates were studied in detail for their ability to release sugars from crude plant biomass. Furthermore, crossing experiments between *M. heterothallica* isolates resulted in offspring with an even higher potential in rapid and efficient enzymatic degradation of plant biomass.

PR8.19

Effective production of Itaconic Acid in A. *niger*

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Itaconic acid is an important building block for the chemical industry that can be produced from sugars in a fermentative process. Currently, *Aspergillus terreus* is most frequently used for the commercial production of itaconic acid. The itaconic acid production pathway in *A terreus* is similar to the citric acid pathway in *Aspergillus niger*. Citric acid is the precursor for *cis*-aconitic acid which can be converted to itaconic acid by decarboxylation. Itaconic acid in *A. terreus* is produced upto a concentration of 80 g/L while citric acid production in *A. niger* reaches concentrations over 200 g/L which show the enormous potential of *A. niger* as a production host for itaconic acid. However, the key-enzyme *cis*-aconitic acid decarboxylase (CadA) in the itaconic acid production pathway is lacking in *A. niger*. Within the genome of *A. terreus* the *cadA* gene is flanked by two putative transporters, a mitochondrial transporter and a plasmamembrane transporter. The expression of the *cadA* gene in an *A. niger* strain optimized for citrate production resulted in the production of itaconic acid. The amount of itaconic acid produced by *A. niger* is further improved by using a codon-optimized version of the *cadA* gene. Still, significant amounts of citrate were produced suggesting that the conversion to itaconic acid is not very efficient. To improve the efficiency and to increase the itaconic acid production both putative transporters are introduced. Introduction of the mitochondrial transporter strongly increased the itaconic acid production.

PR8.20

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

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

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PR8.21

Genetic And Molecular Characterization Of The *Penicillium chrysogenum* PcrsmA Gene, Encoding A Homologue Of The *Aspergillus nidulans* bZIP Transcription Factor RsmA

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Penicillium chrysogenum is the major industrial producer of the ß-lactam antibiotic pencillin. The regulation of cellular processes like secondary metabolism as well as morphogenesis and development in this filamentous fungus is controlled by components of the so called velvet-like complex which was first described in *Aspergillus nidulans*. Previous studies identified *rsmA* (remediator of secondary metabolism) in *A. nidulans* which after overexpression was shown to remediate secondary metabolism defects in knockout strains of the velvet components LaeA and VeA. Hence, it is believed that the putative bZIP transcription factor RsmA may act as a positive regulator of many secondary metabolite pathways. Bioinformatics confirmed the existence of many known and putative bZIP proteins from several fungi such as *P. chrysogenum* and higher eukaryotes which show high homology to RsmA from *A. nidulans*.

Until now, little is known about the biosynthetic regulation of the ß-lactam antibiotic penicillin. Thus, further investigation of putative regulators of secondary metabolism such as PcRsmA, the *P. chrysogenum* homologue to RsmA is necessary to extend the current knowledge of the regulatory network controlling both penicillin biosynthesis and morphogenesis in *P. chrysogenum*. A Δ*Pcku70* strain as recipient for homologous recombination together with the FLP/*FRT* recombination system were used to generate a marker-free Δ*PcrsmA* strain for functional and morphological characterization of PcRsmA. Our results will support deciphering of regulatory networks related to the velvet-like complex in *P. chrysogenum*.

PR8.22

Contamination of peripheral venous catheter associated fungal biofilms

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Introduction: In the hospital, the use of venous catheters for the administration of drugs exposes patients to increased risk for fungal contamination. Yeasts can form biofilms on the surface of catheters. These properties give them resistance to antifungal agents. In an attempt to integrate this clinical reality, we undertook a study in the neonatal unit of EHS-Tlemcen, for aim to isolate yeasts from venous catheters directly after excision of newborns hospitalized. The approach is to test the ability of yeasts isolated to form biofilms and to test their resistance against amphotericin B.

Materiels and Methods: These samples were taken from implanted venous catheters for 72 hours or more. They are removed directly from patients and placed in Sabouraud liquid medium.The tubes were then agitated in a vortex for 1 minute. Purified strains were identified by API Candida (Biomerieux,France)

Results: From 281 samples, 15 yeasts were isolated, colonizing venous catheters implanted in newborns. *Candida albicans, Candida parapsilosis, Cryptococcus néoformans , Candida famata, Trichosporon spp.* and *Saccharomyces cereviseae* were isolated. C*andida* and *Cryptococcus* species have the ability to form biofilms

Conclusion: Sessile *Candida* species and *Cryptococcus neoformans* isolates were less susceptible than the planktonic populations to AmB.

These results suggest contamination of venous catheters with strains isolates able to form biofilms may be associated with infection.

PR8.23

A *velvet*-like complex in *Penicillium chrysogenum*: the two faces of PcVelC

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The filamentous fungus *Penicillium chrysogenum* is the main industrial producer of the pharmaceutically relevant beta-lactam antibiotic penicillin. All three biosynthesis genes are found in a single cluster and the expression of these genes is known to be controlled by a complex network of global regulators. Recently, the velvet complex containing several global regulators of secondary metabolism was described for the model fungus *Aspergillus nidulans*. Next to the founding member VeA, several other velvet-like proteins were meanwhile identified in *A. nidulans* and many other filamentous fungi.

Here we provide a functional analysis of a *velvet*-like complex in *P. chrysogenum* with structurally conserved components that have distinct developmental roles, illustrating the functional plasticity of these regulators in genera other than *Aspergillus*. Data from penicillin bioassays, quantification of conidiospores as well as detailed microscopic investigations of these knockout mutants clearly show that all velvet-like proteins are involved in secondary metabolism and other distinct developmental processes. Interestingly, the velvet-like protein PcVelC seems to be a major regulator of penicillin biosynthesis and conidiation. By protein-protein interaction studies using bimolecular fluorescence complementation, tandem-affinity purification and yeast two-hybrid, we want to extend the analysis of the velvet-like complex in *P. chrysogenum*. These analyses will focus on the velvet-like protein PcVelC to elucidate its opposing roles in the regulation of penicillin biosynthesis and conidiation. Our results widen 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.

PR8.24

New hemicellulolytic enzymes for bioethanol production

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Hemicellulolytic enzymes such as endoxylanases from glycosyl hydrolysis family 11 (GH11) and 10 (GH10) have a major importance in several industrial application such as bioethanol production. They improve the yield of monosaccharides from plant biomass by depolymerization of hemicellulose. This will expand the set monosaccharides available for conversion to bioethanol by yeast.

Most commercially applied fungal endoxylanases are obtained from *Aspergillus* or *Trichoderma* species. However, comparison of fungal genomes identified several species that are much richer in genes encoding these enzymes. One of them is *Podospora anserina*, which has a saprobic life style, and is only found in dung of herbivores [1]

A growth profile of *P. anserina* on various substrates shows a high ability to growth in substrates that are rich in hemicellulose such as wheat straw and Arundo donax. Both substrates are commonly used in ethanol production. Using a combination of comparative genomics and phylogeny new GH10 and 11 enzymes were selected to increase the efficiency of ethanol production.

Reference:

[1] Espagne E, Lespinet O, Malagnac F, Da Silva C, Jaillon O, Porcel BM, Couloux A et al., The genome sequence of the model ascomycete fungus Podospora anserina. Genome Biol. 2008;9 (5) : R77.

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PR8.25

Genetic and biochemical investigations of natural product formation in *Boletales*

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The release of an increasing number of basidiomycete genomic sequences provides valuable insight into their capacity to biosynthesize small molecule natural products. The *Boletales* are represented by *Serpula lacrymans* (dry rot fungus) and *Paxillus involutus* (roll rim mushroom). Assessed by the number of genes encoding polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPSs) in these representative genomes the *Boletales* include particularly prolific producers of secondary metabolites.

This poster presents our current work on genera within the *Boletales* focusing on NRPSs and quinone synthetases, i.e., NRPS-like enzymes. Methodically, we relied on a combined approach of genetic and biochemical methods, complemented by liquid chromatography.

PR8.26

***Aspergillus clavatus* as a potential enzyme source to use in biomass degradation to produce second-generation ethanol: Cloning and expression of hemicelulases after secretomic analysis using different pre-treatment sugar cane bagasse**

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Aspergillus clavatus is a cosmopolitan fungus that had its genome sequenced by The Institute for Genomic Research. A detailed comparative genomic analysis using a large number of fungi database showed that this microorganism is a potential enzymes producer. In addition, there are few studies about this fungus. The plant cell wall polysaccharides are being considered as source of renewable energy such as biofuels owing to the fact that they are the most abundant reserves of carbon. It can be conveniently divided into three groups namely cellulose, pectin and hemicellulose, with hemicellulose being the second most abundant biopolymer component of plant cell wall and composed by xylan, arabinan, mannan, glucomannan, galactomannan, and glucogalactomannan. Due to its complexity a large set of enzymes are necessary to degrade the plant cell wall. In order to study potential enzymes directly involved in degradation of the most abundant brazilian biomass we report in this work, the comparative analysis of the *A. clavatus* secreted in 5 different pre-treatment sugar cane bagasse using glucose as the control, cloning and expression of hemicellulases. The proteomic analyses have identified 135 different proteins where 2% of those are enzymes related with biomass degradation. The relative difference reflect the necessity to use specific enzymatic pool to degrade different pre-treated sugar cane bagasse, because of diference in the sugar compositions. In addition, to perform the heterologous protein expression of *A. clavatus* hemicellulase we used our *Aspergillus nidulans* expression system and the recombinant enzymes were highly expressed and secreted to the culture medium.

PR8.27

Molecular diversity of *Cercospora zeina* on maize in South Africa

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Grey Leaf Spot is a prevalent worldwide maize disease of global economic importance, and is specifically caused by *Cercospora zeina* in South Africa. In order to implement control measures, it is important to understand the population diversity of the fungus. As no sexual stage of *C. zeina* has been observed, we hypothesised that there would be greater variability among isolates from different populations rather than within a population in South Africa. In order to address this question, we analysed *C. zeina* isolates from GLS infected maize from three regions within South Africa viz. Machadodorp, Greytown and Cedara. We collected 40 GLS infected maize leaves from each region, and isolated and maintained cultures from 40 single *C. zeina* conidia from each area. DNA was isolated from fungal cultures, and a subset of 30 isolates from different regions was screened with 36 Simple Sequence Repeats (SSRs) designed from the genome sequence of a US *C. zeina* isolate. Twelve SSRs were found to display polymorphisms across isolates, and six were selected to score samples against. The amplified SSRs were analysed on agarose gels and scored for variability to assess the diversity amongst isolates within South Africa. From these results we could determine that there was variability within and between isolates from different regions. Thus, we observed greater diversity within *C. zeina* populations than originally anticipated.

PR8.28

Identification And Biochemical Characterization Of Putative miRNAs In The Penicillin Producer *Penicillium chrysogenum*

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Since the early 1990s small non-coding regulatory RNAs were identified in a variety of different eukaryotic organisms. In fungi, the transient inactivation of gene expression by homologous sequences was first observed in *Neurospora crassa*. The effect of short interfering RNAs (siRNAs) is mediated by the RNA-induced silencing complex (RISC). Besides the class of exogenous siRNAs a previous study shows that there is another class of regulatory small RNAs, which interact with RISC in filamentous fungi. These endogenous small RNAs are derived from RNA hairpin structures of RNA polymerase II transcripts and show typical characteristics of microRNAs (miRNAs), which play an important role in the regulation of gene expression in plants and metazoan.

The aim of this study is to investigate whether miRNA-like regulatory RNAs can be detected in the penicillin producer *Penicillium chrysogenum*. We performed an *in silico* analysis, based on RNA next-generation sequencing data, to predict putative miRNAs hairpin structures. By this approach, sequences with familiar characteristics of previously identified miRNA-precursors could be identified. To confirm the *in silico* predictions, transcript analysis were done *in vitro*. The statements of the predictions and the results from the transcript analysis confirmed the existence of the small RNAs and their precursors. To provide evidence for a regulatory activity of the putative miRNA-like sequences, mRNA-targets were chosen and inducible overexpression constructs of the miRNA-like sequences were generated. The results of this study suggest the existence of a miRNA based silencing mechanism in *P. chrysogenum*.

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PR8.29

Secretion of Client Proteins in Aspergillus

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Production of pure and high-yield client proteins is an important technology that attends the needs for industrial applications of enzymes as well as basic experiments such as protein crystallization. Client protein expression platforms are available in *Escherichia coli* and the methylotrophic *Pichia pastoris* that result in proteins released to the intracellular cell extract and extracellular medium, respectively. Fungi are utilized in industrial protein production because of their ability to secrete large quantities. In this study we engineer a high-expression-secretion vector, pEXPYR that directs proteins towards the extracellular medium in two *Aspergillii* host strains, examine the effect of maltose overexpression, production time and pH-dependent protein stability in the medium. We describe five client proteins that accumulated 50-100 mg of protein per liter and only one protein was secreted at low quantities. We also test a recyclable genetic marker that allowed secretion of multiple client proteins, enabling the design of an enzyme activity set.

PR8.30

Functional characterisation of Cytochrome P450 genes from the wheat leaf pathogen *Mycosphaerella graminicola*

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The Ascomycete fungus *Mycosaphaerella graminicola* (Anamorph *Septoria tritici*) causes the disease Septoria tritici blotch (STB) in wheat. This pathogen is typical of temperate, high-rainfall environments and is of major international importance owing to its ability to substantially reduce agricultural yields. *M. graminicola* infects both hexaploid bread wheat (*Triticum aestivum*) and tetraploid durum wheat (*T. turgidum*) and is largely controlled by fungicide applications. However *M. graminicola* has evolved resistance to several classes of fungicides highlighting the continued need to identify novel targets for disease intervention.

Little is known about fungal metabolism during plant infection. The cytochrome P450 (CYP450) gene superfamily represents an ideal target for further investigation. Members of this group are (1) known to be present and regulated together with clusters of other genes important for the synthesis of secondary metabolites including mycotoxins, pigments and defence compounds; (2) known to act directly upon potentially harmful xenobiotics such as plant defence compounds and potentially fungicides in order to detoxify them; and (3) known to operate in various cellular locations. As a preliminary to this project, Solexa next generation transcriptome sequence analyses were performed at different time points post inoculation of wheat leaves with the fungus. Analysis of expression of members of the CYP450 gene family has identified many which are specifically expressed early during plant infection and some which may reside within secondary metabolite clusters. These will be further investigated initially through the generation of fungal CYP450 gene deletion strains. It is anticipated that this project will provide new insights into the genetic basis underlying the metabolic changes occurring during *M. graminicola* infection of wheat.

PR8.31

Mutation of Genes *areA*, *wcoA*, *cryA* and *acyA* Affect The Regulation of Fusarin Production in *Fusarium fujikuroi*

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Fusarium fujikuroi is a rice pathogen that produces a diversity of secondary metabolites, that includes terpenoids with biotechnological applications, as carotenoids and gibberellins, and potentially harmful polyketides, as bikaverin and fusarins. In contrast to other compounds, very limited information is available on the biosynthesis and regulation of fusarins in this species. We formerly reported than fusarin production depends on nitrogen availability and is negatively affected by light. To learn more about the genetic basis of this regulation, we have studied the fusarin-producing pattern of targeted mutants on several regulatory genes affecting the synthesis of other metabolites. The loss of the global regulator of the nitrogen metabolism AreA affects fusarin production, but the effect varies depending on the nitrogen source. Our study was extended to two photoreceptor genes, encoding the White Collar protein WcoA and the DASH-cryptochrome CryA, and the gene for the adenylyl cyclase AcyA, that mediates the synthesis of the regulatory signal cAMP. The four classes of mutants investigated exhibit different alterations in the accumulation of fusarins, indicating that this pathway is under control of the regulatory network involved in nitrogen-regulated secondary metabolism. Despite the participation of two putative photoreceptors, the effect of light is mainly explained by light-induced instability of the secreted fusarins.

PR8.32

CefR* Acts As A Regulator Of β-Lactam Transporters In *Acremonium chrysogenum

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Acremonium chrysogenum is an ascomycete filamentous fungus which has been used for the industrial production of cephalosporin-like antibiotics. Our previous investigations about the cephalosporin C biosynthetic pathway led us to discover three genes, *cefT*, *cefP* and *cefM* involved in the secretion of intermediates and therefore in the compartmentalization of this pathway. The genes *cefP* and *cefM* codify membrane proteins that carry the intermediates isopenicillin N and penicillin N, respectively, through the peroxisomal membrane. *CefT* protein takes part in the secretion of hydrophilic β-lactams through the plasma membrane.

However, the regulation of the secretion of the intermediates and the final product of cephalosporin C biosynthetic pathway is still unknown. For this is a reason a search for regulator genes within the early biosynthetic cluster of cephalosporin C was made. A new ORF was found encoding a protein (CefR) which shows homology with other regulatory proteins and bears a “Fungal_trans” domain, characteristic of many fungal regulators. Targeted inactivation of *cefR* diminishes and delays the cephalosporin production but increases the penicillin N secretion. On other side, the overexpression of *cefR* decreases the secretion of penicillin N, preventing the loss of intermediates and then inducing the cephalosporin C production. Northern blot analysis revealed that CefR protein works as a repressor of *cefT* and *cefM* genes, making possible the use of these intermediates in the synthesis of cephalosporin C. In summary, CefR protein represents the first example of a regulator of β-lactam transporters described in *A. chrysogenum.*

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PR8.33

PenV Is A Vacuolar Membrane Protein Related To The Penicillin G Biosynthetic Pathway In Penicillim chrysogneum

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The penicillin G (PenG) biosynthetic pathway is compartmentalized in Penicillium chrysogenum, taking place in the cytosol, where δ-(L-α-aminoadipil-L-cysteinil-D-valine) (ACV) and isopenicillin N (IPN) are formed, and inside peroxisomes, where PenG is synthesized. Transport processes linked to this compartmentalization are largely unknown, but most of them would be mediated by transporter membrane proteins. During the search for this kind of transporters, was found the protein PenV showing a 42% of identical amino acids with the IPN transporter CefP, described in Acremonium chrysogneum. To elucidate the function of the protein PenV, the encoding gene, penV, was silenced by the mechanism of small interfering RNAs. As a consequence of the silencing process an alteration of the transcription levels of the β-lactam biosynthetic genes occurs, together with a drastic decrease of the yield of PenG and the intermediates ACV and IPN. The silencing process also causes alterations in several developmental aspects. The subcellular location of PenV was determined through the expression of the red fluorescent protein PenV-DsRed in the strain Wisconsin 54-1255. Microscopy analysis revealed the presence of PenV, not in peroxisomes but in the vacuolar membrane. Curiously, non ribosomal peptide synthetases appear to be linked to the cytosolic side of the vacuolar membrane. Given these results is purposed the involvement of PenV in the transport of amino acids from the vacuolar lumen to the cytosol, where they would be used as substrates for the synthesis of structural proteins and as precursors of the β-lactam biosynthesis.

PR8.34

The program of iterative fungal PKS-NRPSs

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Fungal hybrid polyketide synthase-non ribosomal peptide synthetases (PKS-NRPSs) are huge enzymes that carry out a large number of chemical reactions in the biosynthesis of the natural product. Examples of natural products are tenellin **1**, desmethylbassianin **2** and bassianin **3** produced by different Beauveria strains. Current knowledge about the programming of these enzymes is very limited. Important questions as how the chain length of a polyketide or the methylation pattern is encoded are as yet unanswered.

Tenellin synthase (TenS) and desmethylbassianin synthase (DMBS) produce together with their tailoring enzymes the similar yet not identical natural products **1** and **2**. These natural products differ in chain length and in methylation pattern. The domain architecture of both enzymes is identical, i.e. KS-AT-DH-CMet- ER^0-KR-ACP-C-A-T and homology is over 85%. Domain swaps between different PKS-NRPSs often fails presumably to incompatibility of protein structures. Here we present the successful swap of domains from two highly similar enzymes.

The poster covers domain swap strategy and outcome. It will also outline the use of the domain swap technology to resurrect the extinct compound **3**

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PR8.35

Three acidic residues Glu31, Asp142 and Asp171 of *Aspergillus oryzae* cutinase CutL1 are required for both interaction with hydrophobin RolA and consequent stimulation of polyester-degradation.

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Hydrophobins are amphipathic proteins, and are ubiquitous among filamentous fungi. When the industrial fungus *Aspergillus oryzae* is grown in a submerged medium containing a biodegradable polyester polybutylene succinate-coadipate (PBSA) as a sole carbon source, cutinase CutL1 and hydrophobin RolA are simultaneously secreted into the medium. RolA attached to the surface of PBSA particles specifically recruits CutL1, resulting in stimulation of PBSA hydrolysis (1). In our previous study, we identified amino acid residues involved in the RolA-CutL1 interaction by means of chemical modification and site-directed mutagenesis of RolA and CutL1. As a result, we found that His32 and Lys34 of RolA and Glu31, Asp142, Asp171 of CutL1 are involved in the RolA-CutL1 interaction. In the present study, to quantitatively elucidate the role of the three acidic amino acid residues of CutL1 in the RolA-CutL1 interaction, we characterized kinetics of the interaction between CutL1 variants of the three residues and wild type RolA by using Quartz crystal microbalance (QCM). The QCM analysis revealed that replacement of the three acidic amino acid residues of CutL1 to serine caused increases in K_D values for interaction with RolA. In conclusion, Glu31, Asp142 and Asp171 of CutL1 are critically required for the RolA-CutL1 interaction by multivalent effect.

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PR8.36

A New Method of Increasing the Hydrolytic Activity of *T.atroviride*

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Trichoderma species are widely used as biological control agents due to their strong antagonistic activity against phytopathogenic fungi and effectiveness in simultaneously promoting plant growth and defense mechanisms. Biocontrol efficiency may result from a direct interaction between the pathogen and *Trichoderma* when lytic enzymes secreted by the latter degrade the cell wall of the pathogen, causing damage of its cells.

It was observed that many, if not all, lytic enzymes secreted by *Trichoderma* were glycosylated. It was postulated that O-glycosylation of these enzymes was closely correlated with their secretion. Taking into account this correlation we decided to improve the biocontrol abilities of *T. atroviride* P1 against plant pathogens by activation of the mevalonate pathway; in this pathway dolichyl phosphate, a carrier of carbohydrate residues in the glycosylation processes, is produced together with many other biologically active molecules, such as sterols, terpenoids and quinones. The new strains of *T. atroviride* were characterized in terms of the activity of overexpressed enzymes, protein secretion, activity of secreted hydrolases and antifungal properties. Our results showed that activation of the mevalonate pathway could result in higher antifungal activity of the studied new strains.

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PR8.37

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

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Trichoderma reesei is a soft rot Ascomycete fungus able to secrete enzymes extremely efficiently. Availability of the complete genome sequence of *T. reesei* has made it possible to utilise genome wide methods to study protein production by the fungus and to utilise the information obtained to develop new strains with better enzyme production qualities. In order to study the co-expression of enzyme genes, a complete list of CAZymes of *T. reesei* needed to be obtained. Novel CAZymes were searched from the genome by mapping *T. reesei* proteome with Blast search to the protein sequences of the CAZY database. New annotation was given to several genes in order to gain more information on the possible function of novel candidate genes and to specify the annotation of previously identified genes. A phylogenetic approach was used to reveal the functional diversification of *T. reesei* enzyme genes within CAZY families and between the gene duplicates. Expression of the hydrolytic system of *T. reesei* Rut-C30 was studied by cultivating the fungus in the presence of different lignocellulose substrates. Cultures were subjected to transcriptional profiling using oligonucleotide microarrays. Differentially expressed genes were identified and expression profiles of genes encoding lignocellulose degrading enzymes were compared to identify co-regulated groups of genes and genes needed for the degradation of specific substrates. Transcriptional profiling revealed a group of genes co-regulated on all of the substrates and genes which expression profiles were more diverse. Also some examples were found from co-regulation of enzyme genes according to genomic localization.

PR8.38

Novel manganese peroxidases of the litter-decomposing fungus *Agrocybe praecox*

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Litter-decomposing fungi (LDF) are the primary decomposers of residual plant materials in forest soil. In spite of the ecological significance of LDF, relatively little is known about the molecular characteristics of their lignocellulose-degrading machinery. *Agrocybe praecox* is a litter-decomposing basidiomycete that is capable of mineralising synthetic lignin as well as decomposing various aromatic compounds. We have characterized the primary structure of two different MnP encoding genes of *A. praecox* which encode extracellular, short-type class II heme-containing peroxidases. *Mnp1* corresponds to previously characterized MnP1 enzyme and interestingly, both MnPs are deficient of one of the three conserved acidic amino acids involved in Mn^{2+} binding. Phylogenetically, the closest homologue to MnP1 is a class II peroxidase of the mycorrhizal, agaric basidiomycete *Laccaria bicolor* that lacks all conserved amino acids that bind Mn^{2+}. MnP2 resembles the hybrid-type of MnPs, as described in the wood-decaying, corticioid white-rot basidiomycete *Phlebia radiata*. When the fungus was grown on forest litter, laccase and MnP activities were detected. In birch leaf litter cultures, the transcript levels of expression of *mnp1* and *mnp2* were similar, whereas in cultures on conifer needle litter, transcription of the *mnp1* gene was up-regulated. Molecular characterization of the new MnP enzymes aims to understand better the physiology of litter and lignocellulose decay by *A. praecox*.

PR8.39

Characterization Of Two Redundant *Aspergillus flavus* Peptide Synthetase-like Enzymes

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LaeA, a regulator of secondary metabolism in fungi, controls expression of two genes encoding NRPS-like enzymes in Aspergillus flavus, termed LnaA and LnbA. Although these enzymes resemble α-aminoadipate reductases (i.e., primary metabolism enzymes), gene deletion and knockdown experiments pointed to a role for LnaA and LnbA in secondary metabolism. Specifically, the biosyntheses of heterocyclic L-tyrosine-derived natural products are dependent on these enzymes. Genetic data is also suggestive of functional redundancy of these two enzymes.

Hexahistidine-tagged LnaA and LnbA were heterologously produced, and assayed using the amino acid-dependent ATP-pyrophosphate exchange method. L-tyrosine was identified as clearly preferred substrate of both enzymes. Further biochemical characterization established divergent temperature and pH-optima, and differences regarding stereospecificity.

Our biochemical experiments prove that the LnaA and LnbA substrate spectrum is compatible with their tentative heterocyclic products. Further, participation of α-aminoadipate reductase-like NRPSs in secondary metabolism and functional redundancy of LnaA and LnbA, anticipated by genetic methods, is supported by the substrate spectrum of these enzymes.

PR8.40

Genome-wide transcriptome and proteome analysis of *Aspergillus oryzae* in the hypoxic stress condition

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

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PR8.41

Autonomously replicating plasmids as a transient expression tool in *Aspergillus niger*

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The possibility of having an autonomously replicating plasmid for filamentous fungi broadens the horizon of genetic engineering. Tools like recombinases or reporter genes can easily be inserted to cells and just as well be expelled when taking away the selective pressure. Such a system can be used as a transient expression tool for genetic engineering purposes in *Aspergillus niger*.

AMA1 is a 6.1 kb DNA fragment that allows extrachromosomal replication in filamentous fungi and strongly enhances the transformation efficiency. However, plasmid construction is hindered by the two palindromic, inverted sequences that flank the 0.3 kb central region of the AMA1 fragment. Hints in literature led to the assumption that the sequence can be shortened without loosing the positive influence on transformation efficiency.

In this study we characterized different plasmids carrying shortened fragments of AMA1. The transformation efficiencies as well as the plasmid stabilities were analyzed. The conducted experiments demonstrate that only one of the palindromic sequences together with the central region of AMA1 are necessary for autonomous replication in *Aspergillus niger*. Further shortening led to a drastic decline of transformation efficiency. Plasmids are lost at the latest in the 3rd generation when the spores are cultivated without antibiotic pressure. On the other hand, integration of the plasmids seems to be possible if antibiotic pressure is sustained.

PR8.42

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

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The filamentous fungus *Aspergillus oryzae* is widely used as a microbial cell factory for large scale heterologous protein production. *A. oryzae* is also known as a natural organic acid producer. However, the metabolism and regulation of organic acid production in *A. oryzae* are poorly characterized. Furthermore, the media composition has a major impact on the performance of this organism and the economic feasibility of an industrial fermentation process. We therefore evaluated the global expression response towards and the utilization of different nitrogen sources by *A. oryzae* in batch fermentations.

In this study, we aim for a deep investigation of the cellular mechanisms of the utilization of different nitrogen sources. Firstly, we performed batch cultivations with two strains (NRRL3488 and DSM1862) on defined and complex nitrogen sources using di-ammonium sulphate and peptone, respectively. In addition, transcriptome analysis was performed on samples from these fermentations to analyze the gene expression under exponential growth conditions (mid-exponential phase; 6h) and in nitrogen starvation (stationary phase; 30h) to further identify key-players in the metabolism and regulation of gene expression.

Cluster analysis revealed a conserved response of both strains and helped to identify regulatory sequences among the co-expressed genes. Furthermore the expression data pointed towards malic acid production as a respond to nitrogen starvation stress.

PR8.43

Proteins secrted by *Heterobasidion irregulare* during growth on spruce wood

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Heterobasidion irregulare is a severe conifer pathogen that causes root and butt rot. The white-rot fungus degrades simultaneously or selectively lignin. The genome of the fungus was established by the JGI (http://genome.jgi-psf.org/Hetan2/Hetan2.info.html) and the annotated genome can be used in proteomic studies. Here, *H. irregulare* was grown in liquid medium with and without *Picea abies* wood. Freely secreted and hyphal sheath associated proteins analyzed by 2D-gel electrophoresis revealed a high diversity between wood supplemented and control cultures. Protein identification by ESI-LC-MS/MS was either performed on single protein spots form 2D-gels or by application of a shot-gun method on complex protein mixtures. Using a MASCOT database with the proteome deduced from the *H. irregulare* genome, in total 118 different secreted proteins have been identified. 64 proteins were present under both culture conditions and only seven proteins were suppressed by wood supplementation. Addition of wood resulted in 47 new proteins secreted into the culture media. Redox-enzymes were represented by 23 proteins and most of them were induced by wood. Expression of laccases (except of one) and alcohol oxidases differed not between the two culture media. However, wood induced secretion of FAD-oxidoreductases and redox-enzymes with unknown function and furthermore secretion of specialized glycanases, lipases and proteases.

This work was supported by the Ministry of Science and Culture in Hannover, Germany (Common Lower-Saxony-Israel Project ZN2043). We acknowledge the genome work conducted by the U.S. Department of Energy Joint Genome Institute (JGI) supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

PR8.44

Optimisation of vectors for transformations in Coprinopsis cinerea

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The genetic transformation of the model fungus Coprinopsis cinerea allows the genomic analysis and manipulation of this organism. Initially, transformations were used to study the structure; functions and regulation of expression of genes; in recent years usage for overexpression of industrially important enzymes are also emerging. For the transfer of genetic material, chromosomal integrative vectors are used. These vectors contain a selectable marker gene and/ or a gene of interest under the control of regulatory sequences such as promoter or terminator. Due to lack of systematic experimental data, little is known about the influence of vectors on transformation frequencies. This work targets at improvement of the transformation vector pCc1001 (1). This pUC9-based vector contains a 6.5 kb PstI genomic fragment of C. cinerea with the tryptophan synthetase gene (trp1) that can be used to complement trp1- defects. The vector however shows a surprising phenomenon. In single transformation it gives only low numbers of transformants whereas efficiencies in co-transformation raise by factors of >100%, yielding several hundreds of transformants per experiment. To investigate this phenomenon further, the vector was modified in length and fragments with the trp1 gene were subcloned into pBluescript KS-. The effects on the transformation efficiency were investigated by using several co-transformation experiments. (1) Binninger DM et al. (1987) DNA-mediated transformation of the basidiomycete Coprinus cinereus. EMBO J 6:835-840

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PR8.45

Genomics of *Aspergillus oryzae* and effective utilization of large scale genomic information.

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Recently, we have determined the genes responsible to the biosynthesis of kojic acid from *A. oryzae*, which is used as a cosmetic whitening agent. Transcriptome analysis and successive gene disruption approach has been successfully applied to the gene identification. We found three genes encoding oxidereductase, transporter and transcription factor, which are localized on the non-syntenic block. It took, however, more than one year for the identification because the target gene prediction was inaccurate and disruption of roughly 20 genes was required before finding the genes. To accelerate identification of the genes responsible to biosynthesis of novel metabolites, we have prepared our pipelines from genome sequencing to gene detection, which includes DNA sequencer, DNA microarray and LC/MS.

We have improved the performance of the *de novo* assembling pipeline for ABI SOLiD, and have optimized it for sequencing of microorganism genomes. Our pipeline generates scaffolds longer than 1 Mb, covering 99% of approximately 40 Mb genome with roughly 100 or less scaffolds in general. Resulted sequences are subjected to annotation, comparative analysis and the highly accurate gene prediction that we have developed. We have found that our pipeline detected the genes for secondary metabolites that have been already known. We are now evaluating the performance in accuracy by disruption and overexpression of some novel genes that have been predicted by the pipeline.

PR8.46

Oxalate decarboxylases of the white-rot fungus *Dichomitus squalens*: expression on wood and in acid-induced cultures

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Oxalate decarboxylase (ODC) is an oxalic-acid decomposing enzyme produced by certain bacteria and fungi with potential for diverse biotechnological applications. Uses of ODC range from assays of oxalate concentration and removal of oxalate salt deposits in industrial processes to transgenic *odc*-expressing crop plants.

In fungi, ODC has an essential role in regulation of toxic concentrations of intra- and extracellular oxalate. Oxalic acid is an important metabolite of basidiomycetous wood-rotting fungi as it assists in wood and lignin degradation by e.g. enhancing the reactions catalyzed by lignin-modifying oxidative enzymes. Moreover, ODC has been suggested to participate in energy production during the fungal vegetative growth.

We found previously that the selectively lignin-degrading white-rot fungus *Dichomitus squalens* secretes oxalic acid during growth in liquid cultures and on spruce wood[1]. The fungus demonstrated intracellular ODC activity after exposure to excess oxalic acid. Mycelial Ds-ODC protein was partially purified, and for the first time for a white-rot polypore species, we succeeded in complete cloning of ODC-encoding gene of *D. squalens*[2].

The whole genome sequence of D. squalens (www.jgi.doe.gov) reveals that the fungus harbours altogether five odc gene models. In this work, we studied the expression of the five Ds-odc genes by real-time RT-qPCR during the fungal growth on solid-state spruce wood and in acid-induced liquid cultures. These results support involvement of differently regulated, individual ODC isoenzymes in primary and secondary metabolism in wood-decaying fungi.

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PR8.47

Transcriptomic And Genomic Approaches To Understand Cellulase Hyper-production In The Filamentous Fungus *Trichoderma reesei*

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The filamentous fungus *Trichoderma reesei* is well-known for its impressive capacity to secrete high amounts of cellulase and hemicellulase enzymes. These enzymes are key components of most cellulosic biomass to biofuel biological processes, and despite huge R&D efforts, their cost is still too high. Most industrial strains in use today have been obtained by random mutagenesis of the original QM6a isolate. Highest performances range from 40 to above 100 g/L proteins production, depending on the strain and process configuration used. While it is not known whether the already impressive performances of these strains can be further enhanced through targeted genetic engineering, there is still a high interest in understanding the genetic mechanisms leading to cellulase (hyper)secretion and to set up genomic tools that could be used to adapt strains to various industrial conditions. Toward this goal our group has been investigating the onset of cellulase production both on the single-gene level and on the whole transcriptome level using dedicated DNA oligonucleotide microarrays. We are currently completing our previous high-throughput sequencing of the NG14 and RUT C30 strain lineage with the sequencing of five other strains. These genome data are also being completed by transcriptome analysis with RNAseq technology. Our objective is to provide a whole picture of the mechanisms involved in cellulase production by *T. reesei* as well as potential new targets for genetic engineering of industrial strains.

PR8.48

Transposition of the miniature inverted-repeat transposable element mimp1 in the wheat pathogen Fusarium culmorum

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The genome of *Fusarium culmorum*, incitant of crown and foot rot on wheat and type B trichothecene producer, is now being sequenced. The number of predicted genes is estimated to exceed 10,000 and for many of them the function is still unknown. Consequently, there is a strong need for a high-throughput method for functional genomic analysis. Our aim was to test the efficacy of a double component system based on the ability of the *impala* transposase to transactivate the miniature inverted-repeat transposable element *mimp1* of *Fusarium oxysporum*. In this paper we report for the first time on the application of a tagging system based on an heterologous transposon and on the application of the splinkerette-PCR to identify *mimp1* flanking regions in the filamentous fungus *F. culmorum*. Similarly to what was previously observed in *Fusarium graminearum*, *mimp1* was shown to transpose in *F. culmorum* by a cut-and-paste mechanism into TA dinucleotides, which are duplicated upon insertion. Our results also show that *mimp1* reinserts in open reading frames in 16.4 % (i.e., 10 of 61) of the strains analysed, spanning throughout the entire genome of *F. culmorum*. Therefore the *mimp1/impala* double-component system is an efficient tool for gene tagging in *F. culmorum* as confirmed phenotypically for a putative aurofusarin gene. This system allowed also to identify two genes putatively involved in oxidative stress coping capabilities in *F.culmorum* as well as a sequence specific to this fungus, thus suggesting the valuable exploratory role of this tool.

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PR8.49

Bioinformatic prediction of *cis*-acting elements in *FUM* gene promoters putatively involved in transcriptional control of fumonisins biosynthesis

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Fumonisins are secondary metabolites produced by the maize pathogen *Fusarium verticillioides*, agent of pink ear rot; these mycotoxins cause agro-economical losses and detrimental health effects in animals and humans. The FUM genes needed for fumonisins biosynthesis are clustered and co-expressed in the fumonisins producers.

In eukaryotes, coordination of gene transcription is mostly attained through transcription factors shared by co-regulated genes, whose specificity relies on the recognition of *cis*-regulatory elements on the promoters of their targets. A bioinformatic analysis of FUM gene promoters in *F. verticillioides* identified a partially degenerated motif potentially involved in the regulation of FUM genes expression, and therefore in fumonisins biosynthesis. The same oligomer was found in the clustered FUM genes of the other fumonisins producers *Fusarium oxysporum* and *Aspergillus niger*; while it is not significantly over-represented in the scattered FUM homologs of the fumonisins non-producing euascomycetes *F. graminearum*, *A. nidulans*, *Magnaporthe grisea* and *Neurospora crassa*.

Comparison of the transcriptional strength of the intact *FUM1* promoter and of a synthetic version, where the motif discovered had been mutated, was carried out *in vivo* and *in planta* by quantifying GFP transcripts in *F. verticillioides* transformants, carrying either promoter upstream of the GFP reporter. Our results show that mutation of the main motif in *FUM1* promoter is sufficient to significantly impair its efficiency, thus validating our *in silico* approach as a discovery tool. The presence of the degenerated 6-mer in all clustered FUM genes suggests that this set of oligomers includes candidate regulatory sequences.

PR8.50

Biosynthesis of Natural Products Through a Fungal Molecular Genetics

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Analysis of fungal genome sequences to date has revealed that ascomycetes possess far greater numbers of gene clusters for biosynthesis of secondary metabolites, polyketides and peptides, than the numbers of natural products that have been isolated from the organisms. Therefore, such cryptic secondary metabolism gene clusters are anticipated to be a source of chemically diverse compounds. However, conventional methodologies in the field of natural products chemistry cannot be applied in hunting of natural products synthesized by enzymes encoded in the fungal genome as silent gene clusters. First, it is necessary to activate the gene cluster to induce biosynthesis of the corresponding compounds. Here, we expressed a gene encoding a transcriptional regulator associated with a target silent gene cluster to induce its natural product biosynthesis. This approach was used successfully to produce two polyketides from *Aspergillus oryzae* and three from *Chaetomium globosum*.

Second, once mRNA can be transcribed from the target gene cluster, cDNA can be synthesized to allow transfer of the cluster genes into budding yeast and achieve heterologous production of compounds. Here, we have developed an innovative method for biosynthesizing bioactive molecules using an engineered *Saccharomyces cerevisiae* strain as a host. We expressed five polyketide synthases and two nonribosomal peptide synthetases from *Aspergillus fumigatus*, *Chaetomium globosum* and *Coprinopsis cinerea*. Subsequent detailed chemical characterizations of the resulting natural products identified six polyketides and two peptides. The methodologies shown in this study can be applied in acquisition of numerous natural products biosynthesized by silent/unknown fungal secondary metabolism gene clusters.

PR8.51

Unravelling the MDR mechanism in new emergent phenotypes of *Mycosphaerella graminicola*

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Multidrug resistance (MDR) is a trait developed by many organisms to counteract the effect of chemicals and/or drugs for the control of these agents. The basic MDR mechanism relies on an overexpressed efflux transport system that actively expulses the compound outside the cell. MDR was observed in field populations of the wheat septoria tritici blotch fungus *Mycosphaerella graminicola* since 2007 in France, Ireland and the UK, which evidently threatens control options. Individual strains with MDR (MDR 6 and 7) were characterized on the basis of their high resistance levels to fungicides belonging to the DMI family and to their cross-resistance with QoIs and SDHIs. Two main strategies were adopted: (a) Investigating the relationship between MDR phenotypes and an efflux transport system: Reversal agents inhibiting ABC/MFS transport systems coupled to C14-radiolabeled Prochloraz shed light on the involvement of at least two different transporters. In addition, the Tolnaftate (thiocarbamate) phenotyping of 140 descendant from a cross of MDR6 x MDR7 confirmed allelism or close linkage of genes involved in the MDR phenotype; (b) Analysis of differentially expressed genes in sensitive and MDR isolates:

RNA sequencing profiling of the MDR6/7 strains *vs.* sensitive strains in untreated and Prochloraz treated conditions are ongoing and the latest data and analyses will be presented.

PR8.52

Correlation of gene expression and protein production rate - a system wide study

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Growth rate is a major determinant of intracellular function. However its effects can only be properly dissected with technically demanding chemostat cultivations in which it can be controlled. Recent work on *Saccharomyces cerevisia*e chemostat cultivations provided the first analysis on genome wide effects of growth rate. In this work we study the filamentous fungus *Trichoderma reesei (Hypocrea jecorina)* that is an industrial protein production host known for its exceptional protein secretion capability. Interestingly, it exhibits a low growth rate protein production phenotype. We have used transcriptomics and proteomics to study the effect of growth rate and cell density on protein production in chemostat cultivations of *T. reesei*. Use of chemostat allowed control of growth rate and exact estimation of the extracellular specific protein production rate (SPPR). We find that major biosynthetic activities are all negatively correlated with SPPR. We also find that expression of many genes of secreted proteins and secondary metabolism, as well as various lineage specific, mostly unknown genes are positively correlated with SPPR. Finally, we enumerate possible regulators and regulatory mechanisms, arising from the data, for this response. Based on these results it appears that in low growth rate protein production energy is very efficiently used primarly for protein production. Also, we propose that flux through early glycolysis or the TCA cycle is a more fundamental determining factor than growth rate for low growth rate protein production and we propose a novel eukaryotic response to this i.e. the lineage specific response (LSR).

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PR8.53

Enzyme production by Trichoderma reesei Rut C-30 followed by enzymatic hydrolysis of different lignocellulosic materials

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The filamentous fungus *Trichoderma reesei* is one of the main sources for cellulose degrading enzymes. We studied the enzyme profile produced during the fungal growth on cellulosic and lignocellulosic substrates and their capacity to hydrolyze cellulosic and lignocellulosic substrates with different chemical and physical properties. The results brought insight into the bottlenecks of enzymatic hydrolysis.

During the enzyme production study, we grew *T. reesei* strain Rut C-30 in submerged fermentations on Avicel PH-101, commercial cellulose, and industrial-like lignocellulosic substrates from spruce. These substrates were produced during the process of sodium hydroxide cooking, used in pulp and paper industry. Additionally we altered the chemical and physical properties of those substrates by drying and rewetting, treatment of sodium hydroxide and sodium chlorite in order to decrease or increase the surface area and delignify, respectively. We measured cellulolytic enzyme activity by enzymatic assays. Proteins were examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and two-dimensional gel electrophoresis.

The enzymes produced were subsequently used for enzymatic hydrolysis of lignocellulosic substrates and compared to enzymatic hydrolysis of model cellulosic substrates, namely, Avicel PH-101, nanocrystaline cellulose, phosphoric acid-swollen cellulose and cotton, which have defined characteristics. The structural properties of the substrates during the different times of hydrolysis were analyzed by solid-state nuclear magnetic resonance (NMR) technique. Dynamics of the hydrolysis was analyzed by quartz crystal microbalance with dissipation (QCM-D) technique. Hydrolysis products were verified by high performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD).

PR8.54

Structural and Functional analyses of Dehydrin-like proteins in the necrotrophic fungus *Alternaria brassicicola*

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Dehydrins (DHN) are a protein subclass of Late Embryogenesis Abundant proteins (LEA) which, in plants, are involved in the salt and hydric stress resistance. In fungi, dehydrin-like proteins have been identified in *Tuber borchii* and *Aspergillus fumigatus* and a signature pattern has been described for these proteins (1,2). Using this sequence motif, we have identified three DHN-encoding genes in the genome of the necrotrophic fungus *Alternaria brassicicola*. Sequence analysis confirmed that they all shared the characteristic features of dehydrins: high glycine, threonine and serine content, low cysteine and tryptophan content, high hydrophilicity, absence of secondary structures and a high proportion of disordered amino acids. Measures of the expression levels of the three DHN genes in conditions previously reported to induce fungal DHNs transcription (low temperature, salinity and oxidative stress) revealed that they were all up regulated in these conditions. To study their subcellular localization, dehydrin-like proteins were fused to eGfp at their carboxy-terminal end and expressed in *A. brassicicola* under control of their own promoters. Fluorescent protein fusions showed that at least one dehydrin was associated with peroxisomes. A functional analysis has been performed by the construction of knockout mutants deficient for each DHN. Although none of the dehydrin-like mutants were found more susceptible to NaCl than the WT strain, they were all characterized by a stronger susceptibility towards oxidative stress (menadione or H_2O_2). A double-mutant strain exhibited reduced virulence on host leaves and decreased seed transmission rates compared to the parental strain, indicating a role of DHNs in pathogenicity.

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PR8.55

Esterases of basidiomycetes as supporting enzymes in degradation of lignocellulosic material

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In contrast to fossil resources, lignocellulose as the main part of wooden plant material is available in almost unlimited amounts. Its main components - cellulose, lignin and hemicellulose – represent an important feedstock used in various industries, such as in paper manufacturing or bioethanol production. Unfortunately, lignocelluloses are recalcitrant materials, and harsh chemical conditions are needed to degrade them. In nature, fungi are capable of breaking up the wooden material by using a diverse set of extracellular enzymes, the so-called secretome. For degradation of the lignin, a number of oxidases including lignin peroxidases, manganese peroxidases, versatile peroxidases, and DyP-type peroxidases and laccases, are secreted which are able to oxidise aromatic and phenolic parts of the lignin structure. As a second part of the lignocellulosic fungal secretome, hydrolytic enzymes, such as cellulases and esterases, are needed for the extraction of sugars and are involved in the degradation of the lignocellulosic structures, respectively. The different cellulases are able to fracture the cellulose structure, whereas esterases (EC 3.1.1.x) are involved in the hydrolysis of several ester bonds. E.g., feruloyl-esterases (EC 3.1.1.73) participate in the breakup of linkages connecting hemi-cellulose (arabinoxylans) and lignin. Nevertheless, the knowledge on basidiomycete esterases is fragmentary and, thus, their biotechnological potential unknown.

In this work, 30 different basidiomycetes were screened for the ability to degrade several esterase substrates. Interesting candidates were cultured in minimal and complete liquid media with and without addition of lignocellulose. Esterase activities of up to 340 U/L were obtained when cultivating the fungi in shaken flasks at 24 °C. The supernatant of interesting fungal candidates was used together with already optimised cellulolytic enzyme cocktails to improve the degradation of lignocellulosic residues regarding the amount of reducing sugars gained by this bioconversion. Promising candidates will be purified, characterised and heterologously expressed in an ascomyceteous host.

PR8.56

Characterization of Cellobiohydrolase I (CBHI) of the White-Rot Fungus *Dichomitus squalens*

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In nature basidiomycetous white-rot fungi are the most efficient wood-decaying organisms. They express extracellular hydrolytic and oxidative enzymes that are needed to degrade all the wood polymers, i.e. cellulose, hemicellulose and lignin, but while their lignin modifying enzymes are well known their cellulolytic enzymes are much less studied. In a large screening of cellobiohydrolases (CBHs) of basidiomycetous fungi from the Fungal Biotechnology Culture Collection (FBCC, University of Helsinki) the white-rot fungus *Dichomitus squalens* strain FBCC312 appeared to be highly cellulolytic and a promising source of novel cellulases. In this work we purified and characterized native CBHI of *D. squalens*. The fungus produced extracellular CBH, endoglucanase, *β*-glucosidase, xylanase and laccase activities in liquid 1% (w/v) microcrystalline cellulose (Avicel) - peptone medium. Ds-CBHI was purified from this culture liquid after 6 to 10 days of cultivation, with anion exchange and size exclusion chromatography. Molecular mass of the purified Ds-CBHI was 45 kDa and p*I* 3.8-4.1, as determined by SDS-PAGE and IEF, respectively. Three internal peptides sequenced by LC-MS/MS were similar with the translated amino acid sequence of the cloned *D. squalens cbhI* gene. The putative polypeptide of Ds-CBHI lacks cellulose binding module and is similar to glycosyl hydrolase family 7 proteins. Ds-CBHI showed wide pH and temperature working ranges with artificial substrate 4-methylumbelliferyl-β-D-lactoside (MULac). Optimum temperature of Ds-CBHI for MULac reaction was +65°C and optimum pH 4.0. Purified Ds-CBHI resembled the known white-rot fungal CBHs by its acidic p*I* and catalytic optimum pH.

PR8.57

Associated biocontrol of cotton pest *Dysdercus peruvianus* by the fungus *Metarhizium anisopliae* and environmental isolates of *Pseudomonas fluorescens*

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Dysdercus peruvianus is an insect pest of cotton culture, which causes serious economic damages. The method currently used to control this plague is by the use of chemical pesticides. However, this practice is increasingly questioned by society, due environmental impact, high costs, low specificity and risk in its handling. *Metarhizium anisopliae* is a filamentous fungus used in biological control with attested effect on several arthropod pests, including *D. peruvianus*. The main barrier to apply this fungus, or any other biocontrol agent, is the time of death of target pest; which is generally greater than the corresponding chemical pesticide. In this work, the isolation, identification and evaluation of environmental bacteria in association with *M. anisopliae* were performed to optimize the biocontrol of the *D. peruvianus*. Four bacteria isolated from soil effectively accelerate the biocontrol of *D. peruvianus*, when associated with the fungus, and the two best bacteria were identified as *Pseudomonas fluorescens*. The formulation containing *M. anisopliae* conidia and bacterial culture of *P. fluorescens* showed efficiency up to 96% in reducing the time of the death of *D. peruvianus*. Besides molecular aspects of this interaction, including the expression of enzymatic arsenal of *M. anisopliae* in association of bacterial isolates were also evaluated. This work attests the efficiency of associated biocontrol applying the fungus *M. anisopliae* and bacterial isolates collected from environment using a specific strategy. Also, this alternative represents a significant increase in efficiency of biological control, increasing the interest in application of this environmentally safe way of pest control.

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PR8.58

Morphological and molecular characterization of Hyphodermella rosae the causal agent of dry fruit rot on plum and peach in Iran

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In June 2011, a new symptoms of dry fruit rot on plum (Prunus domestica) and peach (P.persica) was observed in Mazandaran province of Iran. Initial symptoms, appeared as dark brown, circular necrotic spots on fruits. The fungus Hyphodermella rosae isolated and identified on the basis of morphological characteristics on PDA. Basidiomata were effuse, resupinate, 15 × 10 mm, tubercules small with apical bristles, light orange to greyish orange. Subhymenium composed of vertically arranged, short-celled, non-agglutinated hyphae; subhymenial hyphae were 3-4 µm in diam. Basidiospores were ellipsoid, 7.5× 5.5 µm and their cell walls were thin, hyaline and smooth (1). A CTAB DNA extraction protocol was used to acquire DNA from mycelium culture. The primer pair ITS4 (5-TCCTCCGCTTATTGATATGC-3) and ITS5 (5-GGAAGTAAAAGTCGTAACAA-3) was used to amplify the Internal Transcribed Spacer (ITS) regions including 5.8S from ribosomal DNA (4) and the PCR product was sequenced. The 627-bp and 604bp fragments of Plum and peach isolates was amplified, respectively. After multiple sequence alignment with CLUSTALW software the obtained sequences were compared with the other related sequences of Hyphodermella genus deposited in GenBank. Blast analysis of the MA4099 (plum isolate) and VA1345 (peach isolate) sequences confirmed a 99 and 100% similarity with the sequences of H. rosae (GenBank accession no FN600386.1, FN600385.1) respectively. The pathogenicity of the isolates has been proven. The genus Hyphodermella has been reported causing wood rot on apricot (2), sweet and sour cherry (3). To our knowledge, this is the first report of H. rosae on stone fruit species in the world.

PR8.59

Screening the secondary metabolome of an unidentified basidiomycete for antifungal compounds

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The isolate BY represents an unidentified anamorphic fungus. Based on the analysis of ITS/IGS-sequence data it was tentatively assigned to the russuloid clade of the basidiomycetes. However, its genus remains obscure. As cultures of this isolate exert strong antifungal effects further research into its secondary metabolism was warranted. A series of natural products has been isolated, structurally elucidated, and tested for antifungal activity. Our results suggest that the bioactivity of BY is not related to a single agent but due to several structurally dissimilar compounds.

They possibly originate from different metabolic pathways, as polyene and phenylpropanoid core structures were found, alongside polyketidic scaffolds. To clarify the respective biosynthetic routes, feeding experiments with 1-13C labeled acetate have been initiated, accompanied by genetic screens for natural product biosynthesis genes.

PR8.60

Heterologous expression improvement in filamentous fungi by using RNA interference tool

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Fungi are industrial workhorses of secretion, where commercially useful proteins are targeted for secretion and high-yield accumulation. High-yield protein secretion and accumulation are amino-acid sequence dependent and in many cases, more often than not, high-yield of secretion is not observed. *Aspergillus nidulans* has been recognized and utilized as an excellent host to homologous and heterologous protein production. Researches have studied the protein production, because it shows an excellent extracellular secretion capability for a large amount of proteins compared to described in other microbial secretion systems such as *E. coli* and *S. cerevisiae*. Some post-translational modifications including glycosylation and folding are expected to take place in *A. nidulans*. For these reasons, it is considered one of the most adequate hosts to produce higher eukaryotic proteins. To enhance the protein production ability, it is important to construct the host applicable to multiple rounds of genetic manipulation. Recently, in order to carry out multiple gene silence and expression rapidly and efficiently, we developed *pyrG* marker recycling system. Using this system, successive rounds including concatenated gene silence for proteases respectively in *A. nidulans* were successfully achieved with gene-targeting frequency. Based in proteomics results, the genes encoding proteases, we concatenated five proteases in only one construction to analyze the effect of RNA interference on heterologous protein production by *A. nidulans*. Moreover, based on these data, we also confirmed the improvement of cellobiohydrolase productivity with the protease deficient strain. As a result, we constructed a quintuple protease genes disruptant having enhanced levels of cellobiohydrolase protein productivity.

PR8.61

Heterologous expression of the human peptide hormone obestatin in *Trichoderma reesei*

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The filamentous fungus *Trichoderma reesei* is an efficient expression host widely exploited as an industrial workhorse. However despite being regularly used for proteins and enzyme of larger size its capability of expressing small peptides of (T. reesei as an expression host for peptide production by engineering a strain for the expression of obestatin, a small peptide hormone of 23 amino acids in size that suppresses the appetite and regulates body weight gain in human and other mammals. Thus providing an alternative source for this otherwise naturally scarce and expensive to chemically synthesize peptide hormone. Preliminary expression of obestatin, with HIS tag fused at either N- or C- termini of the peptide, was carried out in *Escherichia coli* and the expression level was evaluated by enzyme immunoassay (EIA). The expression of obestatin was subsequently evaluated in *T. reesei* with a C-terminal purification tag, Hydrophobin I tag (HFBI) which is native to the host. Following the successful expression of obestatin in *T. reesei,* modifications of growth conditions were made to optimize the production of the peptide. Results indicated that it was possible to express the small peptide hormone obestatin in *T. reesei* at a higher concentration than it is in the *E. coli* expression system. It was also possible through strain selection and modification of the culture medium to achieve yield of up to 7µg/ml of obestatin in *T. reesei*.

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PR8.62

Extracellular enzymes from *Trichoderma harzianum*, *Aspergillus terreus* and AM fungus

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Cellulotic enzymes consistitute form the group well described biologically important enzymes,The enzyme cellulase, a multi enzyme complex made up of several proteins, catalyses theconversion of cellulose to glucose in an enzymatic hydrolysis. Which catalyze the transfer of the glycosyl group between oxygen nucleophiles. important role this enzymes in the biology is include degradation celluloses of the biomass fungi and bacteria, degradation of glycolipids in mammalian lysosomes, and the cleavage of glycosylated flavonoids in plants. Fungus Trichoderma and Aspergillus have biotechnologicaly importance, since they are a producer extracellular enzyme. We studies from the new strains selection high cellulotic activities and using them in the biotechnical studies. Studing extracellular enzymes from 3-fungi, Aspergillus terreus, Trichoderma harzianum and Mucorrhiza conducted in minimum ambience, containing wheat bran as single source of the carbon. Enzyme activities were assayed spectrophotometrically by using Samogy-Nelson. Temperature and pH optimum of some purified enzymes were determined also. The isolation and purification cellulotic enzymes we are used the ion exchange chromatography on DEAE TOYOPEARL 650 M gel in the gradient 0,5M NaCl. The test strains of each species ., Aspergillus terreus, Trichoderma harzianum and AM fungi were analyzed on the basis of extent of hydrolyzing ability. Cellulose hydrolysis of all three strains was immensely affected by varying pH and medium. The results indicated statistically significant interaction in all correlating factors of strain, growth medium and pH level

PR8.63

Finding the conserved mushroom developmental pathway

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Mushrooms are important sources of food, medicinal compounds, for industrial applications, waste recycling and environmental health. However, cultivation of most mushrooms is difficult or yet impossible. A major obstacle for improvement of mushroom cultivation technology is the lack of knowledge on molecular genetic mechanisms that underlie mushroom development. Available information is highly scattered, describing specific, unrelated studies in model mushrooms and more recently including individual studies on major cultivated mushrooms.

Despite the high variations in shape, color, composition, substrate and triggers for fruiting induction, mushroom life cycles follow a general course. Within the developmental stages of the mushroom, especially the formation of hyphal knots and primordia seem highly similar between species. We decided to 'access' the general developmental pathway through interspecies comparison of primordium specific gene expression patterns, including model as well as major cultivated species. Promising universal, primordium specific genes will be studied in detail in the model organisms *Coprinopsis cinerea* and *Schizophyllum commune* by means of gene deletion, fusion to fluorescent markers and quantitative PCR. Once confirmed to play a role in mushroom development, these genes will serve as foundation for compilation of a general mushroom development model in two directions; stages preceding, and stages following primordium formation.

At the moment, superSAGE datasets are being assembled and most mushroom species are still under cultivation. We just started comparison of our first four datasets (dikaryotic mycelium versus primordia) in *C. cinerea* and *Polyporus brumalis*. Considering the effectiveness of other expression-based studies for identification of stage specific developmental genes in mushrooms, we expect to reveal several universal genes to start our model in the near future.

PR8.64

Laccase functions in *trichoderma virens*

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Fungal laccases are involved in multiple functions, such as lignin degradation, pigments synthesis and degradation, detoxification and pathogenesis. Furthermore, they are useful biocatalysts for several biotechnological applications. Six laccase genes were previously identified in *Trichoderma virens*, an effective biocontrol agent, and one of them was deleted and proved to be involved in the mycoparasitic activity against *Botrytis cinerea* sclerotia. Laccase activity in some *Trichoderma spp.* is also associated with the production of green pigment in conidial spores. Further investigations on the laccase gene family in *T. virens* were performed in order to explore substrate specificity and mechanisms putatively involved in ligninolysis, conidiogenesis and industrial dyes decolorization. Laccase functions in lignocellulosic process and sporulation mechanisms were studied by growing *T. virens* on two different substrates: wheat straw liquid medium, containing lignocellulose as the only carbon source, or solid Hölker medium, formulated to induce spore formation. Laccase expression analysis induced by multiple substrates is in progress to identify the more effective molecules or the pathways involved in *T. virens*. Further biochemical analyses are going on to search laccase isoforms when fungal cultures are grown on specific substrates. Possible variations of intra/extra-cellular enzymatic levels is also under study. In addition liquid cultures containing twelve commercial textile dyes were set up and *T. virens* efficiently decolorized three of them. In conclusion information is gained about the *T. virens* laccase gene family, involved in physiological processes important for fitness or antagonistic attitude and exploitable in biotechnological applications related to textile dyes decolorization or ligninolysis.

PR8.65

Development of a homologous protein carrier system for heterologous protein production in *Myceliophthora thermophila.*

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Filamentous fungi have proven to produce and secrete large quantities of extracellular enzymes. Species such as *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei* and *Myceliophthora thermophila* C1 are used in industry as work horses for enzyme production. High yields of homologous enzymes can be readily obtained. On the contrary, heterologous proteins are often produced at low levels. One of the main reasons for this is the presence of host proteases that partially or fully degrade the heterologous protein. In addition at the level of transcription, translation and transport and processing through the secretion pathway problems may be encountered. In order to increase the chances of success, protein carriers have been used for improved heterologous protein production in filamentous fungi[1]. It is believed that the carrier protein will "drag/guide" the heterologous protein through the (initial stages) of the secretion pathway protecting it from mis-folding and proteolysis. Previously, we have used this technology successfully in the expression of human antibodies in C1[2]. The carrier protein used in that study was the catalytic domain of *Aspergillus niger* glucoamylase A, which by itselfis a heterologous protein to C1. In the present study we investigated whether the homologous C1 glucoamylase yields higher levels of a heterologous xylanase sensitive to proteolysis. [1] Gouka *et al*. (1997) Efficient production of secreted proteins by *Aspergillus*: progress, limitations and prospects. Appl. Microbiol. Biotechnol. 47: 1-11. [2] Visser *et al*. (2011) Development of a mature fungal technology and production platform for industrial enzymes based on a *Myceliophthora thermophila* isolate, previously known as *Chrysosporium lucknowense* C1. Ind. Biotechnol. 7(3): 214-223.

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PR8.66

Closely related fungi employ diverse enzymatic strategies to degrade plant biomass

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Fungi can grow in many biotopes and on many different carbon sources. In natural biotopes, plant biomass is the predominant carbon source for most fungi. Plant biomass consists largely of polymeric compounds of which polysaccharides are the main components. Fungi cannot take up the intact polysaccharides, but need to degrade them extracellularly to monomeric and small oligomeric compounds. To achieve this, fungi produces diverse enzymatic mixtures that are tailored specifically to the available polysaccharides.

A recent study demonstrated significant differences in the polysaccharide degrading ability of three Aspergilli, while only small differences were detected in their growth on various plant polysaccharides (Coutinho et al, 2009). This suggests that related fungal species may have developed different approaches to plant biomass degradation, employing different enzyme sets. A better understanding of these strategies will not only increase our insight in fungal biodiversity, but will also help in designing more efficient industrial processes for plant biomass degradation.

In this study we have compared the plant biomass degrading potential and strategy of 8 Aspergilli and demonstrate that they have developed a highly diverse approach to using these complex carbon sources. Although all eight species contain the main transcriptional activators involved in plant polysaccharide degradation (AmyR, XlnR, AraR, InuR) the enzymatic sets produced by them differs hugely, suggesting a species specific fine-tuning of plant biomass degradation.

PR8.67

Induction of lignocellulose degrading enzymes in Neurospora crassa by cellodextrins

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Neurospora crassa colonizes burnt grasslands in the wild and metabolizes both cellulose and hemicellulose from plant cell walls. When switched from a favored carbon source such as sucrose to cellulose, N. crassa dramatically upregulates expression and secretion of a wide variety of genes encoding lignocellulolytic enzymes. However, the means by which N. crassa and other filamentous fungi sense the presence of cellulose in the environment remains unclear. Here, we show that a N. crassa mutant carrying deletions of two genes encoding predicted extracellular β-glucosidase enzymes and one intracellular β-glucosidase enzyme (Δ3βG) lacks β-glucosidase activity, but efficiently induces cellulase gene expression and cellulolytic activity in the presence of cellobiose as the sole carbon source. These data indicate that cellobiose, or a modified version of cellobiose, functions as an inducer of lignocellulolytic gene expression and activity in N. crassa. In addition, we have identified two cellodextrin transporters involved in sensing cellulose. A N. crassa mutant carrying deletions for both transporters is unable to induce cellulase gene expression in response to crystalline cellulose. Furthermore, a mutant lacking β-glucosidase enzymes and transporters (Δ3βGΔT) does not induce cellulase gene expression in response to cellobiose. We are currently in the process of characterizing the transport kinetics of each individual transporter in the Δ3βG background with the goal of understanding how the transport of cellodextrins influences cellulose sensing and induction of cellulase gene expression.

PR8.68

A novel group of class II hydrophobins from *Trichoderma* with biased occurrence

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Hydrophobins are small secreted proteins containing eight positionally conserved cysteine residues, are unique to Pezizomycotina (Ascomycota). These proteins assemble in amphiphilic layer on the outer fungal cell wall, where they mediate interactions between the fungus and its environment. The amphiphilic properties have also raised considerable industrial interest in the application of hydrophobins for the processes requiring surface modification. *Trichoderma* spp. possess an amplified arsenal of class II hydrophobins compared to other fungi. In order to exploit this richness for biotechnology, we performed a genus-wide screening for novel *Trichoderma* hydrophobins. Thereby we discovered a group of class II hydrophobins which is restricted to *T. virens* (teleomorph *Hypocrea virens*) and taxa from closely related Harzianum Clade (both section Pachybasium). The intraspecific nucleotide diversity π is in the range <0.062, which is similar to the interspecific diversity of other *Trichoderma* class II hydrophobins, indicating a high rate of evolution. Fishers exact test showed that the gene is under purifying selection and exhibits a high relative synonymous codon usage (>0.8). The expression of these new hydrophobins under different conditions and the characterization of their amphiphilic properties will be reported.

PR8.69

The HFB4 family: novel class II hydrophobins of *Trichoderma* with universal infrageneric distribution and potential for industrial applications

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Hydrophobins are small secreted proteins containing eight positionally conserved cysteine residues, are unique to Ascomycota. They usually assemble in amphiphilic structures on the outer fungal cell wall, thus mediating interactions between the fungus and its environment. These amphiphilic properties have also raised considerable industrial interest in the application of hydrophobins for the modification of surfaces. We have previously shown (Kubicek *et al.,* 2008. BMC Evol Biol) that *Trichoderma* spp. possess an highest diversity of class II hydrophobins compared to other fungi. This variability likely arose by iterating patterns of gene duplications and gene loss processes (”birth and death evolution”). Here we studied the HFB4 family, which comprises the most conserved clade of class II hydrophobins in Trichoderma. HFB4 orthologues occur almost in all infrageneric groups of *Trichoderma*, with the exception of the the *Hypocreanum, Psychrophila* and *Lutea* clades. HFB4 sequences from some species exhibited a significant amino acid sequence variation (e.g. *HFB4s in Trichoderma* section Trichoderma), whereas that of other taxa was identical (e.g. section *Longibrachiatum*). The K_a/K_s ratio of 1.93, as well as Tajima’s test (significantly positive) and Fishers exact test (< 0.3; see ref. above) showed that *T. atroviride (*teleomorph *Hypocrea atroviridis*) HFB4 is apparently under positive selection pressure, whereas the other confirmed the birth and death mechanism. The expression of *hfb4* gene accompanied the conidia formation of *T. atroviride*, *T. reesei* (teleomorph *H. jecorina*) and *T. virens* (teleomorph *H. virens*). The amphiphilic properties of HFB4 were confirmed by overexpression of the protein from the three above mentioned species in *E. coli,* purification and analysis by contact angle measurement after dropping water on the surface of hydrophilic and hydrophobic surfaces, respectively. HFB4 may comprise a new group of class II hydrophobins with potential industrial properties.

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PR8.70

***In Vitro* Assessment of Chitosan on *Ganoderma boninense*, Pathogen of Basal Stem Rot Disease in Oil Palm**

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Chitosan is a natural by-product polymer derived from chitin component of crustaceans, insects and fungi which exhibits antimicrobial properties against various pathogens. This study aims to evaluate the *in vitro* antifungal poperties of chitosan on *Ganoderma boninense*, the causal agent for basal stem rot disease in oil palm. Five concentrations of chitosan (1.0, 1.5, 2.0, 2.5 and 3.0 % w/v) in Potato Dextrose Agar (PDA) media were tested for their efficacy to control the *in vitro* growth of *G. boninense* mycelium during culture for 21 days. All of the concentrations tested significantly reduced mycelial growth compared with the control treatment. Chitosan exhibited a fungistatic effect on mycelial growth of *G. boninense* and markedly reduced radial growth via dose-dependent manner. The highest inhibition of radial growth (PIRG) of 90.09 % was observed with chitosan at 3.0 % (w/v). Chitosan also caused morphological changes in *G. boninense* mycelium including the occurrence of small vesicles due to coagulation of fungal cytoplasm and formation of excessive abnormal hyphal branching at higher concentrations. Spores of *G. boninense* treated with the same five concentrations of chitosan during the 21 days incubation period were unable to germinate.

PR8.71

Post-genomic analyses of fungal lignocellulosic biomass degradation reveal the unexpected potential of the plant pathogen *Ustilago maydis*

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Filamentous fungi are potent biomass degraders due to their ability to thrive in ligno(hemi)cellulose-rich environments. During the last decade, fungal genome sequencing initiatives have yielded abundant information on the genes that are putatively involved in lignocellulose degradation. At present, additional experimental studies are essential to provide insights into the fungal secreted enzymatic pools involved in lignocellulose degradation.

In this study, we performed a wide analysis of 20 filamentous fungi for which genomic data are available to investigate their biomass-hydrolysis potential. A comparison of fungal genomes and secretomes using enzyme activity profiling revealed discrepancies in carbohydrate active enzymes (CAZymes) sets dedicated to plant cell wall. Investigation of the contribution made by each secretome to the saccharification of wheat straw demonstrated that most of them individually supplemented the industrial *Trichoderma reesei* CL847 enzymatic cocktail. Unexpectedly, the most striking effect was obtained with the phytopathogen *Ustilago maydis* that improved the release of total sugars by 57% and of glucose by 22%. Proteomic analyses of the best-performing secretomes indicated a specific enzymatic mechanism of *U. maydis* that is likely to involve oxido-reductases and hemicellulases.

This study provides insight into the lignocellulose-degradation mechanisms by filamentous fungi and allows for the identification of a number of enzymes that are potentially useful to further improve the industrial lignocellulose bioconversion process.

PR8.72

Expression profile of beta-galactosidases in *Penicillium chrysogenum*

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Penicillium chrysogenum is used as industrial producer of penicillin. We investigated the catabolism of lactose, an abundant component of whey that has been used extensively in pencillin fermentation, comparing NRRL 1951 as a wild-type reference with the industrial penicillin-producer ASP-78.

Both strains grew similarly on lactose under batch conditions. The time-profile of sugar depletion concurred with the presence of intra- and extracellular beta-1,4-D-galactosidase (bGal) activities. Upon growth on D-glucose, D-fructose, D-xylose, D-galactose and glycerol, neither extra- nor intracellular bGal could be detected. However, L-arabinose induced activity to about half the values measured on lactose. The measured bGal activities were similar for the two investigated strains.

In silico analysis revealed that P. chrysogenum features at least five putative bGal-encoding genes at the annotated loci Pc22g14540, Pc12g11750, Pc16g12750, Pc14g01510 and Pc06g00600. The first two proteins appear to be orthologs of the Aspergillus nidulans intracellular family 2 glycosyl hydrolases AN3201 and AN3200. The latter three P. chrysogenum proteins feature an N-terminal secretion signal and appear distinct paralogs to the extracellular bGal from Aspergillus niger, LacA.

Transcript analysis of Pc22g14540 and Pc12g11750 showed that they were expressed exclusively in response to lactose but completely repressed on the mixed growth substrate glucose/lactose. Pc16g12750 was seemingly co-expressed with the two putative intracellular bGal genes, while its two paralog genes were apparently not transcribed under any condition tested. This expression profile is distinct from those in other ascomycetes, like Trichoderma reesei or A. nidulans, where bGal genes are induced by the monosaccharides D-galactose and/or L-arabinose.

PR8.73

D-galactose uptake of Aspergillus niger

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The majority of Black Aspergilli (Aspergillus Section Nigri), including *Aspergillus niger*, as well as many other Ascomycetes fail to germinate on D-galactose as a sole carbon source. Here, we provide evidence that the ability of *A. niger* to transport D-galactose is growth stage dependent, being absent in the conidiospores but partially present in the mycelia. Despite earlier claims, we could identify galactokinase activity in growing cells and all genes of the Leloir-pathway (responsible for channeling D-galactose into the EMP-pathway) are well induced on D-galactose (and also on lactose, D-xylose and L-arabinose) in the mycelial stage. Expression of all Leloir pathway genes was also detectable in conidiospores, though *galE* (encoding a galactokinase) and *galD* (encoding a UTP-galactose-1-phosphate uridylyl transferase) were expressed very poorly. These results suggest that the D-galactose-negative phenotype of *A. niger* conidiospores is due to the lack of inducer uptake.

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PR8.74

Approaches for evaluating the performance of lignocellulosic biomass hydrolysates obtained by using fungal enzyme cocktails

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The feedstock for 2nd generation biofuel is lignocellulosic biomass, such as wheat straw, bagasse and corn stover. For microorganisms to utilize the biomass, a pretreatment and a hydrolysis step are needed to release fermentable sugars into the lignocellulosic biomass hydrolysate. Fungal enzyme cocktails are used in the hydrolysis step. The hydrolysates differ in their compositions and effects on growths of yeasts and fungi, due to (i) the biomass type (ii) the pretreatment and hydrolysis method (iii) strain characteristics. To select the best combination of these factors is a key step in conducting 2nd generation biofuel research.

In this study, methods were developed to determine the composition of various hydrolysates, generated from diverse biomass and by different pretreatment and hydrolysis methods. In particular, a HPAEC-MS method was found suitable to identify the limiting and interfering factors to the activities of fungal enzyme cocktails. The results of this analysis allow targeted optimization of these cocktails. The performance of these hydrolysates was screened in Bioscreen C Analyzer using *Saccharomyces cerevisiae* CEN.PK 113-7D as model strain.

The results show that our approaches are effective for evaluating and selecting the most suitable hydrolysate for a specific production purpose. The developed analysis methods have potential to enhance the enzymatic hydrolysis efficiency in the hydrolysates, and enable the systematic study on the hydrolysate inhibitory effects.

PR8.75

Enhance itaconic acid production in Aspergillus via cultivation condition optimization

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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. The transcriptomics analysis from A. terreus identified the most relevant itaconic acid related genes. Subsequently, expressing the specific cis-aconitate decarboxylase (CAD) encoding gene in A niger lead to itaconic acid production in the fermentation medium. To enhance itaconic acid production level via medium improvement, 20 different media were designed based on a reference medium from A. terreus and a 96-well micro-titer plate screening assay was applied for screening . The best medium increased the production level in controlled batch fermentation. This was confirmed showing that various levels of one of the trace elements correlated with itaconic acid production under these conditions. In addition, several other parameters such as pH, temperature and dissolved oxygen tension (D.O.) in controlled batch fermentations were shown to be important for itaconic acid production .

PR8.76

Brazilian mangrove fungi in biological synthesis of silver nanoparticles

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Mangrove is an ecosystem in which there is a high competition for nutrients among microorganisms and where lack of oxygen supplies enables many oxido-reduction reactions to occur. In this context our study has been focused on the biological synthesis of silver nanoparticles (Ag NP) by some fungi isolated from Sao Paulo State's (Brazil) mangrove and evaluation of Ag NP antimicrobial activities. Fourteen fungi were cultivated in Potato Dextrose Broth at 25°C and 150 rpm for 72 h. The biomass was filtered and incubated at the same conditions with water (0.1 g mL^{-1}). The biosynthesis of Ag NP was performed adding $AgNO_3$ (1 mM) into fungal filtrate. The Ag NP formation was confirmed by Plasmon resonance band (λ = 440 nm). The nanoparticles were characterized applying TEM, size, zeta potential, and protein portion adhered to Ag NP was analyzed by SDS-PAGE electrophoresis. Antimicrobial activities were tested against some Gram-negative and Gram-positive bacteria and *Candida* species. The results showed that the fungi coded as L-2-2, R-2BI-4, MGE-201, MGE-202 and R-3BI-10 were able to produce Ag NP in satisfactory yields, pronounced antimicrobial activities, spherical morphology, and size in a range of 10-30 nm. TEM and SDS-PAGE revealed the presence of proteins around the Ag NP with molecular weight in the range of 75 to 328 kDa and further investigations are being performed to characterize these proteins. Fungi were taxonomically identified as *Bionectria ochroleuca* (L-2-2), *Cladosporium* spp (R-2BI-4), *Aspergillus tubingensis* (MGE-201), *A. niger* (MGE-202) and *Fusarium proliferatum* (R-3BI-10), respectively.

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PR8.77

Identification and characterisation of novel antifungal compounds against fungal human pathogens

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Fungal infections represent a serious health problem for immune suppressed patients who can be highly susceptible to life-threatening systemic infections. The increasing number of fungal infections and the development of resistance as well as the significant side effects result in the need for the identification of novel antifungal drugs. To identify, evaluate and optimize new tolerable and potent compounds with antifungal activity we have developed an *in vitro* High-Throughput-Screening Activity-Selectivity Assay (AS-HTS-Assay). This assay mimics the smallest unit of a natural infection by incubating host cells with the pathogen, e.g. *Candida species*, in the presence or absence of antimicrobial compounds. Thereby, it covers all potential targets of pathogen and host simultaneously in one assay and provides the minimal inhibitory concentration of active compounds in a host context and the tolerability of these compounds by the host cells. Using this assay we screened more than 100,000 compounds for antimycotic activity. One hit, a benzimidazole derivative, showed high antifungal activity against Candida spp. and good compatibility with human cells. This compound showed a good tissue penetration, tolerability and efficiency in complex 3D-epithelial tissue models and in multicellular organisms as demonstrated in first nematode models. The results of transcriptional profiling of *Candida albicans* indicated that the compound is a potential inhibitor of the ergosterol pathway. This is in contrast to other benzimidazole-derivatives which target microtubules. To further verify the specific target enzyme in the ergosterol pathway sterol pattern of different Candida spp. were carried out by GLC/MS analysis.

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PR8.78

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

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

PR 8.79

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

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Peptides are widely used in the pharmaceutical industry and are also gaining importance as food ingredients. However, the current state-of-the-art production of oligopeptides is via chemical synthesis, which is expensive especially for long peptides. In addition, peptides can be produced by means of fermentation, wherein multimeric genes encoding tandem repeats of the peptide are fused to a carrier protein. After production of the fusion protein peptides are cleaved from the carrier protein. However the yield of the process is generally low and the fusion protein generally accumulates in inclusion bodies.

We developed a new approach in which peptides are inserted in the variable loops of ankyrin repeats. Ankyrin repeat domains are conserved structures and consists of tandem repeats of a 33 amino acid ankyrin repeat unit. Especially interactions between units are important for stability and folding. Therefore, ankyrin repeat domains usually consist of 4 or 6 units. Interestingly, in between two adjacent ankyrin units inserts up to 47 amino acids has been observed in nature.

We have shown in E.coli and Aspergillus niger that peptides can be inserted in between two adjacent ankyrin repeat units and peptides can be isolated from the protein. In Aspergillus niger, ankyrin repeat proteins comprising peptides were fused to truncated glucoamylase. The chimeric repeat protein comprising peptide was successfully expressed and secreted by Aspergillus niger.

In conclusion, production of peptides by inserting peptides in ankyrin repeat proteins is a successful new approach for fermentative peptide production.

PR8.80

The transcriptional regulator RhaR of *Aspergillus niger* is involved in L-rhamnose catabolism and in degradation of Rhamnogalacturonan-I

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

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

RhaR also appears to regulate L-rhamnose catabolism as growth of *rhaR* disruptant strains on L-rhamnose was abolished and two genes encoding putative L-rhamnose catabolic enzymes were down-regulated in the Δ*rhaR* strain.

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Monday 2 April

Parallel session 9: The Fungal Cell Wall

PS9.1

Advances in fungal cell wall proteomics

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The cell walls surrounding pathogenic fungi represent the first point of contact with their hosts and play an important role in the infection process. Electron microscopy studies on ascomycetous yeasts as well as some other species have shown that two different layers can be distinguished in fungal walls. The inner part of the wall is electron-transparent and mainly consists of carbohydrates. This is surrounded by an electron-dense layer, which is packed with a diversity of covalently-bound cell wall proteins. The protein composition of fungal walls is dynamic and depends on the growth environment. We are interested in studying the role of fungal wall proteins in pathogenesis. As a first step, we perform (comparative) genome-wide *in silico* analyses to identify putative cell wall proteins (and other cell surface proteins). The majority of known fungal cell wall proteins are so-called GPI proteins, which are covalently linked to cell wall β-1,6-glucan through a remnant of their GPI lipid anchor. In a different approach, we have developed techniques to identify and quantify covalently-bound cell wall proteins using advanced mass spectrometry technology. This allows us to identify cell wall proteins whose expression and incorporation is triggered under infection-relevant conditions and therefore may be important for the infection process.

Here, we will discuss our advances in mass spectrometric identification of cell wall proteins, and we will present our recently launched web server named ProFASTA, which facilitates fast genome-wide predictions of fungal cell surface proteins. In addition, we will present examples of functional characterizations of identified infection-relevant proteins in the human pathogen *Candida albicans*.

PS9.2

Interaction of cell wall polysaccharides with amyloid forming proteins

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Fungi secrete a variety of proteins that form amyloid-like fibrils at the cell wall surface. In this way, they fulfill a structural role in growth and development. Hydrophobins are such a class of proteins. In vitro experiments showed that the class I hydrophobin SC3 of *Schizophyllum commune* spontaneously forms amyloid-like fibrils at the water-air interface. In contrast, SC3 is arrested in an intermediate conformation at the interface between water and a hydrophobic solid such as Teflon. This finding prompted us to study conditions that promote assembly of SC3 into amyloid-fibrils. We have shown that amyloid formation at a hydrophobic surface does take place at high concentration (300 µg ml^{-1}) and prolonged incubation (16 h). The concentration of hydrophobin needed for amyloid fibril formation was much lower in the presence of the cell wall components schizophyllan (β-(1-3)β-(1-6)-glucan) and β-(1-3)-glucan. From this it is concluded that SC3 will not only assemble into amyloid-like fibrils at the cell wall of aerial hyphae but also of hyphae in contact with a hydrophobic solid such as the surface of a plant. Experimental data have shown that the resulting amyloid layer forms a molecular sieve and is also an insulating layer that does not allow transfer of electrons unless a mediator is present. The consequences of these properties for fungal growth and development will be discussed.

PS9.3

Microarray analysis of antifungal synergy between inhibitors of chitin synthases and beta-(1,3)-glucan synthase

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Fungi are major agents causing diseases in crops with a strong yield reduction and an alteration of the quality as main consequences. Further knowledge of the infection process can bring new insights to develop novel antifungal strategies. The cell wall of fungi is quite specific and essential for their viability and their aggressiveness towards their hosts. How plant-pathogenic fungi cope with cell wall alterations was explored in *Botrytis cinerea* as a model of necrotrophic and phytopathogenic fungus. Inhibition of the cell wall biosynthesis pathways was assessed using two commercial inhibitors, Nikkomycin Z and Caspofungin, targeting respectively chitin synthases and b-(1,3)-glucan synthase, responsible for the synthesis of the two major polysaccharides of the fungal cell wall. Each compound alone inhibits the *in vitro* growth of *B. cinerea*, and the combination of both inhibitors shows a significant synergistic effect. It was previously reported that inhibition of the synthesis of b-(1,3)-glucans and chitin leads to a compensation phenomenon in several fungi. Our data suggest that, in the case of synergy, *B. cinerea* was not able to compensate all the cell wall deficiencies induced by the application of both inhibitors together. Compensation phenomena are known to involve transcriptional regulations of cell wall related genes or several signalling pathways in other fungi.The effect of both compounds alone or in combination on the *B. cinerea* gene expression are studied using a microarray transcriptomic approach (*B. cinerea*, Nimblegen chip), and results will be presented in the communication.

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PS9.4

Cell Wall Stress Affects Chitin Synthase Delivery And Secretion In The Pathogen *Ustilago maydis*

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Chitin is an essential component of the fungal cell wall, whose composition and structural organization changes in response to the environmental conditions. Chitin synthase-containing vesicles are taken to the growth region by molecular motors, which transport their cargo along the fibres of the cytoskeleton. It was shown that chemical-induced cell wall stress increases the expression of chitin synthases in *Candida albicans*, which increases the amount of chitin in the cell wall and allows survival of the cell (Munro *et al*. (2007) *Mol Microbiol* 63: 1399–1413). In this study we investigate the effect of cell wall stress on the actual delivery of chitin synthase containing vesicles. Using the basidiomycete *Ustilago maydis* we show that Calcofluor White and Caspofungin treatments increase the chitin content in this plant pathogen. The effect on polar delivery, secretion and transport rates of the four polar localized chitin synthases (Chs5, Chs6, Chs7 and Mcs1; Weber *et al*. (2006) *Plant Cell* 18: 225–242) will be presented and wider implications discussed.

PS9.5

Self-assembly at air/water interfaces and chitin-binding properties of the small cell wall protein EPL1 from *Trichoderma atroviride*

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The protein EPL1 from *Trichoderma atroviride* belongs to the cerato-platanin family (IPR010829). Members of this family from other fungi appear to be readily recognized by other organisms, and therefore are associated with the induction of defense responses in plants and allergic reactions in humans. However, also in non-pathogenic fungi the genes of cerato-platanin family members are abundantly transcribed under various growth conditions, but the primary function of this protein family has not been elucidated yet. EPL1 is the major secreted protein in submerged cultivations with glucose as carbon source. The *epl1* gene is expressed under all so far tested growth conditions. We were now for the first time able to show that, similar to hydrophobins, the cerato-platanin protein EPL1 self-assembles at air/water interfaces and forms a protein layer. In contrast to hydrophobins, protein layers of EPL1 can be easily re-dissolved in water. Further, for EPL1 no statistically significant alteration of the surface tension of aqueous solutions was detected. In other fungi it was shown that cerato-platanin proteins are not only secreted into the medium, but also partially cell-wall localized. A potential chitin-oligomer binding site was recently found in structural NMR studies of the cerato-platanin protein CP from *Ceratocystis fimbriata* (de Oliveira *et al.* 2011, JBC). Carbohydrate-binding experiments with EPL1 now yielded biochemical evidence this protein indeed binds to different chitin-preparations, but interestingly not to complex fungal cell wall preparations of *T. atroviride*. This shows that this protein has lectin-like properties and advances our knowledge toward understanding the functional roles of cerato-platanin proteins. *Trichoderma* species have three *ep*l-genes. Transcript analysis revealed that only two of these genes - *epl1* and *epl2* - are expressed under most growth conditions. Single and double gene knockout strains were created in order to analyze the function of these genes in fungal growth and development.

PS9.6

Analysis of the cell wall integrity (CWI) pathway in *Ashbya gossypii*.

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Fungal cells are constantly exposed to rapidly changing environmental conditions, particularly considering their osmotic potential. The fungal cell wall takes on an important function in protecting the cell from external stresses and controlling intracellular osmolarity, but it is also required to maintain regular cell shape. However, cells must still be able to remodel the rigid structure of the cell wall to guarantee cell expansion during cell differentiation processes, but also in response to external cell stresses. While several signaling pathways contribute to the maintenance of the cell wall, it is the cell wall integrity (CWI) pathway that is most important in regulating changes made in the cell wall structure during vegetative growth, morphogenesis or in response to osmotic stress.

To characterize the CWI pathway in the filamentous ascomycete *Ashbya gossypii* we generated deletion mutants of several genes encoding for the most important components of the CWI pathway including potential cell surface sensors (e.g. *AgWSC1*), the coupled downstream protein kinases including a MAPK signaling module (*AgPKC1*, *AgBCK1*, *AgMKK1* and *AgMPK1*), but also downstream effector genes (e.g. *AgRLM1*). An initial characterization of the corresponding mutants is presented. While a mutant in *Agpkc1* shows a strong general growth defect found similarly in corresponding mutants in other fungi, mutants in several other components of the CWI pathway, in particular in the MAPK module, show a noticeable colony lysis phenotype. In addition, preliminary experiments showed that riboflavine production, a typical feature of *A. gossypii*, may be affected in some of the CWI mutants.

PS9.7

Efg1 Shows a Haploinsufficiency Phenotype in Modulating Cell Wall Architecture and Immunogenicity of *Candida albicans*

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The *Candida albicans* transcription factor Efg1 is known to be involved in many different cellular processes, including morphogenesis, general metabolism and virulence. Here, we show that Efg1 besides its manifold roles, also has a prominent effect on cell wall structure and composition, affecting strongly its structural glucan part. Deletion of only one allele of *EFG1* already results in severe phenotypes for cell wall biogenesis, comparable to deletion of both alleles, indicative of a severe haploinsufficiency for *EFG1*. The observed defects in structural setup of the cell wall together with the previously reported alterations in expression of cell surface proteins, result in altered immunogenic properties of strains with compromised Efg1 function. This is shown by interaction studies with macrophages and primary dendritic cells. The structural changes in cell wall carbohydrate meshwork presented here, together with the manifold changes in cell wall protein composition and metabolism reported in other studies, contribute to the altered immune response mounted by innate immune cells and the altered virulence phenotypes observed for strains lacking *EFG1*.

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Poster Category 1: Fungal Cell Biology

PR1.1

Role and spatio-temporal regulation of Rho1 in *Candida albicans*

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Small Rho G proteins such as Rho1 and Cdc42 are key regulators of the actin cytoskeleton. In *Saccharomyces cerevisiae*, Rho1 plays a critical role in cell wall integrity *via* beta-1,3-glucan synthase, protein kinase C (Pkc1) and the actin cytoskeleton. In *Candida albicans* Rho1, which has greater than 80% sequence identity with its *S. cerevisiae* and human counterparts, was shown to be essential. Using mutants, in which the sole copy of *RHO1* is under the control of the repressible Tetracycline promoter, we investigated the role of Rho1 in this organism. *PTEToffRHO1/rho1Δ* cells form hyphae in liquid media containing serum, but are defective in filamentous growth in embedded media and on solid media containing serum, indicating that Rho1 is required for invasive filamentous growth. As both Rho1 and Cdc42 are critical for filamentous growth, we set out to examine the spatio-temporal regulation of these proteins, under different growth conditions. Previous studies in *S. cerevisiae* have identified GTPase binding domains (GBD) that specifically bind activated G-protein: the Pkc1 Rho Interaction Domain (RID) has been used to localize activated Rho1, while Cdc42/Rac-Interactive Binding (CRIB) domain from Gic2 was used to localize activated Cdc42. We used similar GBDs, *i.e* a fusion of the RID from *C. albicans* Pkc1 with GFP to visualize activated Rho1, and the *S. cerevisiae* Gic2 CRIB domain fused to GFP, as Gic2 is absent from the *C. albicans* genome, to visualize activated Cdc42. The localization and dynamics of these sensors in wild-type cells and different mutants will be presented.

PR1.2

Systematic analysis of kinase and phosphatase function in *Candida albicans* yeast to hyphae transition

Vincent Corvest, Christian Schmauch, Bernardo Ramírez-Zavala, Tsvia Gildor, Daniel Kornitzer, Joachim Morschhäuser, Robert Arkowitz
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An important factor in the pathogenicity of *Candida albicans* is its ability to exhibit a large morphological variability in response to changing environmental conditions. In particular, the morphogenetic switch between the yeast and hyphal form is thought to be an important virulence trait, helping the organism to gain access to and to proliferate in new host niches. To elucidate new genes that regulate this yeast to hyphae transition we systematically analyzed all identifiable protein kinases, phosphatases and their regulators in this morphogenetic process. We have used an inducible expression strain library to identify proteins that, when expressed, promote or inhibit the yeast to hyphal transition. The resulting library comprises a total of 224 strains, covering 123 verified and putative kinases, 39 phosphatases, 25 kinase and 6 phosphatase regulators. In addition to these wildtype genes, >30 mutant alleles were generated. After screening we have initially identified 22 different proteins that induce filamentation. Among these 22 were 12 proteins that have been previously shown to be involved in hyphal morphogenesis, including members of different MAP kinase cascades and cell cycle regulators. In addition to these previously characterized genes, our screen has identified 10 genes whose role in *C. albicans* filamentation has not been described. Currently, we are examining the molecular functions of these proteins in the yeast to hyphal transition and whether they function in previously described pathways or define novel pathways. The analyses of 2 of these protein kinases will presented.

PR1.3

Disruption of the *Trichoderma atroviride Eng18B* ENGase gene affects growth, conidiation and antagonistic ability

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[1.] Swedish University of Agricultural Sciences, Forest Mycology and Plant Pathology [2.] University of Copenhagen, Institute of Medicinal Chemistry

The mycoparasitic fungus *Trichoderma atroviride* is known for its ability to antagonize plant-pathogenic fungi and is therefore used as a biocontrol agent in agriculture. The phylogenetic subgroup B5 of fungal glycoside hydrolase family 18 genes (GH18) encodes enzymes with mannosyl glycoprotein endo-*N*-acetyl-β-D-glucosaminidase (ENGase)-type activity. However, the biological roles of these enzymes are not known. The present work is a functional study of the *T. atroviride* Eng18B ENGase. Lack of N-terminal secretion signal peptide, transmembrane domains, or C-terminal GPI-anchor signal suggested a cytosolic localization of Eng18B. Gene expression analysis showed that *Eng18B* was induced in dual cultures with the fungal plant pathogens *Botrytis cinerea* and *Rhizoctonia solani*, although a basal expression was observed in all growth conditions tested. *Eng18B* disruption strains had reduced growth rates, but higher conidiation rates compared to the wild-type. However, growth rates on abiotic stress media were significantly higher in *Eng18B* disruption strains. No difference in spore germination, germ-tube morphology or in hyphal branching was detected. In addition, we determined that Eng18B is required for the antagonistic ability of *T. atroviride* against the grey mould fungus *B. cinerea* in dual cultures and that this reduction in antagonistic ability was partly connected to a secreted factor. These phenotypes were recovered by re-introduction of an intact *Eng18B* gene in *Eng18B* disruption strain. A putative role of Eng18B ENGase activity in the endoplasmatic reticulum associated protein degradation pathway (ERAD) of endogenous glycoproteins in *T. atroviride* is discussed in relation to the observed phenotypes.

PR1.4

Dual targeting of glycolytic enzymes to peroxisomes and cytoplasm is widespread in fungi

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In fungi, peroxisomes are not only important for metabolism of long-chain fatty acids but also for biosynthesis of cofactors and secondary metabolites. We observed that in the basidiomycetous fungus *Ustilago maydis* two core enzymes of glycolysis, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoglycerate kinase (PGK) show dual localization in the cytoplasm and peroxisomes. Both enzymes contain hidden peroxisomal targeting signals (PTS1), which are unveiled by alternative splicing or ribosomal readthrough. *U. maydis* mutants lacking peroxisomal targeting of GAPDH were reduced in virulence. In addition, these mutants displayed a significant growth defect if other NADH dependent dehydrogenases were excluded from the peroxisomes. Dual targeting of glycolytic enzymes via cryptic peroxisomal targeting signals was also observed in other fungi suggesting an evolutionary conserved function of glycolytic enzymes in peroxisomes. Interestingly, a variety of different mechanisms operate in different species to achieve dual targeting.

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PR1.5
Abstract Ref.617
Towards the extent and meaning of fungal alternative splicing
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During gene expression of higher eukaryotes, alternative splicing (AS) can produce various isoforms from one primary transcript. Thus, AS is thought to increase a cell's coding potential from a limited gene inventory. Although AS is common in higher plants and animals, its extent and use in fungi is mostly unknown. We undertook a genome-wide investigation of alternative splicing in 28 fungal species from the three phyla Ascomycota, Basidiomycota and Mucoromycotina, applying current bioinformatics data mining techniques. Our analysis reveals that on average over the investigated fungi, 6.2% of the genes are associated with AS. *Cryptococcus neoformans* and *Coccidioidis immitis* show outstanding rates of 18% and 13%, respectively. Intron retention is the predominant AS type in fungi, whereas exon skipping is very rare. The investigated Basidiomycota have on average higher AS rates (8.6%) and more diverse categories of AS affected genes than the Ascomycota (AS rate 7.0%, excluding yeasts). Contrarily, AS is nearly absent in strict yeasts. We hypothesize that AS is rather common in many fungi and could facilitate mycelial and thallic complexity.

PR1.6
The redox-active fungal metabolite, gliotoxin, induces transcriptional remodelling in *Saccharomyces cerevisiae*.
Stephen Hammel, Jennifer O,Brien, Stephen Carberry, David Fitzpatrick, Sean Doyle, Gary W. Jones
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The opportunistic pathogen *Aspergillus fumigatus* is the cause of invasive aspergillosis and has a significant impact on mortality of immunocomprised patients. The redox active, non-ribosomal peptide, gliotoxin, which is highly toxic towards animal cells and fungi is produced by this organism however the precise mechanism of its anti-fungal activity remains to be elucidated. We have identified and characterised proteins and genes that are up- or down-regulated in *A. fumigatus* and *Saccharomyces cerevisiae* in response to gliotoxin exposure. Proteomic analysis of *A. fumigatus* exposed to gliotoxin (14 micro g/ml) revealed *de novo* expression of a short chain dehydrogenase, eukaryotic translation elongation factor β1 and Cu/Zn super oxide dismutase; as well as a reduction in the expression of the mycelial catalase AFUA_6G10660 was observed.

To assess the effects on global gene expression in *Saccharomyces cerevisiae*following exposure to gliotoxin we applied RNAseq technology. Exposure to 16 or 64 µg/ml gliotoxin caused up- and down-regulation of genes involved in sulphur and carbohydrate metabolism and oxidative stress resistance.

A candidate gene approach identified *S. cerevisiae* mutants that exhibited altered sensitivity to gliotoxin exposure. Strains were chosen, based upon proteomic data which identified proteins showing induction/repression in *A. fumigatus* following exposure to gliotoxin. In contrast to a previous study that screened a *S. cerevisiae* gene deletions, we identified that deletion of the γ-glutamylcysteine synthase 1 gene (*GSH1*), confered resistance to gliotoxin (16 µg/ml) compared to wild type yeast strains. Also, mutants which lacked either the Cu/Zn superoxide dismutase gene (*SOD1*) or the Yeast activating protein (*YAP1*) gene resulted in hypersensitivity in the presence of gliotoxin compared to wild type strain. No growth difference was observed when using Δ*CTT1*, Δ*GSH2* or Δ*GLR1.*

Our data indicates that exposure to gliotoxin causes a complex *in vivo* transcriptional remodelling altering the expression of genes involved in metabolic pathways, while also appearing to induce oxidative stress in fungal cells.

PR1.7

Exploring the early symptomless phase of *Fusarium graminearum* infection of wheat ears

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Globally, *Fusarium graminearum* infections reduce cereal grain yield and contaminate grain with harmful trichothecene mycotoxins. *F. graminearum* forms an intimate intercellular association with the host species wheat whilst infecting the floral tissues (1). *TRI* gene expression required for deoxynivalenol (DON) mycotoxin production is maximal at the advancing hyphal front (2). This finding supports the hypothesis that DON plays a role in inhibiting plant defences. During this latent infection period, extracellular communication between live pathogen and host cells must occur, implying a role for secreted fungal proteins. In the current study, a refined *F. graminearum* secretome was predicted by combining several bioinformatic approaches (3). A comprehensive comparative genomic analysis involving 57 fungal and oomycete genomes revealed that very few predicted *F. graminearum* secreted proteins are species specific (3). A simplified linear system of rachis infection has now been used in conjunction with next generation sequencing (Illumina) and Affymetrix array technologies to explore globally the repertoire of *F. graminearum* genes specifically expressed at the intercellularly advancing hyphal front. To investigate gene function we are using the Barley Stripe Mosaic Virus vector to express transiently *in planta* sequences coding for secreted proteins of most interest.

1. Brown et al. (2010) Fungal Biology 114, 555-571.
2. Brown et al. (2011) Journal of Pathogens, Article ID 626345
3. Brown et al. (2012) PLoS One (in press)

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PR1.8

The Num1 protein in *Ustilago maydis* – a novel connection between splicing and transport

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In the corn smut fungus *Ustilago maydis*, sexual development is initiated by the fusion of two haploid sporidia, resulting in a filamentous dikaryon that is capable to infect the plant. Growth as a dikaryon requires an elaborate coordination of the cell cycle, the migration of the nuclei and polar hyphal growth. We have identified the Num1 protein with a pivotal function during these processes. Num1 is homologous to SPF27, a core component of the evolutionary conserved Prp19/CDC5 complex, an integral component of active spliceosomes. In addition to regulating spliceosome formation and splicing fidelity, the complex is involved in DNA damage repair and cell cycle checkpoint control. Hyphae of *num1* deletion strains exhibit pleiotropic polarity defects and a decreased splicing efficiency was verified upon realtime PCR analyses; additionally, the *num1* mutation affects the cell cycle and cell division as well as survival upon UV-irradiation. Using the Yeast-Two-Hybrid system and Co-immunoprecipitation analyses, we identified Cdc5 and Prp19, two further components of the Prp19/CDC5 complex, as Num1 interactors. Moreover, we identified proteins with functions during vesicle-mediated transport processes; in particular the kinesin 1 motor protein Kin1 was shown to physically interact with Num1. Both *num1* and *kin1* deletion strains exhibit identical phenotypes, corroborating their genetic interaction. As our data connect the splicing machinery and long distant transport we performed an mRNA-Seq experiment to elucidate which specific transcripts are affected for splicing in the *Δnum1* mutant on a global level. We will present our current view how these two disparate mechanisms may be linked.

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PR1.9

The *N. crassa* Bem46 protein is involved in ascospore germination and interacts with the F domain of anthranylate synthase component II

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The bud emergence (BEM) 46 proteins are evolutionarily conserved members of the *α/β*-hydrolase super family. The exact function(s) of the protein remain unknown. Vegetative hyphae, perithecia and ascospores of *Neurospora crassa* RNAi and over-expressing transformants develop normally, but hyphal germination from ascospores is impaired. These results indicate a role of BEM46 in maintaining cell type-specific polarity in *N. crassa*. The protein is localized in the perinuclear endoplasmatic reticulum and also forms spots near to the plasma membrane (Mercker et al. 2009). The use of Lifeact-TagRFP (Lichius & Read pers. comm.) and Bem46-eGFP in heterokaryons of *N. crassa* indicated that the Bem46 protein is not interacting with actin. Likewise, the use of the lipid raft-stainer TexasRed™ showed no co-localization with Bem46-eGFP. We currently analyze the potential co-localization of Bem46 with the eisosomal protein LSP1. To that end we cloned the corresponding *N. crassa* ortholog of *lsp1* and fused it to RFP.

A yeast two-hybrid approach was undertaken using a previously established *N. crassa* two-hybrid library (Seiler pers. comm.). We identified one interacting protein, the anthranylate synthase component II (Walker & DeMoss 1986). Further investigation showed that the BEM46 protein is likely to interact with the F domain of that protein, which is a N-(5'-phosphoribosyl) anthranylate isomerase. The interaction was confirmed *in vivo* by employing bimolecular fluorescence complementation assays.

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PR1.10

***Trichoderma* glutamate decarboxylase plays an important role during germination and hyphae development**

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Glutamate decarboxylase (GAD) catalyzes glutamate decarboxylation to gamma-aminobutyrate (GABA) and is the first enzyme of the GABA shunt cycle. Endogenous changes in GABA levels in fungi correspond with developmental changes during conidiation and/or germination. It was observed previously that GAD activity in *Trichoderma atroviride* F-534 changes during conidiation, early germination and stationary phase of submerged culture. A lack of GAD activity during batch culture on sucrose as a sole carbon source lead to several aberrations in morphology and development: increased formation of chlamydospore-like structures, longer lag-phase, lower germination rate, higher number of bipolar germ tubes and increased hyphae branching. It has been demonstrated before that in vitro analysis of GAD activity in *Trichoderma* decreased in presence of cyclosporine A, a calcineurin signaling pathway inhibitor. Wild type strain in cultures containing various concentrations of cyclosporine A exhibited similar phenotypic characteristics of the delta *gad* strains – increased bipolar germination and hyphae branching. The similar effects of cyclosporine A have been known in other filamentous fungi (*Neurospora, Aspergillus*). Bipolar germination and branching of *delta gad* strains remained unaffected in the presence of cyclosporine A indicating that GAD could function as a downstream effector of calcineurin.

PR1.11

Discovery of a novel peroxisomal function for biotin biosynthesis in *Aspergillus oryzae*

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Peroxisomes, ubiquitous organelles in eukaryotic cells, typically contain enzymes involved in β-oxidation of fatty acids, and play diverse roles in different eukaryotic organisms. In filamentous fungi, for example, they are required for the formation of Woronin body that is an organelle specific to filamentous ascomycetes and functions in the septal pore plugging upon hyphal injury. Furthermore, peroxisomes are reported to play fundamental roles during growth in humans, plants and also filamentous fungi. However, molecular mechanisms responsible for the severe growth defects of peroxisomal deficiency remain unknown.

Here we generated the *Aspergillus oryzae* strains with deleted *Aopex5* and *Aopex7* genes encoding the receptors for peroxisomal targeting signals PTS1 and PTS2, respectively. In addition to their growth defects in the minimal medium containing oleic acid as a carbon source, surprisingly, they exhibited growth defects on the glucose medium. By screening for required nutrient supplements, we found that the addition of biotin restores their growth defects. Genome database searches revealed that BioF protein/KAPA (7-keto-8-aminopelargonic acid) synthase, one of the biotin biosynthetic enzymes, has a PTS1 sequence. Both Δ*AobioF* and *AobioF*Δ*PTS1* strains exhibited growth defects in the absence of biotin, indicating that peroxisomal targeting of BioF is crucial for the biotin biosynthesis.

In conclusion we demonstrate a novel role for peroxisomes in biotin biosynthesis, of which steps are upstream of the terminal reactions in mitochondria.

PR1.12

Endogenous short RNAs generated by Dicer-2 and RNA-dependent RNA polymerase-1 regulate mRNAs in *Mucor circinelloides*

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Endogenous short RNAs (esRNAs) play diverse roles in eukaryotes including viral defence, transposon silencing, heterochromatin formation and post-transcriptional silencing of protein coding genes. Most esRNAs are produced from double stranded RNA (dsRNA) by Dicer and loaded into a RISC complex containing an Argonaute protein. esRNAs are grouped into different classes based on biogenesis and function but not all classes are present in all eukaryotic kingdoms. It is not clear what esRNA classes are present in fungi and whether they regulate the expression of protein coding genes. By deep sequencing of esRNAs in the wild type and silencing mutants we have shown that the opportunistic pathogen *Mucor circinelloides* does not contain microRNAs but produces new classes of esRNAs, which map to exons and regulate the expression of protein coding genes. The main class of these exonic-siRNAs (ex-siRNAs) is generated by RNA-dependent RNA Polymerase-1 (RdRP-1) and Dicer-like-2 (Dcl-2), although there is some redundancy between RdRP-1/RdRP-2 and Dcl-1/Dcl-2 proteins. Ex-siRNAs target the mRNAs of protein coding genes from which they were produced and their accumulation requires the function of the *argonaute-1* (*ago-1*) gene, which suggests that binding of ex-siRNAs to Ago-1 is required for their stabilization. Genes regulated by this class of ex-siRNAs are mainly involved in signal transduction and information storage and processing. Other classes of ex-siRNAs require different combination of Dicer and RdRP proteins for their biogenesis and target different set of genes. Our results expand the range of esRNAs in eukaryotes and reveal a new role for esRNAs in fungi.

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R1.13

Microtubule-dependent co-transport OF mRNPs and endosomes in *Ustilago maydis*

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Long-distance transport of mRNAs is important in determining polarity in eukaryotes. In *U. maydis* this process is mediated by the RNA binding protein Rrm4 which is a key component of large motile ribonucleoprotein complexes (mRNPs) shuttling along the microtubule cytoskeleton. Deletion of *rrm4* disrupts long-distant transport of mRNP and leads to defects in filamentous growth and a reduced virulence. Here we show that the plus end-directed UNC104/Kif1A-like Kinesin 3 as well as the minus end-directed split dynein Dyn1/2 are involved in the shuttling of the Rrm4-containing mRNPs. Kin3 transports the mRNPs to the poles of the growing hyphae, whereas Dyn1/2 mediates the retrograde movement of the mRNPs. Furthermore we demonstrate co-localisation of Rrm4-containing mRNPs with the t-SNARE Yup1 on endosomes, that are transported by the same set of motors. Interfering with endosome function, loss of Kin3 or removal of its lipid-binding pleckstrin homology domain abolishes Rrm4-dependent movement without preventing co-localisation of Rrm4 and Yup1-positive endosomes. This data suggests vesicle hitchhiking as new mechanism of mRNP transport.

PR1.14

Export of staurosporine and glutathione by Neurospora crassa

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ATP-binding cassete (ABC) transporters are membrane proteins that use the energy of ATP hydrolysis to drive the transport of substrates across membranes. The death-inducer drug staurosporine (STS) up-regulates the expression of the ABC transporter protein ABC-3 in Neurospora crassa, as revealed by microarrays, qPCR and western-blotting. Taking advantage of STS fluorescence, a flow cytometry methodology was devised to measure STS intracellular content. The abc-3 deletion mutant revealed to be deficient in STS export, resulting in high intracellular accumulation of the drug, and pointing to a role of the ABC-3 protein as a STS exporter. Accordingly, the mutant was found to be highly sensitive to STS-induced cell death, as revealed by impaired germination and increased DNA fragmentation, and classical inhibitors of ABC transporters enhanced the effects of STS. We also found that STS-induced cell death in N. crassa is accompanied by the export of reduced glutathione (GSH). Surprisingly, the abc-3 null mutant was found to be deficient in STS-induced glutathione export, suggesting an additional role for the ABC-3 protein in the transport of GSH. The hypothesis of a co-transport of STS and GSH by the N. crassa ABC-3 protein is currently under investigation.

PR1.15

Lipidomic-based approach to identify possible lipid compounds able to modify *Fusarium verticillioides* metabolism

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Fusarium verticillioides is one of the most important fungal pathogen to cause ear and stalk rot in maize, even if frequently asymptomatic. The interest for this fungus increased significantly after the discovery of fumonisins, hypothesized to contribute to *F. verticillioides* colonization and confirmed harmful to human and animal health. The understanding of the mechanisms associated with pathogenicity and fumonisin biosynthesis in *F. verticillioides* could help to mitigate *Fusarium* diseases.

Endogenous fungal oxylipins are known for their roles in carrying out pathogenic strategies to successfully colonize their host, reproduce, and synthesize toxins.

A synthetic medium (CDY), amended (FB_1-conducive) or not (FB_1-non conducive) with lyophilized maize, inoculated with *F. verticillioides* has been used for studying the importance of the lipid by-products such as oxylipins and their role in fumonisin B_1 (FB_1) formation. We analysed some molecular and physiological parameters: the expression of genes whose products are related to oxylipin synthesis (such as lipoxygenase, diol synthases and fatty acid oxidases) by relative Real Time PCR, the fatty acids and oxylipin profile using a lipidomic approach, i.e. combining LC-TOF with a robust statistical analysis (i.e. PCA) and the FB_1 biosynthesis by HPLC.

The results obtained indicate that the presence of lyophilised maize induces the up-regulation of all the genes analysed, the modification of lipid profile of *F. verticillioides* and the biosynthesis of FB_1 without significantly affecting fungal growth. The crucial role of lipid compounds in *F. verticillioides* lifestyle emerges also in this study.
Work supported by FIRB-RBFR08JKHI MIUR project

PR1.16

Vegetative hyphal fusion in epichloae endophytic fungi

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Vegetative hyphal fusion has been hypothesized to play an important role in the emergence of fungal diversity, including the generation of interspecies hybrids. The ability to form fused hyphae also appears to be related to fungal sexual reproduction, since genes involved in the former are often required for the latter as well. Here, we used epichloae endophytic fungi as models to study the potential correlation between vegetative hyphal fusion and fungal diversity, since they include both sexual (*Epichloë*) and asexual (*Neotyphodium*) species, with the latter consisting of interspecies hybrids and non-hybrids. Subcellular staining and confocal laser scanning microscopy revealed that the representative *Epichloë festucae* isolate Fl-1 forms complete hyphal fusion with a continuous cytoplasm being established. It also formed hyphal fusion during endophytic growth in a tall fescue plant. Calcofluor White staining and DIC optics revealed that most *Epichloë* isolates form vegetative hyphal fusion in cultures. A majority of *Neotyphodium* isolates were also capable of hyphal fusion; however, a substantial portion of non-hybrid *Neotyphodium* species appear to lack this ability. Our data favor the hypothesis that the ability to undergo hyphal fusion is important for interspecies hybrid formation, and support its importance in the emergence of fungal diversity.

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PR1.17

The role of *Aspergillus nidulans* myosin V, MyoE, in hyphal tip growth

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Aspergillus nidulans has a single myosin V heavy chain gene which we designate *myoE*. *myoE* is not essential but deletion of *myoE* causes abnormal hyphal morphology and branching anterior to the first septum. Tip extension in *myoE* deletants is slower than in controls, but because the hyphal diameter is greater, the growth rate (increase in volume/ unit of time) is only slightly reduced relative to myoE^{+} controls. Growth is extremely slow in the absence of MyoE and microtubules, but not completely inhibited. MyoE-GFP localizes to the Spitzenkörper (a vesicle supply center) and to puncta in the cytoplasm that move bidirectionally. Treatments with inhibitors show that the localization of MyoE at the Spitzenkörper is actin dependent but movement of some of the MyoE puncta in the hyphae is actin independent. Time-lapse imaging of SynA, a v-SNARE, reveals that in *myoEΔ* cells vesicles no longer localize to the Spitzenkörper. Photobleaching experiments with GFP-SynA show that in myoE^{+} cells, the SynA exocytic vesicles move rapidly from the non-bleached area of the hypha to the Spitzenkörper before incorporation into the plasma membrane, whereas in *myoEΔ* cells they do not localize to the Spizenkörper. Rather they move directly to the plasma membrane. Our data indicate strongly that MyoE function is required for the accumulation of vesicles at the Spitzenköper which facilitates faster tip extension and a normal hyphal shape.

PR1.18

MAPK pathways are key regulators of the chemotropic response in *Fusarium oxysporum*

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Fungal hyphae have the capacity to explore the surrounding environment and to grow towards gradients of tropically active cues. Chemotropic responses to nutrients, sex pheromones or host compounds play a crucial role in fungal development and virulence, but the underlying mechanisms remain poorly understood. We used a genetic approach to dissect chemotropism in the soilborne vascular wilt pathogen *Fusarium oxysporum*. A quantitative assay was used to measure directed growth of germ tubes towards different classes of compounds, including carbon and nitrogen sources, sex pheromones, plant secondary metabolites and root exudates. Mutants lacking the mitogen activated protein kinase (MAPK) Fmk1 or the transcription factor Ste12, two components of the conserved Pathogenicity MAPK cascade, were impaired in chemotropism towards nutrients, but fully responsive to α-pheromone and tomato root exudates. By contrast, Rho1 and Mpk1, two components of the cell integrity MAPK cascade, were specifically required for directed growth towards root exudates or α-pheromone. We further identified several transmembrane proteins involved in chemoattractant sensing, including the G-protein coupled receptor Ste2, the signalling mucin Msb2 and the tetraspan adaptor protein Sho1. Our results suggest that distinct MAPK signalling pathways mediate chemotropism of *F. oxysporum* towards nutrients and pheromone-like compounds.

PR1.19

Functional analysis of glycoside hydrolase family 18 and 20 genes in *Neurospora crassa*

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Glycoside hydrolase (GH) family 18 and 20 contain chitinolytic (chitinase and NAGase respectively) enzymes responsible for chitin degradation during various aspects of fungal biology. GH18 are phylogenetically divided into 3 groups (A, B and C), each further divided into subgroups.

Subgroup B5 genes encodes enzymes with ENGase deglycosylation activity. In this study,we investigated the functional role of 10 *N.crassa* genes coding for chitinases, 2 genes encoding ENGases and 1 gene encoding a NAGase, using gene disruption and qPCR techniques. The ENGasedisruption mutant Δ*gh18-10* showed slower growth rate on carbon rich media and on chitin plates when compared with the wildtype (WT), while it grew faster than WT during abiotic stress conditions. *gh18-10* was constitutively expressed during growth on carbon rich media, during carbon starvation conditions, on chitin plates and during fungal–fungal interactions. The function of gh18-10 may be connection with the endoplasmatic reticulum associated protein degradation process (ERAD), a stringent quality-control of protein folding. Furthermore, the two C2 subgroup chitinase genes *gh18-6* and *gh18-8* were both induced during fungal–fungal interactions. However, *gh18-6* was only induced during interspecific interactions, while *gh18-8* displayed the highest expression levels during self–self interactions. *gh18-8* also displayed a unique domain structure including 2 transmembrane domains, indicative of cell wall localization. These data suggest functional differentiation of *N. crassa* C2 chitinases; gh18-6 may function in aggressive interspecific interactions while gh18-8 may be a cell wall modifying enzyme.

PR1.20

Two functionally distinct RNA-dependent RNA Polymerases participate in the Dicer-independent degradation of endogenous mRNAs in *Mucor circinelloides*

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RNA-dependent RNA polymerases (RdRPs) participate in the initiation and/or amplification steps of the RNA silencing mechanism in different organisms. The role of these enzymes is not limited to silence exogenous sequences but they are required for production of endogenous siRNAs that mainly map to repetitive sequences and transposons. We have tested the role of two different *rdrp* genes in exogenous and endogenous silencing in the zygomycete *Mucor circinelloides*, a basal fungus evolutionary distant from other fungal model organisms. Analysis of *rdrp*$^{-}$ disruption mutants indicates a functional diversification of both genes in transgene-induced silencing. Gene *rdrp-1* participates in the initiation of silencing by sense transgenes by producing antisense transcripts derived from the transgenes, but it does not have a role in dsRNA-induced silencing. On the other hand, *rdrp-2* is required for efficient amplification of silencing and accumulation of siRNAs regardless the nature of the silencing trigger. Differences in the phenotype of mutants of each gene highlight the cellular and physiological relevance of the functional diversification. Small RNA analyses in the wild-type strain and mutants affected in silencing genes demonstrate that both *rdrp* genes are involved in a novel degradation process of endogenous mRNAs that is *dicer*-independent. This new regulatory pathway, which does not include discrete sRNA molecules, mainly regulates the level of expression of housekeeping genes by specific degradation of mRNAs. Our results expand the role of RdRPs in gene silencing and reveal a new RNA degradation process that could represent the initial step in the evolution of RNA silencing.

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PR1.21

Two Highly Conserved Arginines at the N-terminus of the NDR Kinase COT1 are Required for its Proper Function Via Interactions with MOB2A/2B

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Nuclear Dbf2-related (NDR) kinases are important for growth and development in *Neurospora crassa* and other filamentous fungi, and require the physical interaction with MPS1-binding (MOB) proteins for proper activity and function. Dysfunction of the *N. crassa* NDR kinase COT1 leads to cessation of tip extension and hyperbranching. In order to further characterize COT1/MOB interactions, we mutated two highly conserved Arg residues (R167 and R203) at the N-terminal region of COT1 (the MOB-binding domain) to Ala and inserted these alleles at the *cot-1* locus. Both point-mutated *cot-1* strains exhibit, at different extents, abnormal hyphal morphology, typified by hyphal swelling, dichotomous branching and cytoplasmic leakage along the aerial hyphae. In addition, these mutants produce less macroconidia. In spite of the mentioned defects, general colony morphology and growth rate of the mutants was only slightly affected. Interestingly, the hyphal and conidiation defects of both arginine mutants were suppressed in a Δ*mob-2b* background. In contrast, the Δ*mob-2a;cot-1*^{R203A} strain grew as a small fluffy colony that produced massive amounts of aerial hyphae with delayed carotenoid biosynthesis. Hyphae in this strain were slow growing, highly dense and hyper-branching was evident. These results establish the involvement of R167 and R203 in the regulation of COT1 function and in the interactions between COT1 and MOB2A/2B. As we have determined the occurrence of a physical interaction between the Arg methyl transferase SKB1 and COT1, it is conceivable that Arg methylation has a role in regulation of COT1 function.

PR1.22

High temperature growth arrest in of *Cryptococcus neoformans* mediated by APE4 an aspartyl amino peptidase involved in authophagy.

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Cryptococcus neoformans is an opportunistic pathogen that can cause fungal meningitis in immune compromised patients. The treatment choices for cryptococcosis are limited and resistant strains have being reported. However, in order to develop new drugs against this mycosis it is necessary to broad the knowledge about this yeast biology. Its ability to grow at high temperature is an important virulence factor and a key feature during pathogenesis. Therefore, we have screened a mutant collection generated by *Agrobacterium* random insertional mutagenesis aiming to uncover new genes that may be involved in high temperature growth. The inactivation and deletion of *APE4*, an aspartyl amino peptidase which localizes to degradation vesicles and is involved in autophagy in *S. cerevisiae*, leads to growth arrest at 37°C. We will present the role of this gene on the virulence and biology of *Cryptococcus neoformans*.

PR1.23

The role of *Cryptococcus neoformans* URA4 on de novo pirimidine biosynthesis pathway and its impact upon high temperature growth.

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Cryptococcus neoformans is an opportunistic fungus that has the ability to infect immune compromised individuals causing mild respiratory infection, pneumonia and meningitis, leading to the death if untreated. The increase of the immune compromised population and isolation of strains resistant to the treatment the knowledge about the biology of this organism, as well as its virulence factors, became desirable. Among its virulence factors, special attention has been dedicated to high temperature growth which is a key feature during pathogenesis. We have created and screened a library generated by *Agrobacterium* random insertional mutagenesis aiming to uncover new genes that may be involved in high temperature growth that could also be a new target for antifungal therapy. We found that the inactivation and deletion of *URA4* gene leads to growth arrest at 37º C. *URA4,* codes for a dihydroorotase involved in the conversion of carbamoyl aspartate to dihydroorotate in the *de novo* pyrimidine biosynthesis pathway. We will present the role of this gene on the virulence and biology of *C. neoformans*.

PR1.24

Functional characterization of *Aspergillus nidulans* RpdA: identification of complex partners and subcellular localization

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

PR1.25

The impact of ornithine and arginine biosynthesis on siderophore production of *Aspergillus fumigatus*

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The opportunistic fungal pathogen *Aspergillus fumigatus* produces extracellular siderophores for iron uptake and intracellular siderophores for storage and distribution of iron. Moreover, *A. fumigatus* employs a second high-affinity iron acquisition system, reductive iron assimilation (RIA). Siderophore biosynthesis (SB) but not RIA is essential for virulence. The main precursor of siderophores, ornithine, can be produced from glutamate in the mitochondria or cytosolic hydrolysis of ornithine-derived arginine.

Here, the impact of inactivation of mitochondrial ornithine biosynthesis (*ΔargEF* mutant lacking N-acetylglutamate kinase/ N-acetylglutamylphosphate reductase) and cytosolic arginine biosynthesis (*ΔargB* mutant lacking ornithine transcarbamoyl transferase) on siderophore production was studied. Both *ΔargEF* and Δ*argB* are arginine auxotrophic. Growth of *ΔargEF* but not *ΔargB* is partially rescued by ornithine supplementation. Blocking RIA by ferrous iron chelation inhibited growth of *ΔargEF* but not *ΔargB.* Siderophore production of *ΔargEF* decreased while that of *ΔargB* increased with declining arginine availability. Taken together, these data indicate that the siderophore system is mainly fueled by mitochondrial rather than cytosolic ornithine production and that mitochondrial ornithine biosynthesis is feedback inhibited by arginine. In agreement with the SB defect, *ΔargEF* displayed a dramatically reduced cellular ornithine content. In contrast, the arginine and polyamine contents were wild type-like, indicating prioritization of the later two biosynthetic pathways over SB. Consistent with cellular balancing of SB and arginine metabolism, arginine was recently identified to allosterically activate the ornithine monooxygenase SidA and consequently SB-mediated ornithine consumption.

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PR1.26

Highly conserved signalling multi subunit protein complexes in the filamentous fungus *Sordaria macrospora*

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The homothallic ascomycet *Sordaria macrospora* can be easily applied as a model organism for research in cellular development. From a set of sterile mutants showing only protoperithecia formation, we were able to characterise the PRO-proteins involved in sexual development. The characterisation of three PRO proteins led to the identification of two different conserved multi subunit complexes involved in signalling during cellular differentiation [1, 2, 3]. PRO40, the homolog of Soft from *Neurospora crassa*, supposedly works as a scaffold in the PRO40-MAPK interaction network conserved in ascomycets. Besides the MAPK cascade kinases and PRO40, the multifunctional protein SPG20 is involved in this complex. For the first time in filamentous fungi the highly conserved striatin interacting phosphatase and kinase (STRIPAK) was characterised in *S. macrospora* via different approaches [4]. PRO11 is the homolog of human striatin and probably is a scaffolding subunit in this complex [1]. PRO22 is also highly conserved among eukaryotes and is homologous to human STRIP ("striatin interacting protein") [3]. As part of the STRIPAK complex, it was possible to show the interaction of PRO22 and the scaffolding subunit PP2AA of the heterotrimeric protein phosphatase PP2A [4]. These two multi modular protein complexes play a crucial rule in signalling during the transition from vegetative growth to sexual development and are possibly linked through scaffolding or anchoring proteins to a single large and highly conserved signalling multi subunit protein complex.

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[3] Bloemendal et al. (2010), Eukaryot Cel, [4] Bloemendal et al. (2011), in prep.

PR1.27

In-Depth analysis of Pectin Degradation and Catabolism by the Model Filamentous Fungus *Neurospora crassa*

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Pectin, e.g. isolated from apple pulp or citrus peel, is a well-known commodity of the food and medical industry. Although in varying amounts, Pectin is present in all plant biomass, being the main constituent of the middle lamella, which is the cell wall layer connecting adjacent cells.

As such, it is also a source of fermentable sugars in the production of biofuels or value-added by-products from plant-based feedstocks. Our institute is using *Neurospora crassa* to study the enzymatic depolymerization of plant cell walls by filamentous fungi, as it is not only an efficient cell wall degrader, but also a well-established model system. In the present study we are analyzing the "toolbox" that *Neurospora* unfolds when growing on Pectin as its sole carbon source. Using a combination of shotgun proteomics and functional genomics we are trying to identify the Pectin-specific secretome as well as all the genes involved in the subsequent catabolism of the resulting sugars. *Neurospora* was found to secrete more than 70 proteins on Pectin and orange peel from at least 25 carbohydrate-active families. RNA sequencing allowed us to screen the entire genome for genes specifically induced by Pectin. Furthermore, strains containing deletions in genes (potentially) involved in Pectin catabolism were analyzed for their phenotype when grown on pectin and any difference in their rate of pectin consumption in comparison to the WT. Our results demonstrate that *Neurospora*, with its well-established genomics resources in combination with proteomic tools, is ideally suited to characterize fungal polysaccharide degradation in unparalleled detail.

PR1.28

Fungal siderophore excretion is mediated by a new fungal-specific subfamily of ABC transporters

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The opportunistic human pathogen *Aspergillus fumigatus* produces extra- and intracellular siderophores for acquisition, storage and intracellular distribution of iron. The siderophore system is essential for virulence and therefore represents an attractive antifungal target. Here we report the functional characterization of three ABC transporters, termed AbcB, AbcC, and AbcD. All three transporters are transcriptionally repressed by iron via the GATA-factor SreA. Moreover, the genes encoding AbcB and AbcC are located within siderophore metabolic gene clusters indicating a function in siderophore metabolism. Enhanced green fluorescent protein (EGFP)-tagging localized AbcB in the plasma membrane. Inactivation of AbcB blocked excretion of the siderophore fusarinine C (FsC) and increased intracellular accumulation of FsC degradation products. Deletion of AbcC decreased excretion of triacetylfusarinine C (TAFC) but increased excretion of its precursor FsC. Inactivation of AbcD reduced siderophore excretion only in AbcB- or AbcC-deficient backgrounds, implying mutual compensation of these ABC-transporters. Inactivation of all three ABC transporters completely blocked siderophore excretion. Consistently, inactivation of AbcB, AbcC, either two of the three, and particularly all three ABC transporters impaired growth during iron starvation but not iron sufficiency. Phylogenetic analysis revealed that AbcB, AbcC and AbcD are members of a new subfamily of the ABC transporter superfamily conserved in all siderophore-producing but not siderophore-lacking fungal species, indicating involvement of all subfamily members in siderophore metabolism.

PR1.29

Identification and Characterization of a Novel Secondary Metabolite Gene Cluster in *Aspergillus nidulans.*

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Fungal secondary metabolites are a rich source of medically useful compounds due to their pharmaceutical and toxic properties. Sequencing of fungal genomes has revealed numerous secondary metabolite gene clusters, yet products of many of these biosynthetic pathways are unknown since the expression of the clustered genes usually remains silent under normal laboratory conditions. We discovered a novel secondary metabolite in *Aspergillus nidulans* by predicting a biosynthetic gene cluster with genomic mining. We identified a Zn(II)2Cys6–type transcription factor and demonstrated its role as a pathway-specific activator for the cluster genes. Quantitative real-time PCR and DNA array analysis showed that overexpression of the putative transcription factor leads to upregulation of seven genes in the predicted cluster area. The GC/MS analysis of the strain with an activated gene cluster revealed production of a novel secondary metabolite for *Aspergillus nidulans*. In addition, we noted an increase in sexual fruiting body formation and a reduction in conidiation in the metabolite producing strain. The change in the fungal morphology seen in the overexpression strain could indicate a link between development and activated secondary metabolism.

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PR1.30

Protein map of cytosolic proteins of the Agaricomycete *Schizophyllum commune*

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The white-rot fungus *Schizophyllum commune* is a model organism for microbiological and molecularbiological studies. Since the genome of *S. commune* has been published, it is easy to perform studies not only on the genome but also on the proteome of this fungus. Therefore, we prepared 2 dimensional protein gels with a pH range from 3 to 11 for cytosolic proteins of the monokaryotic strain 12-43, which acts as the reference strain. More than 700 protein spots were detected on the protein gel and 527 were chosen for mass spectrometry analysis *via* MALDI-TOF.

The reference strain was compared to a second monokaryotic strain 4-39 and a dikaryotic strain, which demonstrates the main mode of life of *S. commune*. Several differences could be ascertained. There are additional proteins in the monokaryotic and dikaryotic strain as well as differences in the expression of proteins compared to the mastergel of strain 12-43. In contrast, a few proteins are missing in the comparison specimen.

With this study we will simplify proteome comparisons of wildtypes and mutants of *S. commune* by publishing the mastergel as protein map with detailed information of the analyzed protein spots.

PR1.31

The role of calmodulin during cell fusion and colony initiation in *Neurospora crassa*

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Calcium signalling is involved in regulating many important processes in filamentous fungi including spore germination, hyphal growth, mechanosensing, stress responses, the cell cycle and circadian rhythms. As the primary intracellular Ca^{2+} receptor, calmodulin (CaM) converts Ca^{2+} signals into enzymatic signals by regulating the activity of numerous target proteins. We have found that both Ca^{2+}-free medium and two CaM antagonists (calmidazolium and trifluoperazine) selectively inhibit conidial anastomosis tube (CAT) fusion during colony initiation in *Neurospora crassa.* These results indicate that Ca^{2+}/CaM signalling is probably involved in regulating this process. In order to further analyse the role of CaM in regulating cell fusion during colony initiation, we have been imaging GFP labelled CaM in living cells. For this purpose we use both the native promoter and P*ccg-1* promoter of *N. crassa* to express N-terminal or C-terminal GFP tagged CaM. In germ tubes and CATs, CaM localized as dynamic dots associated with the plasma membrane and within the cytoplasm. In germ tubes CaM also localized at developing septa (CATs lack septa). However, CATs that were undergoing chemoattraction towards each other showed a distinctly different pattern of localization at their growing tips; CAT tips showed a very pronounced accumulation of Cam whilst germ tube tips did not. We are currently co-localizing GFP labelled CaM with RFP labelled for nuclei, F-actin and microtubules to analyse their dynamic temporal and spatial relationships with each other.

PR1.32

Investigating the role of actin and microtubule cytoskeletons in endocytosis of plant pathogen *Ustilago Maydis*

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Endocytosis is essential for pathogenic development in *Ustilago maydis*. It has been shown that the initiation of pathogenicity relies upon the cell's ability to recognise the pheromone *(a1 or a2)* released from its mating partner and to form conjugation hyphae *(Urban et al., 1996b)*. In yeast the actin cytoskeleton is essential for endocytosis and the absence of actin function can cause blocked internalisation of endocytic cargoes *(Raths et al., 1993)*. We have produced a synthetic, fluorescently labelled pheromone marker and observed its localisation over time in *Ustilago maydis* cells. Our results strongly suggest the intra-cellular movement of the pheromone is driven by receptor-mediated endocytosis. Destabilisation of the cytoskeleton, using either benomyl or latrunculin A, inhibits the translocation of pheromone to the vacuole. Furthermore, we show the microtubule-dependent motors kinesin 3 and dynein are essential in the transport of the pheromone to the vacuole.

PR1.33

HYM1 functions as dual scaffold for NDR and MAP kinase pathways in *Neurospora crassa* to coordinate hyphal polarity with cell communication and sexual development

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HYM1/MO25-type proteins function as general co-activator proteins of germinal center kinases (GCKs), which activate nuclear-DBF2-related (NDR) kinase pathways and thereby regulate cellular morphogenesis and proliferation. Here we show that, in addition to a scaffold function of HYM1 for the POD6-COT1 GCK-NDR kinase complex in *N. crassa*, HYM1 is also critical for the NRC1-STE7-MAK2 MAP kinase pathway, which regulates vegetative cell-cell communication and sexual development. HYM1 interacts with all three kinases of the MAK2 MAP kinase cascade and co-localizes with MAK2 at the apex of growing cells. Deletion of *hym-1* phenocopies all defects observed for MAK2 pathway mutants by abolishing MAK2 activity. A NRC1-STE7 fusion protein reconstitutes MAK2 signaling, while constitutive activation of the individual MAPKKK and MAPKK proteins of the MAK2 pathway does not. These data identify HYM1 as novel scaffold for the NRC1-STE7-MAK2 pathway and establish HYM1 as central player for coordinating NDR and MAP kinase signaling during cell polarity, cell communication and sexual development.

PR1.34

The α-tubulin B-encoding gene and its expression in the phytopathogenic fungus Botrytis cinerea

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The plant pathogen fungus *Botrytis cinerea* is able to infect more than 250 hosts. It is responsible for major losses in crops, and grapes in particular in countries like France. The fungus life cycle relies on distinct developmental stages and specific cellular structures like appressoria, infection cushions, conidiophores and apothecia. Based on the important role that cytoskeleton plays in cellular organization and shape in all eucaryotes, attention was given to tubulins and their putative specific role in the virulence of *B. cinerea*. One β-tubulin and two α-tubulin encoding genes are found in *B. cinerea*'s genome. Phylogenetic analyses revealed that orthologs to the α-tubulin A gene exist in all asco- and basidiomycetous species whereas the α-tubulin B gene seems to be present in some ascomyceta only. In *B. cinerea*, tubulin A and B share 69% identity at the protein level, and the B gene is always more expressed than A. Besides, the expression of both genes follow a similar profile during growth and conidia-derived development. Interestingly, a peak of expression is observed early in development and expression is higher in infection cushions than in mycelium. To understand these changes in expression, transcriptional fusions and successive deletions were used to identify putative regulatory regions in the B gene promoter. In parallel, bioinformatic motifs searches were conducted to find putative DNA sequences involved in this regulation.

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PR1.35

Interrogating the transcriptomes of developmental mutants to identify conidiation-specific genes in *Fusarium graminearum.*

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The vast majority of fungi propagate clonally through the formation of asexual spores (conidia). To produce conidia, fungi must undergo a defined program of morphological development, which presumably requires the coordinated efforts of many individual genes and signal transduction pathways. In the cereal pathogen, *Fusarium graminearum*, the morphological transition from filamentous growth to conidiation is critical for dissemination and infection. In spite of this, very little is known about the genetic regulation of this important developmental process. From genetic screens designed to identify genes regulating specific aspects of morphogenesis in *F. graminearum*, we identified several insertional mutants impaired in conidiation, which variously display a range of loss- and gain-of-function phenotypes. One mutant that fails to produce conidia, designated 8B5, contains an insertion within a putative bi-directional promoter of genes FG_10779 and FG_10780. To further understand the impact of the insertion on gene regulation, genome-wide analyses of gene expression were performed with microarrays [Fusarias520715 Affymetrix GeneChip] on the wild type and 8B5 mutant strain under conditions either favorable or unfavorable for asexual development. A total of 39 genes exhibited altered expression (>4 f.c.) in both wild-type and 8B5 under various culturing conditions. Analysis of these candidate genes revealed the presence of putative transcription factors (such as a white-collar homolog), structural genes (such as a chitin synthase homolog), five orphan genes, as well as two putative gene clusters. Additional findings stemming from our functional and comparative analyses of developmental mutants will be presented.

PR1.36

Identification Of Transcriptional Regulators Involved In Azole Resistance In The Pathogenic Fungus *Aspergillus fumigatus*

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Aspergillus fumigatus is an opportunistic human pathogen responsible for invasive and chronic disease. Treatment of fungal infections is becoming increasingly difficult due to the emergence of resistance particularly to the azole drug class. The key causes of clinical azole resistance are point mutations in the drug target (CYP51) however isolates with non-target resistance mechanisms are being frequently isolated in our patient population. In an attempt to identify some of these novel mechanisms we are exploring the role of transcription factors in itraconazole reistance.

Availability of the complete genome sequence of *A. fumigatus* has enabled us to identify 450 transcription factors. To enable disruption of all 450 of these genes we have devised a systematic approach to gene knockout coupling a 2-step PCR fusion approach to a 96-well transformation method. Our process, from the automated design of gene KO primers to generation of KO cassettes strains and subsequent isolation of mutant isolates takes less than one week per 96 strains. Several of the transcription factors we have identified appear to be critical to the viability of *A. fumigatus* whilst many other mutants exhibit a range of gross phenotypic effects. We are currently analysing our collection of gene deletion mutants to identify those transcription factors which are either resistant or sensitive to azoles and other antifungal agents.

PR1.37

The influence of the conditions of leaf extract on perithecia formation of *Pleospora herbarum*

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Many phytopathogenic fungi have both teleomorph and anamorph, and differentiate from hyphae depending on the conditions in the host plants. The fungi often lose the morphogenic ability and grow only in the hyphae form by the succession of in vitro culture. They, however, recover the ability if the hyphae could infect to the host plants. We have developed the method (ALP medium) that many fungi could form perithecia in vitro using lightly boiled gardenia and hydrangea leaves (Furukawa and Kishi 2000). These facts indicate that some common factors which induce perithecia formation in many fungi must exist in the leaves. We tried to extract the factors and re-investigate physical conditions, especially light wavelength, to induce perithecia. *Pleospora herbarum* (Pers.) Rabenh was used for the examinations. As a result, the activity existed in the oxidized aqueous fraction, which induced the perithecia and conidia abundantly under the dark condition. Fatty acids and the related compounds, which were reported to induce perithecia and conidia in *Aspergillus nidulans* (Calvo et al. 1999, Tsitsigiannis et al 2004, 2007), did not induce perithecia or conidia under light or dark conditions. Blue light, which is essential for conidia formation in *Neurospora crassa* (Olmendo et al. 2010), was not needed for *P. herbarum* on the medium containing oxidized leaf extract, though it was essential for perithecia production on other media. Those imply the morphogenic switch of phytopathogenic fungi is different from that of saprozonic fungi.

PR1.38

Subcellular localization of maltose permease (MalP) in response to carbon sources in *Aspergillus oryzae*

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A gene cluster (*MAL* cluster) involved in maltose utilization is also required for production of amylolytic enzymes in *Aspergillus oryzae*. In particular, maltose permease encoded by the gene *malP* in the *MAL* cluster is essential for uptake of maltose that induces the amylolytic genes through the activation of the transcription factor AmyR. The *malP* gene expression is induced by maltose and repressed by glucose [1]. However, it has not yet been examined how MalP is regulated at the protein level in response to carbon sources, although MalP is thought to be degraded through the endocytic pathway in the presence of glucose as MAL63 in *Saccharomyces cerevisiae*. In this study, we examined the effect of various sugar species on endocytosis of the MalP protein.

MalP fused to sGFP (MalP–GFP) localized at the plasma membrane, when expressed by own promoter in maltose medium. After addition of glucose, MalP–GFP was promptly internalized and delivered to the vacuole. This internalization was prevented by the addition of latrunculin B, an actin depolymerizing agent, indicating that MalP was targeted to the vacuole by endocytosis. Effect of the sugar species including glucose analogs on the internalization of MalP–GFP was investigated by fluorescence microscopy. Consequently, fructose, mannose, and 2-deoxyglucose as well as glucose triggered the internalization of MalP–GFP to the vacuole, whereas xylose, 6-deoxyglucose, and 3-*O*-methylglucose had no effect on MalP–GFP localization.

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PR1.39

Establishment of tools for the analysis of vesicle traffic in *Aspergillus nidulans*

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In order to allow the extreme polar hyphal growth of filamentous fungi membrane lipids and cell wall synthesizing enzymes are needed constantly in the growth area. This is achieved by a continuous flow of secretory vesicles from the Golgi apparatus to the growing tip. For efficient growth, cell end markers and enzymes anchored in the plasma membrane are removed from the plasma membrane subapically by endocytosis. Thus filamentous growth depends on different populations of vesicles, which may use different routes for transportation. In order to investigate the dynamics and role of different vesicles, we studied the localization of different proteins of both the endocytotic and exocytotic pathway. SynA, a v-SNARE protein and clathrin heavy-chain are components of both pathways. In addition, three putative secreted proteins, β-glucosidase with a signal peptide (BglA) and two proteins without a signal peptide, the glucan synthase regulatory protein (GlsA) and the chitin synthase (ChsB), were studied.

By co-localization with fluorescently labeled microtubules we showed transport along these filaments. SynA, BglA, GlsA und ChsB were transported by conventional kinesin towards the Spitzenkörper, were they accumulated. After deletion of the kinesin-1 the anterograde transport was taken over by other motor proteins like kinesin-3.

At the tip the secretory vesicles containing SynA fused with the plasma membrane and were internalized at the subapical region. The internalized vesicles called early endosomes were transported by dynein.

PR1.40

Peroxisomal Localization Of Siderophore Biosynthesis In *Aspergillus* SSP.

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Virtually all organisms require iron as an essential nutrient. Siderophores, low molecular mass, iron-specific chelators, play a central role in iron acquisition, iron storage and virulence of various phyto- and animal-pathogenic fungi (1-3). However, the subcellular localization of siderophore biosynthesis is unknown. *Aspergillus fumigatus* and *Aspergillus nidulans* produce two major siderophores: extracellular triacetylfusarinine C (TAFC) for iron acquisition and intracellular ferricrocin (FC) for iron storage. Interestingly, two TAFC biosynthetic enzymes, SidH (*cis*-anhydromevalonyl CoA-hydratase) and SidF (N^5-hydroxyornithine:*cis*--anhydromevalonyl CoA-N^5 transacylase), possess putative peroxisomal targeting signals type 1 (PTS1), which are highly conserved in their orthologs of *Aspergillus ssp.* . Using N-terminal GFP-tagging of SidH and SidF, we could show that the TAFC biosynthesis is, in part, localized in peroxisomes. Addionally SidH and SidF were localized in peroxin mutant strains of *A. nidulans* to confirm PTS1 dependent import. Peroxins are proteins critical for peroxisome biogenesis (e.g. PexC) or protein targeting into peroxisomes (e.g. PexE). Furthermore peroxin mutant strains were compared to the wild type with respect to siderophore biosynthesis and growth rate during iron-replete and iron depleted conditions to show the role of peroxisomes in iron acquisition.

This is the first description of peroxisomal localization of siderophore biosynthetic steps beside known peroxisomal metabolic pathways as ß-oxidation and ergosterol biosynthesis.

PR1.41

The interplay of vacuolar and siderophore-mediated iron storage in the opportunistic fungal pathogen *A. fumigatus*

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Iron is an essential element for all eukaryotes but its excess is deleterious. Iron homeostasis results from tight regulation of iron acquisition and iron storage. *A. fumigatus* produces the extracellular siderophores triacetylfusarinine C (TAFC) and fusarinine C (FSC) for iron uptake and the intracellular siderophores ferricrocin (FC) and hydroxyferricrocin for iron distribution and storage. Siderophore biosynthesis is important for the adaptation to iron starvation and therefore crucial for virulence. Intracellular iron excess has been shown to increase the content of FC-chelated iron and the expression of AFUA_4g12530, termed CccA, which shows similarity to the vacuolar iron importer Ccc1 of *S. cerevisiae*. These data indicate a role of both the vacuole and FC in iron detoxification.

Green fluorescence protein-tagging confirmed localization of CccA in the vacuolar membrane. During high iron conditions genetic inactivation of CccA impaired growth, in particular in combination with derepressed iron uptake due to deficiency in the iron regulator SreA. In contrast, overproduction of CccA increased iron resistance. Inactivation of FC biosynthesis did not affect iron resistance. Lack of FC, CccA and in particular both, increased the cellular content of iron chelated by FSC/TAFC breakdown products. A delayed release of iron from FSC/TAFC degradation products might represent another iron detoxifying mechanism.

Our data indicate that vacuolar rather than FC-mediated iron storage is the major iron detoxifying mechanism of *A. fumigatus.*

PR1.42

Regulatation of the BUD3-BUD4 landmark complex by the NDR kinases DBF2 and COT1 during septum formation in *Neurospora crassa*

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Cytokinesis is essential for cell proliferation, yet the mechanisms for determining the cell division plane are only poorly understood. Our data indicate that the anillin BUD4 marks septum placement by organizing the RHO4-BUD3-BUD4 GTPase module and that this complex is controlled through two NDR kinase signaling cascades, the septation initiation network (SIN) and the morphogenesis network (MOR). By using a combination of live cell imaging, genetic and biochemical approaches, we show that COT1 and DBF2 localize to the constricting septum and are regulated by the two specific upstream germinal center (GC) kinases POD6 and NCU04096 that phosphorylate the respective NDR kinase at their C-terminal hydrobhobic motif. A third GC kinase, MST1, functions as generic regulator of both NDR kinases. *cot-1* and *dbf-2* mutants display opposite septation defects. Epistasis analysis of *sin*, *mor* and *bud* double mutants places the SIN upstream of the MOR, which in turn inhibits BUD function. We demonstrate that COT1, but not DBF2, binds to and phosphorylates BUD3 and BUD4. Mutational analysis of BUD3 identifies Ser798, located within an amphiphatic helix of BUD3 that seems to be phosphorylated by COT1. Localization of this amphiphatic helix at septa is only possible in its nonphosphorylated form. In summary, our data suggest a preliminary model, in which the MOR kinase COT1 phosphorylates BUD3 and BUD4 and that this phosphorylation inhibits cortical localization and function of the BUD complex.

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PR1.43

ApsB, a component of microtubule-organizing centres resides in microbodies

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In *Aspergillus nidulans* septum-associated microtubule-organizing centres (sMTOCs) together with spindle pole bodies (SPBs) polymerize cytoplasmic microtubules. Previously, we identified a novel MTOC-associated protein, ApsB (*Schizosaccharomyces pombe* mto1), whose absence affected MT formation from sMTOCs more than from SPBs, suggesting that the two protein complexes are organized differently (Suelmann et al., 1998). sMTOCs share at least two further components, gamma-tubulin and GcpC (*S. pombe* Alp6) with SPBs, and ApsB interacts physically with gamma-tubulin. Surprisingly, ApsB contains a peroxisomal targeting sequence, PTS2, which could be replaced by a PTS1 motif at the C-terminus of ApsB (Zekert et al., 2010). Here, we show that fractions of ApsB and gamma-tubulin indeed reside in microbodies. Both proteins co-fractionated with microbodies in sucrose gradients. Protein protection assays revealed that ApsB and gamma-tubulin reside inside microbodies suggesting a novel role for these organelles in microtubule organization. As a further proof for such a function, we created a deletion mutant of *pexC*. PexC is an essential protein for peroxisomal biogenesis (Heiland & Erdmann, 2005). In agreement with our hypothesis, the *ΔpexC* strain partially phenocopied the *ΔapsB* mutation.

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PR1.44

Actin Precedes Myosin in Formation of Contractile Rings in *Aspergillus nidulans*

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

PR1.45

Differential transcriptome and proteome analysis of the plant-pathogenic fungus *Verticillium longisporum*

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Verticillium longisporum is a soil-born host-specific fungal pathogen of *Brassicaceae*. It infects through the roots, colonizes the central cylinder inside the xylem vessels and spreads through the whole plant. The evolutionary origin of the cruciferous fungal pathogen, *V. longisporum* is still a mystery. It is very closely related to both *V. dahliae* and *V. albo-atrum* but possesses some typical characteristics such as long spores, almost double amount of nuclear DNA content and cruciferous host specificity. On the genomic level most of the genes of *V. longisporum* have two copies. In this project we focus on effectors of *V. longisporum* which modulate the interaction of the pathogen with its host *B. napus*. In order to identify such effectors, we started to compare biotrophic with saprophytic growth conditions. As we can't investigate the fungus within the plant yet, we extracted xylem-sap from oilseed rape and used it as growth substrate to mimic biotrophic conditions. We compared them with saprophytic growth media such as simulated xylem medium (SXM) and potato dextrose broth (PDB). All cultures were simultaneously used to analyze the transcriptome by RNA-Seq and the exoproteomes by MALDI-TOF and LC-MSMS. With this experimental setup we could identify several putative effectors which could play a role in the interaction of the fungus with the host. Currently, we are analyzing candidate genes with a knock-down strategy in *V. longisporum* and knock-out strategy in *V. dahliae*.

PR1.46

Localization Analysis of P-bodies and Stress Granules under Different Stress Conditions in the Filamentous Fungus *Aspergillus oryzae*

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Recently, it has become apparent that one aspect of posttranscriptional regulation of gene expression acts on the remodeling of translating mRNAs from polysomes into non-translating mRNPs (messenger ribonucleoproteins). P-bodies (processing bodies) and stress granules are two types of cytoplasmic mRNP granules that are widely observed in eukaryotes. Both of them are implicated in the posttranscriptional regulation of gene expression when cells are exposed to stresses. However, these structures in filamentous fungi including *Aspergillus oryzae* have not yet been elucidated in detail. In this study, the well-known components of P-bodies and stress granules were tagged with fluorescent proteins and analyzed in *A. oryzae* under various stress conditions. AoDcp2-EGFP and AoEdc3-EGFP-labeled P-bodies were observed in the cytoplasm under normal growth condition, and were further induced in response to stresses. AoPab1-EGFP and AoPub1-EGFP showed a dispersed distribution under normal growth condition, and the aggregation was induced to form stress granules under stresses. Most of the stress granules were colocalized or closely associated with P-bodies. Additionally, it has known that *A. oryzae* AoSO protein, a homolog of the *Neurospora crassa* SO, accumulates at septal pore in response to stresses. AoPab1 was also colocalized with the AoSO cytoplasmic foci induced by the high temperature stress, while the AoSO accumulation at the septal pore did not accompany with the AoPab1. The colocalization of the AoSO cytoplasmic foci with stress granules suggested that AoSO may have a novel role in the regulation of mRNP granules.

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PR1.47

Contribution of MAK-1 and MAK-2 MAP kinases to cell wall integrity in *Neurospora crassa*.

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The Mpk1-like MAP kinases control the cell wall integrity that regulates cell wall remodeling during the growth and differentiation in fungi. *Neurospora crassa* has MAK-1 and MAK-2 MAP kinases which are an ortholog of Mpk1 and Fus3 in *S. cerevisiae*, respectively. MAK-2 regulates hyphal fusion and sexual development, whereas the function of MAK-1 is not fully understood. In this study, we examined the contribution of MAK-1 and MAK-2 to cell wall integrity in *N. crassa*. Both the *mak-1* and *mak-2* disruptants were more sensitive to micafungin, a beta-1,3-glucan synthase inhibitor, than the wild-type strain, whereas only *mak-1* disruptant displayed highly sensitivity to polyoxin, a chitin synthesis inhibitor. Western blot analysis revealed that the phosphorylation level of MAK-2 significantly increased by micafungin in wild-type strain. In contrast, the MAK-1 was constitutively phosphorylated from conidial germination to hyphal growth stage in wild type strain. Interestingly, this basal MAK-1 phosphorylation was almost abolished in the *mak-2* disruptant. When the mycelia of *mak-2* disruptant were treated with micafungin, the MAK-1 phosphorylation was significantly induced. These results suggest that both MAK-1 and MAK-2 participate in regulation of cell wall integrity, and there may be cross-talk between these MAP kinase pathways in *N. crassa.*

PR1.48

Genome wide insights into the targets and mechanism of function of LAE1 in *Trichoderma reesei*

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In *Aspergillus* spp., the *laeA* (loss of *aflR* expression) gene acts as global regulator of secondary metabolism. We found that the *T. reesei* orthologue LAE1 regulates cellulase and hemicellulase gene expression (Seiboth, Karimi e*t al.* unpublished data). In order to identify all targets of LAE1 in *T. reesei*, we analysed the transcriptome of *lae1* deletion (*Dlae1*) and LAE1 overexpressing (OE) strains during growth on lactose. 1186 (13 % of the total 9143) genes of *T. reesei* were affected more than 2-fold in the *Dlae1* strain, and 701 in the OE strain, and almost all of the latter (696) were also significantly expressed in the former strain. Amino acid permeases, ankyrins, and G-protein coupled receptors were most strongly downregulated in *Dlae1* and upregulated in OE. Expression of known (PKS, NRPS) or predicted (monooxygenases, dioxgenases, multicopper oxidases) enzymes involved in secondary metabolite production was also affected, with roughly equal numbers of genes being up- and downregulated. Approximately 15 % of the genes presumably regulated by LAE1 occurred in 40 discrete clusters in the *T. reesei* genome. ChIP-seq analyses suggest that LAE1 does not directly alter the balance of an active (H3K4me2) and silencing (H3K9me3) histone modification under our conditions. Conidiation was strongly reduced in the delta-*lae1* strain, both in light and in darkness, and about one third of conidiation genes were affected in the *Dlae1* strain, indicating a major effect of LAE1 on *T. reesei* sporulation. Our data expand the current knowledge of LAE1 function and point to a major involvement in conidiation in *T. reesei*.

PR1.49

LAE1 regulates conidiation in *Trichoderma atroviride*

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Trichoderma atroviride (teleomorph *Hypocrea atroviridis*) exhibits a synchronized manner of sporulation in response to light stimulus, and thus serves as a model organism for photomorphogenetic investigations. However, conidiation of *Trichoderma atroviride* strongly depends on carbon source. Here we show the involvement of the LAE1 protein in light sensing by *T. atroviride* and regulation of its development. Phylogenetic analysis shows that LAE1 from *T. atroviride* is orthologous to LAE1 from *T. reesei* (teleomorph *Hypocrea jecorina*) and they both are orthologous to LaeA from *Aspergillus nidulans* (teleomorph *Emericella nidulans*). In the later fungus this protein is the master regulator of secondary metabolite production. A loss of function of *lae1* in *T. atroviride* dramatically reduces sporulation in general and weakens the induction of conidia formation by light. We compare photostimulation of conidiation of *lae1* mutants of *T. atroviride* P1 (*lae1* knockout and *lae1*OE respectively) and the corresponding *lae1* mutants of *T. reesei* by using a broad set of carbon sources in an optimized BIOLOG Phenotype MicroArray. We also show that the two blue light receptor proteins BLR-1 and BLR-2 interact with LAE1 in the developmental regulation of *T. atroviride*. Last not least, the mechanical injury of mycelia, which usually triggers the conidiation in *T. atroviride*, was not functional in *lae1* knockout mutant as compared to parent and *lae1*OE strains. These data suggest that developmental regulation is a main target of LAE1 function in *T. atroviride*.

PR1.50

Cytological observation of mitotic chromosomes in *Colletotrichum* spp. and its implications for chromosome analysis, genome project and phylogenetics

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In this study, we show that cytological approach is useful to analyze chromosome structure, karyotype, and phylogeny of *Colletotrichum* spp. Seven species, *C. graminicola* (*C-gra*), *C. higginsianum* (*C-hig*), *C. orbiculare* (*C-orb*), *C. gloeosporioides*, *C. lindemuthianum* (*C-lin*), *C. truncatum*, and *C. trifolii* (*C-tri*), were examined here. For visualizing chromosomes, mitotic metaphase specimens were prepared by germ tube burst method, stained with DAPI and PI, and observed by fluorescence microscopy. Summarized results are as follows. (1) Using *C-gra* and *C-hig*, cytological karyotypes were shown to be consistent with optical maps in terms of chromosome number (CN) and relative chromosome size. Even mini-chromosomes of 400-500 kb in optical maps were clearly observed with our method. With the merits of cost and time, cytological karyotyping should be useful to know the outline of genome at the onset of genome project. (2) In *C-orb*, a novel feature of chromosomes was discovered: most chromosomes are distinctly partitioned into highly AT-rich regions that constitute centromeric, constitutive heterochromatin and the remaining highly GC-rich regions. Also chromosome rearrangements were easily detectable between the strains based on morphological features. Thus, *C-orb* is promising as a model for fungal cytogenetics. (3) Comparison of karyotypes and cytological features of interphase nuclei among seven species indicated that *C-orb* and *C-tri* are very close to each other with similar cytological properties unique to them, but are distantly related to other species including *C-lin*. This finding suggests that *C-orb* and *C-tri* diverged recently. Core CN excluding mini-chromosomes were 7 for *C-lin* and 10 for the other species.

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PR1.51

Regulation of the expression of genes encoding glycoside hydrolases in *Penicillium funiculosum*

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Penicillium funiculosum is a filamentous fungus that has the capability to secrete a mixture of enzymes (Rovabio™) that is used as animal feed additive for the enhanced hydrolysis of plant polymers. The aim of this work is to study the networks regulating the production and secretion of the hydrolytic enzymes that constitute the cocktail from *P. funiculosum*. The genome of *P. funiculosum* was sequenced and, following computer-based annotation, a manual annotation has been initiated with a focus on genes likely to encode glycoside hydrolases, regulators and proteins involved in the secretion pathway. Furthermore, we have constructed a Δ*pyrG* strain of *P. funiculosum* to afford gene deletion studies as part of a programme to assess the functionality of target genes. We studied the expression of selected genes encoding glycoside hydrolases that are known to be present in the Rovabio™ cocktail. As a prelude to a genome-wide transcriptional analysis, we studied the transcription of genes encoding a cellobiohydrolase, an arabinofuranosidase and a xylanase using semi-quantitative RT-PCR to determine the level of transcripts from cells grown in presence of two different carbon sources, glucose and wheat straw. For those genes, transcription was repressed in glucose and induced in wheat straw medium. With the tools now assembled, we are in a strong position to investigate the regulation of gene expression in *P. funiculosum*.

PR1.52

Subcellular loclisation of AREA and AREB under different carbon and nitrogen regimes.

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

PR1.53

The fungal plasma membrane: a central player in the toxicity of the *Penicillium chrysogenum* antifungal protein PAF

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The cationic antifungal protein PAF inhibits the growth of sensitive filamentous fungi, e.g. *Aspergillus nidulans*, *Aspergillus fumigatus*, *Neursopora crassa*. The plasma membrane plays a crucial role in binding PAF, regulating ion channels in response to PAF, transmitting signals into the cell and finally also in PAF uptake. Thus the plasma membrane might determine at least in part PAF resistance or sensitivity. A microarray-based gene expression analysis in *A. fumigatus* revealed the deregulation of genes in response to PAF which are directly involved in fatty acid and lipid synthesis, membrane composition and cell signalling. In accordance, the following pathways were identified to be significantly deregulated by PAF treatment (KEGG GSEA, $p<0.05$): glycerophospholipid metabolism (afm00641), inositol phosphate metabolism (afm00562) and phosphatidylinositol signalling (afm04070). A change in the expression of genes involved in the synthesis of the plasma membrane may affect its composition and fluidity and finally its accessibility for PAF pointing towards the attempt of the fungus to survive the antifungal attack. We studied in more detail the interaction of PAF with the plasma membrane and found that PAF binds exclusively to phosphoinositolphosphate (PIP), bis- (PIP_2) and triphosphates (PIP_3) and phosphatidic acid (PA), but not to phospholipids. These properties were further investigated by solution NMR ^{15}N-chemical shift titration (CST) and saturation transfer difference (STD). We could prove by CST that PAF binds to PIP_3 with moderate affinity (K=3.600/M). STD experiments revealed that PAF interacts by its aromatic parts with so far unidentified membrane components (putatively high molecular weight proteins).

PR1.54

New insights into the regulation of candidate effector proteins of the fungal wheat pathogen *Mycosphaerella graminicola*

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The ascomycete *Mycosphaerella graminicola* is an economically important wheat pathogen causing septoria tritici blotch (STB), which is one of the most devastating wheat diseases worldwide. To establish successful infection, this fungus secretes a wide array of small proteins to evade or escape host resistance mechanisms during colonization. Identification and functional characterization of genes involved in effector secretion render new insights in the genetic control of pathogenicity and may lead to the identification of novel effector proteins. Here, we show that *MgWor1* is an important regulatory gene in *M. graminicola* that plays a crucial role in the expression of specific small-secreted proteins (SSPs), either directly or indirectly through links with signal transduction pathways. For instance, we found that expression of several SSPs is controlled by *MgTpk2*, the catalytic subunit of protein kinase A (PKA), or the G protein *MgGpb1* that regulates the cyclic AMP pathway and is required for pathogenicity of *M. graminicola*. Functional analyses of these candidate effectors are ongoing and will be discussed.

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PR1.55

Functional characterization of three HMG-CoA reductase genes in the beta-carotene producing *Mucor circinelloides*

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In fungi, carotenoid biosynthesis branches from the general acetate/mevalonate pathway. 3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase is a key enzyme of this pathway catalysing a rate-limiting step, the conversion of HMG-CoA to mevalonate. The *Mucor circinelloides* genome contains three HMG-CoA reductase genes (named in this study as *hmgR1*, *hmgR2*, *hmgR3*). All of them were cloned based on sequence data available in the *M. circinelloides* genome database (http://genome.jgi-psf.org/Mucci2/Mucci2.home.html) and used in gene expression studies to investigate their role in the carotenogenesis and other physiological and cell biological processes.

Using autonomously replicating plasmids, a set of transformants were constructed, in which the copy number of each of these genes were elevated. Gene-dose effect enhanced the carotenoid production and decreased the sensitivity to statins (inhibitors of the HMG-CoA reductase enzyme) of all types of transformants, but in different extent. Relative transcription levels of the three genes during the life cycle of the fungus and under different cultivation conditions (such as aerobic/anaerobic growth or different carbon sources) were analysed by quantitative real-time PCR. In these studies, *hmgR1* showed a constitutively low, while *hmgR2* showed a constitutively high transcription level. Transcription of *hmgR3* increased significantly under anaerobic growth conditions. Moreover, growth rate of transformants, in which *hmgR3* was silenced, showed a reduced growing rate. Our results suggest that *hmgR2* may play an important role in the general metabolism, while *hmgR3* may have a role in the sensing of the oxygen concentration and the mycelial development.
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PR1.56

Functional analysis of Hsp70 family protein SsaA in *Aspergillus oryzae*

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

PR1.57

Isolation and characterisation of *FcStuA* in *Fusarium culmorum*, causal agent of foot crown rot

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F. culmorum is the causal agent of crown foot rot (CFR) as well as of Fusarium head blight (FBH) on wheat. Here we report on the characterisation of *FcstuA* gene, a APSES protein, in the *F. Culmorum* genome. The *FcstuA* gene was deleted by homologous recombination in two wild-type strains which are able or unable to produce deoxynivalenol *in vitro*, respectively. Both deletion mutants showed complete loss of pathogenicity in a CFR pathosystem on durum wheat being also unable to efficiently colonise different plant tissues (apple, potato and tomato). Fungicidal assays showed that the mutants are equally sensitive to three classes of fungicides as the wild type. Other phenotypes of the mutants include impaired growth on solid substrates, loss of hydrophobicity of the mycelium, higher susceptibility towards oxidative stress, lack of monophialides and altered pigmentation. Toxin production was decreased but not completely inhibited as in *F. Graminearum*. It is argued that toxin production is not essential for determining the loss of pathogenicity by *FcstuA* mutants in CFR. Data obtained by environmental Scanning Electron Microscope analysis suggests that the hyphae of the mutant are unable to penetrate the germinating grain.

PR1.58

To kill a fungus- antifungal activity of a plant defensin

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Plant defensins are small, cysteine-rich proteins involved in the innate immune system of plants. The plant defensin NaD1, from the ornamental tobacco Nicotiana alata is highly expressed in flowers and has a role in fungal protection. Its potent antifungal activity has been used to protect transgenic cotton from fungal infections in the field. The antifungal activity of NaD1 involves a multi-step mode of action whereby NaD1 interacts specifically with the cell wall, permeabilises the plasma membrane and ultimately enters the cytoplasm. The kinetics of cell wall binding and membrane permeabilisation suggest that a cell wall receptor may be required. In an attempt to identify this receptor as well as any other genes that may be required for the antifungal activity of NaD1, we are screening a Fusarium oxysporum mutant library created by random TDNA mutagenesis. Mutants that demonstrate enhanced resistance to NaD1 will be characterised to identify the gene(s) responsible for the resistant phenotype. Mutants will also be examined for changes in the cell wall composition or proteome in order to better understand the mechanism of resistance.

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PR1.59
Rho GTPases: Insights From The *Schizophyllum commune* Genome
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The sequenced genome of the mushroom *Schizophyllum commune* provides a powerful tool to inventory the components of different signalling pathways in filamentous basidiomycetes. Six Rho GTPases could be identified in the *S. commune* genome, namely Rho1, Rho2, Rho3, Rho4, Cdc42, and Rac1. *S. commune* Rho3, Cdc42 and Rac1 have been previously cloned (cDNA sequences with accession numbers AAF61254, AY954038, AAQ88447 in GenBank), while *S. commune* Rho1, Rho2 and Rho4 were annotated as hypothetical Ras-related small GTPases of the Rho subfamily during the genome project. Only one copy of each gene is detected in the genome, as has been suggested previously for *Rho3*, *Cdc42* and *Rac1* by Southern analysis. In addition the *S. commune* genome contains 11 and 12 genes encoding RhoGAP and RhoGEF proteins, respectively, but only one gene for RhoGDI. Ectopic expression of constitutively active *cdc42* in *S. commune* changes the hyphal regions at septa into multinucleate, swollen compartments with disturbed branching. These alterations suggest that Cdc42 is regulating branch formation and growth in *S. commune*. The tips of hyphae at the edge of *S. commune* colonies that are expressing constitutively active *cdc42* have a normal morphology, suggesting that Rac1 rather than Cdc42 regulates the polarized growth of leading hyphae. This hypothesis is now under cell biological investigation in living hyphae. No effect of expression of constitutively active or inactive *Rho3* on hyphal growth was observed but in Northern hybridization with *Rho3* cDNA a signal was obtained in young haploid hyphae and a slight increase during their mating interaction.

PR1.60
Filamentous septin in the corn smut fungus *Ustilago maydis* is specifically recognised by Gap7, a novel Cdc42 specific GTPase-activating protein
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Cell separation in fungi requires the formation of plate-like cross-walls called septa. A key determinant during septum formation is the assembly of septins into a ring-like structure, for which septins need GTP-binding. In the plant pathogenic fungus *Ustilago maydis* septins form collar-like, ring-like and filamentous structures. In the genome of *U. maydis* four septins have been identified: Sep1-4. Interestingly, only Sep4 (the Cdc10 ortholog of S. cerevisiae) is able to assemble in long filaments.

Recently, we have started to investigate a class of proteins, the GTPase-activating proteins called GAPs towards their specificity on the Rho/Rac-like GTPases in *U. maydis*. We found a novel type of GAPs with the ability to bind specifically to filamentous septin Sep4. Overexpression of the GAP-domain of this Gap7 protein is able to switch off active Cdc42 leading to a cell separation defect known for *cdc42* mutants in *U. maydis*. Interestingly, beside the GAP-domain the amino acid sequence of Gap7 contained no additional domain after searching the protein databases.

To narrow down the region which enables septin binding we have started to generate deletion mutants fused to GFP for colocalisation studies with cdc10-RFP. These mutants were also tested in GST-pulldown-assays with GDP- or GTP-bound Sep4. Our data showed that the Gap7-GAP domain was also able to interact with Cdc10. Currently we are investigating if Gap7 is a real GAP for Cdc10 and what is the function of filamentous septin in *U. maydis*.

PR1.61

Insights into the structural determinants for specificity and transport by UreA, the specific urea transporter of *Aspergillus nidulans*

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UreA, is a high-affinity urea/H+ symporter which seems to be the sole transport system specific for urea in*Aspergillus nidulans*. Homologous proteins have been characterized in *Saccharomyces cerevisiae*, *Paxillus involutus*, *Candida albicans,* in the model plant *Arabidopsis thaliana* and more recently in *Oryza sativa* (rice).Paralogues are present in the genomic sequences of all *Aspergilli*. The protein is predicted to consist of 15 transmembrane helical domains (TMSs), with an extracellular N-terminus and an intracellular C-terminus.

Little is known about the structure-function relationship of these membrane proteins. In order to address this subject, we designed a mutational strategy based on 3D homology modelling of UreA and the identification of conserved residues in all known fungal urea transporters. The functionality of the mutant proteins was assayed by growth tests on urea and resistance to its toxic analogue, thiourea. All mutations were introduced on an UreA::GFP fusion construct, which allowed us to follow the sub-cellular localization of mutant fusion proteins. This strategy allowed us to identify a number of key residues involved in the recognition and/or translocation of urea across the membrane. These mutations localize in helixes number 3, 7 or 11 which, according to homology modelling, are predicted to be part of the substrate binding domain. A chemical mutagenesis approach has been also undertaken which allowed for the identification of key residues for the functionality of the protein.

Our work constitutes the first mutational analysis in this family of transporters, providing insights into urea transporters functionality.

PR1.62

The Role of Ornithine Supply in Siderophore Biosynthesis in *Aspergillus fumigatus*

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Background: Iron is an essential nutrient required for a wide range of cellular processes. However, excessive iron accumulation is toxic. Therefore, microorganisms evolved fine-tuned iron uptake and storage mechanisms, such as the siderophore system. The opportunistic fungal pathogen *Aspergillus fumigatus* produces siderophores (low-molecular mass iron-specific chelators) to acquire, store and distribute iron. Past studies indicated coordination of siderophore biosynthesis with supply of its precursor ornithine.

Methods: The role of mitochondrial ornithine production in siderophore biosynthesis of *A. fumigatus* was characterized by analysis of the phenotypic consequences of genetic inactivation of the putative mitochondrial ornithine exporter, AmcA (Afu_8g02760).

Results: Consistent with a role in mitochondrial ornithine export, inactivation of AmcA resulted in a decrease in the cellular ornithine content as well as a decrease in extra- and intracellular siderophore production. In the presence of the iron chelator bathophenanthroline disulfonate, which inhibits siderophore-independent iron uptake, AmcA-deficiency decreased conidiation, indicating increased iron starvation. In contrast to siderophore production, AmcA-deficiency didn't affect the cellular content in polyamines, which are also derived from ornithine via the ornithine decarboxylase. Nevertheless, AmcA-deficiency increased the susceptibility of *A. fumigatus* to eflornithine, an inhibitor of the ornithine decarboxylase, most likely due to the decreased ornithine pool.
Conclusion: Siderophore biosynthesis is mainly fueled by mitochondrial production of ornithine, rather than by conversion of arginine to ornithine in the cytoplasm. There exists coordination between siderophore biosynthesis and its precursor supply. This study also indicates a prioritization of ornithine flux into synthesis of polyamines compared to siderophores, emphasizing the essentiality of polyamines.

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PR1.63

Disruption of the catalytic subunit of calcineurin (*cnaA*) in the grass symbiont *Epichloë festucae* reduces host colonization and induces formation of intrahyphal hyphae both in culture and *in planta*

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Calcineurin is a Ca^{2+}/calmodulin-dependent protein phosphatase (PP2B) comprised of a catalytic subunit (CnaA) and a regulatory subunit (CnaB). Calcineurin signalling, which occurs via the calcineurin-responsive transcription factor CRZ1, is important for regulation of ion stress, cell wall integrity, hyphal growth and a number of other developmental processes including those associated with fungal-plant interactions. The aim of this project is to test whether calcineurin has a signalling role in establishment of the mutualistic symbiotic interaction between *Epichloë festucae* and *Lolium perenne*. Analysis of the genome sequences of *E. festucae* strains Fl1 and E2368 identified one *cnaA* copy in the former and two in the latter. Deletion of *cnaA* in Fl1 resulted in severe defects in culture morphology and growth. TEM revealed a remarkable developmental defect; hyphae of the mutant formed intra-hyphal hyphae. These growth defects were partially remediated by plating mycelia at high density or by growing in the presence of $MgCl_2$. The two copies of *cnaA* in E2368 were shown to be functionally redundant. Both *cnaA1* from Fl1 and *cnaA2* from E2368 were able to complement the D*cnaA* mutant phenotype. Inoculation of *L. perenne* seedlings with the D*cnaA* mutant resulted in a strong hypersensitive response (HR). By contrast, remediated cultures failed to induce an HR but were still defective in host colonization. In the few plants that were colonized hyphal biomass was reduced and intra-hyphal hyphae were observed in the intercellular spaces of the leaves. This work demonstrates that calcineurin signalling is crucial for symbiotic establishment and for normal hyphal tip growth and development.

PR1.64

***Candida albicans* biofilms and their risk in nosocomial infections**

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The hospital can be considered as an ecosystem where the patient is in contact with the microbial world and faces the risk of contracting an infection that is termed the nosocomial. Some yeasts parts of this universe, like *Candida albicans*, are opportunist pathogens. Usage of catheters provides ample opportunity for *C. albicans* biofilms to set up a nidus for disease that is not easily amenable to conventional antifungal therapy.

Nosocomial infections caused by bacteria associated with medical implants, especially catheters, have been the subject of intense researches for a long time in Algeria, but the infections of fungal origin, particularly those caused by *C. albicans* have not been clarified yet. For this framework, our study was done at Maghnia hospital (Algeria), where 51 strains (16.94% of all taken samples) of *C. albicans* were isolated. They were divided into various hospital services with variable rates; the most concerned is the ICU followed by the gynecology department, while that of general surgery came third.

Furthermore, testing the antifungal amphotericin B "AmB" showed clearly that the *C. albicans* sessile cells (in biofilms) are much more resistant than their planktonic counterparts (suspended cells) that the resistance increases during the different phases of biofilm formation until it reaches its threshold at the maturation phase (48 hrs).

PR1.65

Assessment of alterations types of catheters by *Candida spp*. in ICU. First study in Algeria

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Nosocomial candidiasis remain a potential risk in the Intensive Care Units (ICU) where *Candida albicans* is the most responsible; however, non-albicans *Candida* species, especially *C. glabrata*, are involved. These infections are often associated with biofilms that contaminate the medical implants such as catheters; thus, therapeutic failures are due to their increased resistance to antifungal agents.

The diagnosis of catheter-related candidiasis is difficult; however, the differentiation between catheter infection (or other medical implant) and a simple contamination is essential to establish an antifungal treatment.

Our study was conducted between February 2011 and Jun 2011 in the ICU of Sidi Bel Abbes University Hospital Center (Algeria). We evaluated the various types of catheters alterations (contaminations, colonisations and infections) and their corresponding rates, as well as the responsible yeast species. In addition, we have taken images (photography) from a patient (tongue and the breathing tube) showing the spread of biofilms of *C. albicans*. In parallel, we have enhanced our study by conducting a prevalence survey by including several sampling sites.

PR1.66

Dynamics of horizontal chromosome transfer in *Fusarium oxysporum*

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Fusarium oxysporum (*Fo*) is known as a diverse and widely dispersed pathogenic species complex showing a broad host range, including many economically important crops. Comparative genomics revealed lineage-specific (LS) genomic regions in *Fusarium oxysporum f. sp. lycopersici* (*Fol*) that include four entire chromosomes and account for more than 25% of the genome. At least two LS chromosomes can be transferred horizontally to non-pathogenic *Fo* strains, resulting in pathogenicity towards tomato in the recipient.

To unravel the mechanics of horizontal chromosome transfer we will use the live-cell fluorescence system developed by Ruiz-Roldan et al. to first observe nuclear dynamics during hyphal fusion events between chromosome donor and acceptor strains. Subsequently, the split-GFP technique will be established to (i) examine temporal and spatial distribution of hyphal fusion between different donor and acceptor strains and (ii) determine whether LS chromosomes are transferred *via* nuclear fusion or exit the donor and enter the acceptor nucleus.

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PR1.67

Redirection of pigment biosynthesis to isocoumarins in *Fusarium*

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Colonies of *Fusarium* species often appear red due to production of pigments, such as bikaverin or aurofusarin. The entry compounds in the aurofusarin and bikaverin biosynthetic pathways are YWA1 and pre-bikaverin, respectively, in a process catalyzed by two multidomain polyketide synthases (PKSs), which both have a claisen-type cyclase domain (CLC) in their N terminal. Disruption of the CLC domains has previously been shown to result in formation of the lactones citreoisocoumarin and SMA93 instead of YWA1 and pre-bikaverin, respectively.

In the present study we have developed a medium with low nitrogen supply on which the aurofusarin or bikaverin pathways were partially redirected resulting in production of citreoisocoumarin and SMA93, respectively. This is first time that SMA93 is identified in a fungus and we suggest that it is renamed bikisocoumarin, as it is derived from the bikaverin pathway. The redirection of the aurofusarin and bikaverin biosynthetic pathways was reverted by adding nitrate or ammonium to the medium, suggesting that nitrogen starvation induces isocoumarin production. The suppressive influence of nitrate was investigated using *Fusarium graminearum* where even small amount of nitrate (0.1 g/L $NaNO_3$) more than halved the production of citreoisocoumarin and was undetectable at 3 g/L $NaNO_3$.

PR1.68

Effect of conditional expression of CreA and HECT ubiquitin ligase HulA on glucose repression in Aspergillus oryzae

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Aspergillus oryzae has an ability to produce copious amounts of amylolytic enzymes, production of which is repressed in the presence of glucose. Glucose repression in filamentous fungi is mediated by the transcriptional repressor CreA. In Aspergillus nidulans, it has been proposed that ubiquitination and deubiquitination of CreA play a key role in regulating glucose repression, and that HECT ubiquitin ligase HulA is involved in ubiquitination of CreA. Since yeast ortholog (Rsp5) of HulA is an essential factor for cell viability, we speculated that HulA is also essential for cell viability in filamentous fungi. In addition, it has been known that deletion of creA has a detrimental effect on growth, although CreA is not essential for cell viability. Thus, we generated the conditional expression strains for CreA and HulA in A. oryzae to investigate the mechanism of glucose repression. The conditional expression strains were generated by using the promoter of nmtA, expression of which is repressed considerably in the presence of thiamine. The resultant conditional HulA expression strain was defective in conidial formation in thiamine-containing medium, suggesting that HulA is essential for conidiation or cell viability. On the other hand, the growth defect of CreA conditional expression strain in thiamine-containing medium was leaky as compared with the creA null mutant. Northern blot analysis of α-amylase gene showed that glucose repression was relieved in the CreA conditional expression strain. These results suggested that suppression of CreA expression level is highly effective in relieving the glucose repression without growth defect.

PR1.69

Specificity determinants of GTPase recognition by RhoGEFs in *Ustilago maydis*

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Small GTPases of the Rho family act as molecular switches and are involved in the regulation of many important cellular processes. They are activated by specific guanine nucleotide exchange factors (RhoGEFs). In their active GTP bound state RhoGTPases interact with downstream effectors and trigger cellular events. The number of both RhoGEFs and effectors exceeds the number of GTPases, which raises the question how signalling specificity is achieved. In recent years the importance of RhoGEF specificity became more and more evident, as these upstream activators are often connected to their downstream effectors by scaffolding proteins. We analysed all *U.maydis* Cdc42-specific RhoGEFs (Don1, Its1 and Hot1) for their role in Cdc42 signalling both in vivo and in vitro. Interestingly, the GTPase recognition mechanism differs between Hot1 and the other two RhoGEFs. While amino acid at position 56 of Cdc42 is critical for GEF recognition of Don1 and Its1, Hot1 uses a different amino acid to discriminate between Cdc42 and Rac1. We identified additional amino acids which are important for GTPase recognition by Hot1. In future we will try to find out whether orthologs of Hot1 in other organisms use a similar mechanism to discriminate between GTPases.

PR1.70

***Cryptococcus neoformans SEC7-1* is involve in high temperature growth**

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The basidiomycete yeast *Cryptococcus neoformans* is an opportunist pathogen that causes life threatening disease on immune-compromised patients. The treatment of choice for this mycosis is based on azoles; but clinical strains resistant to the treatment have been reported. Therefore, it is desirable that new treatment alternatives are developed and to do so, is necessary to increase the knowledge on this fungus biology. To explore this alternative, a random insertion mutagenesis library was created aiming to uncover genes involved in high temperature growth (37°C) which is an important virulence factor in *C. neoformans*. Among several mutants unable to grow at 37°C we have found *Sec7*. This gene encodes a guanine nucleotide exchange factor protein (GEF) that activates ADP ribosylation factor (ARF) which is a key regulator of vesicular transport in eukaryotic cells. *C. neoforman* genome has two genes that code proteins with a Sec7 domain and both are called Sec7. However, they lie on different chromosomes, 1 and 5 (*Sec7-1* and *Sec7-2*, respectively). Previously, in *S. cerevisiae* two SEC7 coding genes were identified but the deletion of only one of them actually impaired growth at the restrictive temperature. The second gene was able to correct this defect only when over expressed. Our results suggest that deletion of *C. neoformans Sec7-1* gene is sufficient to impair the growth at 37°C.

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PR1.71

Germination of conidia of *Aspergillus niger* is accompanied by major changes in RNA profiles

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The transcriptome of conidia of *Aspergillus niger* was analysed during the first 8 hours of germination. Dormant conidia started to grow isotropically 2 hours after inoculation in liquid medium. Isotropic growth changed to polarized growth after 6 hours, which coincided with one round of mitosis. Dormant conidia contained transcripts from 4626 genes. The number of genes with transcripts decreased to 3557 after 2 hours of germination, after which an increase was observed with 4780 expressed genes 8 h after inoculation. Dormant conidia had the most unique RNA composition. The correlation coefficient between the RNA profiles of t = 0 h and t = 8 h was 0.46. They were between 0.76-0.93 when profiles of 2, 4 and 6 h were compared with that of 8 h. Dormant conidia were characterized by high levels of transcripts of genes involved in the formation of protecting components such as trehalose, mannitol, protective proteins (*e.g.* heat shock proteins and catalase). Transcripts belonging to the Functional Gene Categories (FunCat) protein synthesis, cell cycle and DNA processing and respiration were over-represented in the up-regulated genes at t = 2, whereas metabolism and cell cycle and DNA processing were over-represented in the up-regulated genes at t = 4 h. At t = 6 h and t = 8 h No functional gene classes were over- or under-represented in the differentially expressed genes. Taken together, it is concluded that the transcriptome of conidia changes dramatically during the first two hours and that initiation of protein synthesis and respiration are important during early stages of germination.

PR1.72

Identification and Characterization of a *Trichoderma reesei* Calcofluor-Sensitive Mutant

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Genencor/DuPont

As a filamentous fungus capable of secreting large amounts of extracellular proteins, *Trichoderma reesei* is used for large-scale production of native as well as heterologous proteins. As such, it is of interest to explore changes in the *T. reesei* genome that have the potential to affect its secretion capability. This study presents the characterization of a calcofluor-sensitive mutant obtained by mutagenesis. We identified the mutation responsible for the phenotype using complementation cloning, CGH analysis and genome sequencing. The mutation resides in a gene encoding a protein with a putative function in ER-Golgi trafficking. The findings of this study help better understand the phenotypic consequences of mutations in genes encoding components of the secretion pathway in *T. reesei*.

PR1.73

Genetics of horizontal chromosome transfer in the plant pathogenic fungus *Fusarium oxysporum.*

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The *Fusarium oxysporum* species complex is comprised of many host specific asexual lineages. Interestingly, different lineages that infect a single host, reside in different phylogenetic clades. This distribution of host specificity has been attributed to acquired pathogenicity. Lineages sharing a host share chromosomes and genomic regions carrying effector genes. Transfer of these regions could explain the distribution of host specific lineages within the species complex.

Horizontal transfer of complete chromosomes has been demonstrated under laboratory conditions, and resulted in gain of pathogenicity by the non-pathogenic recipient. Using a genetics approach we aim to uncover the underlying cellular processes, which have so far remained elusive. We are currently creating knockout strains in genes with key functions in processes thought be required for horizontal chromosome transfer.
Furthermore, we will investigate what properties make a chromosome amenable for transfer. A screening method based on random insertion of a marker in the donor strain, will allows us to determine which chromosomes can be transferred. Analysis of these chromosomes should give us better insight into which properties they share.
Combining knowledge of both the properties required for chromosome transfer and the cellular processes involved will help to gain a better understanding of this process and its importance in the evolution of the *F. oxysporum* species complex.

PR1.74

The RNA-binding protein Khd4 - a posttranscriptional regulator for cell morphology and pathogenicity in *Ustilago maydis*

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In the plant-pathogen *Ustilago maydis* evidence is accumulating that posttranscriptional processes play a major role in regulating cell morphology and pathogenicity. Key factors of the posttranscriptional machinery are RNA-binding proteins, which recognize specific motifs within target transcripts to regulate for example translation, localization, or mRNA-stability. We are working with the RNA-binding protein Khd4 that contains at least five K homology domains. Deletion of *khd4* leads to severe consequences: disturbed cell shape, abnormal cell wall composition, cytokinesis defect, and strongly reduced pathogenicity. Interestingly, the KH domains 3 and 4, which recognize the motif AUACCC, are required for Khd4 function since mutations in the conserved motif G-X-X-G lead to the *khd4* deletion phenotype. The motif AUACCC is necessary and sufficient for binding and is most likely a regulatory element since it accumulates in untranslated regions. An independent mRNA expression profiling approach revealed that the binding motif is significantly enriched in transcripts showing altered expression levels in *khd4*Δ strains. Moreover, the vast majority of potential Khd4 target mRNAs exhibit increased amounts in deletion mutants. These findings suggest that Khd4 might function in mRNA-stability processes and is important for the posttranscriptional regulation of cell morphology and pathogenicity in *U. maydis*. In preliminary studies we analyzed truncated versions of Khd4 to learn more about putative domains in this large protein. In addition, we identified one of the regulated candidates that show the same deletion phenotype like the *khd4Δ* strain.

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PR1.75

Analysis of G-protein and MAPK-components of the pheromone signal transduction cascade in *Ashbya gossypii*

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MAP-kinase cascades are highly conserved among eukaryotes and harbour key virulence factors in human- and plant pathogens. In sexually reproducing fungi the pheromone-signal transduction MAPK-cascade regulates mating and sporulation. The genome of the filamentous fungus *Ashbya gossypii* contains homologs of all of the *Saccharomyces cerevisiae* genes involved in this pathway. However, in spite of this high degree of conservation *A. gossypii* is not known to have a sexual cycle. We have deleted the main components of this cascade including the pheromone receptor genes *STE2*, *STE3*, the G-protein components encoded by *STE4*, *GPA1*, the MAP-kinase genes *STE11*, *STE7*, *FUS3*, *KSS1* and the transcription factors *STE12* and *TEC1*. The results show that all the *A. gossypii* mutants except *tec1* grew with wild-type rates and sporulated. This differs from *S. cerevisiae* where deletion of any of these genes leads to sterility and in the case of *GPA1* is lethal. Strikingly, deletion of the major transcription factor activated by the cascade, *STE12*, resulted in a hypersporulation phenotype similar to that of an *Agtec1* mutant indicating that the cascade might have a regulatory role in sporulation. To overactivate the pheromone cascade in *A. gossypii* we introduced two point mutations in the MAPKK Ste7 that mimics phosphorylation with the aim to generate a constitutively active Ste7 allele (Ste7DD). Heterologous expression of *Agste7DD* from a strong promoter together with an inducible allele of the scaffold protein AgSte5 in *S. cerevisiae* resulted in a morphologic response. Here we will present the results from our work in *A. gossypii*.

PR1.76

The landmark protein Sec3 links Rho-GTPase signaling and polarized secretion in *Ustilago maydis*

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To establish their polarity, cells have to transport cell wall material to the growing tip. This is achieved by a directional transport and fusion of vesicles with the plasma membrane. A octameric protein complex, the exocyst, marks the areas where active exocytosis takes place. Two proteins of the exocyst complex, Sec3 and Exo70, serve as landmark proteins for exocytosis, whereas the other subunits tether secretory vesicles and mediate their interaction with SNARE proteins. In *S.cerevisiae* it is known that components of the exocyst complex are effector of small GTPases of the Rho family. For example Sec3 interacts with Cdc42 and Rho1 which are involved in the localization and regulation of this protein. We have analyzed the role of small GTPases for the regulation of the exocyst complex in *U. maydis*. In contrast to *S.cerevisiae*, *U. maydis* contains not only Cdc42, but also another small GTPase, Rac1. Since Rac1, but not Cdc42, is critical for polar growth in *U.maydis*, we tested if it is also involved in the regulation of the exocyst complex. We analyzed components of the secretory machinery using genetic, cell biological and biochemical approaches. Our results indicate that Sec3 acts as a critical regulator of polar growth in *U. maydis*. This is consistent with the idea that Sec3 is a landmark protein for polarized secretion. Our biochemical data indicates that Sec3 can interact with Rac1, which supports the idea that Rac1 as a main regulator of polar growth is also involved in the regulation of the secretory machinery.

PR1.77

Golgi localized PI(4)P is required for *Candida albicans* filamentous growth

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Membrane phospholipids, such as phosphoinositide phosphates are minor components of cellular membranes. In eukaryotes, phosphoinositide phosphates play a crucial role in cell polarity and membrane traffic, for example PI(4)P and PI(4,5)P2 are critical for regulating the cytoskeleton. In *Saccharomyces cerevisiae* there are two essential genes encoding PI-4-kinaases, STT4 and PIK1. Stt4 is localized to the plasma membrane and Pik1 is localized to the Golgi. The role of these two phosphoinositide kinases in fungal pathogens remains largely unknown.

We have been examining the function of Pik1 and the distributions of PI(4)P in *Candida albicans* filamentous growth. We have generated strains in which the level of the Pik1 PI-kinase can be manipulated using the Tetracycline repressible promoter system. In semi-permissive conditions, the *pik1* mutants are defective in filamentous growth in both liquid and solid media. Using different fluorescent lipid associated reporters, we have been following the distribution of PI(4)P during the transition form budding to filamentous growth and in various mutants. Our results indicate that distinct pools of PI(4)P at the Golgi and plasma membrane are critical for the yeast to filamentous growth transition. We are currently examining the dynamics of these PI(4)P pools during filamentous growth and the interconnection of PI(4)P and Arf1 proteins in the Golgi.

PR1.78

Conidiation is the major target of LAE1 in *Trichoderma atroviride*

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Trichoderma atroviride (teleomorph *Hypocrea atroviridis*) exhibits a synchronized manner of sporulation in response to light stimulus, and thus serves as a model organism for photomorphogenetic investigations. However, conidiation of *Trichoderma atroviride* strongly depends on carbon source. Here we show the involvement of the LAE1 protein in light sensing by *T. atroviride* and regulation of its development. Phylogenetic analysis shows that LAE1 from *T. atroviride* is orthologous to LAE1 from *T. reesei* (teleomorph *Hypocrea jecorina*) and they both are orthologous to LaeA from *Aspergillus nidulans* (teleomorph *Emericella nidulans*). In the later fungus this protein is the master regulator of secondary metabolite production. A loss of function of *lae1* in *T. atroviride* dramatically reduces sporulation in general and weakens the induction of conidia formation by light. We compare photostimulation of conidiation of *lae1* mutants of *T. atroviride* P1 (*lae1* knockout and *lae1*OE respectively) and the corresponding *lae1* mutants of *T. reesei* by using a broad set of carbon sources in an optimized BIOLOG Phenotype MicroArray. We also show that the two blue light receptor proteins BLR-1 and BLR-2 interact with LAE1 in the developmental regulation of *T. atroviride*. Last not least, the mechanical injury of mycelia, which usually triggers the conidiation in *T. atroviride*, was not functional in *lae1* knockout mutant as compared to parent and *lae1*OE strains. These data suggest that developmental regulation is a main target of LAE1 function in *T. atroviride*.

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PR1.79

The formation mechanism of apical sterol rich membrane domains (SRDs) and visualization of SRDs by Photoactivated Localization Microscopy in *Aspergillus nidulans*

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

PR1.80

High Throughput *in vivo* Footprinting – an improved Method to detect Protein-DNA Interactions

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A prerequisite for understanding the mechanisms responsible for transcriptional regulation in a cell is the knowledge which bases of a promoter region are targeted by proteins during induction or repression of a gene. With that in mind we improved traditional *in vivo* footprinting via LM-PCR [1] to obtain a high throughput technique to detect Protein-DNA interactions. The basis of our enhanced method is fluorescent 5'-[6-FAM]-labeling of DNA fragments and analysis via capillary gel electrophoresis (CGE) [2]. In addition we developed a graphical user interface that is essential in the automatization of data processing and visualization of results.

This improved protocol can be a powerful tool in obtaining a better insight in the regulation of gene expression and supporting the systematic manipulation of expression patterns. Furthermore this high throughput technology allows a detailed analysis of the switching of protein-DNA interaction events in regulons or even genome wide, consequently supporting a better understanding of regulatory networks within an organism, tissue or cell.

The utilization of this refined method has already led to new insights into the *xyr1*-regulon responsible for (hemi-) cellulase-expression in the industrially important fungus *Trichoderma reesei*.

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PR1.81

Functional analysis of silent polyketide synthase genes in *Penicillium chrysogenum*

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A major challenge in the battle against infectious diseases is the resistance of pathogens against the commonly used antibiotics. This urges the need for the discovery of new antimicrobial compounds based on new chemical structures in order to develop a new generation of antibiotics. The functional analysis of cryptic secondary metabolite gene clusters is a promising tool for novel bioactive compounds discovery as many of these gene clusters are not expressed under laboratory conditions.

Polyketides represent a diverse group of bioactive compounds that are widely used as therapeutics due to their antibiotic and cytostatic properties. Polyketides are produced by multifunctional enzymes – polyketide synthases – that are encoded by associated (PKS) genes. Here we report on the functional analysis of (silent) PKS genes in the filamentous fungi *Penicillium chrysogenum*. Sequencing data showed the presence of 20 putative PKS genes in the genome [1], none of which have been characterized before. The expression of most of these genes is silent under laboratory conditions. To activate their expression two strategies are used: i) promoter replacement and ii) activation of the complete biosynthetic pathway through local regulatory genes deletion or overexpression. Strong promoters were chosen from the *P.chrysogenum* genome and used for cloning in the appropriate plasmids. Growth media of strains with expression of silent PKS genes were obtained and analyzed using HPLC and mass spectrometry to reveal the products of these PKS clusters. We will report on the activation of the silent PKS cluster via local regulatory gene expression.

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PR1.82

The NADPH oxidase complex is required for re-orientation of the cytoskeleton during appressorium-mediated plant infection by the rice blast fungus *Magnaporthe oryzae*

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Recent studies have demonstrated that fungal NADPH oxidases (Nox) play a key role in fungal morphogenesis, cellular differentiation and virulence. In plants, reactive oxygen species (ROS) are implicated in the control of polarised cell growth in root hairs and pollen tubes, and are induced in response to microbial attack. Nox enzymes are flavoenzymes used to generate ROS and have recently been characterised in several eukaryotic organisms including filamentous fungi. How this regulated synthesis of reactive oxygen species by fungal NADPH oxidases plays such an important function in fungal development is, however, not known. Previously we have shown that in the rice blast fungus *Magnaporthe oryzae*, *Mo*Nox1 and *Mo*Nox2 are both independently required for rice blast disease. Here we show that the membrane-bound NADPH complex is necessary for re-organisation of the fungal actin cytoskeleton during plant infection. We demonstrated using Lifeact-RFP gene fusions that the Nox1, Nox2 and NoxR subunits of the complex are independently required for formation of a 5.9 µm diameter actin ring at the base of the specialised appressorium that is necessary for plant infection. Furthermore, we established a further role for Nox in septin ring assembly at the base of the appressorium. We demonstrated using Cdc11-GFP and Chm1-GFP gene fusions that the Nox2 and NoxR subunits of the complex are independently required for initiating phosphorylation of the septin ring and subsequent septin ring assembly. Formation of these sub-cellular structures is an essential prerequisite for the initiation of rice blast disease.

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PR1.83

The characterization of the three MEKs codifying by the *Cryphonectria parasitica* genome reveals the importance of a functional Cpkk2 for Cryphonectria hypovirus 1 (CHV1) accumulation

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Cryphonectria parasitica is the causal agent of chestnut blight, a chestnut tree disease controlled by the widespread presence of mycovirus-containing hypovirulent strains. The biological function(s) of *cpkk1*, *cpkk2* and *cpkk3* genes, encoding the three mitogen-activated protein kinase kinases (MEKs) of *Cryphonectria parasitica* were examined through specific knock-out strains and addressed to putative roles in virulence and hypovirus infection. Cpkk1 is the Mkk1-homologue acting in the phosphorylation cascade essential for cell integrity. Cpkk2 is the Ste7-homologue involved in the pheromone responsive pathway, while Cpkk3 is the Pbs2-homologue, the MEK activated during the response to high osmolarity. Our analyses confirmed MEKs to belong to the proper signalling cascade with typical defects in the null mutants already identified for the homologues of phylogenetically related filamentous fungi with some exceptions: abnormal hyphae with a reduced number of septa and thinner cell walls were observed in *Δcpkk1* strain and a stronger defect on growth and development was shown for *cpkk2*-null mutant. The growth impairment did not allow to perform proper mating assays in *Δcpkk1* and *Δcpkk2* strains, which were instead normal for *Δcpkk3*. Virulence on chestnut cuttings was only affected in *cpkk1*- and *cpkk2*-null mutants. A successful CHV1 infection through natural anastomosis with a virus-donor line was obtained in *Δcpkk1* and *Δcpkk3* with common symptoms associated to hypovirus infection. On the contrary, no infection was possible in *Δcpkk2* by anastomosis or transformation with an infectious clone of CHV1, suggesting its important role for maintaining a proper cellular environment for virus replication.

Poster Category 2: Sex and Sexual Development

PR2.1

The General Transcriptional Repressor Tup1 Is Required For Dimorphism And Virulence In A Fungal Plant Pathogen.

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The transition between a yeast-like growth and the formation of polar filaments is a critical step in the life cycle of many fungal pathogens. This morphological shift, known as dimorphism, is triggered by multiple environmental signals and controlled by complex genetic pathways that ensure successful pathogenic development. The transcriptional repressor Tup1 is one of the best known regulators of dimorphism in animal pathogenic fungi. However, the role of Tup1 in plant pathogens remained unknown until now. Here we show that Tup1 plays a key role in the dimorphic transition of the maize pathogen *Ustilago maydis.* Deletion of *tup1* compromises the mating and filamentation capacities of the fungus, leading to a reduce virulence phenotype. In *U. maydi*s, such processes are controlled by the Prf1 transcription factor through *a* and *b* mating-type loci genes. Interestingly, Δ*tup1* strains show a significant reduction in the expression level of *prf1* and that of Prf1 target genes at both loci. We have observed that Tup1 seems to control Prf1 activity by regulating the expression of the *prf1* transcriptional activators, *rop1* and *hap2*. In addition, we have found a putative novel *prf1* repressor, named Pac2, which seems to be an important target of Tup1 in the control of dimorphism and virulence. Our findings establish Tup1 as a key factor coordinating dimorphism in the phytopathogen *U. maydis*, and support a conserved role for Tup1 in the control of hypha-specific genes among animal and plant fungal pathogens.

PR2.2

Plant Specific Activation Of The Unfolded Protein Response Is Necessary For Biotrophic Development Of *Ustilago maydis*

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In the smut fungus *Ustilago maydis*, pathogenic development is controlled by the *b*-mating type locus, orchestrating a regulatory network consisting of different transcription factors. A key factor for the regulation of this circuit is the Clp1 protein. Via physical interactions with two of the key regulators of the *b*-network, bW and Rbf1, the Clp1 protein promotes the cell cycle release and the progression of fungal development at the onset of biotrophic development. In addition to bW and Rbf1 we identified Cib1, a previously undiscovered bZIP-transcription factor to interact with Clp1. Cib1 protein expression is restricted to the biotrophic development via regulated splicing. In accordance with this we observed that deletion of *cib1* leads to a plant specific phenotype: while filamentous growth and formation of appressoria is not affected, mutant strains fail to colonize the plant tissue. We discovered Cib1 to be the homologue of yeast Hac1p in *U. maydis*. Hac1p represents the central regulator of the unfolded protein response (UPR), a highly conserved signalling pathway to remodel and to align cellular physiology to the demands imposed by enhanced protein synthesis and protein secretion. An enhanced capacity for protein secretion is expected to be of crucial importance for biotrophic growth *U. maydis* and required to establish a compatible interaction with the host plant. Hence, these data shed light on a novel strategy by which UPR signalling is coupled to mating-type controlled development in order to promote the parasitic lifestyle of a biotrophic pathogen.

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PR2.3

Characterization of the mating type (MAT) locus in the *Grosmannia clavigera* (Ophiostomatales, Ascomycetes) and related species revealed the footprint of a homothallic ancestor

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The mating type (MAT) gene evolution from eight heterothallic fungal species in the order Ophiostomatales, including conifer pathogens associated with mountain pine beetles in the genera *Grosmannia* and its the asexual form *Leptographium* has been investigated. We characterized a MAT1-2 idiomorph from the assembled and annotated genome of *G. clavigera*. The MAT locus is flanked by genes of cytoskeleton protein (SLA) and DNA lyase (APN). The synteny of these genes is conserved and consistent to other members in Sordariomycetes. We also identified a truncated MAT1-1-1 gene adjacent to the MAT1-2-1 gene in the MAT1-2 idiomorph. The truncated MAT1-1-1 is homologous to the '*complete*' MAT1-1-1 gene in the opposite mating type, except that the alpha-box domain had been deleted or removed. The MAT genes determined from additional isolates of *G. clavigera* and seven closely related species were shown to have the same pattern, suggesting that the presence of the truncated MAT1-1-1 gene is ancient. We hypothesize that the ancestor of *G. clavigera* and related species was homothallic and retained both *'complete'* MAT1-2-1 and MAT1-1-1 genes at the MAT locus, and the heterothallic species evolved from this ancestor when MAT1-1-1 and MAT1-2-1 genes were translocated and rearranged from the locus. We also suggest that the deletion of alpha-box domain to maintaining a heterothallic life style, and the 1:1 MAT ratio determined in *G. clavigera* populations, can confer an evolutionary benefit by promoting outcrossing and increasing genetic variability within a species.

PR2.4

Mannitol-1-phosphate dehydrogenase is essential for the development of extreme stress resistant fungal ascospores

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

PR2.5

Evidence for sexual recombination in *Penicillium chrysogenum*

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Penicillium chrysogenum is the major industrial producer of the β-Lactam antibiotic penicillin with a world market value of about 600 million € per year. Although no sexual propagation has been reported so far for this filamentous fungus, we recently were able to detect mating type loci in different strains, indicating a sexual lifecycle. Isolates, carrying opposite mating types, were found in near-equal proportion in nature and we observed transcriptional expression of mating type loci as well as pheromone and pheromone receptor genes [1]. Thus *P. chrysogenum* possesses the genetic requirements for heterothallic breeding. For induction of a sexual cycle we performed crossing experiments resulting in the production of cleistothecia and ascospores, which were similar to those described recently for *Eupenicillium crustaceum* [2]. Here we provide evidence for sexual recombination in ascospore progeny. A sexual cycle provides an invaluable tool for classical genetic analyses and extends an insight into the evolution of fungal sexual development.

[1] Hoff B, Pöggeler S, Kück U (2008) Eighty years after its discovery, Fleming`s *Penicillium* strain discloses the secret of its sex. Eukaryot Cell 7: 465-47.

[2] Pöggeler S, O´Gorman CM, Hoff B, Kück U (2011) Molecular organization of the mating-type loci in the homothallic ascomycete *Eupenicillium crustaceum*. Fungal Biol. 115: 615-624.

PR2.6

Cdc42 causes gasteromycete-like basidial anatomy in *Schizophyllum commune*

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Gasteroid fungi are a morphologically defined group of the Agaricomycetidae, characterised by spore formation within enclosed basidiomata and by statismosporic basidia. The underlying genetic processes are widely unknown so far, since gasteroid fungi have not been subject of genetic studies for decades.

Schizophyllum commune is a well studied model fungus for the sexual development of basidiomycete fungi, due to its ability to fulfil its life cycle in two weeks. Despite its unusual cyphelloid fruiting body anatomy, its hymenium and ballistosporic basidia develop in the same fashion as most other representatives of Agaricales. A number of small GTPases such as Cdc42 are known to influence sexual development of many fungi, including *S. commune*. The Rho-protein Cdc42 regulates elongation and adhesive capacities of hyphal cells. It is also responsible for branch site selection and branch development by proper actin cytoskeleton orientation in monokaryotic hyphae, during mating and in clamp formation of dikaryotic mycelium.

Basidiomata of heterozygotic dikaryons of *S. commune*-mutants ectopically expressing constitutively active Cdc-42 are developmentally disturbed. Although later stages of spore formation are lacking, dysfunctional spores are produced. These are borne on divergent sterigmata, which are thick-walled and longer than in the wildtype, and obviously incapable of active spore discharge. In gasteroids, sterigmata are also often likewise divergent, as seen in the exceptionally long, sclerified pedicels of some Lycoperdaceae, or the pleurocarpous basidia of Tulostoma. These features are hypothesized as evolutionary disturbances in the order and magnitude of developmental gene expression, including Cdc42 and its downstream factors.

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PR2.7

The forkhead gene *fkhA* positively regulates sexual development in *Aspergillus nidulans*

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In a homothallic filamentous fungus *Aspergillus nidulans*, sexual developmental process is largely affected by the genetic and environmental factors. To regulate the corresponding genes necessary for the sexual development, tight regulations of transcription factors are required. Here, we identified the *fkhA* gene which encodes a putative forkhead transcription factor homologous to the yeast *FKH1* gene that is involved in sexual development. The *fkhA* deletion resulted in the complete loss of fruiting body formation under all conditions favoring sexual development, indicating that the *fkhA* gene is required for normal sexual development in *A. nidulans*. Furthermore, overexpression of *fkhA* showed enhanced production of fruiting bodies under induction condition not only in the normal condition but also in the inhibiting condition of sexual development. These results suggest that the *fkhA* gene is necessary and sufficient for regulating sexual development in *A. nidulans*. [This work was supported by the NRF grant 2011-0027448]

PR2.8

Evidence of Sexual recombination in the phytopathogen, *Ramularia collo-cygni*

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Ramularia collo-cygni is the main causal agent of the major barley disease, Ramularia Leaf Spot (RLS). Control of this disease is based on chemical control, due to the lack of varietal resistance. The appearance of resistance in this fungus to Quinone outside Inhibitor (QoI) fungicides was observed in 2001. This rapid evolution has now been confirmed by the detection of the G143A mutation, which confers resistance, in a number of *R collo-cygni* isolates from a number of geographically diverse locations. The life cycle of this fungus is only being slowly elucidated. An asteromella stage has been observed on senescent leaf material. The precise nature and function of this structure is now being investigated. The successful transformation of *R. collo-cygni* using Agrobacterium has allowed detailed microscopic examination of the fungus, including the formation of sexual structures *in vitro.* Preliminary experiments indicate that the precursors of spermagonia and ascogonia form *in vitro* and compatable isolates form sclerotial like structures when growing together. In addition to microscopic evaluation, mating type loci are being characterised using information derived from the sequencing of *R. collo-cygni.*

PR2.9

B-Regulated Sexual Development And The Sugar Transporter Sts1 In The Mushroom *Schizophyllum Commune*

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Mushroom development in the basidiomycete *Schizophyllum* commune is normally the end result of a sexual interaction between two individuals differing at what are termed the *A* and *B* mating type loci. *sts1* is a putative sugar transporter gene also implicated in the regulation of mushroom development. Null (Δ) mutant strains lacking functional copies of *sts1* displayed severely attenuated mushroom production. When *Δsts1* strains were outcrossed, some of the *Δsts1* null haploid progeny displayed a “flat” phenotype, suggestive of an inappropriately activated *B* mating type pathway. Test matings and genetic analysis of these haploid “flats” confirmed that this was indeed the case. A complicating factor was the observation that the original *Δsts1* strain did not exhibit a “flat” phenotype. These and other data indicated that the absence of *sts1* is a necessary, but not sufficient condition to activate the *B* mating pathway in these homokaryotic individuals. Fluorescent microscopy of these B-On *sts1* null haploids stained with DAPI/Calcofluor revealed the presence of multiple hook cells at inappropriate locations, with nuclei frequently found within. We have exploited the recent availability of the sequenced *S. commune* genome and have employed a transcriptome analysis approach to identify likely gene targets regulated in the B-On *sts1* null strains. The preliminary results of this analysis will be discussed at the conference.

PR2.10

Analysis of Ras Proteins as Signal Transduction Elements in *Schizophyllum commune*

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The white rot basidiomycete *Schizophyllum commune* has been used as a model organism to study mating and sexual development as well as analysis of cell development.

Subsequent to pheromone recognition, intracellular signal transduction leads to a specific phenotype involving nuclear migration and clamp cell fusion. The *S. commune* genome encodes more than 30 putative signal transduction proteins of the Ras superfamily containing the Ras, Rho, Rab, Ran and Arf subfamilies. The comparison of both proper *S. commune* Ras proteins reveal a low sequence identity of 44 %. Phylogenetic investigation of Ras proteins from various basidiomycetes show that they cluster in two main groups. High sequence similarities between these proteins in basidiomycetes suggesting an ancient duplication event. The role of the small G-proteins Ras1 and Ras2 have been postulated in pheromone response in addition to MAPK signalling.

To investigate the role of Ras1 mutants with constitutively active ras alleles as well as a ΔRasGap1 mutant were analyzed. They show phenotypes with disorientated growth pattern, reduced growth rates and hyperbranching effects. The fungal cytoskeleton, composed of actin and microtubules has been investigated by immunofluorescence microscopy to reveal whether Ras signaling influences the formation of cytoskeleton. The second Ras protein, Ras2, was detected by genome analysis. Its function is analysed in current studies.

PR2.11

Proteins expressed during hyphal aggregation for fruiting body formation in basidiomycetes

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The first visible step in fruiting body development in basidiomycetes is the formation of small hyphal knot by localized intense branching of hyphae of restricted length followed by hyphal aggregation. In *Coprinopsis cinerea*, the first not yet fruiting-specific step of hyphal branching occurs in the dark, the second step requires a light signal. Hyphal aggregation implies cell-cell contacts and protein interactions on the outer cell walls are anticipated. Few protein candidates were identified and discussed in the past for such function, amongst were the galectins in *C. cinerea* and the Aa-Pri1 protein (aegerolysin) in *Agrocybe aegerita* that are specifically expressed during the step of hyphal aggregation as well as during subsequent primordia development. In this study we follow up the distribution of such genes in the steadily growing number of available genomes of basidiomycetes. Neither galectin genes nor *Aa-pri1*-like genes are present in all mushroom species, making an essential role in hyphal aggregation unlikely.

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R2.12

Differential Regulation of Laccases in *Schizophyllum commune*

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Schizophyllum commune, a saprophytic white rot fungus, is involved in the degradation of complex organic molecules including lignin. Previous reports say that this fungus can degrade refractory organic matter from black slate with the help of different exoenzymes. Laccases are multicopper glycoproteins which are able to oxidize a broad spectrum of organic compounds including xenobiotics, synthetic dyes, pesticides and polycyclic aromatic hydrocarbons by a radical catalyzed reaction mechanism using molecular oxygen. *S. commune* able to produce laccases and laccase-like enzymes.

In this study, relative expression of the laccase and laccase-like genes (LO family) in *S. commune* were analysed using quantitative real time PCR (qRT-PCR) under different conditions. Also, laccase enzyme activity was measured using ABTS (2,2´-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid)) as a substrate. Two laccases and four laccase-like genes all of which are well conserved in *S.commune* were selected for studying differential regulation during fungal morphogenesis and in different sexual phases. Regulation of laccases during substrate utilization was tested using powdered black slate and artificial lignin as the core C- sources, both of which can be used by *S. commune* at low growth rates. Higher laccase enzyme activity was seen in black slate cultures.

PR2.13

PRO45, a potential membrane associated protein is a component of the conserved fungal STRIPAK complex

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Sordaria macrospora is an important model organism for developmental biology and permits insights into the complex formation of three-dimensional fruiting bodies. This is mediated by an interaction between developmental proteins and conserved signaling cascades. A prominent role in sexual development of *Sordaria macrospora* plays a complex of striatin-interacting phosphatases and kinases (STRIPAK), which is associated with the vacuolar membrane. This complex contains homologs of striatin, the striatin-interacting protein, PP2A A, Mob3 (monopolar spindle-one-binder) and SLMAP (sarcolemmal membrane-associated protein).

PRO11 and PRO22, important developmental proteins of *Sordaria macrospora*, represent homologs of striatin and striatin interacting protein, respectively [1, 2]. For *Sordaria macrospora* a direct interaction of PRO22 with PRO11 and PP2A A was shown recently. This raises the question whether a STRIPAK-like complex exists in filamentous fungi and whether homologs exist to components of the human STRIPAK comlex.

Here we present the characterization of another component of the fungal STRIPAK complex: PRO45 in *Sordaria macrospora* is a homolog of SLMAP containing both a FHA- and a transmembrane domain. Deletion strains show sterility together with a severe defect in hyphal fusion. Tandem affinity purification (TAP) followed by mass spectrometry and yeast-2-hybrid analysis showed subunits of the STRIPAK-complex as interaction partners. Further, proteins were identified which are taking part in ubiquitination and Golgi-organization. We will present data of localization experiments to approve a relation between STRIPAK and regulatory effectors.

[1] Pöggeler S, Kück U. 2004. Eukaryot. Cell 3: 232-240
[2] Bloemendal S, Lord KM, Rech C, Hoff B, Engh I, Read ND, Kück U. 2010. Eukaryot. Cell 9: 1856–1866

PR2.14

How Sex Influences Carotene Metabolism in Zygomycetes Fungi?

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In Blakeslea trispora, the mating partners develop zygophores that exchange (pro)hormones to co-ordinate the process of sexual reproduction. The hormones comprise a unique cocktail of compounds known as trisporic acids. Apocarotenoids are the bioactive oxidative degradation products of Beta-carotene. We focus on the "chemical interaction" between (+) and (-) zygophores during sexual cycle and how these chemical signals influence the transcription of genes involved in apocarotenogenesis. Dynamics of transcriptional regulation in liquid cultures of individual and mated Blakeslea were analysed for 144 hours by real time quantitative reverse - transcription PCR. Relative quantification of m-RNA for carRA(Phytoene synthase and lycopene cyclase) and tsp3(carotenoid cleavage dioxygenase) were done using act-1(actin) as reference gene. Individual strains were treated with C-13 apocarotenone and the prohormone methyl trisporic acid-C for 60 hours. In vivo co-expression experiments using Beta-carotene overproducing plasmid and tsp3 gene insert in E.Coli, were carried out to identify the apocarotenoid product. Gene expression kinetics pinpoint a trend of upregulation in both carRA and tsp3 from 72-144 hours in mated culture. Methyl trisporic acid C is more active in (+) with a 3.5 fold upregulation over C-13 apocarotenone concerning tsp3 expression. Interestingly, Methyl trisporic acid C had no significant impact on carRA expression either in (+) or (-) strain. C-13 apocarotenone had a 2 fold impact over methyl trisporic acid C with tsp3 expression in (-). LC-MS analysis for the co-expression experiments in E.coli resulted in identification of 12'-apo carotenal as the apocarotenoid product formed by tsp3 in Blakeslea trispora.

PR2.15

Parasexual development in the host – parasite-pair *Absidia glauca* – *Parasitella parasitica* and its use as system for genetic manipulation of zygomycete fungi

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Biotrophic fusion parasitism within zygomycete species often leads to structures resembling sexual morphogenesis, including cytoplasmic fusion and transfer of nuclei between the interacting individuals. Microscopy of fixed and sectioned infection structures reveals complex interactions between the partner hyphae, with altered cell wall morphology in contact regions and well defined pores of 2-3 µm in diameter, which will easily allow the transfer of the 1 µm sized nuclei from one compartment to the next. The parasexual interaction between *Parasitella parasitica* and its multiple host species also involves the zygomycete trisporic acid-based sexual recognition system. We are aiming at establishing this fusion reaction as a tool for genetic manipulation within this group of fungi.

Methionine auxotrophy in a strain of *Absidia glauca* was found to be the result of an insertion disrupting the single copy *HAT* gene coding for homoserine acetyltransferase. This strain was infected with methionine prototrophic *P. parasitica* containing two different *HAT* genes but defective in adenine biosynthesis and displaying pink colored mycelium. Spores from the infection plates were screened for the ability to grow on minimal medium, where only progeny with recombinant phenotypes will survive. Primary gene transfer events occured with a frequency at least two orders of magnitude higher than spontaneous reversion of the mutation but were found to be mitotically unstable over the subsequent sporulation cycles. Hybridization analyses revealed that successful transformation and complementation is possible also with the wild type *A. glauca HAT* gene which is maintained as autonomously replicating plasmid in the recipient mutant strain.

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PR2.16

Functional Characterization of predicted genes in the Aβ mating-type locus of *Schizophyllum commune*

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Sexual development in the basidiomycete fungus *Schizophyllum commune* is controlled in part by a complex system of interacting homeodomain proteins that function in non-self combinations to regulate the initial events of sexual development. Two functionally redundant "A" mating type loci (Aα and Aβ) encode these interacting proteins. While Aα has been well characterized, little is known about the functional nature of the more complex Aβ locus. The purpose of this investigation is to accomplish a functional characterization of the predicted homeodomain genes encoded in Aβ. Predicted genes (*V6, U6, T6, S6, R6,* and *Q6*) were amplified by PCR and cloned into suitable transformation vectors. Transformants were analyzed for activation of A mating-type developmental events. Our results to date indicate that strains transformed with *V6* show evidence of *V6* specific developmental activity in appropriate mating reactions and that *U6* transformants do not. Analysis of the remaining cloned genes is ongoing. Our initial results indicate that activation of sexual development by the homeodomain genes encoded in the Aβ locus is likely to be complex and may be dependent on the mating-type identity of the transformed strain.

PR2.17

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

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In *Aspergillus nidulans,* light is the main signal that influences the decision if cells undergo asexual (conidiation) or sexual (cleistothecia) development. In all eukaryotes nitric oxide (NO) is an important signalling and defence molecule and we have shown previously that the short-lived nitrogen oxide radical is generated during the nitrate assimilation process, and detoxified by flavohemoglobin proteins FhbA and FhbB.

Here we report that the metabolism of NO is additionally regulated by light. We found that the expression of the flavohemoglobin gene *fhbB* is induced by light and that this regulation depends on the photoreceptor complex. Our data show that conidiation is gradually repressed by increasing NO levels and at the same time formation of cleistothecia is promoted. We also found that other metabolic genes which potentially affect NO formation or consumption are regulated by light and thus may participate in the fine-balanced regulation of developmental decisions in *A. nidulans.*

PR2.18

Mub1 protein regulates mating differentiation and yeast cell morphogenesis in *Cryptococcus neoformans*

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Cryptococcus neoformans is a heterothallic basidiomycete that grows vegetatively as yeast cells and produces mating filaments in the sexual state. Mating initiates when *MAT***a** and *MAT*α cells conjugate and fuse, and then dikaryotic sexual filaments are subsequently produced. Generation of final fruiting structures basidia and meiotic progeny basidiospores leads to completion of the sexual cycle. Prior studies have revealed that *C. neoformans* Cwc1 and Cwc2 proteins are two central photoregulators which form a complex to inhibit the production of sexual filaments upon blue light irradiation. To reveal the detailed light response networks, a genome wide mutagenesis screen was conducted and components involved in light-mediated filamentation pathway have been identified. In this study, a suppressor mutant EE24 was characterized and T-DNA is found to insert at the upstream regulatory region of *C. neoformans MUB1* gene, a homologue of *Saccharomyces cerevisiae MUB1* gene. In *S. cerevisiae,* Mub1p is a MYND domain-containing protein required for ubiquitination and turnover of Rpn4p, a transcription factor of proteasome genes. *mub1* mutant shows a multiple-budding phenotype. Deletion of *C. neoformans MUB1* gene caused compromised growth at 37°C. *mub1* mutants similarly displayed the multiple-budding phenotype and altered structure of bud scars were observed. Morphogenesis of dikaryotic sexual filaments and generation of basidiospores were defective in the *mub1* bilateral cross. Interestingly, same sex mating was also regulated by *C. neoformans* Mub1. Our studies demonstrate that *C. neoformans MUB1* is an important gene that regulates yeast cell morphogenesis and mating differentiation.

PR2.19

Do you recognize me? – Orphan receptors in the basidiomycete *Schizophyllum commune*

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The filamentous fungus *S. commune* is a model organism for sexual development of basidiomycetes. Numerous studies revealed the importance of two gene loci, *A* and *B*, responsible for mating and sexual development. While *A* codes for homeodomain transcription factors, *B* codes for a pheromone/receptor system. Both verify compatibility or abortion of mating. The *B*-receptors (Ste3-like, seven transmembrane domains, G-protein coupled) recognize pheromones of non-self specificity and induce signal transduction pathways and specific gene regulation. After sequencing of strain H4-8 four new Ste3-like GPCRs, homologous to the known *Bα* and *Bβ* specific ones, were found. Three of the four are located close to the *B* locus. Their function is unknown, because a *B*-locus defective strain without any interactions seen in *B*-dependent development still contains those four GPCRs, which obviously do not respond to any wild type pheromone. However, our results indicate their importance since sequence identity – analyzed by PCR, cloning and sequencing – between unrelated strains was found arguing for conservation of these genes. Gene expression was observed with Reverse Transcriptase PCR and also with quantitative Real Time PCR during mating interaction and in monokaryotic strains, which showed comparable results between gene *brl4* and the mating receptor *bar2*. Overexpression of the gene *brl2* under control of *tef1*-promoter is performed to give insights into the function of this new class of pheromone receptor-like genes. Furthermore, the *Agrobacterium tumefaciens* mediated transformation is established for this basidiomycete, to improve efficiency of transformation and increase the number of homologous recombination events.

PR2.20

Blue light acts a double-edged sword in regulating sexual development of *Hypocrea jecorina* (*Trichoderma reesei*)

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Trichoderma reesei, an industrially important cellulolytic filamentous fungus, is an anamorph of the pantropical ascomycete *Hypocrea jecorina*. *H. jecorina* undergoes a heterothallic reproductive cycle, and the mating yields fertilized perithecia imbedded in stroma containing dechexads with 16 linearly arranged ascospores. Here, we investigated the mechanism and environmental regulation of *H. jecorina* sexual development by applying white light or blue light (440-460 nm). We show that visible light is dispensable for *H. jecorina* sexual development. The experiments on mutant analysis revealed that blue-light photoreceptor BLR1 and BLR2 have both positive and negative regulatory roles in stroma formation during early sexual development, and that the photoadaptation protein ENV1 dampens the light-dependent inhibitory effect in response to changes in illumination. Our results suggest that blue light acts a double-edged sword in regulating *H. jecorina* sexual development.

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Poster Category 3: Genomes and Genome Evolution

PR3.1

Development of DNA Barcodes to Identify Edible Mushrooms

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Basidiomycetes as one of the largest groups of edible mushrooms have become more important in recent times for their medicinal and nutritional properties. For many years, species of this family have been mainly classified by their common phenotypic traits, however, taxonomic identification based solely on morphological features can be misleading and unreliable. In contrast, DNA based identification provides a powerful and reliable method for taxonomic discrimination of fungi, it can be performed at any growth stages using parts of the fruit body, mono- and dikaryotic mycelia, or any other organic fungal. In the current study, three different DNA and c-DNA molecular markers including Internal Transcribed Spacer (ITS) I and II, Intergenic Spacer (IGS) I, and mitochondrial COXI gene were developed to identify mushroom species and individuals. Phylogenetic trees could clearly distinguish the species of Basidiomycetes by showing distinct clades. Species differentiations were re-confirmed by AMOVA analysis, nucleotide divergence, haplotyping and P values. Moreover, the designed primers were perfectly matched with the used species, can be employed in phylogenetic studies of other *Basidiomycetes*. Polymorphism occurred throughout the regions of interest due to insertion-deletion and point mutations, and can be clearly differentiated within the families as well as genera. This study proved that the three developed molecular markers can be used as the consensus DNA and/or c-DNA barcodes for taxonomic identification of *Basidiomycetes*.

PR3.2

Genomic and molecular characterization of a model ascomycete that is ancestral to mutualistic and pathogen-rich fungal lineages (Strain A95 *Sarcinomyces petricola*, Chaetothyriales)

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Melanised micro-colonial fungi (MCF) that colonise bare rock surfaces survive in extreme environments. Phylogenetically, rock-inhabiting MCF are ancestors of lichens as well as important animal and plant pathogens. MCF may thus have been a “stepping-stone” to colonisation of other extreme environments including animals. A meristematic black yeast species *Sarcinomyces petricola* (strain A95) was selected as a model strain. The estimated 29 Mbp DNA sequence is being assembled and annotated. As with all MCF that possess the characteristic stress-tolerant morphology (including thick, melanised cell-walls) disruption of the cellular structure is problematic. Nevertheless an efficient procedure for protoplast formation was developed using various hydrolytic enzymes. Protoplast formation is an important prerequisite for both the development of an appropriate transformation system for A95 and application of other tools to characterise the genome. For example, the number of chromosomes will be ascertained by pulsed-field gel electrophoresis of the protoplasts. Karyotyping A95 in this way will provide an additional check on the estimated genome size (and thus the depth of sequencing required to close the genome) and permit the isolation of mitochondrial DNA and plasmids (if A95 contains them).

PR3.3

Grey mould isolates from commercial strawberry fields show multiple fungicide resistance and represent a novel clade between *B. cinerea* and *B. fabae*

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Botrytis cinerea is a destructive pathogen of many fruit and vegetable crops worldwide, which needs to be controlled by fungicide treatments. Previously, we have observed high frequency occurrence of *B. cinerea* strains with three different multidrug resistance (MDR) types, MDR1-3, in French and German vineyards, and identified the underlying mutations leading to drug efflux transporter overexpression. We have extended our analysis to grey mould populations from German strawberry fields which receive weekly fungicide sprayings during flowering. The strawberry populations carried a combination of fungicide-specific (target site) and nonspecific (MDR type) resistances and showed high frequencies of multiple resistance to currently used fungicides. A stronger variant of the MDR1 phenotype, called MDR1^{h}, was discovered which contributes to reduced control efficiency of two major botryticides. Surprisingly, the majority of German strawberry isolates, called Botrytis group 3, were found be genetically distinct, but otherwise indistinguishable, from *B. cinerea*. Based on sequence comparisons of multiple genes, Botrytis group 3 isolates were identified as a novel clade intermediate between *B. cinerea,* which attacks more than 200 host plants, and *B. fabae*, a species that only infects legume hosts. Population studies revealed that group 3 isolates are almost absent from vineyards, possibly due to reduced sporulation efficiency on grape berries, but common on other fruit and vegetable crops in different European countries. To analyse the genomic basis of evolution, host specificity and biology of the *B. cinerea* species complex and related clades, we are currently performing genome sequencing, interspecific crosses and systematic comparative phenotypic studies.

PR3.4

Similar Is Not The Same: Differences In The Function Of The (Hemi-) Cellulolytic Regulator XlnR (Xlr1/Xyr1) In Filamentous Fungi

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The (hemi-) cellulolytic regulator XlnR (Xlr1/Xyr1) is a major factor in fungal xylan and cellulose degradation as well as in the utilization of D-xylose via the pentose catabolic pathway (PCP).

XlnR homologues are commonly found in filamentous ascomycetes and often assumed to have the same function in different fungi. However, a comparison of the saprobe *Aspergillus niger* and the plant pathogen *Magnaporthe oryzae* showed different phenotypes for deletion strains of XlnR.

In this study wild type and *xlnR/xlr1/xyr1* mutants of six fungi were compared: *Fusarium graminearum*, *M. oryzae*, *Trichoderma reesei*, *A. niger*, *Aspergillus nidulans* and *Aspergillus oryzae*.

The comparison included growth profiling on relevant substrates and detailed analysis of protein profiles of extracellular enzymes and extracellular enzyme activities. The data resulting from this comparison demonstrate significant differences in the influence of XlnR and its orthologs on plant polysaccharide degradation by these fungi. Highlights of the study will be presented.

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PR3.5

Twin research in fungi – Phenotype vs. Genotype

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Trichoderma atroviride is a filamentous fungus used widely for biological control of major plant diseases. A strain of this species (LU132) has been developed into a commercial biocontrol agent (BCA) for use in New Zealand. Its broad spectrum of antagonistic activity is linked to a wide range of biological parameters but we have limited knowledge of what specific attributes make this strain particularly effective as a BCA. The development of a molecular marker for LU132 failed, because all known marker genes had identical sequences with *T. atroviride* LU140, a strain that was isolated at the same time from the same paddock but had a different phenotype to LU132. To identify the level of genetic similarity between the two strains, the whole genomes were re-sequenced via Next Generation Sequencing with a surprising result: only 2 Single Nucleotide Polymorphisms (SNPs) could be found between the genomes of LU132 and LU140. In the present study the strains were confirmed to be two different individuals by comparing the phenotypes and confirming the SNPs. Based on those results, five genes were selected whose gene expression was studied. The results are presented here and their impact on the relationship between molecular changes and phenotypic changes in *T. atroviride* are discussed.

PR3.6

Transcriptomics of *Agaricus bisporus* reveals changes in carbon metabolism in different growth stages

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Carbon source utilisation is an important aspect of fungal physiology. Many fungi are exposed to mixtures of carbon sources, which enable them to make choices to use the most favourable substrate. *Agaricus bisporus* is commonly grown on compost, which consists mainly of straw and horse manure. This means that the majority of the carbon source is present as plant-based polysaccharides, which themselves consist of many different monomeric components. The major components of these polysaccharides are glucose, xylose, and arabinose, while smaller amounts of galactose, galacturonic acid, rhamnose and mannose are also present.

In this study we evaluated the expression of genes involved in the catabolism of different sugars during different stages of growth of *A. bisporus*. Clear differences in the expression of genes from different catabolic pathways were observed between mycelium grown on plates, in compost or in casing-soil, and in fruiting bodies, suggesting a high level of specialization.

PR3.7

Homolog searching and RNA-SEQ in *Pleurotus Ostreatus*

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Pleourotus Ostreatus is an edible basidiomycete of great interest in the field of food and bioremediation. In 2009 the genome sequence of the two haplotypes of *Pleurotus* N001 strain were released. The two haplotypes can be individualized permitting the two gene complements of a sexually mature individual to be analyzed simultaneously for first time.

In order to better understanding the mechanisms of ligning degradation and metabolic function of this fungus we realiced three RNA-SEQ experiments in the two haplotypes and the dicariotic strain at three temperatures in liquid and solid grow medium.

For this purpose was of great interest to know the corresponding allele in each haplotype. In each haplotype two sequences were considered alleles if their e-value were less then e-20, their aligment identity percentages were greater than 80% and the aligment covered at least the 80% of each gene. Additional checks were performed, as the best hit was the same in both directions and the positions of both genes corresponded to homologous chromosomes.

The RNA-SEQ experiments were done with SOLID plataform from Applied Biosystems, and then the data were analyzed with the software TopHat and Cufflinks. Read position distribution and the changes in relative isoform abundance determined with Cufflinks were used to perform a genome annotation curation tool.

PR3.8

Genomic context of effector genes in *Fusarium oxysporum* enables prediction of novel effector candidates

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Fusarium oxysporum is a soilborne fungus that causes Fusarium wilt disease in many plant species by colonizing and eventually blocking the host xylem vessels. Virulence depends crucially on effector proteins, which are secreted by the fungus in the xylem sap (Six (Secreted in xylem) proteins). In the tomato wilt strain *F. oxysporum* f. sp. *lycopersici* (*Fol*) Six proteins are encoded on lineage-specific (LS) chromosomes. We analysed the genomic context of *SIX* genes in *Fol* and found that all *SIX* genes harbour a particular transposable element - a mimp (miniature impala) - in their promoters. To investigate the impact of the mimp on *SIX* gene expression, we made partial promoter deletions for two *SIX* gene promoters. The gene products of these two *SIX* genes are recognized by tomato resistance genes. This recognition is abolished in some promoter deletion strains, while in others recognition is still mediated. However, in all promoter deletion strains, *SIX* genes are expressed *in vitro*. We hypothesize that the *SIX* gene promoters are usually in a repressed state and are only transcriptionally released upon plant infection. Partial deletion of the promoters appears to reduce repression of transcription.

Using our current knowledge of *SIX* gene structure and genomic context we developed a bioinformatic pipeline for the prediction of novel effector candidates in *Fol*. We identified 15 of these genes. Currently, we are making knock-out mutant strains to explore whether these genes are important for virulence.

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PR3.9

Fungal glutathione transferases:targets for evolutionary innovations

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Glutathione transferases (GST) are detoxification enzymes, which genes are highly duplicated in fungal genomes. These enzymes could display the classical conjugation of glutathione to toxic compounds but also many other activities, making them excellent examples of how multiple gene duplication events has resulted in groups of enzymes with a large panel of functions. The study was here focused on two specific classes Ure2p and GTE, which are extended in wood-decaying fungi. By developing high throughput screening assays in combination with structural data we found that these classes are highly divergent in their enzymatic activities or ligand recognition in spite of strong primary structure homology. The number of Ure2p isoforms varies between *Phanerochaete chrysosporium* and *Phanerochate carnosa* (9 and 20 isoforms respectively) and could be related to different local adaptation rather than genetic background, since both species are taxonomically very close. The GTE class is composed of 5 isoforms. So far homologues of this family were only found in bacteria and are known to degrade the beta-aryl linkage, which is the most abundant intermolecular link in lignin. Functional and structural data revealed various activities and strong wood compounds specificities between duplicates.
Ure2p and GTE could have thus played a major role in fungal adaptation to their environment through their huge versatility. Both classes are microorganism specific and could be excellent markers of fungal diversity. Their capacities of catalytic promiscuity and neo-functionalization make these enzymes excellent sinks of new functions in environmental adaptation process and are excellent models for protein engineering.

PR3.10

Evidence for Extensive Recent Intron Transposition in Closely Related Fungi

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Though spliceosomal introns are a major structural component of most eukaryotic genes and intron density varies by more than three orders of magnitude among eukaryotes, the origins of introns are poorly understood, and only a few cases of unambiguous intron gain are known. We utilized population genomic comparisons of three closely related fungi to identify crucial transitory phases of intron gain and loss. We found 74 intron positions showing intraspecific presence-absence polymorphisms (PAPs) for the entire intron. Population genetic analyses identified intron PAPs at different stages of fixation. We found direct support for extensive intron transposition among unrelated genes. A substantial proportion of highly similar introns in the genome either were recently gained or showed a transient phase of intron PAP. We also identified an intron transfer among paralogous genes that created a new intron. Intron loss was due mainly to homologous recombination involving reverse-transcribed mRNA. The large number of intron positions in transient phases of either intron gain or loss shows that intron evolution is much faster than previously thought and provides an excellent model to study molecular mechanisms of intron gain.

PR3.11

Genotypic Analysis Of *Fusarium* Species Associated With *Allium cepa* In The UK Including Whole Genome Analysis Of *F. oxysporum* f. sp. *cepae*

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This project set out to identify *Fusarium* species associated with onions in the UK and to develop quick molecular methods to identify *Fusarium oxysporum* f. sp. *cepae* (FOC) causing onion basal rot.
More than 500 isolates representing diverse *Fusarium* species, including 171 isolates associated with onions obtained from 12 different sites in the UK were collected. Five species associated with diseased onions have been identified, of these *F. proliferatum, F. solani, F. redolens* and *F. avenaceum* have not previously been reported in association with onion crops in the UK. Assays under controlled conditions confirmed the ability of the *F. proliferatum* isolates to cause disease on onions.

F. oxysporum was by far the most common (143 isolates) and these isolates belong to at least two different genotypes based on sequence comparison of several "housekeeping" genes and overall, appear to be polyphyletic. None of the housekeeping genes studied correlate with pathogenicity. Secreted in xylem (SIX) genes offer more promise for the specific identification of *F. oxysporum formae speciales* (Lievens *et al*., 2009). FOC isolates were screened for the presence of seven SIX genes (SIX1-7). A homologue of SIX7 gene was found in a few FOC isolates which suggests that SIX7 is not necessary for pathogenicity. Whole genome sequencing of a FOC isolate was completed at our university in order to understand pathogenicity and identify novel effector genes. Screening of 11 candidate effector genes suggest that FOC isolates have different gene sets.

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PR3.12

The evolution of Zygomycetes, the most basal terrestrial fungi: lessons from new genome projects

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Zygomycetes, formerly described as class within the fungal kingdom, are polyphyletic, and therefore, split into five distinct subphyla, which are the Entomophthoromycotina, Mucoromycotina, Mortierellomycotina, Kickxellomycotina and Zoopagomycotina [1, 2]. The former two subphyla contain species, which are human pathogenic causing infections with diverse predisposition and etiologies. They encompass ubiquitously distributed insect-killing, saprotrophic soil- or dead plant material-inhabiting fungi of the orders Entomophthorales and Mucorales, respectively. Human pathogenic species inhabit different growth temperature optima ranging from 33 °C to 42 °C, just members of these two orders are capable of causing diseases, entomophthoromycosis and mucormycosis, in immunocompromised and immuncompetent humans, respectively. Two new genome projects were initiated on *Conidiobolus coronatus* and *Lichtheimia corymbifera* and the results are discussed with respect to the evolution of single genes involved in the development of pathogenicity. The data are embedded in a phylogenomic study comprising all publicly available fungal genomes and EST databases and analysed with Bayesian inference and maximum likelihood approaches [3]. moreover, the novel genome projects are discussed with respect to the 1000 Fungal Genome Project which has been newly launched last year (http://1000.fungalgenomes.org/home/).

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PS3.13

Variation in distribution and evolution of fusarin gene cluster in Fusarium

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Fusarins are mycotoxins produced by a wide range of species in the genus Fusarium. The putative fusarin biosynthetic gene (FUS) cluster in the maize pathogen F. verticillioides consists of nine genes (FUS1 – FUS9), including a hybrid polyketide synthase-non ribosomal peptide synthetase gene (FUS1). Here, we examined sequence variation in FUS cluster genes and in regions flanking the cluster in several fusaria from the Fusarium lineages the Gibberella fujikuroi species complex (GFSC), the trichothecene producing clade (TRC), and the F. tricinctum species complex (FTSC). The results indicate that the order and orientation of genes in the FUS cluster are uniform throughout Fusarium, even though the cluster has been lost from most American-clade species of GFSC and from most strains of F. incarnatum-F. equiseti species complex (FIESC). In contrast, genes flanking the cluster differ in most species, indicating the genomic context of the cluster differs in all but the most closely related species. The phylogenetic relationships of FUS genes are correlated with the relationship of Fusarium species in which the genes occur. An exception to this is F. scirpi strain FRC R-06979. Although this strain is part of the TRC, its FUS genes are more closely related to FUS genes in the FTSC. In contrast, the FUS1 gene in other related strains is more similar to FUS1 in other TRC species. These results suggest that the Fusarium lineage that gave rise to R-06979 lost the FUS cluster that it inherited from a TRC ancestor but acquired a FUS cluster from a species within FTSC by horizontal gene transfer.

PR3.14

Alternaria Leafspot Pathogens: Genetics, Evolutionary History And Diagnostics

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Alternaria black spot diseases on pome fruits are economically important in Asia and the Americas. Movement of infected apple and pear material risks establishment of these and other host specific *Alternaria* diseases in Europe. EC countries are obliged to take action on intercepted non-native pathogenic *Alternaria*. However, identification based on morphology is unreliable and conventional genetic loci show little resolution between species. Fast and reliable diagnostics need to be developed to identify *Alternaria* pathogens.

Disease is linked to the presence of an additional small chromosome containing genes responsible for the production of host-specific toxins.

Phylogenetic and morphological studies were performed on the UK culture collection. Genes sequenced were: ITS, EndoPG, mating type genes and three novel loci. Host-specific toxin genes on the pathogenicity chromosome were detected using PCR.

Isolates carrying the pear specific pathogenicity chromosome were phylogenetically distinct. Isolates carrying the apple specific pathogenicity chromosome were not distinct and were polyphyletic. This may indicate that presence of toxin genes is a better molecular marker for pathogenicity than morphology (primarily sporophore shape) or phylogeny. Future work will look for new diagnostics based on more direct indicators of pathogenicity, such as presence of toxin genes and pathogenicity chromosomes.

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PR3.15

Recent Developments at the Aspergillus and Candida Genome Databases

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The *Aspergillus* and *Candida* Genome Databases (AspGD, http://www.aspgd.org and CGD, http://www.candidagenome.org/) are freely available, web-based resources for researchers studying the molecular biology of these fungi. The interfaces and navigation functionality of both web sites and databases are recently upgraded, providing streamlined, ortholog-based navigation of the annotation for multiple species concurrently. We have now completed manual curation of the entire published literature about multiple species, including *A. nidulans*, *A. fumigatus*, *A. oryzae*, *C. albicans*, and *C. glabrata*. We also provide resources to foster interaction and dissemination of community information, tools, and data. We collect and provide large-scale datasets with a full-featured genomics viewer to facilitate comparative genomics analysis. AspGD is funded by grant R01 AI077599 from the National Institute of Allergy and Infectious Diseases, and CGD is funded by R01 DE015873 from the National Institute of Dental and Craniofacial Research at the US National Institutes of Health. We welcome, encourage, and appreciate your questions or suggestions, and AspGD and CGD curators can be reached at aspergillus-curator@lists.stanford.edu and candida-curator@lists.stanford.edu, respectively.

PR3.16

Functional diversity of *Trichoderma* mycoparasitism

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Many species of the fungal genus *Trichoderma* (teleomorph *Hypocrea*) are mycoparasites, what is widely used for the biological control of plant's pests. Here we studied common responses of ecologically diverse *Trichoderma* spp. to the presence of a potential fungal prey, using the cosmopolitan soil species and powerful mycoparasites *Trichoderma atroviride* (*Ta,* teleomorph *Hypocrea atroviridis*) and *T. virens* (*Tv,* teleomorph *Hypocrea virens*), and mainly saprotrophic tropical species *T. reesei* (*Tr,* teleomorph *Hypocrea jecorina*). We analyzed transcriptional responses during three stages of interaction with *Rhizoctonia solani* (teleomorph *Thanatephorus* spp.)*, i.e.* prior the contact, during the initial stage of interaction, and shortly after the contact was established. *Tv* completely arrested the growth of the prey while *Ta* did not stop the growth of *R. solani*, but continuously overgrew it. *Tr* did not attack the prey but built up a dense hyphal front, which was not permeable for hyphae of *R. solani.* In total 666, 303 and 424 genes were >2-fold regulated in *Ta, Tv* and *Tr*, respectively. The majority of them was down-regulated in *Ta* and *Tv*, but not in *Tr*. 34 % of the up-regulated genes in *Ta* (in contrast to only 6 % in *Tv*) comprised secreted proteins dominated by subtilisin-like and aspartyl proteases. It suggests that *Ta* mainly acts by a hydrolytic attack. In contrast, the majority of up-regulated genes in *Tv* comprised were those involved in the biosynthesis of gliotoxin and its precursor amino acids. Expression in *Tr* was characterized by a massive up-regulation of ribosomal genes indicative for a strong shift in protein synthesis. In contrast, genes encoding permeases of the major facilitator subfamily, PKS and NRPS and Zn2Cy6 transcription factors were down-regulated in all three species. Our data show that in *Trichoderma* the functional stimulation caused by the prey exhibits diverse patterns, whereas the suppression of gene function in *Trichoderma* mycoparasitism is evolutionary conserved.

PR3.17

The interaction of the plant-pathogen *Verticillium longosporum* and its host *Brassica napus* and insights into the evolutionary origin of the fungal hybrid.

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Verticillium longisporum is a soil-borne fungal pathogen of oilseed rape (*Brassica napus*). Infection is initiated by hyphae from germinating microsclerotia which invade the plant vascular system through penetration of the fine roots. We investigated the reaction of the fungus to xylem sap of the host-plant by differential expression of proteins related to reactive oxygen stress [1]. Knockdowns of the catalase-peroxidase of *V. longisporum* were inhibited in the late phase of disease development. The evolutionary origin of the cruciferous fungal pathogen, *V. longisporum* is still a mystery. It is very closely related to both *V. dahliae* and *V. albo-atrum* but possesses some typical characteristics such as long spores, almost double amount of nuclear DNA content and cruciferous host specificity. *V. longisporum* is an example for an early stage of speciation and we show clear evidences for the origin of the fungus. To clarify the hybrid status, we undertook molecular sequence analyses of the internal transcribed spacer (ITS) and intergenic spacer (IGS) regions of rDNA of putative ancestors of *V. longisporum*. In addition a number of other structural genes were analyzed. We found one gene encoding a putative zinc finger transcription factor with two distinct sequences carrying different markers supporting the hybrid origin detection of the fungus. One of these sequences is almost identical to that of *V. dahliae* and the other is highly similar to the sequence of *V. albo-atrum*. Currently we are sequencing *V. longisporum* to determine which rearrangements occurred during and after the hybridization.

1. S Singh, SA Braus-Stromeyer, C Timpner, O Valerius, Av Tiedemann, P Karlovsky, C Druebert, A Polle, and GH. Braus, Molecular. Plant-Microbe Interactions, accepted (2011), DOI: 10.1094/MPMI-08-11-0217

PR3.18

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

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

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PR3.19

How apomixis shaped the genome of *Arnium arizonense*, a homothallic Sordariomycetes?

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Arnium arizonense is a fungus closely related to the heterothallic fungus *Podospora anserina* but displays several unique features. It is apomictic, *i.e.*dikaryotic croziers are formed inside the perithecia but neither karyogamy nor meiosis take place in the asci, although morphological changes in both chromosomes and spindle pole bodies are reminiscent of those associated with meiosis in heteromictic Pezizomycotina. Instead of meiosis, the two nuclei undergo two mitoses and the resulting eight nuclei are enclosed in uninucleate ascospores, among which four mature normally, and four abort. Arrangement of the two ascospore types in individual asci is random (Mainwaring and Wilson, 1968, Trans Br mycol Soc, 51, 663). Another peculiarity of *A. arizonense* is its low number of chromosomes: the haploid number is two, while most fungi in this group have seven chromosomes. Analysis of the genome sequence reveals that *A. arizonense* contains linked counterparts of the *P. anserina* mating-type genes, a structure that is typical of homothallic life style. Deletion of the mating-type locus results in the loss of perithecium formation, thus confirming the role of the mating-type genes in the fruiting-body development. Mating-type target genes have been recently identified in *P. anserina*(Bidard et al, 2011, PLoS ONE, 6, e21476). Search for their orthologs in *A. arizonense* reveals that some of them are pseudo-genes. The genome of *A. arizonense* is currently under analysis to find candidate mutations for the loss of karyogamy and meiosis, and to determine how apomixis has shaped the genome structure.

PR3.20

Comparative QTL mapping of multiple disease resistance traits in the cultivated mushroom *Agaricus bisporus*

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1. INRA 2. CTC

Biotic stresses lead to severe crop losses in *Agaricus bisporus* cultures. The development of resistant cultivars is the most effective, economical and environmentally friendly approach to manage disease control. The understanding of the genetic architecture of resistance to diseases is a prerequisite for breeding. Bacterial brown blotch (caused by *Pseudomonas tolaasii),* dry bubble (caused by *Lecanicillium fungicola)* and green mould (caused by the fungal competitor *Trichoderma aggressivum)* are the most detrimental disorders affecting yield and quality of the button mushroom throughout the world. Independent studies investigating quantitative trait loci (QTL) of resistance for each of these diseases have been done in our laboratory (Foulongne-Oriol et al. 2011, 2012; Mocquet et al. 1999). The present work proposes, as an integrative approach, to compare the location of these QTL: Are there any evidence for multiple disease resistance loci? Do common or different mechanisms underlie each resistance? Consequences for breeding of multi-resistant cultivar in *A. bisporus* will be discussed. Foulongne-Oriol M *et al.* (2012) Relationship between yield components and partial resistance to *Lecanicillium fungicola* in the button mushroom *Agaricus bisporus* assessed by QTL mapping. AEM (in press) Foulongne-Oriol M *et al.* (2011) QTL for resistance to *Trichoderma* lytic enzymes and metabolites in *Agaricus bisporus*. In: Proceedings of the 7th ICMBMP. Vol 2. pp 17-25. Moquet F *et al.* (1999) A quantitative trait locus of *Agaricus bisporus* resistance to *Pseudomonas tolaasii* is closely linked to natural cap color. FGB 28:34-42

PR3.21

Characterization of farA and other zinc finger transcription factor genes for fatty acid metabolism in *Aspergillus oryzae*

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The zinc finger transcription factor FarA in *Aspergillus nidulans* up-regulates genes required for growth on fatty acids (Hynes et al, 2006). Ctf1 and Por1, orthologs of FarA of *A. nidulans* are required for growth on fatty acids in *Candida albicans* and also for the essential transcriptional activation of genes involved in beta-oxidation and peroxisomal biogenesis in *Yarrowia lipolytica*, respectivel (Ramirez and Lorenz, 2009; Poopanitpan et al, 2010). FarA transcriptional factor is also found in the *Aspergillus oryzae* which has 83% homology of all the amino acid sequences and 97.5% homology of Zn_2Cys_6 motifs with the *A. nidulans.* In this study, *farA* disruptant in *A. oryzae* was characterized and expression levels of genes for fatty acid metabolism were also determined.

Interestingly,*A.oryzae farA* disruptants showed indistinguishable growth in fatty acid sources compared to thewild-type, inconsistent with the growth phenotype of the *A. nidulans* counterpart. In contrast, expressions of some genes for fatty acid metabolism were significantly reduced in the *farA* disruptants. These contradicting results suggested that FarA may act not only the primary transcriptional activator for fatty acid utilization in *A. oryzae* and that another transcriptional factor(s) may regulate other fatty acid metabolic genes which can be accounted to the differences on the number of genes between these two *Aspergilli*. We then proceeded to screen other zinc finger transcription factor gene disruptants from the disruptant library of *A.oryzae* for fatty acid metabolism. Characterization and implication of these disruptants in response to different fatty acid substrates and fatty acid gene expressions are currently underway.

PS3.22

Locus-specific analysis of the *in vivo* spontaneous mutagenesis in the human fungal pathogen *Candida albicans*

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The human fungal pathogen *Candida albicans* displays a very high degree of plasticity, including the types of genomic changes frequently observed with cancer cells, such as gross chromosomal rearrangements, aneuploidy, and loss of heterozygosity. Despite its relevance to every aspect of genetics and evolution of this pathogen, our understanding of the mutation process remains quite limited. This is especially important since *C. albicans* may have evolved mechanisms not only to tolerate but also to generate genetic variation as a means of adaptation. Here, we have estimated the gene-specific mutation frequency of *HIS4*, CEN4 and *EST2* in *C. albicans* by using direct DNA sequencing, indirect gene-specific approaches (*HIS4* reversion) and MA lines. We found that frequency of mutation at these loci is similar to that observed for *S. cerevisiae* at homolog loci following the direct DNA sequencing approach, but was slightly lower at the CEN4. Analysis of the spectrum of spontaneous mutagenesis in both fungi revealed a transition bias. Although absence of the homologous recombination protein Rad52 did not show increased mutation frequency within these loci after 20 generations, an *in vivo* mutational accumulation was observed after 800 generations in *C. albicans*; especially for the *HIS4* locus. This was supported by an increased frequency of *HIS4* reversion in absence of Rad52. However, the fact that no mutation become fixed on these loci after those generations either in absence or presence of Rad52 suggest that *C. albicans* genome is relatively stable in terms of mutational accumulation even in the absence of Rad52.

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PR3.23

Evolution and function of Argonaute-proteins in the wheat-pathogen *Mycosphaerella graminicola*

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Important components of the RNA interference (RNAi) machinery are Argonaute-proteins which bind to single-stranded micro RNAs and influence the transcription of complementary messenger RNAs. During defense reactions RNAi fulfills a crucial role in the plant immune system. The role of RNAi in fungal pathogens is poorly understood. We use the fungal wheat pathogen *Mycosphaerella graminicola* to elucidate the role of Argonaute proteins in the evolution of host specificities and virulence. Two Argonaute-proteins, *Mgr 38035* and *Mgr 90232* were identified and selected based on their regulatory behavior *in planta.* We compared sequences of the two genes in different *M. graminicola* isolates to assess patterns of evolution. While *Mgr 35038* was highly conserved at the nucleotide level, the Argonaute gene *Mgr 90232* showed a strong differentiation at the nucleotide level between isolates. The gene has undergone both strong structural changes as well as mutations in the sequence composition. We speculate that the different level of conservation reflects different roles of the two Argonaute genes. We hypothesize that *Mgr 90232* is an evolutionary hotspot in the host-adaption of *M. graminicola* to wheat and plays an important role during infection. Future studies will try to elucidate the function of these genes with a focus on infection behavior via the generation of deletion mutants.

PR3.24

Recent Development In the Taxonomy and Phylogeny of *Aspergillus* and *Penicillium*: implications for Full Genome Sequence Initiatives

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The family of *Trichocomaceae* harbors various economically important genera, such as *Aspergillus*, *Penicillium* and *Paecilomyces*. The importance of these genera is illustrated by the high number of undertaken full genome sequencing projects. Recently, new insights in the taxonomy of *Aspergillus* and *Penicillium* have led to numerous new species and name changes of existing species. In this study, the phylogeny of the full genome sequenced strains is investigated using the protein coding genes *RPB1*, *RPB2*, *Cct8* and *Tsr1*. Furthermore, the impact of recent developments in the taxonomy of these strains is addressed.

Phylogenetic analysis shows a close relationship between the full genome sequenced strains (or representatives of the same species) of *Aspergillus*, *Penicillium sensu stricto*, *Monascus* and *Xeromyces*. *Talaromyces stipitatus* and *Penicillium marneffei* appear to be distantly related to *Aspergillus* and *Penicillium sensu stricto.* As a consequence, *Talaromyces* is re-defined and the combination *Talaromyces marneffei* (= *P. marneffei*) is proposed, leaving *P. chrysogenum* as the sole full genome sequenced species in *Penicillium*. Furthermore, *Talaromyces emersonii* is transferred to the new genus *Rasamsonia* and *Talaromyces thermophilus* will be transferred to *Thermomyces*. The new insights in the relationship among *Aspergillus*, *Penicillium* and related genera will help to interpret the results generated with comparative genomics studies or other studies dealing with evolution of e.g. mating type loci, virulence genes and secondary metabolite biosynthetic gene clusters.

PR3.25

Sequencing of the fungal pathogen *Ramularia collo-cygni,* why and how?

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The fungus *Ramularia collo-cygni* is the major biotic agent involved in Ramularia Leaf Spot (RLS). It was first identified as a pathogen of spring barley in Scotland in 1998 and since then has increased in its importance throughout the whole of the UK. Results from testing of the Rothamsted Hoosfield spring barley archive using quantitative real-time PCR indicated a significant increase in pathogen levels since the 1990's (Fountaine and Fraaije, 2009). RLS has recently been re-classed as a major barley disease in the UK. *R. collo-cygni* is currently classified as a member of the *Mycosphaerella* genera and sequence data derived at SAC suggests a genetic similarity between *R. collo-cygni, Mycosphaerella graminicola* and *M. fijiensis*. These sequences focus primarily on the genes associated with the target sites for fungicides, such as Beta tubulin, Cytochrome *b* and Succinate dehydrogenase, eburicol 14ά-demethylase (CYP51) genes. This paper will highlight previous sequence work and outline a new project using next generation sequencing by the combined approach of illumiina/solexa and Roche/454 sequencing. The use of these combined approaches will help with the assembly of sequence data which can then be used for comparative genetic studies to address the biology of *R. collo-cygni* in areas such as population genetics, fungicide resistance and pathogenicity. These advances should assist in the development of environmentally sound strategies to control this important disease of barley production systems.

PR3.26

Study on Genetic Structure of *Fusarium solani* Populations from Various Host Plants

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One hundred and forty nine *Fusarium solani* isolates were analyzed for their fluorescent AFLP patterns. The isolates were 60 from potato, 30 of *F. s.* f.sp. *cucurbitae*, 29 of *F. s.* f.sp. *phaseoli* and 30 of *F. s.* f.sp. *pisi* collected from different areas of Iran. After DNA extraction, digestion by *Eco*RI and *Mse*I, and ligation of adapters to DNA fragment, preamplification and selective amplification PCR, the products were separated by capillary electrophoresis. Analysis was performed based on 151 polymorphic markers. In general, 92% of observed molecular variance was related to within host populations and 8% variation was found among them. Genetic similarity between four host populations was more than 92%. High degree of similarity between populations may be due to gene flow among them. For determination of phylogenetic relationships, 110 isolates out of 149 isolates were examined based on DNA sequences from rDNA-ITS, a region of the nuclear rDNA-LSU and an intron-rich portion of the translation elongation factor 1 alpha (*TEF*) gene. The analyzed isolates were 59 from potato, 18 of *F. s.* f. sp. *cucurbitae*, 16 of *F. s.* f. sp. *phaseoli* and 17 of *F. s.* f. sp. *pisi.* Phylogenetic trees were inferred from three mentioned loci based on three methods, maximum parsimony, neighbor-joining and UPGMA. Major and minor clades with strong bootstrap, repeated in all trees and confirmed each other. The analyzed DNA sequences similarity of 110 isolates was more than 98%. The results of genomic fingerprinting and DNA sequencing of three loci confirmed each other. Although there are high variations and distinct phylogenetic groups among the Iranian isolates of *F. solani,* high degree of genetic similarity between four host populations presents that these populations are belonged to one clonal lineage.

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PR3.27

Diversity of Fusarium Species in the Phytopathogenic Gibberella fujikuroi Complex in Iran

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Gibberella fujikuroi complex (GFC) which contains Fusarium section Liseola, is composed of at least ten reproductively biological species (mating populations) denoted by letters A through J. These Fusarium species causes destructive disease on different agricultural and horticultural plants and also produce harmful mycotoxins and secondary metabolites that contaminate animal and human feed and food worldwide. We examined 46 isolates collected from maize, rice, sugarcane and onion in different areas of Iran, on the basis of morphological and biological characteristics as well as phylogenetic analysis. Based on morphological characteristics, 45 Fusarium isolates identified as F. verticilioides, F. sacchari, F. fujikuroi, F. proliferatum, and F. thapsinum. Mating studies based on crosses of these isolates with tester strains of GFC belonging to mating populations A to I, revealed that the isolates were belonged to mating populations A (F. verticilioides), B (F. sacchari, C (F. fujikuroi), D (F. proliferatum) and F (F. thapsinum). The results presented accordance between morphological and biological characteristics. We sequenced portions of translation elongation factor 1 alpha (tef) protein coding gene as well as internal transcribed spacer (ITS) of the nuclear ribosomal genes of 22 isolates out of 46 isolates. Molecular sequence analysis revealed that 22 isolates representing all five species identified on the basis of morohological and biological analysis. There were an example of discordance between species identified based on phylogenetic analysis and morphogical/biological analysis. Isolates belonged to F. verticilioides from sugarcane identified as F. andiyazi and isolates belonged to F. verticilioides and F. proliferatum from rice identified as F. fujikuroi based on phylogenetic analysis. This study represents, six Fusarium species from GFC are already identified in Iran. In contrast with previous reports, F. fujikuroi is the only species that causes foot rot on rice in Iran.

PR3.28

Analysis of the lignocellulose-degrading transcriptome and systematics of the white rot basidiomycete *Phlebia radiata*

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Phlebia radiata Fr. is a saprobic basidiomycete species belonging to the family Corticiaceae in the class Agaricomycetes. It is able to cause a white rot type of decay both in dead hardwood (conifers) and softwood (angiosperms). The species is capable of degrading wood lignin and lignocellulose by secreting a number of lignin-modifying enzymes - class II heme-including peroxidases and laccases. Our molecular systematic analyses indicate that *P. radiata* is more near related to *P. acerina* than to most of the other species allocated in the genus *Phlebia*. As a result of the ongoing whole genome sequencing of the Finnish *P. radiata* isolate 79, we have generated over 1.7 million expressed sequence tags (ESTs) from cDNA constructed from malt extract liquid culture mycelia of *P. radiata*. ds-cDNA was made from purified polyA$^+$ RNA and ESTs were sequenced with 454 sequencer using GS FLX Titanium series reagents, and assembled using gsAssembler (v2.6) (454 Life Sciences). The 10 875 contigs and 6 064 isotigs were subjected to Galaxy Megablast analysis. The Blast results with E-value cutoff of 1x e^{-03}, we found hits to protein coding sequences of gene transcripts functioning in the plant cell wall biodegradation, i.e. to several laccases, manganese peroxidases, lignin peroxidases and cellobiohydrolases. Our data indicate that *P. radiata* expresses a versatile array of oxidoreductases and polysaccharide-degrading enzymes already when growing on complex liquid medium.

PR3.29

Ensembl Fungi: an integrative resource for genome-scale data from fungal species

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Ensembl Fungi (http://fungi.ensembl.org) is a portal offering access to genome-scale data from fungal species, using the Ensembl Genome Analysis system. Access to the data is provided through a common set of interfaces (shared with non-fungal species also represented in the Ensembl system) including a web-based genome browser, a Perl API, a public MySQL server and a query orientated data warehouse (BioMart). Core data provided for all species includes genome sequence, sequence patterns, annotation of protein and non-coding genes and functional annotation imported from direct curation, UniProt and InterPro. The platform also supports integration of additional information including regulation, variation and comparative analysis. The current releaseprovides access to 23 fungal genomes across 9 different taxonomical orders. Extensive comparative analyses are performed across all the fungal species, and across the wider taxonomy. Protein alignments are used to reconstruct evolutionary trees and infer homology relationships, while pairwise alignments between DNA sequences are performed between closely related species. Ensembl Fungi also provides access to polymorphism data in the context of reference genome sequences for *Saccharomyces cerevisiae* and the phytopathogen *Gibberella zeae*. Ensembl Fungi gives access to the manually curated data from the fungal model organisms *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, which are imported from Saccharomyces Genome Database and Pombase respectively, while genes from plant pathogen data is being integrated with information about infectious (derived from PHI-base (http://www.phibase.org) through a new targetted resource PhytoPath (http://www.phytopathdb.org).

PR3.30

Comparative analysis of Aspergilli to facilitate novel strategies in fungal biotechnology

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Aspergillus is not only one of the most important fungi in biotechnology, it is also one of the most commonly found groups of fungi in environments worldwide and one of the most severe opportunistic human pathogens. Due to its relevance for industry and medicine, and their use as model systems for fungal biology, *Aspergillus* has one of the largest research communities in the fungal field. Indeed, this has resulted in this genus being one of the most intensively studied fungi with respect to genomics, with genome sequences for eleven species publicly available. The availability of this set of genomes in combination with the tools developed for *Aspergillus* genomics (e.g. AspGD and CADRE) enables comparative genomics at a highly detailed level.

In 2011 the JGI approved sequencing of an additional 8 Aspergilli and Penicillia, to further advance comparative genomics in this group of fungi. These species include additional industrial species, but also species that are distantly related to those for which genome sequences are already available.

A large consortium of researchers has been established to perform comparative genomics on the newly generated and already available Eurotiales genomes (32 in total). In addition, this analysis will be supported with experimental data to validate the differences found through bioinformatics. Details on the project and current status will be presented.

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PR3.31

Sequencing Of The *Taphrina betulina* Genome

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Taphrina are plant pathogenic yeasts, which cause the growth of tumors on mostly woody plant species. *Taphrina* are early diverging Ascomycetes, related to the model organism Schizosaccharomyces *pombe* and the human pathogen *Pneumocystis jirovecii*. There are a variety of *Taphrina* species reflecting the wide host range of this genus. Notable among them are the economically important peach leaf curl causing pathogen *T. deformans,* and the pathogen of the model tree poplar, *T. populina*. Also, *T. betulina* is a pathogen of birch trees and the causative agent of witch's broom that remodels the birch into tumorous brooms to create its living environment. Indeed, all of these *Taphrina* cause leaf curling and other tumor like developmental changes in their hosts. *Taphrina* are biotrophic plant pathogens, i.e. they depend upon evading defence responses in living host cells for their survival. We are developing the interaction between *T. betulina* and Birch (*Betula pendula*) into a model pathosystem. In order to discover genes for molecular work the genome of *T. betulina* is being sequenced. Our preliminary characterization of *Taphrina* species and analysis of the *T. betulina* draft genome will be presented.

PR3.32

The use of transposons as molecular tools for random mutagenesis in filamentous fungi

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The mobile and mostly also repetitive genetic elements named transposons are found in all eukaryotic genomes. Their impact on gene expression as well as their ability to cause major mutations within the genome or single genes makes them an interesting tool for random mutagenesis. Using transposable elements in filamentous fungi would aid gene characterization for instance in pathogenic or biotechnological important strains (1, 2, 3). At current we are employing two types of transposons, i.e. *Restless* from *Tolypocladium inflatum* (4) and *Vader* from *Aspergillus niger* (5). Each transposable element was inserted between the promoter and the open reading frame of the hygromycin phosphotransferase (*hph*) gene and transformed into *Neurospora crassa* or Aspergilli, respectively. We use hygromycin selection to identify transposition events and observed a *Vader* excision frequency of about 1 in 2.2 x 10^5 spores. All colonies analyzed showed an excision event on the DNA level. *Vader* footprints were found and after performing TAIL-PCRs the reintegration sites of 21 independent excision events were determined. *Vader* mostly integrates within or very close to genes, thus it appears to be a useful tool for transposon-mediated mutagenesis in *A. niger* (6). At current we try to improve the *Vader* tool and analyze its function in the heterologous host *A. nidulans.* Furthermore we analyse the performance of *Restless* in *N. crassa*.

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PR3.33

Phytopath – A new bioinformatics resource for plant pathogens

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PhytoPath (http://phytopathdb.org), a new bioinformatics resource launched in 2012, integrates genome scale data from important plant pathogenic species with literature-curated information about the phenotypes of host infection. It provides access to complete genome assemblies, gene models, supporting alignments and genomic polymorphism from priority species of fungi and oomycete pathogens of crop and model plants (using the Ensembl genome browser interface). This information is directly linked to the peer-reviewed information about infectious phenotypes curated within the PHI-base (Pathogen-Host Interactions) database. Nine fungal and four oomycete pathogen genomes are available in the first release including Mycosphaerella graminicola, Puccinia graminis f. sp. tritici and Phytophthora infestans. New releases will be made at 2 monthly intervals. Comparative analysis is provided at many levels, including DNA and protein sequence alignments (between pathogens themselves, and with other fungal species) and the differential role of genes involved in pathogenesis in different hosts.

The PhytoPath project and the PHI-base National Capability are supported by grants from the Biotechnology and Biological Sciences Research Council (BBSRC).

PR3.34

Annotation of 8 Aspergillus genomes derived by the multi-genome Gnomon pipeline

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NCBI

Recent advances in genome sequencing technologies allow generating whole genomes for many organisms in a fast and cost-effective way. Accurate annotation of the genomes is still a challenge. Large-scale sequencing projects usually provide additional experimental data (EST, full-length cDNA) that can be utilized in the annotation process to improve the quality of gene models. More recently sequencing efforts are concentrated on pathogens and model organisms from Fungi and Protozoa organism groups and are focused on sequencing of genomes of closely related organisms for evolution, genetics and comparative studies. These genomes are relatively small but often lack additional transcript or protein data. Using comparative multi-genome approach can greatly improve the accuracy of gene prediction compared to single genome method. The multi-genome Gnomon approach allows utilizing the transcript and protein data from closely related organisms in a single multi-genome annotation run. This method starts from a single genome Gnomon gene prediction and then uses a comparative analysis among multiple genomes to gradually improve the annotation through an iterative process. At each iteration the best models are selected and used as a training set and evidence for the next step. Transcript and protein alignments are used to guide gene model predictions. The most recent version of Gnomon can utilize RNA-Seq data giving more support to the splice junctions. Eight *Aspergillus* genomes have been annotated simultaneously using this method. Four of these genomes have RNA-Seq data available. It was found that the multi-genome approach successfully used the indirect RNA-Seq data to improve the annotation of genomes without such data. The resulting annotation has proven to be more consistent across the genomes than the annotation of the individual genomes.

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PR3.35

Fungal subtilisin-like serine proteases in a novel division into subfamilies

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Subtilisin-like serine proteases (subtilases) are a superfamily of enzymes covering different essential activities from removing the start methionin from a peptide sequence to degrading substrate proteins extracellularly. Due to the wide variety of roles, it is difficult to predict a function based on an amino acid sequence. Subtilases are widespread in fungi, and the growing number of sequenced fungal genomes makes it important to have better tools to analyze these enzymes. Here, we apply PPR, a new non-alignment based analytical approach defining protein subfamilies. It allows both prediction of function based on comparative analysis of sequence and the design of PCR primers that can be used to discover novel members of such subfamilies (Busk and Lange, 2011). We have assembled over 4000 subtilase sequences of various organisms and defined subfamilies that do not correspond to published, alignment-based subtilase subfamilies. We are currently investigating, whether fungi of specific physiological life forms are characterized by a specific set of subtilase PPR-subfamilies. Here, the focus lies on Basidiomycota, in particular wood rotting and mycorrhiza basidiomycetes. Our hypothesis is that such sets of subtilases will unravel functional information about PPR-subfamilies. As a consequence, determining the subfamilies of novel subtilisin-like serine proteases will facilitate their annotation, enabling a short cut to a functionally targeted experimental discovery strategy.

Busk P.K. and Lange L. (2011) A novel method of providing a library of n-mers or biopolymers. Patent application EP11152232.2.

PR3.36

Can mechanisms of host specificity in smut fungi be inferred from genome data?

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Smut fungi parasite more than 4000 flowering plants, including important crop plants like maize, sugar cane, sorghum grass and barley. Typically, smut fungi have a narrow host range, and only one host species is infected. In addition to the two published genomes of *Ustilago maydis* and *Sporisorium relianium* 5-1, both parasitizing maize, three additional smut genome sequences have been determined and manually annotated (*U. hordei* parasitizing barley, *S. reilianum* H2 parasitizing sorghum and *S. scitamineum* parasitizing sugar cane). The narrow host range and the genome availability of closely related species make smut fungi a particularly interesting model to study host specificity.

To identify putative genes involved in host specificity, we follow a computational approach relaying on two strategies. One strategy follows the idea that genes unique for each species (so called orphans) are involved in host specificity. The other idea is to build families of homologous proteins and to scan these families for signatures of species-specific positive selection. This is based on the idea that proteins serving as pathogenicity factors on a particular host will show high rates of adaptive substitutions, since they are involved in a co-evolutionary arms race between parasite and host. To identify candidates for both strategies, we built gene families and alignments based on similarity criteria and clustering techniques. The members of each family of interest were subsequently analyzed with regards to positive selection, ontology categorization, or potential secretion, respectively.

PR3.37

Fusion of two divergent fungal individuals led to the recent emergence of a new widespread pathogen species

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In genome sequences of five individuals of the ascomycete fungus *Zymoseptoria pseudotritici,* a close relative of the wheat pathogen *Z. tritici* (synonym *Mycosphaerella graminicola*), we observed peculiar diversity patterns. On aligned chromosomes long fragments up to 100 kilobases without variation alternate with similarly long fragments of high variability. The variable segments in the genome alignment are organized into two main haplotype groups, ~3% divergent from each other. The genome patterns in *Z. pseudotritici* are consistent with a hybrid speciation event resulting from a cross between two divergent haploid individuals, and the resulting hybrids formed the new species without backcrossing to the parents. The segments without variation is the result of a strong population bottleneck following the hybridization. We observe no variation in 54% of the genome in the five individuals and estimate a complete loss of variation for at least 25% of the genome in the entire species. Variable segments in the *Z. pseudotritici* genome represent the two haplotypes contributed by the parental individuals. From our previously estimated recombination map of *Z. tritici* and the size distribution of regions with two haplotypes we estimate that the hybridization occurred ~675 sexual generations ago. We show that the amount of variation lost is explained by genetic drift during the bottleneck, and by natural selection as evidenced by the correlation of variation presence/absence with gene density and recombination rate. The successful spread of this new reproductively isolated pathogen highlights the accelerating potential of hybridization in the evolutionary emergence of pathogen species with sexual reproduction.

PR3.38

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

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A set of 43 *Aspergillus niger* and *A. awamori* strains isolated from grapes cultivated in Mediterranean area was tested to evaluate genetic differences in fumonisin B_2 (FB_2) biosynthetic gene cluster between toxin producing and non-producing strains.

The ability to produce FB_2 was related to *fum* genes occurrence, evaluated by PCR assyas, using primer sets designed to amplify fragments of *fum1*, *fum3, fum6, fum7, fum8, fum10, fum13, fum14, fum15,* and some relative intergenic regions.

A.niger and *A. awamori* FB_2 producing strains and *A. niger* FB_2 non-producing strains arose amplicon for all tested *fum* genes, while the *A. awamori* FB2 non-producing strains arose amplicon only for few of tested *fum* genes.
Maximum parsimony analysis based on the calmodulin gene sequences indicated that the presence/absence of *fum* genes in the isolates is not correlated with phylogenetic relationship among strains. This is the first report correlating the presence of multiple fumonisin biosynthetic genes with fumonisin production in *A. niger and A. awamori*. The results suggest that the absence of FB_2 production in *A. awamori* can result from the absence of at least one gene of the cluster, while in *A. niger* it should involve other regulator gene/s probably out of the cluster, or concern variations in a regulatory sequence essential for cluster expression.

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PR3.39

Pooled-segregant Whole-genome Analysis Reveals Genetic Basis of High Thermotolerance in Yeast

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Thermotolerance of *Saccharomyces cerevisiae* has long been studied not only because of its industrial application potential, but also its nature as a complex trait that can be influenced by different genomic loci and strain backgrounds. In this study we have performed a genetic mapping of thermotolerance by using pooled-segregant whole-genome sequence analysis. A mixture of genomic DNA from a total of 58 thermotolerant segregants selected from a cross between a highly thermotolerant yeast strain and the control lab strain BY4742 has been sequenced by illumina technology. The ratio of SNPs that represents the abundance of the thermotolerant strain DNA versus the control strain DNA in this segregant population has been plotted against the SNP position along the chromosomes. Afterwards genotyping of the individual thermotolerant segregants has been performed at the loci that show a clear biased deviation from 50% in the plot, and based on this, an FDR statistic analysis has been applied which confirmed that four loci have a significant linkage to the phenotype. A previously identified gene (*MKT1*) located in the locus with the strongest linkage was also identified in this study as having a contribution to high thermotolerance by reciprocal hemizygosity analysis. In addition and surprisingly, one locus with a preference for BY4742 DNA has been confirmed as showing significant linkage. This implies that besides a possible genome dosage effect, the higher thermotolerance of the diploid compared to the haploid parents (thermotolernt haploid and BY4742) may be due to a contribution of its non thermotolerant parent (BY4742).

PR3.40

The FUNG-GROWTH database: linking growth to genome

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Fungal genome sequences demonstrate the potential to utilize a variety of different carbon sources. Natural carbon sources for many fungi are based on plant biomass and often consist of polymeric compounds, such as polysaccharides. They cannot be taken up by the fungal cell and are extracellularly degraded by a complex mixture of enzymes. Plant polysaccharide degrading enzymes have been studied for decades due to their applications in food and feed, paper and pulp, beverages, detergents, textile and biofuels. These enzymes have been classified based on amino acid sequence modules (www.cazy.org).

Based on the hypothesis that fungal genomes have evolved to suit their ecological niche, we have performed a comparative study using >120 fungal species. In this study we have compared growth profiles on 35 different carbon sources (consisting of mono-, oligo- and polysaccharides, lignin, protein and crude plant biomass) to the CAZy annotation of the genomes to identify correlations between growth and genomic potential.

Highlights of these comparisons will be presented as well as the public database in which the growth data is stored and the developments of the database anticipated for the next two years.

PR3.41

***Colletotrichum acutatum*: a model system for studing evolution in filamentous fungi**

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Colletotrichum acutatum is an important pathogen causing economically significant losses of crops and an interesting organism in its own right. *C. acutatum* has a wide plant host range in both crops and natural ecosystems, and its capability to infect different types of host, such as insects, has also been described. *C. acutatum* is able to develop three different types of interaction with plant host: covering biotrophic, necrotrophic and hemibiotrophic infections. It is also capable of growing as a non-pathogen. The life styles of *Colletotrichum* species can include sexual (both homothallic and heterothallic [teleomorph Glomerella]) and asexual states. Furthermore, sexual behaviour in Glomerella is more complicated than in most ascomycetes and strains within the same species do not show a typical MAT1-1/2 system. Globally, *C. acutatum* populations display considerable genotypic and phenotypic diversity. Our previous results suggest the existence of *C. acutatum* populations potentially undergoing speciation processes, related to their reproductive behavior and host association patterns
All this evidence and complexity suggest *C. acutatum* is a suitable system for studying evolution and speciation process trhough whole genome comparisons. One isolate has been sequenced by NGS and more are planned.

PR3.42

The role of cell wall degrading enzymes during the evolution of *Zymoseptoria tritici*

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Plant cell wall degrading enzymes (PCWDEs) of plant pathogens are receiving increasing interest for their potential to trigger plant defense reactions. In an antagonistic co-evolutionary arms race between host and pathogen, PCWDEs could be under strong selection. In a first population genetic study, we tested the hypothesis that PCWDEs in the fungal wheat pathogen *Zymoseptoria tritici* have been positively selected by analyzing ratios of non- synonymous and synonymous nucleotide changes in the genes encoding these enzymes. Analyses of five PCWDEs demonstrated that one (ß-xylosidase) has been under strong positive selection and experienced an accelerated rate of evolution. In contrast, PCWDEs in the closest relatives of *Z. tritici* collected from wild grasses did not show evidence for selection or deviation from a molecular clock. Since the genealogical divergence of *Z. tritici* from these latter species coincided with the onset of agriculture, we hypothesize that the recent domestication of the host plant and/or agricultural practices triggered positive selection in ß-xylosidase and that this enzyme played a key role in the emergence of a host-specialized pathogen. Using an extended second approach based on comparative genomics, we assessed molecular patterns of adaptation and/or selection of all orthologous PCWDEs on a population genomic scale between *Z. tritici* and its closest relatives.

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PR3.43

Comparative molecular evolution of *Trichoderma* chitinases

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Certain fungi from the anamorphic genus *Trichoderma* are known for their ability to antagonize plant-pathogenic fungi and are therefore used as biocontrol agents in agriculture. In *Trichoderma*, whole-genome sequencing reveal between 20 and 36 different family 18 glycoside hydrolase (GH18) genes, and several of these genes have been shown to be induced during the mycoparasitic attack. Sequences of *Trichoderma* GH18 chitinase genes *chi18-5*, *chi18-13*, *chi18-15* and *chi18-17*, that all exhibit specific expression during mycoparasitism-related conditions, were determined from up to 13 different taxa and studied with regard to their evolutionary patterns. *Chi18-13* contained two codons that evolve under positive selection and seven co-evolutionary site networks. Regions of high amino acid variability were preferentially localized to substrate- or product side of the catalytic cleft. *Chi18-15* displayed a unique codon-usage and contained five codons that evolve under positive selection and three co-evolutionary site networks. Regions of high amino acid variability were preferentially localized to coil-regions adjacent to certain alpha-helixes, suggesting structural adaptations of enzyme architecture. In addition, differences in amino acid variability/conservation patterns, indicative of type 1 functional divergence, were observed between *Trichoderma* chi18-15 orthologs and a bacterial ortholog, *Streptomyces* chiJ. These observations show that *Trichoderma* chitinases *chi18-13* and *chi18-15* evolve in a manner consistent with rapid co-evolutionary interactions and identifies putative target regions involved in determining substrate-specificity and structural modifications of the family 18 chitinase TIM-barrel structure. Our results suggest that fungal/fungal interactions can drive adaptive changes in enzymatic properties as a response to specific ecological contexts of different *Trichoderma* species.

PR3.44

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

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Sequencing of *A. flavus* NRRL3357 showed that its 36-Mb genome contains 13,488 genes including predicted 55 secondary metabolite gene cluster. We sequenced cDNA fragments obtained from Poly(A)-enriched total RNA samples extracted from mycelium grown under 3 conditions: (i) PMS medium, 30 C, 24h, no toxin; (ii) GMS medium, 30 C, 24h, make toxin; and (iii) GMS medium, 37 C, 24h, no toxin. Two cDNA libraries from each treatment were sequenced using the Illumina (SOLEXA) short-read technology. Over 5 Million 100 nt reads were sequenced for each cDNA prep, which were combined to generate a powerful high resolution map of the *A. flavus* transcriptome. The analysis detected expression in at least 50 % of the genes for each condition and contributed to our understanding of the genetic basis of the aflatoxin regulation. This study demonstrates that the aflatoxin pathway gene cluster consisting of 30 genes are tightly regulated. High temperature turns down aflatoxin gene transcription by turning down transcription of the two regulatory genes, the *aflR* and *aflS* (old name: *aflJ*). Further, the change in gene expression ratio of *aflS* to *aflR* renders *aflR* non-functional for activation of aflatoxin pathway gene transcription.

PR3.45

Sequencing and assembly of a fungal genome for less than $1000?

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Next Generation Sequencing technology has opened up a whole way of looking at organisms, and it is now possible for a small lab to have the genome of a fungus completely sequenced, and the genes predicted for less than a couple of thousand dollars; however, access to high performance computing facilities as well as some biocomputational skills are required. The results of a project for sequencing the genomes of several ascomycetes is presented, as well as the use of the data to uncover mating type genes and infer the presence of sexual reproduction in populations.

PR3.46

Heterochromatin influences the secondary metabolite profile in the plant pathogen *Fusarium graminearum*

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Chromatin modifications and heterochromatic marks have been shown to be involved in the regulation of secondary metabolism gene clusters in the fungal model system *Aspergillus nidulans*. We examine here the role of HEP1, the heterochromatin protein homolog of *Fusarium graminearum*, for the production of secondary metabolites. Deletion of *Hep1* in a PH-1 background strongly influences expression of genes required for the production of aurofusarin and the main tricothecene metabolite DON. In the *Hep1* deletion strains AUR genes are highly up-regulated and aurofusarin production is greatly enhanced suggesting a repressive role for heterochromatin on gene expression of this cluster. Unexpectedly, gene expression and metabolites are lower for the trichothecene cluster suggesting a positive function of *Hep1* for DON biosynthesis. However, analysis of histone modifications in chromatin of AUR and DON gene promoters reveals that in both gene clusters the H3K9me3 heterochromatic mark is strongly reduced in the *Hep1* deletion strain. This, and the finding that a DON-cluster flanking gene is up-regulated, suggests that the DON biosynthetic cluster is repressed by HEP1 directly and indirectly. Results from this study point to a conserved mode of secondary metabolite (SM) biosynthesis regulation in fungi by chromatin modifications and the formation of facultative heterochromatin.

PR3.47

Effectors in fruit scab fungi: *Venturia inaequalis* and *V. pirina*: a comparative genomics approach

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The hemi-biotrophic fungi, *Venturia inaequalis* and *V. pirina,* cause scab disease on apples and pears respectively. The pathogens are host-specific, with non-overlapping host ranges: *V. inaequalis* is limited to infecting hosts within the Maloideae; and *V. pirina* infects pear (*Pyrus* spp). The genetics of the finer interactions between these fungi and their respective host cultivars follow the gene-for-gene model; effectors (pathogen proteins required for infection) are presumably secreted into the plant/pathogen interface during the infection cycle where a subset can be recognised by plant resistance gene products to induce a hypersensitive response. Whole genome sequencing of *V. inaequalis* and *V. pirina* isolates has been performed. Orthologues of several fungal effector genes were identified in both genomes; including *Ecp6*, *AvrLm6* and *Avr-Pita*. *AvrLm6* is represented by an expanded family of over 25 genes in both *V. inaequalis* and *V. pirina* (*Leptosphaeria maculans* has 2 orthologues). The *Venturia* AvrLm6 orthologues share up to 32% amino acid sequence identity with *L. maculans* AvrLm6. Most of these orthologues are also represented in the *V. inaequalis* transcriptome, with eight upregulated by more than two fold during infection. These, and other effector candidates, are currently being functionally characterized using gene silencing/disruption and GFP fusions. The secretomes, assessed using a proteomics and Illumina sequencing approach, of both pathogens are being compared to reveal *Venturia*-specific elicitors as well as species-specific elicitor candidates that may determine host range. Sequencing of the closely related *V. nashicola* as well as *formae speciales* of *V. inaequalis* from loquat is also planned.

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PR3.48

The *Didymellaceae*: insights into the genomes of key pathogens of legume crops

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The *Didymellaceae* family includes some of the most important pathogens of legume crops: the causal agents of Ascochyta blight in chickpea, pea, lentil, and faba beans among others [1]. Despite their substantial economic impact, little is known about the molecular aspects of pathogenicity in these closely related species. At this meeting, the first genome assemblies of *Ascochyta rabiei*, *Peyronellaea pinodes* (syn. *A. pinodes*), and *Phoma medicaginis* (pathogen of the model legume *Medicago truncatula*) will be presented. Untrained *in silico* annotation resulted in the identification of 9,000-12,000 genes per species, of which 91-97% are complete gene models. The sequence data served to: pinpoint areas of synteny and clusters of genes associated with reproduction and adaptation; identify pathogenicity-related gene-candidates through proteomic, transcriptomic and *in silico* comparative analyses; and design DNA makers for diagnostics and studies in population structure. Among the pathogenicity related genes, we have identified potential necrotrophic effectors that seem to operate similarly to those found in *Stagonospora nodorum* [2]. Detailed understanding of the molecular mechanism involved in fungal adaptation in general, and pathogenicity in particular, is facilitating the development of novel tools and strategies in crop protection.

1. Aveskamp, M.M., et al. (2010) Highlights of the *Didymellaceae*: A polyphasic approach to characterise *Phoma* and related pleosporalean genera. Studies in Mycology **65**(1): p. 1-60.
2. Oliver, R. (2009) Plant breeding for disease resistance in the age of effectors. Phytoparasitica **37**(1): p. 1-5.

PR3.49

Intersterility loci of *Heterobasidion occidentale* and *H. irregulare* has increased recombination rates and affects virulence, speed of growth and wood decay

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The closely related basidiomycete species *H. occidentale* and *H. irregulare* are the major pathogens on industrial forestry of conifers in North America. Although being separate, intersterile species, a degree of interfertility has been observed. This is controlled by a set of five intersterility loci. Mutual + alleles in at least one of these loci are required for compatible mating between *Heterobasidion* mycelia to occur. Using a dense genetic linkage map, the positions for three intersterility loci have been determined. Cross-comparisons with other analyzed traits suggest these regions to be fundamental for the fungal life cycle, as they also carry QTLs for virulence, wood decay and speed of growth on agar medium. The linkage map further reveals that these regions have an increased recombination rate, between 10 and 20 times higher than the genome wide average. As has been described for other species, this could indicate that these regions are in fact flanked by spots for high recombination rate, to prevent recombination and disruption within the crucial regions themselves.

PR3.50

Host specialization in the *Rhynchosporium* genus – a genomics approach

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Based on phylogenetic criteria fungi of the *Rhynchosporium* genus were recently assigned to four species, each exhibiting a specific host spectrum. *R. secalis* grows on rye (*Secale cereale*) and triticale, *R. commune* on *Hordeum* species including cultivated barley and on brome grass (*Bromus* spp.), and *R. agropyri* on couch grass (*Agropyron* spp.). Previously, these morphologically indistinguishable species were all referred to as *R. secalis*. *R. orthosporum* constitutes the fourth species, which is characterized by a different spore shape and its host species, cocksfoot (*Dactylis glomerata*). DNA sequencing of five isolates from the four *Rhynchosporium* species yielded mitochondrial genomes of 69 kb (*R. orthosporum* 49 kb) and nuclear genomes of 50-55 Mb carrying about 12,000 genes. Following a comparative genomics approach, we are now aiming at identifying the factors that underlie host colonization and specialization. Using molecular and proteomics techniques along with targeted deletion we are currently focusing on genes encoding fungal effector proteins as well as polyketide synthesizing enzymes.

PR3.51

The *Hyaloperonospora arabidopsidis* species complex – a new model system for investigating the evolutionary and ecological genomics of plant pathogens

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The *Hyaloperonospora arabidopsidis* species complex contains about 10 phylogenetically and physiologically distinct lineages of obligate biotrophic oomycetes. These include H. arabidopsidis, the downy mildew pathogen of *Arabidopsis thaliana* and *H. cardaminopsidis*, which is parasitic to *Arabidopsis arenosa*. The genomes of both *Arabidopsis thaliana* and *H. arabidopsidis* are available, and this pathosystem has recently become a model system for investigating the molecular basis of plant-oomycete interaction, in which several resistance proteins and some corresponding effectors with avirulence activity have been described. However, little is known about the evolution of the pathosystem on biogeographic scale, and even less about the functional radiation of pathogens on unrelated hosts or their coevolution with closely related hosts.

Here we propose the *Hyaloperonospora arabidopsidis* species complex as a model to dissect the functional evolutionary dynamics of plant pathogens on a landscape and biogeography level.

During the past four years, extensive collections of plants and pathogens throughout the distribution range of *Arabidopsis thaliana* and the annual Brassicaceae *Microthlaspi perfoliatum* have been done, encompassing several hundred populations. The ongoing studies show positive selection comparing known effector genes from the two species, as well as intraspecific variation in both pathogens and hosts with respect to several traits, including pathogenicity and resistance, respectively.

Genome projects for both *H. thlaspeos-perfoliati* and *Microthlaspi perfoliatum* are underway, and first results already helped to reveal some pattern related to effector evolution that will be shortly discussed.

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PR3.52

'Exploring the genome of Fusarium fujikuroi and related species with emphasis on secondary metabolism gene clusters'

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The fungi of the *Gibberella fujikuroi* species complex synthesize a vast array of secondary metabolites and specifically infect a broad spectrum of hosts.

In this work we present the fully annotated genome sequence of *Fusarium fujikuroi*. Due to the availability of other new sequenced genomes, representing every geographic subclade of the species complex, an extensive comparative approach with regards on unique features of the single species and subclades is possible. To determine the factors that contribute to host specificity we explored the make-up of PKS, NRPS, terpene and isoprenoid based secondary metabolite clusters.

Using DNA chip and ChIPseq experiment data we studied the expression of gene clusters under different environmental conditions as well as the influence of chromatin modifications. In addition we revealed transcription factor based regulation in predicting cluster specific transcription factor binding sites. Beyond these key players in plant-pathogen interaction other unique features like secreted proteins or unique transcription factors of the genomes are of interest.

Poster Category 4: Organismic Interactions

PR4.1

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

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During their natural life cycle most fungi encounter other microorganisms and live in mixed communities with complex interactions, such as symbiosis or competition. Industrial fermentations, on purpose or by accident, can also result in mixed cultures. Fungal co-cultivations have been previously described for the production of specific enzymes, however, little is known about the interactions between two species that are grown together. *A. niger* and *A. oryzae* are two of the most important industrial fungi worldwide and both have a long history of strain improvement to optimize enzyme and metabolite production.

Co-cultivation of these two Aspergilli with each other and with the ascomycete phytopathogen *Magnaporthe grisea*, and the basidiomycete white rot fungus *Phanerochaete chrysosporium*, has recently been described by our group (Hu et al, 2010). Total secreted protein, enzymatic activities related to plant biomass degradation and growth phenotype were analyzed from cultures on wheat bran demonstrating positive effects of the co-cultivation compared to the individual cultivations. In a follow-up study the morphology and mechanism of the interaction is addressed using microscopy and proteomics. Data from this study will be presented.

Reference

Hu *et al*. International Biodeterioration & Biodegradation 65 (2011)

PR4.2

A novel effector secreted by the anthracnose pathogen *Colletotrichum truncatum* is required for the transition from biotrophy to necrotrophy in fungal pathogens

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The *in planta* transition from biotrophy to necrotrophy known as the biotrophy-necrotrophy switch (BNS) in hemibiotrophic fungal phytopathogens is critical in disease development. We herein report a novel effector gene *CtNU* from *Colletotrichum truncatum*, the causal agent of anthracnose on pulse crops that exclusively expresses precisely before the BNS and elicits severe hypersensitive response (HR)-like cell death in tobacco leaves transiently expressing the effector. Cell death triggered by CtNU requires its accumulation at the host cell plasma membrane, indicating that the effector may cause perturbation in cell surface dynamics. Overexpression of *CtNU* in *C. truncatum* and *Magnaporthe oryzae*, the rice blast pathogen resulted in incompatibility with host plants lentil and barley, respectively by causing a HR-like response during the biotrophic mode of fungal growth. These results provide compelling evidence that hemibiotrophic fungal phytopathogens deliver *CtNU* effectors to the host cell plasma membrane to promote pathogenesis by causing massive cell death during the *in planta* differentiation of necrotrophic hyphae from biotrophic hyphae.

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PR4.3

Cryptococcus adeliensis the causative agent of stem canker of stone fruit trees

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In attempts made to isolate the previously recorded causative bacteria (Pseudomonas syringae pv. Syringae (PSS) or Xanthomonas arboricola pv. Pruni (Xap) from cankers on stone fruit trees in July 2011 in Khorasan province, yeast-like isolates were consistently recovered from the symptomatic branches. Dark brown to black sunken lesions, predominantly accompanied with exudation of gum turned to typical stem cankers following their expansion. The isolated yeast cell was circular and ca 6.5 µm in diameter and formed round, white to light-cream colored, mucoid colonies with entire margins on sucrose nutrient agar. The internal transcribed spacer (ITS) region of the rDNA of a representative isolate of the recovered yeast was amplified in PCR using primer pair ITS4 (5-TCCTCCGCTTATTGATATGC-3) and ITS5 (5-GGAAGTAAAAGTCGTAACAA-3) and the PCR product sequenced. The sequence was aligned and compared with the nucleotide sequences deposited in GenBank using CLUSTAL w software. The ITS-sequence(NCBI # JQ039907) showed 100% identity with those of Cryptococcus adeliensis isolates. Several recovered isolates of the yeast were inoculated on peach (Prunus persicae) and nectarine (P.persica var.nucipersica) budlings and typical cankers were produced on the inoculated branches. The respective strains were re-isolated from the inoculated plants. The isolates also produced a hypersensitive reaction on geranium (Pelargonium × hortorum). C. Adeliensis has been isolated from decaying algae in the Antarctica and from a patient suffering from meningitis. The stem canker caused by C. Adeliensis appears to be a newly emerged disease of stone fruit trees.

PR4.4

The ATF/CREP transcription factor Atf1 is essential for full virulence, deoxynivalenol production and stress tolerance in the plant pathogen *Fusarium graminearum*

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In a previous study we characterized the stress-activated MAP-kinase FgOS-2 (*Saccharomyces cerevisiae* HOG1) as a central regulator in the life cycle of the cereal pathogen *Fusarium graminearum.* Grains infected with *F. graminearum* accumulate high amounts of mycotoxins, most prominent of which are deoxynivalenol (DON) and zearalenone (ZEA). We showed that FgOS-2 regulates DON- and ZEA-production to different extents depending on growth conditions. Here, we present data on the functional characterization of a putative downstream regulator, the ATF/CREP transcription factor Fgatf1. Like FgOS-2, Fgatf1 is mainly involved in osmotic stress response. Deletion mutants in Fgatf1 (ΔFgatf1) strains are sensitive to osmotic stress (e.g. mediated by NaCl) and less sensitive to oxidative stress mediated by H_2O_2 compared to wild type. Furthermore, sexual reproduction is delayed: perithecia develop slower and frequently remain immature. ΔFgatf1 strains show an increased DON-production under *in vitro* induction conditions compared to wild type. However, during plant infection, DON-production is strongly reduced. Expression of genes encoding for key enzymes in the DON-biosynthesis pathway are regulated accordingly. In infection assays on wheat and maize, the ΔFgatf1 strains show a reduced virulence compared to wild type. Interestingly, overexpression of atf1 ($atf1^{oe}$) leads to hypervirulence on wheat and *Brachypodium distachyon*. The infection proceeds faster and continues into the stalk. Moreover, overexpression of atf1 in ΔFgOS-2 partially complements ΔFgOS-2-phenotypes regarding growth on osmotic-stress medium and virulence towards wheat and maize. Taken together, these results provide new insights in the stress response signalling cascades of *F. graminearum* and assign the transcription factor Fgatf1 a central role in pathogenic development.

PR4.5

The stress-activated protein kinase FgOS-2 is a key regulator in the life cycle of the cereal pathogen *Fusarium graminearum*

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Fusarium graminearum is an ascomycetous plant pathogen and the causal agent of Fusarium head blight disease in small grain cereals and of cob rot disease in maize. Infection with *F. graminearum* leads to yield losses and mycotoxin contamination. Zearalenone (ZEA) and deoxynivalenol (DON) are hazardous mycotoxins. The latter is necessary for virulence towards wheat. Deletion mutants of the *F. graminearum* orthologue of the *Saccharomyces cerevisiae* Hog1 stress-activated protein kinase, FgOS-2 (ΔFgOS-2) showed drastically reduced *in planta* DON and ZEA production. However, ΔFgOS-2 produced even more DON than the wild type under *in vitro* conditions, whereas ZEA production was similar to that of the wild type. ΔFgOS-2 showed a dramatically reduced pathogenicity towards maize and wheat. We constitutively expressed the fluorescent protein dsRed in the deletion strain and the wild type. Microscopic analysis revealed that ΔFgOS-2 is unable to reach the rachis node at the base of wheat spikelets. Vegetative growth was retarded upon osmotic treatment. Also the germination of mutant conidia on osmotic media was severely impaired. Germ tubes were swollen and contained multiple nuclei. The deletion mutants completely failed to produce perithecia and ascospores. Furthermore, FgOS-2 plays a role in reactive oxygen species (ROS)-related signalling. The transcription and activity of fungal catalases is modulated by FgOS-2. Among the genes regulated by FgOS-2 we found a putative calcium-dependent NADPH-oxidase (*noxC*) and the transcriptional regulator of ROS metabolism, *atf1*. The present study describes new aspects of stress-activated protein kinase signalling in *F. graminearum*.

PR4.6

Identification of candidate *AvrRvi1* effector genes from *Venturia inaequalis* by transcriptome analysis

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The 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 secreted into the plant/pathogen interface early in the infection cycle to suppress defence/enhance infection. A subset of effectors can be recognised by plant resistance gene (*R*) products to induce resistance. For example, on host genotypes with the *R* gene *Rvi1, V. inaequalis* isolate MNH120 causes disease, inferring the mutation or absence of the cognate effector AvrRvi1, whereas isolate 1066 is incompatible, hence AvrRvi1 is functional. Previously the gene encoding this effector has been localised to a 330 kb BAC contig[a]. This contig has a suite of 54 predicted genes. Comparison of RNA sequencing reads from an early time-point of infection from compatible interactions with both isolates has enabled candidate *AvrRvi1* genes from this suite to be identified. The expression of four genes is up-regulated during 1066 infection compared with MNH120 infection. Two of these genes encode putative cytochrome p450 enzymes with a log-fold increase in expression of 6 and 5, respectively. A third gene has similarity to a putative phospholipase and the fourth is predicted to encode a small protein, lacking a putative signal peptide or similarity with known proteins, with a 1 and 5 log-fold increase in expression in 1066, respectively. Functional characterisation of these genes is currently being carried out.

[a]Broggini et al. (2007) *Fungal Genetics and Biology 44: 44-51*

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PR4.7

Ammonium Transport in the Arbuscular Mycorrhiza Symbiosis

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Eighty percent of all land plants are presumed to undergo root symbiosis with obligate biotrophic arbuscular mycorrhizal (AM) fungi, which provide the often growth limiting inorganic nutrients phosphorous and nitrogen to the plant. In return, plants invest up to 20% of their photosynthetically fixed carbon to feed the fungal symbiont. While nutrient transporters on the plant side of AM are widely investigated, on the fungal side respective knowledge remains still in its infancies.

The best candidate for the nitrogen source being transferred to the plant is ammonium. To estimate the role of ammonium in the symbiosis, we focused on the identification and characterization of ammonium transporters (AMTs) in AM fungi. Six proteins with homology to fungal AMTs have been identified from two AM species, *Glomus intraradices*-like BEG195 (now: *Rhizophagus irregularis*) and *Geosiphon pyriformis*. While most of these proteins functionally complement ammonium uptake deficient yeast mutants, one AMT homolog does not seem to transport ammonium. We hypothesize that it might play a role in ammonium sensing at the symbiotic interface between fungus and plant instead.

To investigate the functional relevance of the AMT homologs we are performing *in situ* expression and localization analyses. By immuno localization studies in *R. irregularis*, we were able to detect all three AMT homologs inside plant roots in storage organs (spores and vesicles), but not in the arbuscular membrane. Therefore, the primary role of AMTs might be in the re-uptake of ammonium passively released across the fungal plasma membrane.

PR4.8

Functional characterization of early expressed *in planta* genes by the phytopathogenic fungus *Botrytis cinerea*

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Botrytis cinerea is a phytopathogenic fungus able to infect more than 200 plant species, causing the grey mould disease, and great economic losses in the agricultural sector.

New approaches to achieve an effective control of the disease involve the elucidation of the molecular mechanisms during host-plant interaction. Thanks to microarray studies, we have identified more than 150 fungal genes which are expressed during the early stages of infection (germination and penetration), but not in conidia. This fact could indicate that these genes could be important in the first steps of the disease. Moreover, most of the proteins encoded by them have unknown function, although transmembrane domains or signal peptides are predicted for several ones by bioinformatic analysis.

In a first step, their high expression was corroborated by real time PCR, showing, in some cases, a 10,000-fold upregulation at 12 hours post inoculation compared to the expression in conidia. We are currently working with 14 of these genes, knocking them out and checking the phenotype of the mutant strains. We are also considering knocking out gene clusters whose genes are expressed *in planta* during the same conditions. The results of these studies will be reported.

PR4.9

A *WOR1*- Like Protein Regulates Pathogenicity and Reproduction in the Phytopathogenic Fungus *Fusarium graminearum*

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WOR1 encodes a conserved fungal regulatory protein controlling the dimorphic switch and pathogenicity in *Candida albicans* and its ortholog *SGE1* in the plant pathogen *Fusarium oxysporum* is required for pathogenicity and expression of plant effector proteins. *F. graminearum*, an important toxigenic pathogen of cereals, is not known to employ switching or effector proteins during infection and so the potential role of this gene in pathogenesis was further tested. Deletion of the *WOR1* ortholog (called *FGP1*) in *F. graminearum* results in greatly reduced pathogenicity and loss of trichothecene toxin accumulation in infected wheat plants and *in vitro*. The loss of toxin accumulation alone is sufficient to explain the loss of pathogenicity to wheat. Under toxin-inducing conditions *in vitro* or *in planta*, expression of genes for trichothecene biosynthesis and many other genes are not detected or detected at lower levels in Δ*fgp1* strains. *FGP1* is also involved in the developmental processes of conidium formation and sexual reproduction and modulates a morphological change that accompanies mycotoxin production *in vitro*. The Wor1-like proteins in *Fusarium* species have highly conserved N-terminal regions and remarkably divergent C-termini. Interchanging the N- and C terminal portions of proteins from *F. oxysporum* and *F. graminearum* resulted in partial to complete loss of function. Wor1-like proteins are conserved but have evolved to regulate pathogenicity in a range of fungi, likely by adaptations to the C-terminal portion of the protein.

PR4.10

Uncovering the biotrophic and saprophytic proteomes of the plant pathogen *Verticillium longisporum*

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The plant pathogenic fungus *Verticillium longisporum* is the causal agent of early senescence and ripening in rapeseed (*Brassica napus*) and other crucifer crops. Despite the significant economical importance of this pathogen, the factors for host specificity are still unknown and the set of virulence factors (effectors) is poorly analyzed. Therefore, we investigated the extra- and intracellular proteome compositions of *V. longisporum* grown in biotrophic medium (xylem sap of non-infected oilseed rape plants) in comparison to saprophytic growth medium (potato-dextrose broth (PDB) and simulated xylem sap medium (SXM)). Procedures for the isolation and purification of proteins were optimized for *Verticillium* samples. Protein extracts were separated by one- and two-dimensional gel electrophoresis and peptide samples were analyzed by MALDI-TOF and LC-MSMS. Using the draft genome sequence of *V. longisporum* 43 we are currently assembling and annotating, we demonstrate that proteomics experiments deliver valuable data for the improvement of genome annotation and serve as powerful screening techniques to identify potential effectors and factors for host specificity. Proteomic analyses of the intracellular compartment build the basis for the reconstruction of biochemical pathways. We show that exoproteomes vary to a great extent depending on growth phase and media composition. We could identify a broad range of putative effectors such as adhesins and different groups of carbohydrate active enzymes, which might be involved in the attachment to the plant and degradation of structurally complex cell wall molecules of the host. Furthermore potential LysM effectors, necrosis and ethylene inducing like proteins (NLPs) and cysteine-rich proteins were detected, which might be involved in pathogenicity. Comparison of the exoproteome of biotrophic and saprophytic growth demonstrated among others elevated protein abundance in xylem sap of metalloproteinases, NLPs and several conserved proteins with so far unknown function. Therefore resulting candidate genes and proteins might be of specific importance for the *Verticillium-B. napus* interaction and are currently analyzed using knockout and silencing mutants.

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PR4.11

What have you been eating? A perspective on fungal nutrition during plant infection

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Pathogens derive all their nutrients from their hosts. If such processes could be interrupted, a general approach to prevent disease would be at hand. A range of approaches have been used to identify the nutrients acquired by fungal pathogens from their plant hosts. These methods include infection tests on auxotrophic mutants, screening for metabolic genes in genome sequences and transcriptomic studies of infection.

Non-obligate pathogens can grow on artificial media and in most cases can grow on defined media containing only glucose, nitrate, oxidised P, N and S and some minerals. It was therefore reasonably assumed that fungi would use these substrates in planta. A combination of approaches has revealed a more complex and interesting picture. Nitrate assimilation seems to be unnecessary; instead the direct N-sources are mainly asparagine and glutamate and possibly GABA. We have recently shown that mutants in pantothenate biosynthesis are fully pathogenic indicating that the plant supplies and the fungus takes up pantothenate or a later intermediate in the CoA pathway. Furthermore although mutants in aminolevulinate (ALA) synthesis were non-pathogenic when infected onto intact plants they could infect wounded plants indicating that ALA or a later intermediate can be assimilated. Thus it appears that fungal pathogens can and do assimilate more complex molecules that was previously thought to be the case. Such an insight might open the door to a new approach for fungicide design.

Ipcho SVS, Hane JK, Antoni EA, Ahren D, Henrissat B, Friesen TL, Solomon PS & Oliver RP (2012) Transcriptome analysis of the wheat pathogen *Stagonospora nodorum;* gene model validation, effector candidate genes, intensive host regulation of metabolism and dispensability of pantothenate metabolism. Molecular Plant Pathology

PR4.12

Recognition and response to heterospecific non self: Podospora anserina as a model for the fungal immune response

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The ability to detect and react to pathogens is essential to the development of any organism. In plants and mammals pathogen recognition relies on polymorphic Pattern Recognition Receptors (PRRs) belonging to the STAND class of proteins involved in signal transduction, essentially of the NB-LRR type. Pathogen driven evolution of PRR encoding genes can lead to auto-immune diseases. No such fungal immune systems have been described so far and *NB-LRR* encoding genes are absent from fungal genomes. In *Podospora anserina* Vegetative Incompatibility (VI), a conspecific non self recognition process (between individuals of the same species), leads to cell death and autophagy. VI is determined by interaction of *het-c*, encoding a glycolipid transfer protein, and members of the *hnwd* gene family encoding for STAND proteins. *hnwd* gene family members display the hallmarks of PRR encoding genes, including fast evolution promoting production of a repertoire of receptors and ability to initiate a cell death reaction. *het-c* is also showing signs of fast evolution. We recently hypothesized that these genes are involved in pathogen recognition and that the VI reaction is an autoimmune disease. This hypothesis gained experimental support. *P. anserina* WT strains initiate a strong reaction when confronted with another fungal species, *Epiccocum nigrum*. Crucially this reaction is not observed when mutants suppressed for the VI reaction are confronted to *E. nigrum*. We are investigating the function of HET-C, HNWD and additional STAND proteins in *P. anserina* response to *E. nigrum*. We also investigate the cellular response to heterospecific non self.

PR4.13

The functional characterization of candidate genes involved in host specialization of Mycosphaerella grass pathogens

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The ascomycete fungus *Mycosphaerella graminicola* emerged as a new pathogen of cultivated wheat during crop domestication about 11.000 years ago. To understand the molecular basis of host specialization in this pathogen we have sequenced complete genomes of *M. graminicola* and closely related species infecting wild grasses.
Evolutionary genomic analyses allowed us to identify 17 genes that show strong evidence of positive selection between *M. graminicola* and the closely related sister species S1. We hypothesize these evolved in a co-evolutionary arms race with different hosts. None of the genes encode proteins with known function. In this project we focused on three candidate genes and investigated their role in *M. graminicola* and its two closest relatives S1 and S2 during host infection.

Quantitative Real time PCR experiments from the three fungal species infecting four different grass species show that the three genes are strongly up-regulated in planta and that candidate gene expression differs over a time course of 28 days supporting a role in host pathogen interaction. In addition we show that three different host species differentially induce gene expression in the fungi.

Confocal Laser Scanning Microscopy conducted at different time points also show clear differences between species during infection and fungal development. Deletion strains for each candidate gene have been created by *Agrobacterium tumefaciens* mediated transformation. The single deletion of two candidate genes led to a reduced virulence of *M. graminicola* on wheat. We show that genes involved in host specialization can be identified based on footprints of natural selection.

PR4.14

***Penicillium expansum* Glucose Oxidase-Encoding Gene*, GOX2,* a Key Factor for Gluconic Acid Production and Acidification During Pathogenicity of Deciduous Fruits**

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Penicillium expansum (Pe), the causal agent of blue mold rot, causes severe postharvest maceration of fruit, through secretion of total D-gluconic acid (GA). Two glucose oxidase-encoding genes – *GOX1*and *GOX2* – present in *P. expansum*, were analyzed. Glucose oxidase (GOX) activity and GA accumulation were strongly related to *GOX2* expression, which increased with pH to a maximum at pH 7.0, whereas *GOX1* was expressed at pH 4.0, where no GOX activity or extracellular GA was detected. This differential expression was also observed at the leading edge of the decaying tissue, where *GOX2* expression was dominant. The roles of the *GOX* genes in pathogenicity were further studied through: i. development of *goxRNAi* mutants exhibiting differential down-regulation of *GOX2*; ii. heterologous expression of the Pe- *GOX2* gene in the non-deciduous host-pathogen *P. chrysogenum*; and iii. modulation of GA production by $FeSO_4$ chelation. Interestingly, in *P. expansum* pH and GA production elicited opposite effects on germination and biomass accumulation: 26% of spores germinated at pH 7.0 when GOX activity and GA were highest, whereas in *P. chrysogenum* at the same pH, when GA did not accumulate, 72% of spores germinated. Moreover, heterologous expression of Pe-*GOX2* in *P. chrysogenum* resulted in enhanced GA production and reduced germination, suggesting negative regulation of spore germination and GA production. These results demonstrate that pH modulation, mediated by GA accumulation, is an important factor in generating the initial signal(s) for fungal development leading to host-tissue colonization by *P. expansum.*

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PR4.15

Functional genomic tools to decipher the pathogenicity mechanisms of the necrotrophic fungus *Plectosphaerella cucumerina*, a natural pathogen of *Arabidopsis thaliana*

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The analysis of the interaction between *Arabidopsis thaliana* and adapted (*PcBMM)* and non-adapted (*Pc2127)* isolates of the necrotrophic fungus *Plectosphaerella cucumerina* has contributed to the identification of molecular mechanisms controlling plant resistance to necrotrophs. To characterise the initial stages of Arabidopsis colonization by *PcBMM* and *Pc2127* we used *Agrobacterium tumefaciens*-mediated transformation (ATMT) to generate fungal transformants constitutively expressing the Green Fluorescence Protein (*PcBMM-GFP* and *Pc2127-GFP*). Using confocal microscopy we found that *PcBMM-GFP* colonized *Arabidopsis* wild-type plants by successive degradation of leaf cell layers without forming appressorium or penetrating into host cells, as described for other necrotrophic fungi. By comparing *PcBMM-GFP* colonization process in wild-type plants, and hypersusceptible (*agb1*-1 and *cyp79B2cyp79B3)* and resistant (*irx1-6*) *Arabidopsis* mutants, we found that plant susceptibility to the fungus correlated with the time-course of spore germination and hyphal growth on leaf surface, and that the resistance response was established at 12-18 hours post inoculation (hpi). This result was supported by the observation that hyphal growth of the nonadapted *Pc2127-GFP* on the leaves of wild-type plants was arrested at 12-16 hpi. We generated a collection of random T-DNA insertional transformants by ATMT, and we screened a subset of them to test their virulence in *Arabidopsis* wild-type and *agb1-1* plants. In this screening we identified several fungal transformants with altered virulence in comparison with the wild-type *PcBMM.* The *P. cucumerina* functional genomics platform presented here will be a valuable tool to characterize the molecular bases of necrotrophic fungi pathogenicity.

PR4.16

A refined predicted protein secretome for the wheat leaf pathogen *Mycosphaerella graminicola*

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Mycosphaerella graminicola infection of wheat leaves involves an initial extended period of symptomless intercellular colonisation prior to the development of disease lesions. Previous functional genomics and gene expression profiling studies have implicated the production of secreted virulence effector proteins as a key component facilitating the initial symptomless growth phase[1,2]. With a view to identifying further candidate virulence effectors, we have re-analysed the predicted protein secretome from this pathogen, by combining several bioinformatic approaches aimed to increase the probability of identifying truly secreted proteins. An initial secretome of 970 proteins was predicted. A refined prediction of 556 was made based upon further stringent selection criteria deriving from WolfPsort protein localisation prediction. Of these, 298 possess some functional annotation (based upon PFam; KOG or the CDD databases) leaving 258 with no functional annotation. Further characterisation of the un-annotated proteins included the analysis of features associated with known fungal effectors, for example, small size, cysteine-rich, and Blastp searches performed against other sequenced fungal genomes. Finally evidence in support of gene prediction was derived from gene expression profiling during fungal growth *in vitro* and *in planta*. Subsets of candidate genes are currently being subjected to sequence analysis, reverse genetics and BSMV-mediated overexpression in wheat leaves.

[1] Marshall et al., (2011). *Plant Physiol***.** 156**,** 756-769. [2] Rudd et al., (2010) *Fungal Genet Biol.* 47**,** p19-32.

PR4.17

Role of phenylalanine ammonia-lyase and Pathogenesis-related genes in defense mechanisms of some rice cultivars against *Rhizoctonia solani*

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The *Pathogenesis- related genes* (*PR genes*) are among the most important genes in rice in response to pathogens. In this study we focused on the role of *PR* genes and *PAL* in defense mechanisms of rice against *Rhizoctonia solani,* the causal agent of rice sheath blight. For this purpose Binam and Khazar cultivar (Cv) as resistant and susceptible cultivars, respectively, were used. In morphological assessment, the size of lesions was measured 2 weeks after inoculation with isolates of the fungus. The length of lesions in cv. Binam was half as high as those on Khazar cv. Analysis of data by Student's t test showed significant difference between two cultivars (P values of <0.05). In molecular studying, the expression rate of defense genes was evaluated in 2 weeks old seedling after challenging with *R. solani*. The expression patterns of *PR* gens (*PR3*, *PR10*, *PR13*), *PAL* and *NH1* were considered in seedlings by Quantative Real Time PCR method at different time courses (from 0 to 100 hours post inoculation). The expression rate of PAL, PR3, PR10 and PR13, 12 hour after inoculation (hai) in cv. Binam evaluated 10, 7.5, 10.5 and 10 times, respectively, compared to cv.Khazar. The peak of NH1 were 24 times, 24 hai in cv. Binam compared to cv. Khazar. The results of this study suggest that the genes understudy involving in defense mechanisms of rice against sheath blight agent, *R. solani*. Here with this results it seems that the expression of PAL and PR gens are independent of NH1.

PR4.18

Morphological and molecular characterization of *Rhizoctonia solani* AG2-1 the causal agent of canola stem canker in Iran

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In June 2011, Canker-like symptoms, a newly emerged disease were observed on canola (*Brassica napus*) in Mazandaran and Golestan provinces in Iran. Round to elongated, light to dark brown sunken lesion were formed on rapeseed stems. Canker were scattered all along the height of the stem but were more predominant on parts closer to the stem apices. *Rhizoctonia* -like fungi were isolated from the cankered areas about 5 days following plating surface-disinfected segments on Potato dextrose agar (PDA). All isolates were multinucleus and were identified as *Rhizoctonia solani* AG2-1 in anastomosis assay. The internal transcribed spacer (ITS) rDNA region was amplified using the primers ITS4 (5-TCCTCCGCTTATTGATATGC-3) and ITS5 (5-GGAAGTAAAAGTCGTAACAA-3) and the PCR product was sequenced. The sequence alignment of the 661 bp fragment was subjected to genetic distance analyse. After multiple sequence alignment with PHYLIP software the obtained sequences were compared with the other related sequences with the same region of *Rhizoctonia* genus in GenBank. Isolates from *canola* were all clustered with the representative isolates of AG2-1. The PCR products were digested with *Hinf*I, *Hinc*II, *Ava*I, and *TagI* restriction endonuclease enzymes, and different PCR-RFLP patterns were obtained for the isolates. To the basis of our knowledge this appears to be the first report of canola stem canker caused by R.solani AG 2-1 in the world.

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PR4.19

Identification of the *Rhizoctonia solani* AG-4 the causal agent of safflower stem canker based on morphological and genetic characteristics

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A new disease symptoms was observed on safflower stems in Golestan province of Iran during 2010 and 2011. Linear brown lesions which gradually become canker was the main symptoms of this disease. The lesions may girdle the stem or large cankers can interfere with movement of nutrients from the leaves to the roots and eventually extending the canker can lead to seedling Damping-off. *Rhizoctonia* like fungus isolated from the collected samples cultured on Potato Dextrose Agar (PDA) medium after 4 days at 28°C. All isolates were multinucleus and were identified as *Rhizoctonia solani* AG4 in anastomosis reaction. The teleomorph stage of the isolated fungus was produced in vitro and identified *Thanatephorus cucumeris* based on morphological characteristics. After obtaining the genomic DNA of the isolates, an approximately 500 bp amplification product of the ITS4-5.8S-ITS5 region was obtained with PCR, using ITS4 and ITS5 universal primers. After multiple sequence alignment with MEGA5 software the obtained sequences compared with the other related sequences of *Rhizoctonia* genus in GenBank (NCBI). The PCR products were digested with *Mse*I, *Hin*cII, *Ava*II, and *MfeI* restriction endonucleases enzymes and different PCR-RFLP patterns were obtained. This is the first report of occurrence of safflower stem canker caused by *R .solani* in Iran and based on our knowledge this is the first research on genetically characterization of safflower stem canker agent in the world.

PR4.20

Phenylalanine Ammonia Lyase and Pathogenesis related genes involved in sheath blight disease resistance in Tarom, Iranian rice cultivar

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Plants are encountered with the biotic and abiotic stresses in different ways. Among them PAL and PR genes plays a crucial role in plant microbe interactions. Tarom and Khazar as the Iranian resistant and susceptible cultivars (cv), respectively, were used to analyze the expression patterns of defense genes in response to *Rhizoctonia solani* the causal agent of rice sheath blight disease on 2 weeks old seedlings. The length of lesions was measured 2 weeks after inoculation with isolates of fungus. The size of lesions in cv.Tarom was half as high as those on Khazar. Analysis of data by Students t test showed significant difference between two cultivars (P values of < 0.05) . The role of NPR1, PAL, and Pathogenesis- related genes (PR3, PR10, PR13 in the defense mechanism were considered in inoculated plants using Quantative Real Time PCR technique. The expression rate of PAL, PR3, PR10, and PR13 evaluated at different time courses (from 0 to 100 hours post inoculation) and the peak of the genes understudy were 10, 7.5, 10.5 and 10 times 12 hours after inoculation (hai), respectively, in cv.Tarom compared to cv.Khazar.The expression level of *NPR1* rised 27 times in Tarom compared with Khazar at 24 hai. The results indicated that all of the considered genes involved in sheath blight disease resistance in Tarom cultivar, and the pathway of PR genes expression is independent of NPR1 in *R.solani*- rice interactions.

PR4.21

T-DNA-mediated insertional mutagenesis in *Botrytis cinerea* reveals less virulent mutants that are affected in light-dependent development

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By using an *Agrobacterium tumefaciens*-mediated transformation (ATMT) approach in the plant pathogen *B. cinerea*, we generated a library with 2,350 transformants carrying random integrations of a hygromycin resistance cassette. A first virulence screen of all transformants on detached tomato leaves resulted in the identification of 560 less virulent strains (Giesbert et al., in press). 231 of these have been undergone a second screening on primary leaves of *Phaseolus vulgaris*, and the less virulent phenotype has been confirmed for 169 strains. The phenotypes of 30 less virulent ATMT mutants were further characterized by analysing the response to ROS, the formation of ROS and oxalic acid and the light-dependent differentiation. Interestingly, many of the less virulent mutants are affected in light-dependent development as they are either impaired in conidiation, sclerotia formation or both. One less virulent ATMT mutant that sporulates in the dark does not produce oxalic acid and carries the T-DNA insertion 1608 bp upstream of an ORF encoding a GATA transcription factor. Remarkably, two other less virulent ATMT mutants have been identified that are tagged in the same gene locus. Another less virulent ATMT mutant is severely reduced in growth, produces ROS in great quantities and shows a fluffy phenotype when incubated in the dark. TAIL-PCR analyses revealed that the T-DNA is inserted 1307 bp upstream of an ORF encoding a helix-loop-helix transcription factor. The detailed analysis of these transcription factors by analysing deletion and overexpression mutants is currently in progress.

PR4.22

The ectomycorrhizal fungus *Paxillus involutus* express a large diversity of peptidases when degrading protein substrates and plant litter material

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A majority of the nitrogen (N) present in forest soils is bound in organic forms including proteins. Though a poorly characterized process, this N is mobilized and becomes available to plants due to the activity of ectomycorrhizal (ECM) fungi. We have examined the extracellular proteolytic activity and the underlying gene expression patterns in the ECM fungus *Paxillus involutus* when degrading various protein sources (BSA, gliadin and pollen) and plant litter material. During N-deprived conditions, all substrates induced an extracellular proteolytic activity. The activity had acidic pH optimum (2.5-3.0), and it was mainly due to aspartic peptidases with minor contributions of metallo- and serine proteases. The activity was partly repressed by low concentrations of ammonium (1mg/L), but not nitrate in the medium. The transcriptomes expressed by the fungus was analyzed by 454 pyrosequencing and microarray experiments. The sequencing yielded 2,029,605 reads that were assembled into a set of 12,873 contigs. In total 232 of these contigs were annotated to peptidases including 90 metallo- , 26 aspartic-, 38 serine- and 23 cysteine- peptidases. In total, 61 of these transcripts (i.e. 26 %) were significantly upregulated (> 2 fold) during growth on the protein substrates and the litter extracts. Highest expression levels were found for transcripts of aspartic, metallo, and serine proteases, but the expression patterns differed depending on the medium. We suggest that the expression levels of the extracellular peptidases machinery of *P. involutus* can be tuned to different proteins sources and environmental conditions.

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PR4.23

Antagonistic *Fusarium oxysporum*: pathogenicity gene expression modulated by ectosymbiont bacteria.

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Fusarium oxysporum is a soilborne pathogen comprising over 120 *formae speciales*, responsible for vascular wilts in several plant species.

An antagonistic strain was isolated in Piedmont (northern Italy) from suppressive soils and named MSA35. The hyphae of this organism are colonized by an ectosymbiont bacterial consortium mostly including gamma proteobacteria (Minerdi et al., 2008). The removal of this consortium (CU: cured strain) re-establishes the pathogenicity of the organism. It was demonstrated that genes such as *fmk1*, *chsV* e *p/1* are repressed in the wild type strain (WT) and that volatile organic compounds with antimicrobial properties are produced.

In this work, the potential antagonistic activity of soluble compounds secreted by MSA35WT in the surrounding environment was evaluated. Experiments were performed in liquid cultures. Two pathogens (*F. oxysporum* f.sp. *lactucae* e *F. proliferatum*) were grown in a filtered medium were the antagonist strain was previously cultivated. Mycelial growth, measured as wet and dry weight, was significantly reduced in both cases as well as conidial germination. These results indicate that soluble compounds secreted by MSA30WT affect organisms which grow in close contact with the strain.

The expression of key genes was also evaluated on both MSA35WT and MSA35CU: *fmk1, fgb1*, *fga2* and *fhk1* are not expressed in the antagonist strain. Expression analysis of these genes on pathogens grown in the medium of MSA35WT and identification of secreted compounds are in progress.

PR4.24

Unraveling the effects of a bacterial metabolite in the model organisms *Saccharomyces cerevisiae* and *Neurospora crassa*

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Naturally occurring antifungal compounds are abundant and very diverse and are mostly considered to be produced to regulate the growth of competing organisms in environments such as the rhizosphere of plants. However, in recent years the concept of antibiotics as signalling molecules has emerged and receives rising attention. In this study we are investigating the effects of the secondary metabolite 2,4-diacetylphloroglucinol (DAPG), which is produced by a few *Pseudomonas* spp.. This metabolite exhibits a broad spectrum of antimicrobial activity but little is known about its cellular targets or possible fungal resistance mechanisms. We are using two model organisms, *Saccharomyces cerevisiae* and *Neurospora crassa*, to address these questions. DAPG treatment impairs cell growth in both organisms and specifically causes loss of membrane potential in mitochondria suggesting that electron transport is a target. A screen of the yeast deletion library revealed that alterations of several different processes, such as protein biosynthesis and DNA repair, can confer resistance. We also found that in both *S. cervisiae* and *N. crassa,* DAPG induces a transient cytoplasmic Ca^{2+} signal. The relevance of this signal is part of our current investigations but it may indicate a possible role of DAPG as a signal. The outcomes of this study could facilitate understanding the mode of action of antifungals/antibiotics and their role in inter-and intra-species communication but also help exploitation of this metabolite for agri-biotech and other applications.

PR4.25

Development of a hemibiotroph within its host: Transcriptomic and histological studies of the *Colletotrichum graminicola*-maize pathosystem

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Hemibiotrophic plant pathogens first establish a biotrophic interaction with the host plant, and later switch to a destructive necrotrophic lifestyle. The molecular mechanisms and the biochemical events involved in this process are poorly understood. Studies of biotrophic pathogens have shown that they actively suppress plant defenses after they have penetrated the host cell. To determine whether *C. graminicola* also suppresses host defenses during its biotrophic stage, we performed comprehensive transcriptomic, histological and biochemical studies of the early stages of *C. graminicola* infection of maize leaves, a model pathosystem for the study of hemibiotrophy. We identified novel putative fungal effectors differentially expressed during host colonization. Our findings also show the presence of a fungal respiratory burst in fungal tips during the transition from biotrophic into necrotrophic lifestyle. Additionally, time-course experiments revealed a strong induction of defense-related genes, as well as the accumulation of reactive oxygen species and antimicrobial compounds in host cells during the biotrophic stage. We demonstrate the production of maize-derived vesicular bodies containing H_2O_2 targeted to the fungal hyphae. These results demonstrate a strong induction of defense mechanisms occurring in maize cells during *C. graminicola* infection, even during the biotrophic development of the pathogen. Overall, these results demonstrate a complex molecular and metabolic interaction between *C. graminicola* and maize cells, with a strong induction on plant defense mechanisms at early stages of infection. We hypothesize that the switch into the necrotrophic lifestyle is an adaptive response by the fungus that enables it to evade the host immune system, kill the host cells and complete its life-cycle after host infection.

PR4.26

Identification and functional characterization of CgEP1, a novel pathogenicity factor from *Colletotrichum graminicola*

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Fungi secrete effectors that alter the host's structure and function. *Colletotrichum graminicola* is the causal agent of maize anthracnose, which causes severe crop losses worldwide. To better understand disease development, we are studying novel effector proteins that are expressed during infection. We identified a gene encoding a putative effector protein by searching for proteins that contain secretion signal peptides as well as nuclear localization signals (NLS). In addition to the signal peptide and NLS, the protein has a highly basic isoelectric point and seven nearly identical internal repeated motifs. A BLAST search of the predicted protein sequence found no homologs in public databases, suggesting that it is unique to *C. graminicola*. We performed time-course assays for transcript abundance using RT-PCR as well as *in situ* experiments using transcriptional fusions of the promoter with *gfp* as a reporter gene. These studies revealed that the gene is expressed at early stages of host colonization, mainly in primary hyphae. We constructed null mutants by gene replacement and performed pathogenicity assays on maize seedlings. Anthracnose development was severely impaired on maize plants inoculated with mutant strains demonstrating that the gene is crucial for full pathogenicity of *C. graminicola* on maize. Based on our findings, we conclude that the gene encodes a novel fungal pathogenicity factor that we call CgEP1 (*C. graminicola* Effector Protein 1.) Results of *in planta* subcellular localization of the mature protein will be also presented.

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PR4.27

Pathogen-caused release of poly-unsaturated free fatty acid suppresses plant defense by inhibition of callose synthesis in wheat

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The precise function of callose in papillae has not been shown unequivocally. We demonstrate that upon infection of wheat spikes with the fungal plant pathogen *Fusarium graminearum*, callose synthase activity and callose deposition are suppressed, and wheat is susceptible to fungal spreading. The secreted lipase FGL1 is an important virulence factor for *F. graminearum*. In contrast to *F. graminearum* wild-type, the lipase-deficient *Δfgl1* mutant is unable to suppress wheat callose synthesis. Wheat spikes are resistant to colonization by this mutant. Long-chain unsaturated free fatty acids (FFA) inhibit plant callose synthesis in vitro and in planta; and the previously observed resistance of the wheat spike to *Δfgl1* is broken. The lipase-deficient fungal mutant is able to colonize the spike. Analysis of the FFA level in wheat spikes during infection revealed an elevated linolenic acid concentration during *F. graminearum* wild-type compared to *Δfgl1* infection.

We conclude that linolenic acid plays a decisive role in callose synthesis suppression during wheat - *F. graminearum* interaction. A proposed model explains this novel mechanism of plant defense suppression by pathogen-caused increase in FFA due to lipase secretion.

PR4.28

Discovering host specificity candidate genes of *Sporisorium reilianum* by genotyping mixed-variety offspring

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Sporisorium reilianum is a biotrophic plant pathogenic basidiomycete that causes head smut of maize and sorghum. The fungus exists in two varieties with different host specificity. The sorghum variety (*SRS*) is fully virulent on sorghum. *SRS* infection of maize leads to weak symptoms, such as phyllody of the floral parts. The maize variety (*SRZ*) is fully virulent on maize, but does not show symptoms on sorghum inflorescences. Instead, *SRZ* infection of sorghum leads to the formation of red spots containing phytoalexins on leaves.
This different behavior challenged us to find factors responsible for host specificity. We analyze segregants of a mixed-variety infection both phenotypically and genotypically. Approximately 100 offspring of a cross of *SRZ* x *SRS* are tested for virulence on maize and sorghum. Strains that do not lead to disease symptoms on sorghum and those showing full virulence on sorghum are subjected to genotypic analysis by performing species-specific PCRs as well as an NGS approach. Genomic regions stemming from the *SRZ* parent in non-virulent offspring and from the *SRS* parent in virulent offspring are expected to contain candidate genes for host specificity. This way, we identified the beginning of chromosome 7 as one region of interest. This region harbors an *SRZ*-specific gene (*hsc1*) that, when introduced into *SRS*, was shown to positively contribute to the aggressiveness of the recombinant strains on maize and negatively on sorghum.

This shows that genotyping of mixed-variety offspring is a powerful tool to discover candidate genes involved in host specificity.

PR4.29

Isolation and characterization of *F. graminearum* mutants altered in virulence

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The ascomycete *Fusarium graminearum*, the major causal agent of Fusarium head blight, can infect many important grain cereals. To identify genes that are involved in virulence of *F. graminearum*, an effective *mimp1* transposable element-mediated mutagenesis approach was developed that allowed us to identify several mutants showing reduced virulence (Dufresne et al. FGB 2009). One of these transposon mutants is *ebr1*, which was disrupted in a novel Zn_2Cys_6 transcription factor, showing reduced radial growth and reduced virulence. Knocking out *EBR1* in *F. graminearum* strain PH-1 by homologous confirmed reduction of both radial growth and virulence. The conidia of knock-out strain PH-1*Δebr1* germinated faster than those of wild-type PH-1, but its conidiation was significantly reduced. Detailed analysis showed that the reduced radial growth might be due to reduced apical dominance of the hyphal tip leading to increased hyphal branching. Inoculation assays on wheat heads with a GFP-labeled PH-1*Δebr1* mutant showed that it was unable to penetrate the rachis of the spikelets. Protein fusion with GFP showed that EBR1 is localized in the nucleus of both conidia and hyphae. Knocking out the orthologous gene, FOXG_05408, in *F. oxysporum* f. sp. *lycopersici* caused a much weaker phenotype than the PH-1*Δebr1* mutant. Transformation of FOXG_05408 into PH-1*Δebr1* restored the mutant phenotype. Expression analysis by RNA-seq suggests that in the PH-1*Δebr1* mutant protein synthesis is reduced significantly.

PR4.30

***Piriformospora indica* small secreted proteins**

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Transcriptional analysis provided first insights into *Piriformospora indica* root colonization strategies. This included the expression of small secreted proteins (SSP < 300 aa) during the early biotrophic colonization of barley. Among the SSPs several lectin-like proteins and *P. indica*-specific effector proteins were identified which represented about 10% of the fungal genes induced during biotrophy. In analogy to other fungi where effector genes are upregulated during host colonization, *P. indica* genes encoding SSPs are likely to play a role in determining the success of endophytic interaction which involves host cell penetration, suppression of plant immunity and growth within living cells. Most of the plant responsive SSPs are *P. indica* specific and poorly related to each other. Some of them are cysteine-rich, as describe for other effector molecules in mutualistic and pathogenic systems, other possess distinctive features such as a regular pattern of histidine and alanine residues. A search for motifs in the amino acid sequence of these histidine-alanine rich proteins identified a highly conserved pattern of seven amino acids "RSIDELD" at the C-terminus of 29 putative ORFs. *In planta* expression of two of the DELD proteins using the fusion plasmid 35S::SP_Dld1:mCherry has shown that these proteins are secreted and seem to localize in the apoplast. Bioinformatic and functional analyses of these effector-like proteins will be discussed.

PR4.31

Influence of indole-derivatives on the establishment of the biotrophic interaction of *Piriformospora indica* with barley roots.

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Colonization of barley roots by *P. indica* is accompanied by changes in phytohormone homeostasis, such as induction of plant genes involved in ABA and auxin metabolism and signalling during the biotrophic colonization phase (Schäfer et al., 2009). *P. indica* produces the phytohormones indole-3-acetic acid (IAA) and indole-3-lactate (ILA), upon tryptophan feeding, through the intermediate indole-3-pyruvic acid (IPA) and indole-3-acetalaldehyde (IAD). Analysis of the underlying biosynthetic pathways for auxin identified the piTam1 gene as a key player. Transcriptional analysis in barley colonized roots showed that piTam1 gene is induced during the biotrophic phase. Congruent with previous reports that auxin renders the plant more susceptible to colonization by biotrophic microorganisms (Bari and Jones, 2009), *P. indica* transformants carrying the RNAi construct for the pi*Tam1* gene displayed decreased colonization of barley roots in the biotrophic phase, but not in the cell death associated phase. Local increased auxin levels in barley, measured by IC-MS/MS, at the early colonization phase additionally hints towards involvement of auxin in the establishment of early biotrophy.

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PR4.32

Transcriptome of *Penicillium digitatum* During the Infection of Oranges

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Penicillium digitatum is the causal agent of green mould rot of citrus fruit, and represents the major postharvest pathogen of citrus fruit in Mediterranean regions. In this study, with the aim of better understanding the infection process on oranges, we used massive parallel pyrosequencing with 454 Titanium technology to perform a global RNA-Seq transcriptomic analysis of *P. digitatum* in time series from 0 to 48 h after pathogen inoculation, where first symptoms of disease appeared. To identify the putative origin of the reads, two reference genomes were used: (i) the *Citrus sinensis* Genome Assembly (JGI v1.0; these sequence data were produced by the US Department of Energy Joint Genome Institute http://www.jgi.doe.gov/ in collaboration with the user community), and (ii) the first draft of the *P. digitatum* genome with a 20X genome coverage, elaborated in house. All sequence reads from a total of four libraries were assembled in a reference transcriptome containing 24410 contigs or putative genes. About 30% of those putative genes were assigned to *P. digitatum* and about 70% to *C. sinensis*. The number of *P. digitatum* putative genes increased as the infection progressed, whereas citrus genes showed the opposite trend. Quantitative reverse transcription PCR profiling of selected fungal genes revealed dynamic expression patterns during infection of orange fruits.

PR4.33

Experimental evidence for Crozier's paradox: somatic fusion in fungi as a model for the evolution of cooperation and kin recognition

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Cooperative behaviors, behaviors that benefit other individuals, are widespread. However, to understand cooperation we have to explain how cheating, i.e. profiting without contributing, is kept at low frequency. Kin selection is the predominant solution for this problem. Kin selection requires that cooperation is preferentially directed towards related individuals, and one way to achieve this is via genetic kin recognition. However, Crozier argued that in the short term positive frequency dependent selection will eliminate the genetic polymorphism required for such recognition, since common genotypes will experience more cooperation and thereby increase in frequency. Here we study somatic fusion as a model for cooperation and kin recognition. Sharing somatic tissue via fusion seems to be an extreme form of cooperation. The potential for such fusion is widespread, but the fitness consequences of fusion are unknown. In fungi, successful somatic fusion is usually restricted to clonally related individuals regulated by highly polymorphic recognition loci. We study somatic fusion between mycelia of the fungal species *Neurospora crassa*.

First we show that there is a highly significant positive correlation between total fitness and the degree of successful fusion. This result demonstrates that fusion between genetically identical mycelia is net beneficial (*i.e. B-C*>0) and thus cooperative. We then show experimental evidence for Crozier's theoretical prediction that, in the short term, positive frequency dependent selection acts against polymorphism of recognition alleles. With these findings we discuss which counteracting evolutionary forces maintain the extensive recognition polymorphism observed in nature.

PR4.34

Inhibition of ecto-phosphatase activity in conidia reduces adhesion and virulence of *Metarhizium anisopliae* on the insect host *Dysdercus peruvianus*

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Metarhizium anisopliae is an entomopathogenic fungus and one of the most important and best studied biological control agents in world. This fungus has the ability to infect a broad range of arthropods, from ticks and agricultural insect pests to vectors of human diseases. Entomopathogenic fungi have evolved distinct strategies for their attachment to hosts, varying considerably in their modes of action, virulence and degree of host specificity. In this work, we describe the characterisation of phosphatase activity directly on the conidia surface of *M. anisopliae* and its relevance in the host interaction process. The activity of this enzyme was linear with time and cell density, and only 20% of the total activity was secreted to the extracellular medium. The optimum pH was in the acidic range and divalent metals, such as Cu^{2+}, Cd^{2+} and Zn^{2+}, inhibited ecto-phosphatase activity, while Co^{2+}, Ca^{2+}, Sr^{2+} Mg^{2+} and Fe^{2+} had no effect. The activity was also reduced by sodium fluoride, sodium molybdate, sodium orthovanadate and inorganic phosphate. Importantly, the inhibition of phosphatase activity in conidia reduced the adhesion to *D. peruvianus* tegument and, consequently, *M. anisopliae* virulence. The results herein presented show, for the first time, the importance of ecto-phosphatase activity in *M. anisopliae* conidia and provide the first evidence of its direct involvement in adhesion and host infection.

PR4.35

Beyond nitrogen and pH: in search for new inducers of deoxynivalenol biosynthesis in F*usarium graminearum*

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The fungal pathogen *Fusarium graminearum* is the causal agent of Fusarium head blight (FHB) of small grain cereals and cob rot disease of maize. During infection, the fungus produces the trichothecene deoxynivalenol (DON). Contaminated grain is highly toxic to mammals and, thus, not suitable for food and feed production. The key enzyme in the biosynthesis of DON is the trichodiene synthase (Tri5), which is essential for virulence on wheat. We recently created a transgenic reporter strain (Ilgen *et al.* 2009), in which the promoter of the trichodiene synthase is fused to eGFP. Using this construct we are able to perform live cell imaging to check for putative DON-inductive conditions, especially during host invasion. Firstly, we were able to show that in axenic culture, ammoniumsulfate ($(NH_4)_2SO_4$) is strongly inducing eGFP, while, in contrast, sodiumnitrate ($NaNO_3$) elicited no visible eGFP fluorescence. However, this effect is rather due to a drop of pH in the $(NH_4)_2SO_4$ medium than due to the nitrogen source, since we were able to induce DON production in a $NaNO_3$ medium buffered to a low pH using citrate buffer. However, the question remains, how these *in vitro* results fit to the situation in the host-pathogen interaction. Thus, we started to investigate plant-derived substances for their pH-independent DON-inducing potential in a combined *in-planta* and *in-vitro* approach. Preliminary results indicate a supervisory inductive effect of certain plant compounds.

Ilgen, P., Hadeler, B., Maier, F.J., and Schäfer, W. 2009. MPMI 22:899-908.

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PR4.36

Characterization of a high affinity ammonium transporter from the root endophytic symbiont *Piriformospora indica*

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The root endophyte *Piriformospora indica* displays a biphasic lifestyle during colonization of barley roots with an early biotrophic phase followed by a cell death associated phase. The interaction of *P. indica* with barley roots results in growth promotion and in the induction of plant genes involved in nitrate and ammonium transport. Comparative analysis revealed the presence of two ammonium transporters (AMT) in the *P. indica* genome and the absence of nitrate transporters (NTR). One of the piAMT transporters was proven to be plant responsive by microarray and qPCR analysis. Its ammonium import function was verified by yeast complementation. *P. indicaAMT1* knock down mutants show reduced growth phenotype on media containing low amount of ammonium and display an altered colonization pattern in barley roots. Our results suggest that ammonium plays an important role in the interaction with barley. Further analyses are ongoing to better characterize the impact of different nitrogen sources on symbiosis.

PR4.37

Do secreted chorismate mutases have a conserved role as enzymatic effectors in the fungal kingdom?

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A successful colonization of plants by pathogens requires active effector-mediated suppression of defense responses. Recent findings showed that the biotrophic fungus *Ustilago maydis* secretes an enzymatically active, non-allosterically regulated chorismate mutase Cmu1. This enzyme is taken up locally by infected plant cells and then spreads to neighboring cells. Metabolic data of infected maize leafs indicate that Cmu1 is involved in the rechanneling of chorismate into the phenylpropanoid pathway away from the competing salicylic acid biosynthesis pathway. Secreted chorismate mutases were identified in many other fungi mostly associated with plants. Their sequence relationship indicates that several independent events led to the occurrence of secreted chorismate mutases. Using *U. maydis* we are currently testing by complementation of an *U. maydis cmu1* mutant whether other fungal secreted chorismate mutases predicted to be secreted have the potential to act as effectors in a Cmu1-like manner. If not other functions have to be postulated for the predicted secreted chorismate mutases which could indicate a neofunctionalization of this branching enzyme in the shikimate pathway.

PR4.38

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

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The recent finding that the histone acetyltransferase (HAT) complex SAGA/ADA mediates the response of the fungus *Aspergillus nidulans* to the bacterium *Streptomyces rapamycinicus* (1) opens up a number of questions. It was shown that the SAGA/ADA complex is involved in the regulation of the orsellenic/ lecanoric acid biosynthesis gene cluster, as deletion of its HAT-encoding gene *gcnE* resulted in the lack of *orsA* transcription during co-cultivation.

In order to investigate the SAGA/ADA complex in *A. nidulans*, the complex subunits GcnE and AdaB were tagged and purified by tandem affinity purification (TAP). This method is especially suited for the purification of protein complexes under native conditions. Therefore, the TAP-tag method represents an appropriate system for the investigation and analysis of the SAGA/ADA complex composition under various conditions. The TAP-tag constructs were assembled by fusion PCR and transformed directly into *A. nidulans* via homologues recombination. Western blotting was performed to monitor the purification procedure. For *adaB-C-TAP* and *gcnE-N-TAP* bands of the expected size were detected. However, further optimisation of the purification procedure is required. Furthermore, the amount of purified target protein needs to be increased to allow detection by mass spectrometry.

In this study, a first step on the investigation of SAGA/ADA in *A. nidulans* was achieved by the successful tagging of the two subunits GcnE and AdaB with the TAP-tag. Furthermore, it is now possible to elucidate the structure and function of this complex during the interaction of *A. nidulans* and *S. rapamycinicus*.

(1) Nützmann et al. (2011) *PNAS*

PR4.39

In vitro Effect of Chitosan on Growth and Enzyme Production in Ganoderma sp.

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Ganoderma boninense is the causal agent of oil palm basal stem rot disease (BSR). Lignin degradation which involves the secretion of ligninolytic enzymes by Ganoderma plays an important role in pathogenesis of BSR. The aim of the study is to evaluate the efficacy of chitosan on the effect on the growth of *G. boninense* and production of ligninolytic enzymes. Four types of chitosan (low viscosity, high viscosity, low molecular weight, high molecular weight) at 1.0, 2.0 and 3.0 % concentrations were tested for their efficacy to control mycelia growth of the pathogenic GBLS isolate after 20 days of incubation *in vitro*. All of the concentrations of different chitosan tested significantly reduced mycelial growth compared with control treatment. Microscopic observations of the changes and alterations in surface morphology of *G. boninense* mycelium imputes possible mode of action of chitosan on fungal growth. GBLS produced a combination of laccase and Manganese proxidase (MnP) as lignin degrading enzymes (LDE) in semi solid state and liquid culture medium. Chitosan at the different concentrations 1.0, 2.0 and 3.0 % evaluated significantly inhibited the production of lignin degrading enzymes of laccase and MnP by GBLS isolate under solid state culture medium supplemented with rubber wood chips. This observation suggests that ability of chitosan to act as chelating agent facilitates the inhibition of LDE produced by GBLS.

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PR4.40

Identification and analysis of *Penicillium digitatum* genes putatively involved in pathogenicity towards citrus fruits

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Penicillium digitatum, the causal agent of citrus green mould, is the major pathogen of citrus fruit during postharvest handling and storage in Mediterranean climate regions. It is a necrotrophic fungus that penetrates the fruit through wounds. Despite the economical relevance of this fungus our knowledge on its pathogenicity mechanisms is still very limited. In this communication we present the identification and analysis of genes putatively involved in pathogenicity.

We have obtained a subtractive cDNA library enriched in *P. digitatum* genes that are up-regulated during infection of citrus fruit, which in combination with macroarray hybridization, has allowed the identification of several fungal genes with a potential role in pathogenicity. Time course expression analysis of several selected genes confirmed that most of them are up-regulated during infection of citrus fruits. We have cloned and sequenced the coding and flanking sequences of several candidate genes that have been isolated from a genomic DNA library prepared in the fosmid PCC2FOS. In order to determine the role of these genes in pathogenicity we have employed *Agrobacterium tumefaciens*-mediated transformation to obtain *P. digitatum* null mutants lacking the genes coding for two different polygalacturonases, a pectin lyase and a Rieske protein. *P. digitatum* mutants lacking any of the three pectin degrading enzymes showed a reduction in infection capability when compared with either the wild type strain or an ectopic transformant, whereas the mutant strain lacking the Rieske protein was not affected in virulence.

PR4.41

Pathogenicity gene variations within the order Entomophthorales

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Fungi within the order Entomophthorales (subphylum Entomophthoromycotina) are obligate biotrophic pathogens of arthropods with a remarkable narrow host range. Infection takes place through the cuticle when conidia hit a susceptible host, facilitated by enzymatic and mechanical mechanisms. In the hemolymph, they proliferate as hyphal bodies or cell wall-less protoplasts for easy nutrient uptake and host immune response avoidance. Entomophthoralean fungi often manipulate their host to seek an elevated position shortly before host death in order to optimize disease transmission. After host death, conidia are produced and discharged when humidity gets high—usually during night. In an earlier secretome study of field-collected grain aphids (*Sitobion avenae*) infected with entomophthoralean fungi, a number of pathogenesis-related, secreted enzymes were discovered (Fungal Genetics and Biology 2011, vol. 48, 343–352). Among these were cuticle degrading serine proteases and chitinases, involved in fungal penetration of the aphid cuticle, and a number of lipases most likely involved in nutrient acquisition. In the current study, we are investigating the distribution and variation of selected pathogenicity genes within genera *Entomophthora* and *Pandora*, using fungal genomic DNA originating from field-collected, infected insect host species of dipteran (flies, mosquitoes) or hemipteran (aphid) origin.

PR4.42
retro – a retrotransposon of Tricholoma vaccinum
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The basidiomycete *Tricholoma vaccinum* is an ectomycorrhizal fungus, forming symbiosis with spruce and pine. During symbiosis the mycelium colonizes the intercellular spaces of the root cortex to exchange nutrients and for the benefit of both individuals.

In differential display analyses using ectomycorrhiza a retrotransposon was identified showing gag and pol genes with protease, RTase, RNaseH and integrase domains. Transposition of retrotransposons occurs with an RNA intermediate.

In this work the retrotransposon *retro* of *Tricholoma vaccinum* is studied for its ability to induce its transposition by checking expression and copynumber under stressing conditions, like in mycorrhizal interaction of *Tricholoma vaccinum* and *Picea abies* in axenic dual cultures or using additives for *Tricholoma vaccinum* liquid cultures like butanol and ethanol.

Since laboratory strains and new isolates of *Tricholoma vaccinum, Tricholoma imbricatum*, *Tricholoma fracticum* and *Tricholoma fulvum* have developed under different conditions they will likely be different in their number of *retro* signals, which will be investigated in Southern blot analyses.

PR4.43
Ectomycorrhizal Symbiosis: Influenced by fungal aldehyde dehydrogenase
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In forests various organisms live in close interaction with other species and mycorrhiza is an ubiquitous kind of symbiosis in this habitat. The mutualistic symbiosis between the basidiomycete fungus *Tricholoma vaccinum* and the specific host spruce (*Picea abies*) is called ectomycorrhiza. Important role in ecosystem functioning is attributed, particularly improved plant growth by advanced nutrient and water supply and also a phytosanitory effect on the plant against pathogens by fungal activity was shown.

The molecular level of this association is so far slightly understood and we intent to investigate the molecular mechanisms of interaction.

Ald1 - coding for a fungal aldehyde dehydrogenase - was identified in differential display analyses using ectomycorrhiza. Ald1 has a function in the detoxification of alcohols and aldehydes occurring in mycorrhizal biotopes and is involved in phytohormone production.

Agrobacterium tumefaciens mediated transformation was used to produce Ald1 overexpressing transformants of *T. vaccinum* and functional analysis is investigated in ongoing experiments: we ask for the impact of different supplements on the phenotypic character of an *ald1* transformant and the wildtype in mycorrhiza.

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PR4.44

Suppression of plant immunity by the *Ustilago maydis* effector protein Pep1

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Plant-associated organisms secrete proteins and other molecules to modulate plant defenses and enable colonization of plant tissue. The colonization of maize plants by *Ustilago maydis* is initiated by a direct penetration of the cuticle and cell wall of the host epidermis. The secreted effector protein Pep1 is specifically expressed during pathogenic development of *Ustilago maydis*. *pep1* deletion mutants show no defect during saprophytic growth but are arrested upon the point of penetration and elicit a hypersensitive response of the plant. Thus, the establishment of a biotrophic interaction fails and the affected plant tissue shows various defense responses, particularly the production of reactive oxygen species (ROS).

We identified Pep1 being an efficient inhibitor of early plant defense response. In particular, the effector inhibits peroxidase activity and thereby suppresses the generation of ROS. Moreover we could show the direct interaction of Pep1 to a single peroxidase, which is upregulated during infection with the Δ*pep1*-mutant. Consequently, silencing of the maize peroxidase gene lead to partial complementation of the Δ*pep1*-virulence. Here, we present recent data from biochemical and microscopic approaches to specify the peroxidase interaction of Pep1 and the consequences of Pep1 function for early plant defense reactions.

PR4.45

Cell death suppression during the interaction of *Ustilago* and barley

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In plants programmed cell death (PCD) is an essential defense mechanism during pathogen attack. Prevention of PCD is therefore essential to biotrophic plant pathogens such as *Ustilago hordei.* During the compatible interaction of this basidiomycetous fungus with its host plant barley, PCD is fully prevented and no macroscopic symptoms of infection are visible, whereas deletion mutants of the secreted effector protein Pep1 cause PCD comparable to the non-host resistance reaction after infection with *U. maydis.*

Microscopical analyses revealed that plants overexpressing the conserved cell death suppressor Bax Inhibitor-1 (BI-1) show an increased susceptibility to the non-host pathogen *U. maydis*. Interestingly, BI-1 seems to play no role in the interaction with the *U. hordei* or *U. maydis pep1* deletion mutant, respectively, indicating that the induced cell death reaction by the effector mutant is obviously mediated by a BI-1 independent pathway. Preliminary results point towards a role of apoptosis-like cell death during non-host responses, while autophagy might be performed during interactions independent from BI-1. With a combination of microscopic and enzymatic approaches, we try to dissect the different modes of programmed cell death triggered by the described fungal strains.

In a parallel approach, we are aiming to identify cell death suppressing proteins which enable the establishment of the biotrophic interaction of *U. hordei* and barley. Microarray experiments show differentially regulated genes during the early phase of *U. hordei* infection. Up-regulated secreted effectors serve as candidate genes to be used in different screening approaches for which recent progress will be presented.

PR4.46

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

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Aspergillus fumigatus is the most important air-borne human fungal pathogen. The genome of this filamentous fungus exhibits far more gene clusters predicted to encode secondary metabolites than compounds known. Because these unidentified metabolites could have important biological functions and possibly represent drug candidates, it is desirable to activate their often silent biosyntheses.

Our aim was to mimic physiological conditions under which secondary metabolite gene clusters could be activated. Previously, we demonstrated activation of a silent secondary metabolite gene cluster of *Aspergillus nidulans* by co-cultivation with *Streptomyces rapamycinicus* which led to formation of orsellinic and lecanoric acid. Interestingly, as shown here, the bacterium is also able to activate silent gene clusters in the human-pathogenic fungus *A. fumigatus*. Co-culturing of *A. fumigatus* with this streptomycete triggered the specific activation of a so far silent fungal secondary metabolite gene cluster leading to the production of a novel secondary metabolite.

Moreover, overexpression of a pathway-specific regulatory gene demonstrated its function as regulator of the newly identified gene cluster that also includes a polyketide synthase gene.

PR4.47

Identification and analysis of virulence factors in the *Colletotrichum higginsianum Arabidopsis thaliana* pathosystem

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The hemibiotrophic ascomycete fungus *C. higginsianum* causes anthracnose disease on cruciferous plants. In order to identify genes involved in pathogenicity, we generated T-DNA insertion mutants of *C. higginsianum* using *Agrobacterium tumefaciens* mediated transformation. Over 7000 mutants were screened for virulence against the model host *A. thaliana*. 79 T-DNA insertion mutants (about 1%) reproducibly showed pathogenicity phenotypes. This collection contains mutants which are affected in appressoria formation, host penetration or hyphal growth. Mutants are currently being analyzed by Southern Blot and GenomeWalker PCR to determine their T-DNA insertion sites. For confirmation of the corresponding pathogenicity phenotypes, an approach for targeted gene knockout was implemented using a KU80 deficient strain impaired in the non-homologous end-joining pathway. This method was used to knockout several independent genes identified in the mutant screen, which are for example involved in metabolism, cell cycle or membrane transport. Initial results of these studies will be presented.

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PR4.48

The Benzoate p-Hydroxylase of *Cochliobolus lunatus* – Search for an Antifungal Lead Compound

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There is a constant need for new fungicides for (crop) disease control due to appearing resistances and new registration demands. Compounds with new mode of action and more favorable (eco) toxicological properties are necessary. Benzoate p-hydroxylase or CYP53A15, a cytochrome P450 identified in the pathogenic filamentous ascomycete *Cochliobolus lunatus*, is capable of hydroxylation of benzoate, a key intermediate in the metabolism of aromatic compounds in fungi which is basically toxic to the organism. CYP53 family is a promising antifungal target since it seems to be distributed in most of filamentous fungi and have no homolog in higher eukaryotes. Our experiments revealed that naturally occurring phenolic compounds inhibit CYP53A15. A very reliable structural homology model was built to perform initial virtual screening of library of compounds and molecular docking in active site. Compounds were selected from diverse commercial sources to construct a chemical library of over million compounds. A more manageable size of the library was generated by filtering for descriptors: molecular weight, number of ring systems, hetero atoms, H-bond donors or acceptors. Only solutions predicting interactions with the heme iron were considered. About 100,000 compounds were docked into the enzyme active site and ranked. From those, 40 compounds were obtained and evaluated in functional assays *in vitro*: CO-differential spectrum, substrate binding spectrum, HPLC analysis, and in growth inhibition assay *in vivo*.Results of this research have identified some inhibitors which could serve as lead compound for further development of (phyto) pharmaceuticals with different mode of action.

PR4.49

Mechanisms of detoxification in the ectomycorrhizal fungus *Tricholoma vaccinum*

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Toxic substances, e. g. xenobiotics, heavy metals and aldehydes, are found in ectomycorrhizal habitats, like woods, gardens and parks. Mechanisms to prevent cells from toxins, mostly reported from yeast or filamentous ascomycetes, are the modification of compounds by enzymes, extracellular chelation by excreted ligands, cell wall binding, reduced influx across the plasma membrane, enhanced efflux, intracellular chelation by metallothioneins or glutathione, transport into subcellular compartments like the vacuole, protection against toxic metal-induced oxidative stress by thioredoxins and superoxide dismutases and filter function of the mycelial mantle.

Lab experiments were performed using axenic co-culture systems of the basidiomycete *Tricholoma vaccinum* and spruce, and genes *ald1* and *mte1,* upregulated in ectomycorrhiza, were identified. Both are involved in detoxification mechanisms and were investigated in more detail.

The fungal aldehyde dehydrogenase Ald1 catalyzes the conversion of different aldehydes to the corresponding carboxylic acids. By using competitive and real-time RT-PCR, *ald1* was shown to be induced in response to alcohol- and aldehyde-related stress. Ald1 overexpressing mutants of *T. vaccinum* showed increased ethanol stress tolerance in comparison to wildtype.

Mte1 of the multidrug and toxic compound extrusion (MATE) family exports different compounds. By heterologous expression in *Saccharomyces cerevisiae*, different metals, xenobiotics like DNA-intercalating dyes and fungicides were identified as substrates for this specific transporter.

PR4.50

The role of Stp1, a secreted effector of *Ustilago maydis* during pathogenesis

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Secreted effectors play crucial roles during establishment of a successful biotrophic interaction. In the smut fungus *Ustilago maydis*, one of the essential effectors is *stp1*. *stp1* mutants are non-pathogenic and arrest shortly after penetration. Deletion analysis revealed that the N- and C-terminal domains of Stp1 are essential for protein function while the large central region is dispensable. In addition, co-expression of separated N- and C-terminal domains of Stp1 could restore pathogenicity of a *Δstp1* strain. To determine whether Stp1 acts in apoplast or is translocated into plant cells, we are performing uptake assays of Stp1-mcherry-NLS fusion protein. To elucidate the function and the localization of Stp1, we have identified interactors by yeast two-hybrid screening. 23 putative interactors from infected maize leaves were identified, coding for apoplastic as well as cytoplasmic plant proteins. Full length cDNA clones of 8 putative interactors were isolated and their interactions with full length Stp1 as well as truncated Stp1, N-terminal and C-terminal of Stp1 were tested. Stp1 protein purified from *E. coli* could inhibit the activity of a cysteine protease isolated as apoplastic interactor of Stp1. For other interesting interactors, we are verifying the interaction with Stp1 by other biochemical and functional assays and will present the latest results.

PR4.51

Identification of micro-organisms fighting *T. aggresivum* and other "Weed" fungi in mushroom compost

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The cultivation of mushrooms is susceptible to unwanted growth of fungi, like *Trichoderma aggresivum* and *Penicillium*. The growth of these fungi cause substantial decrease of yields to the growers or even destroy the harvest completely. Successful methods to control damage of mushroom beds by these weed fungi are still rare. Microbes inhabiting the mature mushroom compost might play an important role in stimulating or disturbing growth of mushrooms. In this study we have identified micro-organisms present in the compost that are able to inhibit growth of fungi like *T. aggresivum* and *Penicillium* leaving the mycelial growth of the commercial mushroom *Agaricus bisporus* unaffected. Furthermore, we have shown that a *Pseudomonas* strain derived from natural soil is very effective in inhibiting weed fungi. Future research will be focused on the potential of these bacterial strains as biocontrol agents.

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PR4.52

Eukaryotic translation initiation factor 5A is a central regulator in *Fusarium graminearum*

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Activation of the eukaryotic translation initiation factor 5A (eIF5A) requires a posttranslational modification, forming the unique amino acid hypusine. This activation is mediated by two enzymes, deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH). The activated protein transports mRNAs from the nucleus to the ribosomes, where it initiates protein biosynthesis. This system is conserved from archea to humans and has been shown to be instrumental in diseases as diverse as HIV infection, malaria, cancer and diabetes. For the first time, we evaluate its importance in a pathogenic fungus by over-expression of the enzymes that control hypusination of eIF5A. Over-expression of DOHH prevents infectivity of *Fusarium graminearum* to wheat and maize and leads to an over-production of reactive oxygen species (ROS). In addition, it reduces production of the mycotoxin deoxynivalenol *in vitro* and *in planta*. In contrast, over-expression of DHS leads to an increase of virulence to wheat and a decrease of ROS. Simultaneous over-expression of both enzymes results in infectivity and levels of ROS comparable to the wild type strain. Over-expression of DHS, as well as the simultaneous over-expression of both enzymes results in deoxynivalenol levels comparable to the wild type strain. We constitutively expressed the fluorescent protein GFP in the over-expressing mutants and the wild type. Analysis revealed that the over-expressed DOHH mutant is unable to reach the rachis node at the basis of wheat spikelets. Over-expressing DHS results in a faster wheat tissue invasion compared to wild type or the double over-expressing mutant. For the first time, we identified the signalling pathway controlling expression of DHS and DOHH. Our results suggest that transcriptional balance between the two activating genes is important for the correct function of eIF5A. This will be discussed in the context of results obtained in mamalian cells.

PR4.53

Antifungal potential and ecophysiological characterization of indigenous *Trichoderma* spp. isolated from Nile Valley (Egypt)

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Plant pathogenic fungi are the most devastating threats to crop production in Egypt. The need for effective and environmentally friendly control for agriculture (biocontrol) as an alternative to the use of chemical fungicides puts the genus of highly mycoparasitic filamentous fungus – *Trichoderma* in a special focus. The antagonistic potential of 62 Egyptian *Trichoderma* strains, which were identified by means of multiloci DNA barcodes, was assessed in dual confrontation tests with plant pathogenic fungi *Alternaria alternate*, *Botrytis cinrea*, *Fusarium solani*, *Macrophomina phaseolina*, *Rhizoctonia solani* and *Sclerotium rolfsii* . Antagonistic behavior of aggressive *Trichoderma* strains against each of the concerned pathogens was further studied on low nutrition medium and examined microscopically. Moreover, testes for volatiles and inhibitors were carried to emphasize the biological interaction. Among all tested *Trichoderma* strains, the dominant *T.* cf. *harzianum* phylotypes revealed the strongest inhibitory effect against all tested pathogens (66.3% - 100 % growth inhibition). These strains were able to completely overgrow the pathogens and were the best to inhibit the production of sclerotia by *R. solani* and *S. rolfsii*. The other tested species (*T. asperelloides*, *T. brevicompactum*, *T. ghanense, T. longibrachiatum*, *T. citrinoviride* and the two putatively new species) varied in their antagonistic potential depending on the host (prey) species. To understand the information stored in the genome of the highly mycoparasitic *T.* cf. *harzianum* strains we characterized their metabolism using BIOLOG Phenotype MicroArray (PM) technology.

PR4.54

Functional Analysis of *Ustilago maydis* effector Tin3 of Cluster 19A

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Ustilago maydis is a biotrophic fungal plant pathogen that causes smut disease in its host plant maize. Previous genomic studies revealed that *Ustilago maydis* depends on a variety of novel secreted effector proteins to establish a compatible interaction with its host plant. With respect to tumor formation cluster 19A encoding 23 effectors is of special interest, as cluster mutants still proliferate inside the plant tissue but fail to produce tumors. Sub-deletions and single gene deletions of cluster 19A have shown that the major effectors reside in the leftmost half. Especially *tin3* (*tumor inducing 3*), a unique gene, contributes significantly to tumor formation. Using fluorescence microscopy and immunoblot-analysis it was shown that Tin3 is secreted into the apoplastic space and that it accumulates at hyphal tips and cell-to-cell passages. In Yeast-two hybrid approaches two interesting interaction partners for Tin3 were identified: Mir3, a plant defense related cysteine protease and Beclin1, an autophagy related protein of maize. We have present evidence that Tin3 is inhibiting the protease activity of Mir3 in in-vitro protease-assays and in plant lysates of infected maize plants. In addition, we are currently investigating the influence of Tin3 on autophagy induced by pathogen infection via yeast-complementation assays coupled with fluorescence microscopy. With these studies we hope to identify the role of Tin3 during pathogenic development of *U. maydis*.

PR4.55

Proteinaceous elicitor from ascomycete pathogen *Leptosphaeria maculans* induces resistance in oilseed rape

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During the interaction of pathogen and its host comes to considerable exchange of molecular information between both participants. Molecules originating from either pathogen or host capable of inducing resistance response of host are referred as elicitors. Different types of elicitor molecules, including oligosaccharides, glycoproteins and peptides, and phospholipids have been identified.

Studying interaction of Dothideomycete *Leptosphaeria maculans* with its natural host *Brassica napus* we are searching for elicitors produced during in vitro cultivation. Application of medium filtrate after 10 days of *L. maculans* cultivation protects *B. napus* plants from subsequent *L. maculans* infection. Using gene expression analysis by RT-qPCR the biological effect of the filtrate is supported by detection of elevated expression levels of defence marker genes in *B. napus*. Incubation of elicitor with proteinase K lowers the biological effect indicating the proteinaceous character of the elicitor. Moreover, the elicitor was precipitated with ammonium sulphate. Using the isoelectric focusing in combination with ion exchange chromatography we are identifying the elicitor.

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PR5.56

Host-Induced Gene Silencing in Phytopathogenic Fungi Attacking Cereals

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Functional genomics in obligate biotrophic phytopathogenic fungi are severly hampered by the fact that they cannot be grown on artificial media. This makes it difficult to generate, select and cultivate transformants or mutants which are affected in their ability to infect the host plant.

Recently we discovered host-induced gene silencing (HIGS) as a new method to knock down the transcript level of individual genes in *Blumeria graminis*. This was achieved by expressing RNA interference (RNAi) constructs targeting fungal sequences in the host plant. So far the mechanism how the RNAi mediating RNA molecules are transferred into the fungus and whether small interfering RNA or larger RNA would be responsible for the effect is not known.

To illustrate the effect we knocked down two different ß-1,3-glucanosyltransferase genes of *Blumeria graminis* by HIGS using transient systems as well as stably transformed plants. With this approach we could show that both genes may have different functions in cell-wall biology of *B. graminis* but are both important for the colonization of the host plant.

Currently we are also exploring HIGS in *Fusarium culmorum*, one of the causal organisms of the serious *Fusarium* head-blight disease.

PR4.57

ABC transporters in mycoparasitic fungi Trichoderma atroviride P1 and Trichoderma sp. BRM

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Many Trichoderma spp. Have been applied in biological control of phytopathogenic fungi and used as biocontrol agents (BCA) in agriculture and forestry. Although mycoparasitic and biocontrol properties in Trichoderma spp. Have been under scrutiny for a while, the mocecular aspects of mycoparasitic activity and key genes remain largely unknown. Trichoderma spp produce secondary metabolites during mycoparasitic attack in order to harm a prey. In the same time, they increase the resistance against antibiotics of other fungal species. One of many genes responsible for resistance against antifungal compounds was recently identified as Taabc2, a gene encoding an ABC transporter in T. Atroviride P1. TAABC2 belongs to PDR transporters, an ABC transporter subfamily consisting of 8 genes of full-length and one gene of half-size. In our work we analyzed transcriptional changes of PDR transporters named TAPDR5 and TAPDR12 which are homologous to yeast Pdr5p and Pdr12p. The expression profiles of Tapdr5 and Tapdr12 were obtained for T. Atroviride P1, Trichoderma sp. BRM (benomyl-resistant mutant) and Trichoderma sp. T6, a strain prepared by protoplast fusion of T. Atroviride P1 and Trichoderma sp. BRM. The transcription profile was obtained under various culture conditions and in presence of antifungal compounds, organic acids, cell wall and mycelia of fungal pathogens. T6 strain exhibited significantly increased mycoparasitic abilities than parental strains. An individual gene expression 175rofile was observed for each Trichoderma strain.

PR4.58

Functional and molecular analysis of newly identified sulfate transporter in pathogenic *Fusarium* species with respect to their virulence and ability to infect potato.

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AstA protein (alternative sulfate transporter) represents a little known type of sulfate trasporter, belonging to an extensive and poorly characterized family of allantoate permeases Dal5. In *Aspergillus nidulans* the *astA* gene is under control of Sulfur Metabolite Repression (SMR). The closest homologs of *astA* are frequent in evolutionarily distant fungi belonging to the *Pezizomycotina* subphylum (orders *Sordariales* and *Eurotiales*) which exhibit similar plant pathogenicity. They are mostly crop pathogens and are represented by the following sequenced species: *Fusarium graminearum*, *F. verticillioides*, *F. oxysporum*, *Nectria haematococca*, *Verticillium albo-atrum*, *V. dahliae and Leptosphaeria maculans*. The AstA homolog is also present in: cellulolytic *Chaetomium globosum* detrimental to paper industry, opportunistic human pathogen *Neosartorya fischeri*, harmless *Podospora anserina* inhabiting cattle dung, as well as in entomobiocontrol fungi like *Cordyceps militaris* and *Metarhizium anisopliae*.

Fusarium sp. fungi, like *F. solani*, *F. oxysporum* and *F. sambucinum,* contribute to serious devastation of potato crops and increase the cost of cultivation due to application of pesticides. The main problem in the fight with plant pathogenic fungi lies in their metabolic and protein similarity with the host. The aim of this project is to investigate the function of AstA upon infection and colonization of plant host by fungal pathogens, like *Fusarium sambucinum*. The study will involve infection of potato tubers with *astA* deletion mutant of *F. sambucinum*.

PR4.59

Exploring the basidiomycete defensome using RNA sequencing

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Fungi undergo complex symbiotic and antagonistic interactions with other organisms in nature. As an example, fungal mycelium (M) and fruiting bodies (FBs) are preyed by fungivorous vertebrates and invertebrates. As defense against their predators, fungi produce secondary metabolites, peptides and proteins that interfere with the development or the growth of these organisms. In the inky cap mushroom *Coprinopsis cinerea*, several defense lectins and protease inhibitors have previously been found to be specifically and constitutively expressed in FBs. In this project, we aim at the identification of protein-encoding genes in *C. cinerea* whose expression is induced in the vegetative mycelium upon predation by fungal-feeding nematodes using RNA Sequencing by Oligonucleotide Ligation and Detection (SOLiD). As a first step towards this goal, we compared the transcriptomes of young FBs and unchallenged M to identify differentially expressed genes. The first striking observation was that most of the protein-encoding genes were expressed under these conditions (88% and 91% in FBs and M, respectively). Second, the fruiting body-specific expression of genes coding for previously identified defense proteins, including *C. cinerea* lectin 2 (CCL2), *Coprinopsis* galectin 2 (CGL2) and protease inhibitor of *Coprinopsis* 1 (PIC1), was confirmed. Third, several genes encoding cytoplasmic and secreted proteins with a predicted RicinB fold were shown to be differentially expressed. Currently, the transcriptomes of *C. cinerea* mycelium challenged with various fungivorous nematodes or subjected to various stress conditions, including oxidative stress, starvation, heat shock and mechanical damage, are sequenced. A preliminary analysis of this data will be presented.

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PR4.60

Differential responses of sorghum to inoculation with two varieties of *Sporisorium reilianum*

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Sporisorium reilianum is a biotrophic pathogen of maize and sorghum that causes head smut. This pathogen exists in two varieties (SRS and SRZ) that cause spore formation preferentially on sorghum (SRS), or only on maize (SRZ). To understand the different disease capacities, we investigated the infection process of the two varieties on sorghum. Microscopy of infection sites showed that both varieties are able to penetrate and ramify in leaves. Whereas SRS successfully spreads inside the plant from leaves to the floral meristems, hyphae of SRZ are only observed in leaf blades and leaf sheaths, but not in stems or meristems. To find out whether SRZ encounters more or stronger plant defense reactions, we investigated H_2O_2 production, callose deposition and phytoalexin generation. A strong H_2O_2 reaction could be detected at penetration sites of SRZ but not of SRS at one day after inoculation (dai). Callose was also found to accumulate in higher levels at 2 dai in plant cells that were colonized by SRZ than by SRS. Red pigmented phytolaexins were produced at 3 dai only when sorghum was inoculated with SRZ. Interestingly, in leaf regions containing phytoalexins, hyphae were short and displayed an unusual morphology, suggesting an important role of this defense mechanism against *S. reilianum*.

PR4.61

Characterization of two putative Salicylate hydroxylases from *Ustilago maydis*

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Plants have evolved a complex system to defend themselves against pathogens. One of the key regulators for the defense against pathogens is salicylic acid (SA). As a biotrophic pathogen the fungus *Ustilago maydis* must actively suppress the SA production of its host *Zea mays*. One effector involved in this is Cmu1, a secreted chorismate mutase. Cmu1 is translocated into the host cytosol where it interferes with the SA biosynthesis pathway of the plant.

This study focuses on the characterization of two putative *U. maydis* effectors, Um03408 and Um05230, which may also be involved in controlling SA levels. Both proteins are bioinformatically predicted to act as salicylate hydroxylases degrading salicylate to catechol. Real-time PCR analyses showed that *um03408* and *um05230* are strongly upregulated in the early phase of plant infection. By performing salicylate hydroxylase activity assays the SA hydroxylase activity of Um05230 could be verified. However, strains with a deletion of both genes showed no attenuation in virulence. As *um03408*, *um05230*, and *cmu1* could all contribute to virulence, the importance of these genes for the pathogenic development of *U. maydis* may become evident only when all three genes are deleted. Such a strain is under construction and first results on its phenotype will be presented.

PR4.62

A Novel Organ Specific Effector Involved in the *Ustilago maydis* – Maize Interaction

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Ustilago maydis is a biotrophic smut fungus which causes formation of plant tumors in maize. The infection is commonly observed on all the vegetative and floral organs of the host plant. Given the fundamental differences between the different maize organs that are colonized by *U. maydis*, we hypothesized that the fungus deploys organ specific effectors to manipulate physiology and development of specific host organs (1). In the present study, we identified a novel secreted protein, termed See1 (Seedling efficient effector 1) that is organ-specifically regulated and is strongly induced in leaves and weakly in tassels. *U. maydis* deletion mutants for *see1* are found to show a strong reduction of tumor formation in maize seedlings. Mutant hyphae successfully enter the leave tissue but are arrested during the proliferation stage. In contrast, the *Δsee1* mutant induces normal tumor formation in tassels. To localize See1 during the disease progression we applied confocal microscopy using mCherry-tagged See1 protein. At present, we are aiming for the functional characterization of See1 to elucidate the organ-specific function of this effector.

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PR4.63

Fungal-insect interactions with respect to secondary metabolism

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Fungi synthesize an astonishing variety of secondary metabolites, some of which belong to the most toxic compounds in the living world. Even though little is known about the benefit of these metabolites, the ability to regulate the secondary metabolism might be seen as an evolutionary adaptation. Presumably fungi regulate secondary metabolites (e.g. mycotoxine) in response to confrontation with natural competitors like insects to guarantee efficient exploitation of environmental resources (1-3). Admittedly it should be mentioned that secondary metabolites are not the only defence mechanisms of fungi (4).

In order to enlighten the biological function of these secondary metabolites with reference to chemical defence reactions of insect-fungal interactions, we utilized complementary approaches of experimental ecology and functional genomic techniques. A further aspect was to investigate the influence of these competitors at trophic interactions.

In our current research the vinegar fly *Drosophila melanogaster* and its natural antagonist *Aspergillus nidulans* are used as an ecology model system. To analyse fungal up- or down regulated target genes in the interaction of *A. nidulans* with *Drosophila* larvae microarray analysis was performed. Quantitative RT-PCR confirms up-regulation of the global regulator *laeA* as well as of *aflR*. Moreover several other genes are up-regulated under competing conditions. Candidate genes are being used for reporter gene analysis and RNAi constructs are being used for competition experiments.

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PR4.64

Chemical interaction in the mycorrhizosphere: impact of fungal hormones on *Tricholoma vaccinum* and its host

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The ectomycorrhiza between the widespread basidiomycete *Tricholoma vaccinum* and its tree host spruce (*Picea abies*) represents a model system for mutualistic plant-fungus interaction. Besides the mycorrhiza forming basidiomycetes saprophytic fungi inhabit the soil and produce morphogenic substances, which modulate plant root development. Zygomycetes belonging to the *Mucorales* communicate *via* a system based on apocarotinoids. The earliest intermediate after the cleavage of β-carotene is a C18-ketone, called D'orenone, which was shown to stop root hair formation in *Arabidopsis thaliana*. If D'orenone modulates the morphology and physiology of *Picea abies* is still unknown. However, *T. vaccinum* produces the phytohormone indole-3-acidic acid (IAA) which also leads to an extensive hyphal branching and an increased and faster Hartig' net formation during mycorrhization. In this work the effect of D'orenone on the root system will be tested in axenic cultures and in co-cultivation with *T. vaccinum* - wildtype and IAA overexpressing mutants - *via* differential display. Moreover, the receptors for D'orenone perception in *T. vaccinum* will be studied with radioactive labeled D'orenone or FISH. Additionally, co-cultures of Zygomycetes with *T. vaccinum* and with the mycorrhized tree will be done to study the interaction of all three partners. This could improve the knowledge about communication between unrelated fungi which live closely together in the mycorrhizosphere and give hints to interacting "mycohormones", affecting tree morphology, mycorrhization rate, and, thereby, influence tree health and whole ecosystem fitness.

PR4.65

Investigation of unconventionally secreted proteins in *Ustilago maydis*

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Secreted fungal proteins play a crucial role during the biotrophic interaction between the smut fungus *Ustilago maydis* and its host plant *Zea mays*. In the last decade it has been well established that proteins without a signal peptide can also be targeted to the outside of the cell in an ER/ Golgi independent manner. We want to identify such unconventionally secreted proteins in *U. maydis* and investigate their potential function as pathogenicity factors. Our approach is based on affinity purification of tagged candidate proteins, previously detected in the apoplastic fluid of infected maize leaves. Four of the twelve candidate proteins tested so far could be detected in culture supernatants. One candidate protein, Um02959, is a homolog to the *Dictyostelium discoideum* acyl-CoA-binding protein AcbA. In *D. discoideum* AcbA is unconventionally secreted as full-length protein and extracellularly processed into a small peptide (SDF-2) that triggers terminal spore differentiation upon interaction with a membrane receptor. We were able to locate the *U. maydis* protein Acb1 in the culture supernatant of fungal hyphae only in the presence of a protease inhibitor, suggesting extracellular processing. Trypsin digested purified UmAcb1 protein as well as hyphal culture supernatant triggered spore formation in *D. discoideum* indicating the presence of an SDF-2-like peptide in *U. maydis*. Interestingly deletion of the *acb1* gene in *U. maydis* resulted in a late virulence defect. We are currently attempting to rescue this phenotype by extracellular Um-SDF-2 peptide.

PR4.66

The Putative E3 Ubiquitin Ligase Ubl1 is a Central Regulator of Growth, Morphogenesis, and Virulence in *Fusarium* spp.

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Fusarium verticillioides is a ubiquitous and destructive pathogen of maize. In addition to reducing yields, *F. verticillioides* produces fumonisins, a group of polyketide-derived toxins linked to cancer and birth defects in humans, as well as acute and chronic toxicoses in livestock. Recently, we identified *UBL1* in *Fusarium verticillioides* via a forward genetic screen aimed at identifying novel genes involved in pathogenesis. *UBL1* is predicted to encode a UBR1-like E3 ubiquitin ligase. Among eukaryotes, the UBR1-like E3 ubiquitin ligase is a broadly conserved component of the N-end rule proteolytic pathway; however, its role in filamentous fungi has not been extensively investigated. Targeted disruption of *UBL1* in the wild-type strain resulted in a highly pleiotropic phenotype, including reduced conidiation, altered hyphal morphology, increased pigment production, and impaired amylolitic activity. Additionally, disruption of *UBL1* led to a drastic reduction in virulence on maize kernels; however, fumonisin B1 biosynthesis per unit growth was not significantly different from the wild-type strain. Yeast two-hybrid assays revealed that Ubl1 interacts with components of G-protein signaling known to regulate pathogenesis. To investigate the possibility that *UBL1* plays a conserved role in fungal virulence, we deleted *UBL1* orthologs in the closely related pathogens *Fusarium graminearum* and *Fusarium oxysporum*. Interestingly, these mutants revealed that *UBL1* orthologs regulate diverse components of pathogenesis among *Fusarium* spp. This study directly implicates *UBL1* in growth and development in *Fusarium* spp. and provides one of the first links between a UBR1-like E3 ubiquitin ligase and virulence in plant pathogenic fungi.

PR4.67

Fungal ecology in a petri dish – establishment of a laboratory model system for assessing the role of fruiting body lectins in the fungal defense against predators

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Lectins are proteins that specifically and reversibly bind carbohydrates. Among their diverse physiological roles, one universally described function is their participation as recognition and effector molecules in the innate defense of multicellular organisms against all kinds of predators.

In the fungal kingdom, a large number of lectins with different specificities have been isolated from fruiting and resting (sclerotia) bodies of multicellular representatives of the phyla Basidiomycota and Ascomycota. Various lines of evidence suggest that these lectins play a role as effector molecules in the defense against predators. Upon ingestion of the host cytoplasm, the fungal lectins bind to specific glycoconjugates in the digestive tract of the predators which leads to inhibition of development and eventually killing of the predator by a yet unknown mechanism. Toxicity of fruiting body lectins was so far assayed using model organisms e.g. *Caenorhabditis elegans* for nematodes.

To demonstrate the ecological significance of the observed toxicities, we established a laboratory model system for assaying the toxicity of these lectins towards fungivorous nematodes such as *Aphelenchus avenae*. In this system, we use the filamentous fungus, *Ashbya gossypii*, as a host. This fungus can easily be manipulated to express single or multiple exogenous lectins in the vegetative mycelium. Using this system, we could demonstrate a severe reduction in population growth of *Aphelenchus avenae* as a result of the expression of a single fruiting body lectin in the mycelium of the host fungus. Our results show that fungivores are susceptible to at least some of the fruiting body lectins.

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PR4.68

Characterization of function and kinetics of Dicer proteins in the wheat pathogen *Mycosphaerella graminicola* during host infection

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We investigate the role of RNA interference (RNAi) in the wheat pathogen *Mycosphaerella graminicola* during host infection by deletion of Dicer and Drosha proteins. A main goal is to determine if RNAi plays a role in pathogenicity and host specificity. For the transformation of *M. graminicola* we use an *Agrobacterium tumefaciens* mediated approach and we evaluate and compare wild type and mutant phenotypes in plant assays with the two hosts *Triticum aestivum* and *Brachypodium distachyon*. The transformation experiments are currently undergoing.
We have verified the computational predictions of microRNAs in the genome of *M. graminicola* by demonstrating the presence of small (26nt) RNAs using polyacrylamide gel electrophoresis. Small RNAs are expressed in rich medium as well as in plant tissue suggesting that RNAi is not restricted to the host-pathogen interaction but also play a role in the basic growth of the fungus. However, by describing the kinetics of the Dicer and Drosha encoding genes during three different time points of infection we show significant higher levels of transcript abundance during plant infection. Furthermore, we demonstrate that transcript abundance is dependent on host species indicating that RNAi in *M. graminicola* may affect both pathogenicity and host specificity.

PR4.69

Carbon Acquisition In The Ustilago/Maize Pathosystem

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Ustilago maydis is a fungal plant pathogen that infects maize plants. Investigating mechanisms for fungal carbon acquisition that are critical for the biotrophic interaction of *U. maydis* with its host, we identified Hxt1 (hexose transporter 1), a high affinity monosaccharide transporter. *Ustilago maydis Δhxt*-strains cause decreased disease symptoms, and growth in axenic culture is reduced on glucose, fructose and mannose. Surprisingly, these strains show increased growth on media containing xylose and galactose, the latter causing even a growth-inhibiting effects on wildtype strains.

In the *S. cerevisiae* hexose sensor proteins Snf3 and Rgt2, mutation of a conserved arginine residue results in a constitutively active signaling pathway. Interestingly, over-expression of a Hxt1-derivative carrying an analogous mutation decreased the virulence of *Δhxt1* strains even more.

In accordance with the significance of Hxt1 for pathogenic development we found expression of two maize glucose transporters up-regulated after infection with *Ustilago maydis*. These transporters belong to a recently identified family of sugar efflux carriers whose members in some cases can be induced by bacterial as well as fungal pathogens.

We propose that Hxt1 is required for uptake of glucose that is provided to the site of infection by plant efflux carriers, which are specifically induced after infection with *Ustilago maydis*. Further, we speculate on an additional sensor function of Hxt1 that could be most important to sense galactose and xylose levels within the plant that may be indicative for the physiological status of the host cells.

PR4.70

A surface hydrophobin in ectomycorrhiza interaction

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Hydrophobins are small-secreted proteins with low sequence homology. However, all proteins contain eight cysteines, which form disulfide bridges. They are divided into two classes, depending on their solubility and have a broad range of functions such as involvement in growth and development of filamentous fungi, *e.g.* formation of aerial structures. Mutual symbiosis like ectomycorrhiza is based on differential gene expression. Which was shown for hydrophobin *tthyd1*, that is up-regulated in the Hartig'net in the interaction of *Tricholoma terreum* with pine.

We investigate hydrophobins in *Tricholoma vaccinum*, a wide-spread basidiomycete (agaricales – tricholomataceae) which forms ectomycorrhiza with spruce. We want to show in our study in which stage of the life cycle respectively symbiotic interaction hydrophobins are produced, what kind of role they play with respect to function in the symbiotic tissue and whether they are regulated under heavy metal stress. Furthermore, the regulation of the four known hydrophobins (*tvhyd1*, *1b*, *2* and *3*) of *T. vaccinum* will be analyzed by RNAi experiments and heterologous expression in *Schizophyllum commune*.

PR4.71

Expression Profiling of *Solanum lycopersicoides* to Identify Mechanisms Underlying Resistance to *Botrytis cinerea*

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The necrotrophic fungal pathogen, *Botrytis cinerea*, causes grey mold on a wide range of hosts, and is of major economic concern for tomato production. Tomato (*Solanum lycopersicum*; *Sl*) is highly susceptible to *B. cinerea*; however, *Solanum lycopersicoides* (*Slo*), a wild relative of tomato, is highly resistant. The overarching goal of this study is to characterize the molecular mechanisms underlying resistance to *B. cinerea* in *Slo* and identify novel target genes for improving resistance in tomato. To this end, we generated gene expression profiles from *Slo* 24 and 48 hours after inoculation with *B. cinerea*, as well as a pre-infection baseline, via high-throughput RNA-sequencing (Roche-454). Analyses of the transcriptomes revealed that numerous genes were differentially expressed in *Slo* in response to *B. cinerea*, including pathogenesis related proteins, peroxidases, osmotins, and genes involved in biosynthesis of secondary metabolites. These differentially regulated genes provide novel targets for expression analyses in tomato. Genes that are regulated differently between *Sl* and *Slo* in response to infection by *B. cinerea* are currently being characterized to determine their role in resistance. By identifying genes that confer resistance in Slo, this work will substantially refined the current understanding of defense against necrotrophic pathogens and provided targets for improving *B. cinerea* resistance in tomato.

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PR4.72

Characterization Of Three Putative Transcription Factor-Coding Genes For Pathogenesis In The Plant-Pathogenic Fungus *Alternaria brassicicola*

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The necrotrophic fungus *Alternaria brassicicola* causes black spot disease of brassicaceous plants, including green cabbage (*Brassica oleracea*) and the oil-producing *B. napus*. Several transcription factors (TFs) have been associated with pathogenesis in phytopathogenic fungi. We know relatively little, however, about the mechanisms of regulating pathogenesis. To learn more about how pathogenesis is regulated in fungi, we have produced targeted gene knockout mutants for ~200 TF-coding genes and of research interests. We identified two genes whose mutants were putatively nonpathogenic, eleven genes whose mutants produced significantly smaller lesions compared to the wild type in preliminary pathogenicity tests, and one gene whose mutants unexpectedly produced lesions twice as large as the wild type. To clarify the functions of these genes, we studied the phenotypes of mutants for 3 of the 14 genes. The phenotypes of mutants for one gene, *Δabvf01*, were indistinguishable from the wild type in general, but mycelial growth after penetration was greatly reduced. Mutants of another gene, *Δabvf19*, grew slower with pectin as a major carbon source *in vitro* and expressed fewer transcripts than the wild type for a subset of genes that encode putative cell wall-degrading enzymes during pathogenesis. Conversely, mutants of the gene with increased virulence expressed more transcripts for these enzymes. Our study supports the importance of the coordinated regulation of genes putatively associated with using nutrients from their host plants during pathogenesis. A better understanding of how fungi regulate pathogenesis may identify specific transcription factors as targets for efficient disease management.

PR4.73

The role of secreted LysM domain proteins during the biotrophic development of *Ustilago maydis*

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Previous studies revealed that *Ustilago maydis* depends on a variety of novel secreted effector proteins to establish a compatible, biotrophic interaction with its host plant maize. In this context, two putatively secreted LysM-containing proteins (*um11464*, *um05087*) have been identified. LysM-containing proteins are very common in pathogenic fungi and have been shown to contribute to virulence. Using homologous recombination in the solopathogenic strain SG200 we have generated deletion mutants of *um11464* and *um05087*. While the deletion of *um05087* did not show any phenotype in comparison to the progenitor strain SG200, *um11464* deletion mutants often formed cell chains, developed lateral buds and displayed bipolar growth in axenic culture. Surprisingly we observed that plant infections with *um11464* deletion mutants lead to hypervirulence. Since the mutant is able to form significantly more appressoria on the leaf surface we consider that hypervirulence is a result of the increased number of appressoria-forming filaments. *In situ* immunolocalization studies revealed that Um11464 is located predominantly at the cell surface. Therefore we assume that the protein accumulates at the fungal cell wall after secretion. Preliminary results indicate that a domain within the C-terminus of the protein might be responsible for the attachment to the fungal cell wall. Interestingly, the LysM domains which are supposed to bind carbohydrates are not involved in the binding. Currently, functional analyses are performed to investigate the biological role of Um11464 and how it affects pathogenesis negatively.

PR4.74

Functional analysis of a novel pathogenicity-associated gene *CoPRF1* in the anthracnose fungus *Colletotrichum orbiculare*

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Plant pathogens have co-evolved with their host plants which have evolved the defense system against their pathogens. It is known that plants express the basal immunity by the recognition of the pathogen-associated molecular patterns, but compatible pathogens suppress the plant basal defence by secreting the effector protein. *Agrobacterium tumefaciens* - mediated transformation (AtMT) was used to generate pathogenicity deficient insertional mutants in *Colletotrichum orbiculare* that causes anthracnose disease of cucumber, and a mutant named YK4524 was isolated which showed reduced pathogenicity. Genetic analysis of this mutant indicated that the insert was placed in a gene which presumably codes for an extracellular protein with signal peptide sequence, and significant homologous genes could not be recognized. So we named this gene *CoPRF1* (Pathogenesis-related factor 1). In this study, we performed functional analysis of *CoPRF1* to identify and characterize the novel pathogenicity-associated gene. Target gene disruption mutants obtained by AtMT showed significant reduction in virulence on the host leaves, while characteristics such as germination, appressorium formation and penetration hyphae formation of *coprf1* disruption mutants *in vitro* were normal, indicating that *CoPRF1* is not involved in the infection related morphogenesis. On the other hand, penetration ability of mutants was attenuated on intact cucumber cotyledons, and the elongation of its invasive hyphae was slower compared with the wild type. Thus, it was suggested that *CoPRF1* would engage in establishment of host infection.

PR4.75

Functional analysis of the tumor and anthocyanin-inducing effector protein Tin2 of Ustilago maydis

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The fungus Ustilago maydis is the causal agent of smut disease in maize. The interaction with the host is governed by secreted effectors and many of the respective genes reside in clusters in the genome. Cluster19A is the largest of these clusters carrying 24 genes for putatively secreted effector proteins. Deletion mutants of the left half of cluster 19A (19A_1) show dramatic reduction of tumor formation and loss of anthocyanin induction, although the mutant retains the ability to grow inside the plant tissue. We demonstrate that the tin2 effector encoded in this region is secreted and expressed exclusively during biotrophic growth. Introduction of the tin2 gene into the 19A_1 mutant partially rescued tumor formation and fully restored anthocyanin induction. A Tin2 protein lacking the C-terminal 5 amino acids had lost this ability. In line with this, Tin2 mutant protein could not interact with cytoplasmic maize protein kinase ZmABP which was identified by yeast two hybrid screening as Tin2 interactor. Transient expression assays in Nicotiana benthamiana revealed that ZmABP was degraded proteasome-dependently. Interestingly, co-expression with Tin2 stabilized ZmABP. Tin2-binding region of ZmABP contains the phosphodegron-like motif DSGxS. When ZmABP carrying mutations in this motif was transiently expressed, the mutant protein proved more stable than the wild type protein. Therefore, it is likely that Tin2 effector masks the phosphodegron motif of ZmABP, which stabilizes functional full-length ZmABP kinase in plant cell, resulting in signal transduction leading to anthocyanin biosynthesys and tumor induction.

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PR4.76

Molecular analysis of the regulation of the *stp1* effector gene in *Ustilago maydis*

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The biotrophic maize pathogen *Ustilago maydis* relies on the secretion of effectors for successful colonization of plant tissues. *stp1* encodes an effector which is absolutely required for fungal development *in planta*. The expression of *stp1* starts during plant penetration and continues throughout all stages of biotrophic development.
The aim of this project is to identify the regulators responsible for the plant specific expression pattern of *stp1*. We first performed a promoter deletion analysis: *stp1* promoter sequences harboring various deletions were fused to the reporter *eGFP* and inserted into the *cbx* locus of strain SG200. The comparison of GFP expression levels in the resulting strains led to the identification of a short sequence potentially involved in *stp1* up-regulation *in planta*. This sequence contains two conserved elements, A1 and B, which are both required for full expression. A1 is related to the binding sites of two previously described transcription factors (TF), Mzr1 and Biz1, while B is unknown. When overexpressed, Mzr1 and Biz1 activate *stp1* expression (Zhen *et al.*, 2008; Flor-Parra *et al.,* 2006). Using a one-hybrid approach, we could show that both TFs bind to A1 but not B. As B is also required for *stp1* expression, this indicates that additional TFs are involved in the regulation of *stp1*.

To confirm these results and identify new TFs binding to the *stp1* promoter, we now plan to use magnetic beads coated with the target DNA to isolate TFs of interest from a nuclear extract of infected maize leaves. Initial results will be presented.

PR4.77

Biosynthesis of indole-3-acetic acid in basidiomycetes

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Mycorrhiza is a fundamental phenomenon in the interaction of fungi and plants. Most important for formation and functionality of this symbiosis is a vital communication of both symbionts. A key player in this process is indole-3-acetic acid (IAA), a well known growth hormone of plants that is also able to coordinate growth promotion and branching of hyphae in some fungi. Furthermore IAA can be synthezised in both interaction partners by several potential pathways. In preliminary work a tryptophane (Tryp) dependent pathway was postulated for *Tricholoma vaccinum*.

Since there are several potential ways to synthesize IAA in a Tryp dependent matter we want to get further insights into the cellular mechanism. Therefore we want to perform growth experiments with several plant interacting fungi (*Tricholoma vaccinum*, *Paxillus involutus, Armillaria mellea*), and saprophytes (*Schizophyllum commune*, *Heterobasidium annosum, Leucoagaricus leucothites, Lyophyllum loricatum*) in dependence of different potential intermediates of IAA-production (Tryp, indole-3-pryuvate [IPA], indole-3-acetaldehyde [IAAld]) as well as IAA itself. In case of *S. commune* a mono- and dikaryon and a IAA-synthesizing mutant are tested. To this aim length and branching degrees of hyphae will be analyzed.

In addition the expression of acetaldehyde-dehydrogenase will be tested by qPCR. This enzyme catalyzes the conversion of aldehydes to acids and in our case the of IAAld to IAA.

PR4.78

Functional analysis of MiSSPs (Mycorrhiza induced Small Secreted Proteins) from the mutualistic fungal symbion*t Laccaria bicolor.*

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Boreal and temperate forest ecosystems depend on ectomycorrhizal symbiosis for trees nutrition, productivity and stress resistance. Ectomycorrhizal (ECM) symbioses appear several times during fungi evolution and ECM fungi likely derived from saprotrophic ancestors. Despite their ecological importance, very little is concerning the molecular dialogue that occurs between tree roots and ECM fungi to sustain the development of symbiosis. Recently, *L. bicolor* Mycorrhizal induced Small Secreted Protein7 (MiSSP7) has been proved to be the first symbiotic effector required for the development of symbiosis due to its targeting to the plant nucleus (Plett et al., 2011). Transcriptomic analyses reveal the presence of several MiSSPs within the genome of the symbiotic fungus *L. bicolor* (Martin et al., 2008). We have performed functional analysis of several MiSSPs in order to (i) demonstrate that MiSSPs are required for symbiosis development and (ii) to identify which plant compartment / proteins are targeted by MiSSPs. We will present and discuss our last results with regards to the putative role of MiSSPs as fungal effectors.

Plett et al. 2011, Current Biology, 21(14):1197-203 ; Martin et al., 2008 Nature 452, 88-92

PR4.79

Analysis Of The *Fusarium graminearum* Species Complex From Grain Crops Provides Evidence Of Species-Specific Differences In Host Preference

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Species identity and trichothecene toxin potential of 560 members of the *Fusarium graminearum* species complex (FGSC) collected from diseased wheat, barley and maize in South Africa was determined using a microsphere-based multilocus genotyping assay. Although three trichothecene types (3-ADON, 15-ADON and NIV) were represented among these isolates, strains with the 15-ADON type predominated on all three hosts. A significant difference, however, was identified in the composition of FGSC pathogens associated with Gibberella ear rot (GER) of maize as compared to Fusarium head blight (FHB) of wheat or barley ($P < 0.001$). *Fusarium graminearum* accounted for more than 85% of the FGSC isolates associated with FHB of wheat and barley ($N = 425$), and was also the dominant species among isolates from maize roots ($N = 35$). However, with the exception of a single isolate identified as an interspecific hybrid between *F. boothii* and *F. graminearum*, GER of maize ($N = 100$) was exclusively associated with *F. boothii*. The predominance of *F. graminearum* among FHB isolates, and the near exclusivity of *F. boothiii* among GER isolates, was observed across all cultivars, collection dates, and provinces sampled. Because these results suggest a difference in host preference among species of the FGSC, we hypothesize that *F. graminearum* may be less well adapted to infect maize ears than other members of the FGSC.

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PR4.80

Symptom formation in *Sporisorium reilianum* is modulated by effector proteins

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Sporisorium reilianum and *Ustilago maydis* are closely related biotrophic pathogens that cause different symptoms on maize. Following fungal inoculation of seedlings, *U. maydis* induces tumors on leaves near the infection site within a few days, whereas symptoms of *S. reilianum* are visible only at flowering time, and include the formation of spores and leaf-like structures in inflorescences. To identify genes responsible for species-specific symptom generation, we compared the highly syntenic genomes of both fungi and discovered the presence of genomic regions of weakly conserved genes mainly encoding secreted proteins [1]. Deletion of the largest divergence region of about 30 genes in *S. reilianum* dramatically reduced virulence, and led to wilting of inoculated leaves. By subdeletion analysis we identified a region encoding three related secreted effector proteins as responsible for the early leaf wilting phenotype. Only two of the three genes are up-regulated during biotrophic growth of *S. reilianum* as determined by qRT-PCR analysis. Individual gene deletion indicated that both up-regulated genes contribute equally to symptom formation in *S. reilianum*. This shows that symptom formation of *S. reilianum* is modulated by fungal effector proteins.

[1] Schirawski et al., 2010. Science 330: 1546-1548.

PR4.81

High-resolution crystal structure of the LysM effector Ecp6 of the fungal tomato pathogen *Cladosporium fulvum*

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Plants induce defence responses upon recognition of chitin, the primary structural component of fungal cell walls. To prevent the induction of host defense responses, the plant pathogenic fungus *Cladosporium fulvum* secretes large amounts of ECP6 protein which binds chitin with high affinity and thus prevents their recognition by plant receptors. ECP6 is a Lysin motif (LysM)-containing effector protein with orthologs, known as LysM effectors, that are widely distributed in the fungal kingdom. LysM effectors of the Septoria tritici blotch pathogen *Mycosphaerella graminicola* and the rice blast pathogen *Magnaporthe oryzae* scavenge chitin molecules in a similar fashion as *C. fulvum* ECP6, demonstrating the importance of LysM effectors in fungal pathogenicity. LysM domains are highly conserved in many proteins produced by prokaryotes and eukaryotes which bind to peptidoglycan and chitin. However, the specific interactions of LysM domains with their substrates have not yet been elucidated. Here, we present a high-resolution crystal structure of the LysM effector ECP6. The structure revealed that each of the three LysM domains from ECP6 adopts an αββα tertiary structure, in which the chitin-recognition site is localized in the highly conserved region in the loop between the first β-sheet and the first α-helix. The close interaction that occurs between the first and the third LysM domain of an Ecp6 monomer forms a high-affinity binding site for single chitin molecule.

PR4.82

LysM effectors of fungal plant pathogens contribute to virulence in various manners

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LysM effector genes are found in the genomes of a wide range of fungal species. LysM effectors are secreted proteins that contain a varying number of LysM domains and no other recognizable protein domains. LysM domains are carbohydrate-binding modules that occur in various proteins that are produced by a variety of organisms. Ecp6 is the first characterized LysM effector that was isolated from the tomato leaf mould fungus *Cladosporium fulvum* and that is instrumental for fungal virulence. Carbohydrate binding assays demonstrated that Ecp6 specifically binds chitin, the major constituent of fungal cell walls that acts as microbial-associated molecular pattern (MAMP) that triggers immune responses upon recognition by the host. We demonstrated that the chitin-binding effector Ecp6 can compete with plant receptors for chitin binding, and thus prevents the activation of immune responses. Two orthologues of Ecp6 were identified in the fungal wheat pathogen *Mycosphaerella graminicola*, of which one suppresses chitin-induced immune responses in a similar fashion as Ecp6. Interestingly, unlike Ecp6, both *M. graminicola* LysM effectors were able to inhibit degradation of fungal hyphae by plant chitinases. Many fungal genomes carry multiple LysM effector genes that share only low sequence conservation and encode varying LysM domain numbers per molecule. Therefore, we hypothesize that different fungal LysM effectors are likely to bind different carbohydrate substrates, exert other functions, or are active in other stages of the fungal life cycle than plant infection. We will report on our most recent findings on LysM effector substrates and functions.

R4.83

The emergence of *Botryosphaeria* sp. as a wheat necrotrophic pathogen in Australia

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Necrotrophic pathogens of wheat have a considerable impact on yield globally. The primary pathogens in this class include *Pyrenophora tritici-repentis*, *Stagonospora nodorum* and *Mycosphaerella graminicola*. Over the last decade, increasing levels of *Botryosphaeria* sp. have been identified on mature wheat grain in Australia. The infection of the grain by *Botryosphaeria* sp. results in slightly shrivelled grain with a pale colour closely resembling that of *Fusarium graminearum* – infected wheat. As a consequence of the infection, *Botryosphaeria* sp-infected grain is downgraded for animal feed only, resulting in considerable economic losses. Almost nothing is known about the pathogen or resulting disease and it is unclear though if the infected grain is actually harmful to human health. A project has recently started in the Solomon laboratory to look at the mycotoxin producing potential of *Botryosphaeria* sp. To do this, commercially grown infected wheat grain will be analysed using a variety of mass spectrometry techniques to determine the presence of known mycotoxins. In this poster, preliminary data will be presented on the growth and phenotyping of the *Botryosphaeria* sp. strains identified thus far as well some of the initial mass spectrometry analysis.

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PR4.84

Fungal phenotypic plasticity in response to fungivores

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Secondary metabolites are natural products, principally synthesized by plants, bacteria and fungi among other organisms. Secondary metabolites are not essential for organism survival but may have other functions including adaptation to different environments. Although many beneficial as well as toxic secondary metabolites have been investigated intensively, their role in enabling fungi to explore and conquer new ecological niches remains elusive. A common threat fungi are exposed to in their natural habitat is the attack by fungivorous insects. In this context, we hypothesize that the model fungus *Aspergillus nidulans* produces secondary metabolites, e.g. the mycotoxins sterigmatocystin, as part of an adaptive response to ward off or harm fungivorous insects. We confront fungi with insects to investigate induction of changes in fungal development, secondary metabolite formation, and gene expression. By means of qRT PCR, we currently focus on changes in the expression of laeA (encodes a methyltransferase-domain protein that functions as a regulator of secondary metabolism and development) and aflR (encodes a Zn2Cys6-type sequence-specific DNA-binding protein that is thought to be necessary for expression of most of the genes in the sterigmatocystin gene cluster). On all levels of organization we observe significant alterations in fungal traits that finally modify the outcome of insect-fungus interactions.

PR4.85

Plant resistance inducer β-aminobutyric acid inhibits spore germination and growth of the ascomycete *Leptosphaeria maculans* by interfering with nitrogen metabolism

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β-aminobutyric acid (BABA) is for decades known as an agent protecting plants from infection by broad range of pathogens. It is widely accepted that BABA has no antifungal activity and its effect is based solely on stimulation of plant immune system. Here we demonstrate that BABA displays strong *in vitro* antifungal activity against *L. maculans* with EC_{50} similar to the fungicide tebuconazole. Both spore germination and hyphal growth are affected. Unlike other resistance inducer benzothiadiazol, BABA reduced disease development on *Brassica napus* plants also when applied after inoculation. Suppression of disease progression in plants and antifungal activity *in vitro* was weaker for α-aminobutyric acid and negligible for γ-aminobutyric acid. These facts indicate that the mechanism by which BABA protects *B. napus* plants from *L. maculans* infection is based on antifungal activity. In contrast to standard antifungal assays, the medium used in our study contained no organic nitrogen. Trypton added into the medium at only 6 ppm completely reverted the effect of 2 μM BABA. Similar effect can be also achieved by addition of some proteinogenic amino acids. We hypothesised that BABA might inhibit inorganic nitrogen assimilation or might interfere with amino acid metabolism. While we have not conclusively demonstrated how BABA suppresses the disease progression, our results do indicate that antifungal activity is another mechanism by which BABA can protect plants from infection.

PR4.86

Sequential Delivery of Host-Induced Virulence Effectors by Appressoria and Intracellular Hyphae of the Phytopathogen *Colletotrichum higginsianum*

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Phytopathogens secrete effector proteins to manipulate their hosts for effective colonization. Hemibiotrophic fungi must maintain host viability during initial biotrophic growth and elicit host death for subsequent necrotrophic growth. To identify effectors mediating these opposing processes, we deeply sequenced the transcriptome of *Colletotrichum higginsianum* infecting *Arabidopsis*. We found that expression of many effector genes is plant-induced and that distinct sets of effectors are deployed in successive waves by particular fungal cell-types. Using fluorescent protein tagging and TEM-immunogold labelling, effectors were localized to stage-specific compartments at the host-pathogen interface. Early-expressed effectors are focally secreted from appressorial penetration pores before host invasion. These proteins may function to suppress early plant defense responses, which we found to be activated before fungal entry. Later-expressed effectors accumulate in structures formed at the interface between biotrophic primary hyphae and living host cells, implicating these specialized hyphae in effector delivery. By transient expression in *Nicotiana benthamiana* leaves, we identified effectors either inducing or suppressing plant cell death. Our findings reveal new functions for appressoria and biotrophic hyphae and suggest that hemibiotrophy in *Colletotrichum* is orchestrated through the coordinated expression of antagonistic effectors supporting either cell viability or cell death.

PR4.87

Manipulation of trehalose biosynthesis in *Laccaria bicolor*

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The disaccharide trehalose is a key regulator of glycolysis in higher plants, animals, and certain fungi. Based on gene expression and metabolite analysis, trehalose is also supposed to be a storage carbohydrate in ectomycorrhizal basidiomycotic fungi. Under symbiotic conditions, a crucial function of trehalose biosynthesis is carbohydrate sink formation, which is thought to enable a continuous fungal sugar support by a plant host. In this work manipulation of trehalose biosynthesis was initiated and a first glance of the impact of reduced trehalose formation on ectomycorrhizal fungal physiology will be given.

Successful gene knock out by homologous recombination has not been archived for higher basidiomycetes yet. Therefore, an RNAi strategy was followed to suppress genes involved in trehalose formation and break down using the ectomycorrhizal model fungus *Laccaria bicolor*. *Laccaria* transformation was performed using an *Agrobacterium*-based strategy. Monocaryotic mycelia were used to increase RNAi efficiency and the manipulation was performed with two different strains to compare the impact of the genetic background on fungal physiology. Suppression of trehalose-6-phosphate synthase gene expression was observed for all investigated transformants. Surprisingly, both transformed *Laccaria* strains differed in their growth behaviour. While the growth speed was increased by 10 % in one strain, it was inhibited by 10-45 % in the other. As trehalose is furthermore well known as stress protectant, temperature stress is also currently under investigation. These results together with metabolite content will be presented.

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PR4.88

Expression studies of Candidate Effector Genes in different *Magnaporthe* species

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The causal agent of rice blast disease, *Magnaporthe oryzae,* is the economically most important threat in rice cultivation. While isolates of this species establish a host interaction with wheat or barley, isolates of *Magnaporthe grisea* derived from *Digitaria sanguinalis* establish a nonhost interaction with both crop plants.

Interestingly co-inoculation of barley plants with isolates of both species enabled the nonhost isolate to partly overcome nonhost resistance in epidermal cells simultaneously attacked by the adapted host isolate. Thereby providing evidence that host`s defence is actively manipulated by the adapted isolate.

Aiming at identifying pathogen-derived molecules orchestrating this scenario we performed transcription profiling in barley epidermal peels after inoculation with adapted and non-adapted isolates (24 h p.i.). Time course studies of candidate gene expression revealed that *HEGs* (hypothetical effector genes) can be grouped according to their maximal transcript abundance. A first group of *HEGs* was up-regulated during the early infection process when the fungus had not yet entered a host cell. Expression of those early *HEGs* could also be detected in fungal infection structures formed *in vitro*. A second group exhibited expression maxima during the biotrophic infection phase. At later stages, possibly correlated with the switch of the pathogen to necrotrophy, *HEG* transcript abundance was down-regulated. Currently functional analyses and localization studies of *HEGs* are in progress. Additionally we would like to investigate expression and function of *HEG* homologs in the interaction of different *Magnaporthe* species, e.g. *M. grisea*, with their cognate hosts.

Poster Category 5: Mitochondria

PR5.1

Dancing With The Right Partner: Interaction Of Porin Pore Associated Factors With Inner Mitochondrial Membrane Carriers Selectively Facilitates Metabolite Transport

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The diverse functions and metabolic processes in mitochondria require numerous transport processes through and between the two mitochondrial (mt) membranes. It is widely assumed that the metabolite and ion transport through the mt outer membrane (MOM) is mediated by the porin pore, whereas in the mt inner membrane (MIM) specific carriers are responsible for the transport processes. However, the coordination between the two membrane transport processes is largely unknown. Here we provide experimental evidence that in the yeast *Saccharomyces cerevisiae* the two MOM proteins Om14p and Om45p associate with subpopulations of independently assembled porin pores. These complexes interact with carriers of the MIM, as shown by preparative TAP followed by MS identification. We propose that interaction of the two MOM proteins with the porin pore allows binding to specific channels in the MIM, thereby facilitating directed transport of metabolites.

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Poster Category 6: ROS, Autophagy and Apoptosis

PR6.1

The role of the NADPH oxidase complex in the biotrophic interaction of *Claviceps purpurea* and *Secale cereale*

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Claviceps purpurea is an obligate biotrophic pathogen on diverse monocots. We are interested in the impact of the Nox complex on the interaction of the fungus and its host *Secale cereale*.

C. purpurea encodes two homologues of the mammalian gp91^{phox}, *cpnox1* and *cpnox2*. Cpnox1 is a virulence factor in *C. purpurea*: the knockout mutant shows drastically reduced infection rates compared to the wild type. Formation of honeydew, the first macroscopic sign of infection, is strongly retarded and mature sclerotia, typical fungal resting structures, have never been observed[1]. In contrast, the knockout mutant Δcpnox2 is not affected in early colonization stages as it shows significantly enhanced and prolonged production of honeydew compared to the wild type, while sclerotia are even less developed than in the Cpnox1 deletion strain. These data indicate that both NADPH oxidase catalytic subunits have impact on the biotrophic interaction of *C. purpurea* and rye. Cpnox1 plays a major role in early colonization of plant tissue while Cpnox2 is involved in the metabolic switch leading to development of sclerotia.

Recently, we were able to obtain a deletion mutant of the regulatory subunit CpnoxR. In pathogenicity assays on rye it shows strong production of honeydew, but sclerotia are very small and not fully mature, comparable to the Cpnox1 deletion mutant.

We are also interested in the composition and recruitment of the Nox complexes. Yeast two-hybrid experiments already showed that CpnoxR interacts with the small GTPase Rac, suggesting that Rac is involved in regulation of the complexes.

PR6.2

New aspects of the regulatory functions of the MAP kinase BcSak1 of *Botrytis cinerea* during stress and pathogenesis

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The mitogen-activated protein kinase (MAPK) BcSak1 of *Botrytis cinerea* is activated upon exposure to H_2O_2 and hence might be involved in coping with oxidative stress during infection. However, beside osmotic and oxidative stress sensitivity Δbcsak1 mutants have a pleiotropic phenotype as they do not produce conidia and are unable to penetrate unwounded host tissue.

In this study the role of BcSak1 was investigated in the stress response and during infection of French beans by *Botrytis cinerea*. Using a macroarray approach it was shown that BcSak1 is only marginally involved in the specific oxidative stress response. In fact, the induction of several genes after oxidative stress treatment is BcSak1-dependent, but most of these genes are also induced under conditions of osmotic stress. The majority of genes regulated by BcSak1 are not involved in the stress response at all. Using a translational fusion of BcSak1 to GFP, it was shown clearly that the localization of this MAPK depends on the type of stress being applied: it associates rapidly to the nucleus only under osmotic stress. Interestingly, the MAPK is also involved in the regulation of secondary metabolism, as the major phytotoxins secreted by this fungus are reduced in the Δbcsak1 deletion mutant. Experiments done *in planta* underlined the essential role of BcSak1 in the early stages of infection when it translocates to the nucleus and then changes to cytosolic distribution during hyphal growth within the tissue.

PR6.3

Identical ROS production of sterile nox and pro mutants in *Sordaria macrospora*.

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The ascomycete *Sordaria macrospora* is an excellent model organism to study the complex cell differentiation process in sexual development [1]. In this process, simple structured, vegetative hyphae change their direction of growth and begin to form three dimensional, sexual structures. The regulation of this differentiation is dependent on multiple signaling factors like pheromones, nutrient sufficiency and reactive oxygen species (ROS). To date only few data are available, that explain the interplay and regulation of different signaling factors.

In the last few years, we identified important players involved in sexual development. Among them are the pro mutants, which show an arrest in sexual development after protoperithecia formation and a severe defect in hyphal fusion. Here we present data on knockout mutants carrying a deletion of the NAD(P)H oxidase genes *noxA* and their regulator *noxR,* which correspond to the two above described phenotypes. Interestingly, both the nox and pro mutants show also a strong increase of ROS production in distinct cellular structures. Regarding this data we hypothesize coherence between hyphal fusion, fertility and ROS production.

References:

[1] Engh I, Nowrousian M, Kück U (2010) Eur J Cell Biol 89(12):864-72

PR6.4

Protective role of thiamine (vitamin B_1) in baker's yeast cells exposed to hydrogen peroxide.

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Thiamine diphosphate (TDP) serves as a cofactor for main metabolic pathways in all cells. Moreover, numerous recent reports have suggested other biological functions of thiamine independent of its cofactor role, such as involvement in the response of plants and bacteria to stress conditions. However, similar data on *Saccharomyces cerevisiae*, a model eukaryotic organism which is also able to synthesize vitamin B_1 *de novo* are scarce.

The aim of this study was to analyze thiamine biosynthesis in a baker's yeast wild type strain under oxidative stress conditions and to compare its response to that of mutants with disrupted thiamine biosynthesis (*thi4Δ*, *thi6Δ*) or transport (*thi7Δ*). Additionally, we studied a strain with damaged *YAP1*-dependent stress response system which is based on hydrogen peroxide sensing.

Our results showed that thiamine biosynthesis was up-regulated in the wild type strain under oxidative stress. The *thi4Δ* mutated strain was characterized by elevated activity of superoxide dismutase and thiamine pyrophosphokinase under control conditions, while under hydrogen-peroxide treatment the process of thiamine activation to TDP was accelerated 2 fold stronger. The *thi6Δ* mutant with catalytically defective thiamine monophosphate synthase showed a decreased stress response and rate of TDP-dependent pentose phosphate pathway. On the other hand, the *YAP1* mutant presented almost 3 fold higher level of thiamine activation and utilization, suggesting some compensation of disrupted defence system by thiamine biosynthesis, thus confirming a hypothesis of a protective role of thiamine in baker's yeast against oxidative stress.

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PR6.5

***Aspergillus fumigatus* counteracts nitric oxide stress**

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Aspergillus fumigatus is a saprophytic living mold that can cause life-threatening infections in immunocompromised patients. To improve diagnosis and therapy a detailed knowledge of the processes related to host-pathogen interaction is required. In the lung, inhaled conidia are confronted with immune effector cells. After recognition of the conidia are phagocytosed and attacked by host-derived reactive oxygen species (ROS) and antimicrobial proteins. In recent studies it was found that macrophages and neutrophil granulocytes can also form nitric oxide intermediates (RNI) that are putatively involved in killing of the fungus. Because both radicals are present in infected tissue it is very likely that they interact to form highly reactive intermediates like peroxynitrite. *A. fumigatus* produces several enzymes potentially involved in RNI detoxification. Two flavohemoglobins, FhpA and FhpB, convert NO to nitrate, and the S-nitrosoglutathion (GSNO) reductase, GnoA, reduces GSNO to ammonium and glutathion disulphide (GSSG). To elucidate the role of these enzymes in detoxification of RNI single and double deletion mutants of FhpA, FhpB and GnoA encoding genes were generated. Mutant strains revealed enhanced sensitivity against the NO donor DETA-NO. Furthermore, Δ*gnoA* mutants were negatively affected in germination. To investigate the role of RNI and its detoxification in fungal pathogenicity virulence of the D*gnoA* mutant was analysed in a cortisone acetate murine infection model for invasive aspergillosis. However, no difference in pathogenicity was detectable compared to the wild type and complemented strains. Therefore, the ability to detoxify host-derived RNI does not have a major influence on virulence of the human pathogenic fungus *A. fumigatus*.

PR6.6

The Thioredoxin System of *Botrytis cinerea* Has a Severe Impact on Virulence

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In the course of infection the pathogenic grey mould fungus *Botrytis cinerea* triggers an oxidative burst as early plant defense reaction. This leads to an active release of reactive oxygen species (ROS), which are on the one hand known to be responsible for molecular damages of biological molecules, but on the other hand they are also involved in cell signaling pathways. We are particularly interested in the influence of ROS on pathogen-host interaction and development. In order to investigate the maintenance of the fungal intracellular redox state, we focused on the thioredoxin system, which is composed of two enzymes, the thioredoxin (BcTrx) and the thioredoxin reductase (BcTrr). Knock-out and complementation approaches of *bctrx1* and *bctrr1* revealed a severe impact on pathogenicity. The mutants were able to penetrate, but only caused small necrotic lesions that were not able to spread. Furthermore, Δ*bctrr1* and Δ*bctrx1* showed a strong sensitivity to oxidative stress; in addition an enhanced H_2O_2 production of Δ*bctrr1* and generally retarded growth compared to Δ*bctrx1* and the wild-type was striking. Northern analyses showed that oxidative stress response genes were constitutively expressed in the Δ*bctrr1* mutant, while the transcriptional level of these genes was not altered in the Δ*bctrx1* mutant. Consequently, the thioredoxin system seems to be essential for the detoxification of ROS, fungal pathogenesis and the development of *B. cinerea*.

PR6.7

Regulation of cAMP levels during *neurospora crassa* conidiation

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Conidiation involves three morphogenetic transitions: growing hyphae to adhered hyphae, adhered hyphae to aerial hyphae and aerial hyphae to conidia. A hyperoxidant state develops at the start of each of these morphogenetic transitions. The *ras-1^{bd}* and a *Δsod-1* strain exhibit cyclic conidiation that is suppressed by N-acetyl-cysteine. Paraquat shortens its period in both strains. This behavior suggests a cyclic oxidative stress. We investigated how RAS-1 controls the switch between growth and conidiation.

Adenylate cyclase (AC) has a predicted RAS association domain and cAMP is involved in mycelial morphology, aerial hyphae formation and conidia development. Adenylate cyclase null mutant strains do not form aerial hyphae but conidiate profusely. *N. crassa* has a high (PDE_H) and a low affinity (PDE_L) phosphodiesterases. D*pde$_H$* strain grows slow and does not conidiate; there is no evident phenotype for the D*pde$_L$* strain.

We found that oxidative stress and RAS-1 determine cAMP levels during the first two hyperoxidant states of the conidiation process; higher levels than *Wt* were observed in *ras-1^{bd}*. In both strains, a rapid decrease in cAMP at the start of the first two hyperoxidant states was due to activation of PDE_L. PDE_H was important for maintenance of initial cAMP levels, once oxidative stress was compensated. During oxidative stress of the second morphogenetic transition both phosphodiesterases participate in decreasing cAMP and mainly PDE_L is used to restore initial cAMP levels. Thus, RAS influences the level of cAMP, probably though AC activation, but more critical for development is the activation of phosphodiesterases during oxidative stress.

PR6.8

Jasmonic acid and methyl jasmonate induce defense response in grape berries against postharvest gray mould caused by *Botrytis cinerea*.

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Botrytis cinerea, a necrotorphic pathogen, causes serious losses in both yield and quality in grapes (*Vitis vinefera* L.). Jasmonic acid (JA) and its derivatives incuding methyl jasmonate (MeJA) occur naturally in host plant tissues and have signalling roles in defense against necrotrophs and as well as induce systemic resistance (ISR) against disease. The present study investigates the effect of exogenous JA and MeJA, on the suppression of postharvest gray mould in green grape cultivar 'Thompson' and red grape cultivar 'Flame'. The surface sterilized grape bunches (15 grapes/bunch and three replicate treatments) were spray-treated with 0.2 mM of JA or 1mM of MeJA, air dried for 3 hours. Three days after the JA or MeJA treatment, each of the grape berries in the bunch was wounded with a needle and inoculated with 1 x 10^4 spores of *B. cinerea* B05.10 and incubated in the dark at 12 ºC and 85% RH. Control treatment did not receive JA or MeJA. The lesion diameter was recorded at 7 and 14 days after inoculation. Both jasmonic acid and methyl jasmonate induced defense response by significantly suppressing the *Botrytis* gray mould disease in the green grape cultivar 'Thompson', and in the red grape cultivar, 'Flame'. Investigations on the mechanisms of how JA and MeJA induce defenses of grape berries against Botrytis are underway. Postharvest treatment with jasmonic acid or methyl jasmonate may be incorporated as the potential tools in the grape postharvest disease management strategies.

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Poster Category 7: Sensing and Responding

PR7.1

Interplay between metabolism and regulation of cell wall structure in *Candida albicans*: application

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The cell wall of the opportunistic human pathogen *Candida albicans* is the point of direct interactions of this fungus with host cells, especially with macrophages. It is a layered structure of polysaccharides. However, how changes in carbohydrate metabolism affect the cell wall composition is not yet understood.

We investigated the effects of the cytochrome *c* reductase inhibitors myxothiazol A and antimycin A on the metabolism and transcriptome of *C. albicans*. Treatment with either inhibitor led to the formation of reactive oxygen species and to fermentative growth, as was visible from changes in the metabolite profile. Transcriptome analysis revealed changes in the expression of genes related to carbon metabolism and stress-response. Moreover, a wide range of cell wall-building genes were differentially expressed. Flow cytometry confirmed changes in the exposure of beta-glucans and mannans, which were in accordance with an increase in phagocytosis of *C. albicans* by murine macrophages.

To gain insight into the regulation of the differentially expressed cell wall genes, computational analysis of a dataset comprising expression data for 5214 genes from 317 different conditions was conducted, focusing on a group of mannosyl-transferase and glycosylase genes. A group of likely regulators contained, for example, the transcription factor Efg1p, which is connected to morphogenesis, carbon metabolism, and resistance to antimycin A. The roles of these regulators in cell wall construction and susceptibility to cytochrome *c* reductase inhibitors were evaluated by gene expression and metabolite analysis of the respective deletion mutants, indicating new connections between regulation of cell wall structure and metabolism.

PR7.2

The bZIP-type transcription factor FlbB: A versatile regulator of *Aspergillus nidulans* asexual development.

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The early stages of asexual development in the model fungus *Aspergillus nidulans* are controlled at the molecular level by a discrete number of regulatory proteins that includes the bZIP-type transcription factor (TF) FlbB. Vegetative hyphae contain two main pools of FlbB, one at the tip and the other at the most apical nucleus. The apical pool requires the interaction with the positional regulator FlbE at or in the proximity of the *Spitzenkörper*. This interaction requires in FlbB a functional bZIP domain, specific central regions and a highly conserved Cys residue. Nuclear FlbB is renewed after each mitotic cycle and under appropriate conditions, activates the cMyb-type TF FlbD. Both factors, in turn, jointly activate the expression of the conidiation-specific TF *brlA*.

A 2D-PAGE screening of proteins in wild type and Δ*flbB* strains showed that the concentration of specific stress-response proteins was controlled through FlbB. *gmcA*, a previously uncharacterized glucose-methanol-choline oxidoreductase coding gene, shows miss-scheduled expression in a Δ*flbB* genetic background and the derived protein is required during development under alkaline pH conditions. Sequencing of mRNA from both vegetative and asexual samples provides for a wide overview on the genes and pathways under the hypothetic transcriptional control of FlbB activity. Preliminary results obtained in the functional characterization of some of these genes are also presented.

Overall, the functional versatility of FlbB provides for a new outlook on morphogenetic change and focuses our future work on the study of the molecular mechanisms through which this TF regulates different cellular processes during development.

PR7.3

Calcipressin – an activator of calcineurin-dependent signal transduction in *Botrytis cinerea*?

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The Ca^{2+}/calmodulin-dependent phosphatase calcineurin (CN) is a conserved protein that plays a critical role in calcium signaling and stress response in eukaryotic cells. CN activity itself is regulated by a class of conserved proteins termed Calcipressins.

Deletion mutants of *bccnA* encoding the catalytic subunit of CN in *B. cinerea* exhibit severe growth and development defects and form small, compact colonies. In comparison, deletion of the only calcipressin homologous gene, *bcrcn1*, affects vegetative growth in a similar way.

While *bccnA* deletion mutants are completely apathogenic, virulence of *bcrcn1* deletion mutants on bean plants appears to be only reduced.

Studies with the inhibitor of CN Cyclosporine A (CsA) showed that the expression of several genes, e.g. those involved in phytotoxin biosynthesis, is regulated by CN and the CN-responsive transcription factor CRZ1 in *B. cinerea*. Interestingly, the same set of genes is down-regulated in the Δ*bcrcn1*mutants indicating that BcRcn1 functions as an activator of CN.

Studies based on fluorescent microscopy showed equal distribution of BcRcn1 in the cytoplasm, but accumulation in close proximity to the nucleus connecting the influence on gene regulation directly with its intracellular localization.

Further characterizations identified motifs in the BcRcn1 sequence probably essential for activation of this regulator and for binding to CN.

PR7.4

Signaling Pathways Involved In Response To Environmental Iron In The Human Fungal Pathogen *Candida Albicans*

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Candida albicans is one of the most frequent fungal pathogens in humans with a compromised immune system. Iron uptake during infection is considered as a virulence factor for *C. albicans*. As host and pathogenic microorganisms are in permanent competition for iron, the host sequesters iron through proteins, which bind this metal ion with high affinity. To overcome this barrier, pathogens have developed different strategies in order to acquire iron from host proteins.

We established a rapid protein extraction method that allows semi-quantitative determination of representatives of multicopper ferroxidases (MCFOs) present in the *C. albicans* cell membrane. Members of this protein family are required for high affinity iron uptake under iron limiting conditions. Removal of ferrous iron from YPD medium by the chelator bathophenanthrolinedisulfonate (BPS) led to induction of MCFOs compared to a control lacking BPS. Moreover, addition of $FeCl_3$ as well as $FeSO_4$ to iron-free RPMI medium (buffered to pH 7.3) induced flocculation of *C. albicans* cells in an iron concentration-dependent manner.

A combination of the iron induced flocculation of cells and the MCFOs protein extraction method mentioned above is used to identify signalling pathways involved in the response to iron availability or drugs interfering with high affinity iron acquisition pathways. This could be accomplished by using different homozygous single gene deletion mutant strains where MCFOs and the flocculation process play the role of “reporters” for reduced or intact response to iron respectively.

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PR7.5

Does Fgap1 Regulate *Tri* Gene Expression And Trichothecene B Production In Response To An Oxidative Stress In *Fusarium Graminearum*?

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The filamentous fungus *Fusarium graminearum* infects cereals and corn. It is one of the main causal agent of "Fusarium Head Blight" and "Maize Ear Rot". During infection, it produces mycotoxins belonging to the trichothecenes family which accumulate in the grains. Although the biosynthetic pathway and involvement of the genes (*Tri* genes) have been elucidated, the global regulation of the toxin biosynthesis remains enigmatic. We previously showed that, an H_2O_2 oxidative stress enhances the production of toxins in liquid cultures of *Fusarium graminearum* and increases *Tri* gene expression (Ponts et al, 2007, FEBS Lett 581: 443-447).

In the yeast *Saccharomyces cerevisiae,* the transcription factor Yap1p is required for tolerance to oxidative stress and for the expression of genes coding for detoxification enzymes. In this study, we analysed the role of the corresponding factor in *Fusarium graminearum,* Fgap1, in response to an oxidative stress and its eventual interference with the regulation of trichothecene production. A deleted mutant and a strain expressing a constitutively activated form of the Fgap1 factor in *F. graminearum* PH1 were constructed. To mimic an oxidative stress, we cultured these mutants in GYEP liquid medium supplemented with H_2O_2 or diamid to evaluate their sensitivity and analyse their toxin production. Expression profiles of genes encoding detoxification enzymes controlled by Fgap1 and of genes involved in the biosynthesis of type B trichothecenes were analysed by Q-RT-PCR.

PR7.6

Transcriptional links between light and development in *Neurospora crassa*

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The ascomycete fungus *Neurospora crassa* grows as a branched mycelium with interconnected hyphae during the vegetative phase of its life cycle. Several environmental cues, including blue light, promote a developmental transition that leads to the development of conidiophores and the production of conidia. Several mutants have been isolated that are blocked at different stages of conidiation. One of them, *fluffy*, has been investigated in some detail. The FLUFFY (FL) protein is a 792-aminoacid polypeptide containing a Zn2Cys6 binuclear zinc cluster domain belonging to the Gal4p family. Blue light activates *fl*, and light regulation requires the products of genes *wc-1* and *wc-2* that bind transiently to the promoter of *fl*. The activation by light of key regulatory genes may explain the activation by light of conidiation in *Neurospora*. In *Aspergillus nidulans,* another ascomycete, several genes responsible for the formation of conidiophores are activated by light and their *Neurospora* homologs have been identified in the *Neurospora* genome. We have investigated the regulation by light of these putative regulatory genes in the *Neurospora crassa* wild type and Δ*fl* strains and we have found that deletion of *fl* promotes a light-dependent accumulation of mRNA of some of these putative developmental genes. Our results suggest an interaction between FL and the White-Collar complex in the promoter of light and developmentally regulated genes.

PR.7.7

Shuttling of entire MAPK module from membrane to nuclear envelope links fungal development to secondary metabolism

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

PR7.8

Redundant Nuclear Localization Signals Mediate Nuclear Import Of The *Aspergillus nidulans* Transcription Activator Of Nitrogen Metabolic Genes AreA

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The *Aspergillus nidulans* GATA transcription factor AreA activates transcription of genes for uptake and metabolism of nitrogen nutrients. AreA accumulates in the nucleus during nitrogen starvation but not in the presence of nitrogen sources. The AreA protein contains five putative classical SV40 Large T Antigen-type nuclear localization sequences (NLSs) and one putative non-canonical bipartite NLS conserved with mammalian GATA4. We have used two approaches to determine which of these predicted NLSs are functional. First, we fused DNA sequences encoding the putative AreA NLSs to the Green Fluorescent Protein (GFP) gene and introduced these constructs into *A. nidulans*. We determined which of the predicted NLSs are sufficient to direct GFP to the nucleus. UV-fluorescence microscopy showed that the bipartite NLS strongly directs GFP to the nucleus, one of the classical NLSs weakly directs GFP to the nucleus and the other four classical NLSs collaborate to direct GFP to the nucleus. Second, we constructed epitope-tagged gene replacement *areA* mutants affected in individual NLSs and combinations of NLSs to identify by immunofluorescence microscopy sequences required for nuclear localization. Deletion of all five classical NLSs did not affect utilization of nitrogen sources and did not prevent AreA nuclear localization. Mutation of the bipartite NLS conferred inability to utilize alternative nitrogen sources but did not prevent AreA nuclear localization. We determined the effect of this bipartite NLS mutation with combinations of deletions of the five classical NLSs on nuclear localization. Our results indicate redundancy among the AreA NLSs.

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PR7.9

Glutamine Synthetase In *Fusarium Fujikuroi* – An Enzyme With Major Impact On The Regulation Of The Secondary Metabolism

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The rice pathogen *Fusarium fujikuroi* produces a broad spectrum of secondary metabolites. The biosynthesis of two of them, gibberellins and bikaverin, is subject to nitrogen metabolite repression, while the mycotoxin fusarin-C is exclusively synthesized with high amounts of nitrogen. Glutamine is regarded as one of the primary effectors in the nitrogen regulation network. The deletion of the glutamine synthetase (GS) – encoding gene *gln1* was performed to deregulate the expression of the nitrogen repressed secondary metabolite gene clusters. To our surprise the deletion lead to complete loss of the expression of the nitrogen repressed as well as nitrogen induced secondary metabolite genes, indicating an additional regulatory function of the GS.

To better understand this regulatory function we studied the expression of the transcription factors AreA and AreB and the ammonium permeases MepB and MepC under different nitrogen conditions, which were all shown to be affected by the GS. To examine whether it is possible to separate the enzymatic and regulatory functions of the GS, we complemented the deletion mutant with *gln1* alleles created by random and side directed mutagenesis. These mutants were screened for the ability to grow without glutamine and produce certain secondary metabolites. In addition, heterologous GS-genes from other species were transformed into the mutant and examined likewise. While in most cases glutamine auxotrophy corresponds with loss of secondary metabolism, in some rare cases the regulatory and enzymatic functions could be separated. The achieved results confirm an important, direct regulatory role of the GS in the nitrogen regulation network.

PR7.10

Light controls *Fusarium fujikuroi* secondary metabolism via distinctive pathways

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Fusarium fujikuroi is best known as a pathogen of rice that causes hyper elongation the plants due to production of gibberellic acids (GAs). Besides GAs, *F. fujikuroi* may also synthesize other natural products such as carotenoids and the mycotoxins fusarin C and bikaverin. Production of these secondary metabolites is influenced by several environmental stimuli including ambient pH and nitrogen availability. Furthermore, the fungal-specific *Velvet*-like complex was shown to affect secondary metabolism in *F. fujikuroi*.

Currently, we focus on investigating light-dependent regulation of secondary metabolism in *F. fujikuroi*. Expression of genes responsible for carotenoid, bikaverin and fusarin C production is regulated by light in distinct ways. Homologous proteins of the *Neurospora crassa* GATA-type transcription factors white collar (WC)-1 and -2 interact in the nucleus and deletion of either encoding gene results in loss of early light-dependent *car* gene induction. Since carotenoid production is maintained in the deletion mutants in constant light, we expected additive effects of additional light sensors. The sequenced *Fusarium* genomes reveal the existence of three cryptochromes/photolyases: Additionally to the genes coding for the class I CPD photolyase Phr1 and the Cry-DASH cryptochrome, *Fusarium* spp. posses a homolog of Phl1 from *Cercospora zeae-maydis* closely related to diatome 6-4 photolyases with regulatory functions. Comparison of *phl1*, *vel1* and WC mutants regarding control of light-dependent secondary metabolites, suggests common and distinct regulatory roles in *F. fujikuroi*. Since carotenoid production is maintained in WC/Phl1 double mutants we postulate a light-independent induction mechanism triggered by reactive oxygen species.

PR7.11

Identification of Novel Interactors of the Trichoderma atroviride Gpr1 7-Transmembrane Receptor

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By transducing extracellular signals to intracellular downstream effector proteins, eukaryotic seven-transmembrane (7-TM) receptors, also known as G protein-coupled receptors (GPCRs), function as sensors. The classical paradigm of 7-TM receptor signalling is based on a physical interaction of the receptor with an intracellular Galpha subunit. In recent years however, evidence accumulated of signal transmission by 7-TM receptors through mechanisms involving intracellular partners other than heterotrimeric G proteins. In fungi, heterotrimeric G proteins are essential for regulating growth, sexual and asexual development, and virulence, but the involvement of 7-TM receptors in these processes remains to be shown for most fungi. Our recent functional characterization of Trichoderma atroviride Gpr1revealed a prominent role of this 7-TM receptor in the antagonistic interaction of the mycoparasite. gpr1-silenced transformants showed an avirulent phenotype probably resulting from their inability to respond to the presence of living host fungi. When analyzing possible interactions between Gpr1 and the three Galpha subunits of T. atroviride, we could not observe any physical interaction between the receptor and Tga1, Tga2 or Tga3. Here we provide the isolation and identification of novel interactors of Gpr1 by screening a T. atroviride cDNA library using the membrane-based split-ubiquitin yeast two-hybrid assay. After selecting for growth on drop-out medium and testing for positive interactions by re-transformation and beta-galactosidase assays, inserts representing 15 different genes could be isolated from the initially obtained 115 colonies. The majority of the encoded proteins were identified to be related to transport processes and ten of them were shown to harbor transmembrane domains.

PR7.12

Conditional expression of the phospho-transmitter gene *ypdA* and the interaction of YpdA with response regulators in *Aspergillus nidulans*.

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Eukaryotic histidine-to-aspartate (His-Asp) phosphorelay systems consist of three types of common signal transducers: His-kinase (HK), a response regulator (RR), and a histidine-containing phosphotransfer intermediate (HPt). In general, HPt acts as an intermediate between HK and RR and is indispensable for inducing appropriate responses to environmental stresses through His-Asp phosphotransfer signaling. Although HPt is thought to be essential among *Aspergillus* species, the molecular mechanism underlying the HPtA essentiality remains unclear. In the present study, we constructed *Aspergillus nidulans* mutant in which expression of the HPt gene *ypdA* is conditionally regulated under the control of the *A. nidulans alcA* promoter (C*ypdA* strain). When C*ypdA* was cultured in CD medium (repressed condition), the transcript level of the *ypdA* gene was decreased to 5% that of the wild type (ABPU1). In CD medium, C*ypdA* showed remarkable growth retardation and formed abnormal hyphae, suggesting that YpdA is an essential component. Downregulation of *ypdA* expression resulted in induction of the *catA* and *gfdB* genes, which are upregulated downstream of the active HogA mitogen-activated protein kinase (MAPK) cascade. We then constructed mutant strain from C*ypdA* by deleting the response regulator gene *srrA* (C*ypdA/ΔsrrA*). When *ypdA* was downregulated, C*ypdA/ΔsrrA* unexpectedly showed more severe growth retardation than the parent C*ypdA*. We are also trying to isolate a mutant from C*ypdA* by deleting another response regulator gene *sskA*. Here, we discuss two-component signaling under the inhibitory conditions of signaling between YpdA and response regulators.

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PR7.13

The fungicide fludioxonil induces expression of ABC-transporters Cdr1p and Cdr2p in *Candida albicans* and increases the resistance to fluconazole

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The fungicide fludioxonil is widely used in agriculture, especially to protect grapes and berries from fungal diseases. Residua of this fungicide are occasionally detected in fruits and can therefore be ingested by humans.

The human fungal pathogen *C. albicans* expresses the target of fludioxonil, a type III histidine kinase, called Nik1p. Thus, we investigated the effects of fludioxonil on the growth and transcriptome of *C. albicans*. While inhibition of growth was strongly dependent on the genetic background of the strain, such that growth of the major test strain of this study (SC5314) was hardly affected, we observed a concentration-dependent induction of the expression of the ABC-transporter genes *CDR1* and *CDR2*. The induction of ABC-transporter genes was independent of the presence of the target of fludioxonil, as induction was also observed in a deletion mutant. Deletion of *CDR1* caused a decrease in resistance against fludioxonil, indicating that the fungicide was discharged from the cell by Cdr1p.

The effect of concurrent exposition to fludioxonil and known cargoes of the induced ABC-transporters on the growth of *C. albicans* and the extrusion of the cargo were thus examined. The presence of fludioxonil decreased the export of rhodamine 6G. A synergistic growth inhibitory effect of both compounds was detected. The resistance against fluconazole, however, was increased by fludioxonil. This effect was independent of the presence of Nik1p.
Therefore, it may be concluded that the increase in resistance to fluconazole in the presence of fludioxonil was caused by the induction of the ABC-transporter genes.

PR7.14

The CryA DASH-cryptochrome Of *Fusarium fujikuroi* Is An Active Photoreceptor

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The ascomycete *Fusarium fujikuroi* has a complex secondary metabolism whose regulation has been object of detail attention. In this work we analyze the functional role and biochemical properties of CryA, a DASH-cryptochrome of this fungus that belongs to the photolyase/cryptochrome family. DNA repair and light sensing activities, formerly found in other fungal cry-DASH proteins, have been investigated in CryA. Biochemical data shows that CryA binds the typical cofactors flavin-adenin-dinucleotide (FAD) and methenyl-tetrahydrofolate (MTHF), and that FAD undergoes photoreduction, a previous step required for DNA repair. CryA was able to bind damaged DNA in single and doubled stranded DNA probes. The protein was also able to repair cyclobutane pyrimidine dimers, tested in ss DNA. Regarding its *in vivo* function, *cryA* transcript levels are strongly induced by light, reaching the maximum after 60 minutes of illumination. Δ*cryA* mutants display light-dependent phenotypic alterations when they are grown under nitrogen starvation. Bikaverin biosynthesis is enhanced in illuminated Δ*cryA* liquid cultures, which acquire an intense reddish pigmentation, but this increment is not accompanied by enhanced expression of the specific polyketide synthase gene *bikA*. In addition, these mutants produce a different type of conidia called macroconidia, whose production is rarely detected in the wild type, and which differ from the normal microconidia in size and morphology. Our data suggest that CryA is a light-dependent repressor involved in the regulation of bikaverin production and conidiation. We propose that CryA has a dual function in *F. fujikuroi* as DNA-repair enzyme and photoreceptor.

PR7.15

Transcript response of polyketide synthases from the chickpea pathogen *Ascochyta rabiei* grown under the influence of divalent cations

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Polyketides compounds are natural products that are involved in the development of fungal structures and plant disease. The phytotoxin solanapyrone, a polyketide, has been greatly associated with *Ascochyta rabiei* aggressiveness. The production of solanapyrone in liquid cultures of *A. rabiei* is dependent on the divalent cations calcium, cobalt, copper, manganese, and zinc. Therefore, we hypothesized that certain polyketide synthases (PKSs) in *A. rabiei* may be regulated by these divalent cations. Using bioinformatics, we predicted thirteen PKSs in the genome of *A. rabiei* using fungal-based profile hidden Markov models. Ten of these were predicted as reducing and three as non-reducing. We conducted organic extractions to isolate polyketide compounds from liquid cultures of *A. rabiei* grown under presence or absence of light and/or under the influence of the aforementioned cations. These organic extracts were inoculated on liquid cultures of the single-cell algae *Chlamydomonas reinhardtii* in order to assess their phytotoxic potential; showing that the presence or absence of light during incubation had no effect on phytotoxicity. However, divalent cations added to the liquid media induced the production of phytotoxic compounds. Transcript analysis showed that the PKS genes *ArPKS02*, *ArPKS03*, *ArPKS04*, *ArPKS08*, and *ArPKS09* were expressed only when these five divalent cations were added to the liquid culture. Through BLASTP analysis, ARPKS09 was found to be a homolog to the solanapyrone synthase (*SOL1*) from *Alternaria solani*. Furthermore, all six genes of the *ARPKS09* gene cluster were highly similar and in synteny to the genes of the *SOL1* gene cluster, suggesting horizontal gene transfer.

PR7.16

Production of the polyketide phomenoic acid, by *Leptosphaeria maculans*

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The epipolythiodioxipiperazine, sirodesmin PL, and the polyketide phomenoic acid are secreted in abundance in culture by *Leptosphaeria maculans,* the dothideomycete that causes blackleg of canola. The biosynthetic gene cluster for sirodesmin PL is well-established, but that of phomenoic acid, is unknown. Both molecules have antifungal activity which may allow *L. maculans* to outcompete other fungi in their niche. Domain modelling and comparative genomics with *L. biglobosa* and *Stagonospora nodorum* (neither of which have been reported to make phomenoic acid), was used to predict a candidate polyketide synthase (PKS) for phomenoic acid biosynthesis. A reducing PKS with the following domains was predicted: KS - keto-synthase; AT - acyltransferase; DH - dehydratase; MT- methyltransferase; ER - enoylreductase; KR -ketoreductase; ACP- acyl carrier protein. Of the 14 PKSs in the genome of *L. maculans*, five had reciprocal best hits in *L. biglobosa* 'canadensis' and seven had close matches to PKSs in *S. nodorum*. Seven PKSs had the above domain structure, but only one (PKS2) was highly expressed by *L. maculans* in culture. Expression of PKS2 was considerably reduced by gene silencing in two different genetic backgrounds resulting in significantly reduced levels of phomenoic acid in culture. Four genes flanking PKS2 shared a similar transcriptional profile, suggestive of their being part of the phomenoic acid gene cluster.

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PR7.17

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

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The filamentous fungus *Aspergillus fumigatus* is present in diverse habitats and therefore confronted with a wide variety of environmental stimuli. Due to its ability to grow on numerous nutrients, *A. fumigatus* is also able to colonise the human body. In immunocompromised patients, severe life-threatening infections like invasive aspergillosis can occur, thus making *A. fumigatus* the most important airborne fungal pathogen.

To be able to sense and respond to changing environmental conditions during infection, *A. fumigatus* contains a large array of sensing and signaling mechanisms including G protein-coupled receptors (GPCRs). GPCRs form the largest group of membrane receptors among eukaryotic organisms. Until now, little is known about the stimuli and signal transduction mechanisms of the 15 GPCRs predicted to be encoded by the genome of *A. fumigatus*. Therefore, to understand their impact on fungal growth, development and pathogenicity, it is of major importance to investigate their function in detail and to identify their possible contribution to pathogenicity.

We created a collection comprising single knock-out strains of almost all *A. fumigatus* GPRCs and started to investigate their phenotypes. First results show that some mutant strains show reduced growth and production of conidia, which gave first hints on the importance of functional signaling pathways for survival of *A. fumigatus*. The ongoing analysis will define the function of different GPCRs in a filamentous fungus.

PR7.18

Functional analysis of gene *cutA* of *F. fujikuroi*, encoding a protein of the haloacid dehalogenase family.

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The gibberellin-producing fungus *Fusarium fujikuroi* combines its phytopathogenic capacity with a saprophytic style of life. In nature, the fungi have to survive to changing environmental conditions during their life cycles, and appropriate regulatory networks are necessary to overcome a huge variety of stresses. In this work, we analyze the role and regulation of gene *cutA* of *F. fujikuroi*, orthologous to *cut-1* from *N. crassa*. This gene codes for an enzyme of the haloacid dehalogenase family (HAD), which includes different classes of phosphatases. Targeted deletion of *cutA* results in normal morphology and pigmentation, but reduced capability to grow in osmotic stress conditions. Interestingly, *cutA* is clustered in a head-to-head arrangement with *gldA*, which putatively encodes a glycerol dehydrogenase. *F. fujikuroi* responds to osmotic stress triggering a fast accumulation of glycerol inside the cell, while the strains lacking a functional *cutA* gene are unable to raise the intracellular glycerol content. Both genes seem to be co-regulated, as their mRNA levels increase transiently after heat shock or osmotic stress treatments. In contrast to *N. crassa*, where *cut-1* is down-regulated by light, *cutA* and *gldA* mRNA levels increase after illumination. Mutants of genes *wcoA* and *cryA*, encoding a WC-1 protein and a DASH-cryptochrome, also show this photoresponse, suggesting that these photoreceptors do not participate in *cutA* and *gldA* photoinduction. The stimulation by light of the expression of these genes in *F. fujikuroi* is consistent with a regulatory association between illumination and osmotic stress.

PR7.19

Comparative mRNAs expression patterns between vegetative growth and asexual development in *Aspergillus nidulans*.

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Using the high resolution RNA-Seq, we have analyzed the different mRNA expression patterns between vegetative and asexually developing mycelia of *Aspergillus nidulans*. The pathways and genes that are activated and repressed during vegetative hyphal growth and conidiophore production have been identified. We also describe a substantial number of novel transcripts that are controlled by *U*pstream *D*evelopmental *A*ctivators (*UDA*s, especially FlbB) at different stages of development. Genes involved in secondary metabolism (like polyketide synthases, see poster Rodríguez-Urra *et al*.), increased oxidoreductase activity and/or transcription factors (principally binuclear zinc clusters) are examples of groups regulated by FlbB.

Our analysis indicates that during asexual development the 4% of the transcriptome is modified comparing with vegetative growth, including more genes than previously were anticipated. Of these, FlbB regulates the 60% directly or indirectly. These results may provide a blueprint for further study of the *Aspergillus nidulans* development.

PR7.20

Involvement of saga complex components in transcriptional regulation in *aspergillus nidulans*

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The SAGA complex is highly conserved from yeast to humans. in yeast it is invloved in the regulation of highly regulated genes that respond to environmental stresses, such as metabolic starvation, DNA damage and heat. Bioninformatic and proteomic analyses have shown that the components of the SAGA complex are also present in *Aspergillus nidulans*.

In *A. nidulans*, acetate is a repressing carbon source that leads to similar levels of CreA mediate repression as glucose. *acdX* was identified in a mutation screen in *A. nidulans* to identify genes invloved 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 complex may have a role in acetate repression in *A. nidulans*, since Spt8 is a component of the SAGA complex.

CreA has been shown to repress the expression of the *alc* regulon, which is required for the ethanol utilization pathway, and two mechanisms have been identified: (i) by the direct repression of the *alcR* gene, which results in almost no expression of the *alcA* and *aldA* genes or (ii) by the mechanism in which CreA represses both the *alcR* gene and the *alcA* gene indepenently of one another.

We report results of experiments undertaken to confirm whether AcdX and/or SptC, which is the *A. nidulans* homologue of the *S. cerevisiae* SAGA complex component Spt3, are invlolved in the transcriptional regulation of the *alc* regulon in glucose or acetate repressing conditions.

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PR7.21

Aquaporin and aquaglyceroporins in *Aspergillus nidulans* are dispensable in osmotic stress responses.

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Aquaporin is a water channel protein found in almost all organisms from bacteria to human. More than 200 members of this family were identified up to date. There are two major categories of Major Intrinsic Protein (MIP) channels, aquaporins and glycerol facilitators, which facilitate the diffusion across biological membranes of water or glycerol and other uncharged compounds, respectively. The full genome sequencing of various fungal species revealed that there are 3 to 5 aquaporins in their genome. However, no functional characteristics were studied so far in Aspergillus sp. In *Aspergillus nidulans*, one orthodox aquaporin (*aqpA*) and four aquaglyceroporins (*aqpB~E*) were found in the genome. Knock-out of each aquaporin or aquaplyceroporin didn't show obvious phenotypic change in osmotic stress, suggesting that the function of the genes may be redundant or not be related in osmotic stress responses. However, resistance of fluconazol has been changed in some mutants, indicating that the function of aquaporins play roles in susceptibility of antifungal reagent. [This work was supported by NRF Korea (2009-0072920)]

PR7.22

A Novel Transcriptional Regulator, ClbR, Controls the Expressions of Cellulose- and Hemicellulose-degrading Enzyme Genes by Two Distinct Mechanisms in *Aspergillus aculeatus*

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The cellobiose- and cellulose-induction of the FIII-avicelase (*cbhI*) and FII-carboxymethyl cellulase (*cmc2*) genes were regulated by XlnR-independent pathway in *Aspergillus aculeatus*, which suggests that this fungus possesses an unknown cellulase-genes-activating pathway. To identify the novel *trans*-acting regulatory factors controlling their expressions, we established a positive screening system to monitor the inducibility of the *cbhI* promoter using the orotidine 5'-phosphate decarboxylase gene (*pyrG*) as a reporter. Gene disruption library was constructed by T-DNA insertion using *Agrobacterium tumefaciens*-mediated transformation, and transformants were selected for 5-fluoroorotic acid (5-FOA) resistant under the cellulase-inducing condition. Of the ~6,000 transformants that we screened, one 5-FOA resistant, S4-22, grew poorly on cellulose media and reduced the cellobiose-induced expression of *cbhI*. Southern blot analysis and nucleotide sequence of the flanking regions of the T-DNA inserted in S4-22 indicated that the T-DNA located within the coding region of a putative $Zn(II)_2Cys_6$-transcription factor designated as the cellobiose response regulator (ClbR). Interestingly, the *clbR* disruption resulted in reduced expression of not only *cbhI* and *cmc2* but also genes regulated by XlnR in the presence of cellulose. However, the *clbR* disruption did not affect for XlnR-dependent induction in response to D-xylose and L-arabinose. The *clbR* overexpression led to sustainable cellulase and xylanase production for 10 days, which increased their production by 2- and 5-fold, respectively. These data demonstrate that ClbR participates in cellulose signaling pathway regulated by both the XlnR-dependent and the XlnR-independent pathways.

PR7.23

The plasma membrane receptors Sho1 and Msb2 prime *Ustilago maydis* for biotrophic development

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The dimorphic fungus *Ustilago maydis* switches from budding to hyphal growth on the plant surface. In response to hydrophobicity and hydroxy fatty acids *U. maydis* develops infection structures called appressoria. These structures enable the fungus to penetrate the plant epidermis. After penetration a biotrophic interaction between *U. mayids* and its host plant maize is established. Here we report on the transmembrane proteins Sho1 and Msb2, which are essential for appressorium formation in response to the hydrophobic stimulus. Epistasis analysis revealed that Sho1 and Msb2 act upstream of Kpp2 and Kpp6, two MAP-kinases essential for appressorium formation and plant cuticle penetration, respectively. To unravel the impact of Sho1 and Msb2 on gene regulation we performed genome-wide transcriptional profiling under appressorium-inducing *in vitro* conditions. We found that *sho1* and *msb2* are specifically required for the expression of genes encoding putative secreted proteins. Some of these proteins have been previously shown to be required for the establishment and maintenance of the biotrophic interaction. Our data indicate that Sho1 and Msb2 are sensors of plant surface cues acting upstream of the pathogenicity-related MAP-kinase cascade. This signaling cascade has the capacity to prime *U. maydis* for biotrophic development when hyphae are growing on the plant surface.

PR7.24

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

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Aspergillus fumigatus is a saprophytic mould normally inhabiting the soil. The fungus also represents a medically important human pathogen causing severe systemic infections in immunocompromised patients. To survive in these entirely different habitats, the fungus needs mechanisms to sense environmental signals and transduce them intracellularly. One of these signal transduction pathways is the cAMP dependent protein kinase A (PKA) pathway. For *A. fumigatus*, components of this generally well conserved signaling cascade have been characterized in detail and its significance for virulence was shown. To identify target genes of PKA, we performed microarray analyses using a mutant strain overproducing the PKA catalytic subunit in comparison to the corresponding wild type. Following this approach, we were able to find 282 up and 343 down regulated genes involved in different cellular processes like carbon and nitrogen metabolism, cell cycle regulation and ribosome biogenesis. Among these genes potentially regulated by PKA, 23 transcription factors were found of which 21 have been not yet functionally characterized in *A. fumigatus*. 15 of these putative transcriptional regulators were deleted and the mutant phenotypes were characterized under different cultivation conditions. A C6 finger domain protein that shows highest upregulation of all identified transcription factors is located in a potential secondary metabolite gene cluster. Because a gene deletion resulted only in minor phenotypical changes, an overexpression mutant of this transcription factor was created to gain deeper insights into its function.

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PR7.25

The *Ustilago maydis* MAP Kinase signaling pathway: Identification of direct MAP kinase targets by phospho-proteomics.

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The basidiomycete fungus *Ustilago maydis* is a plant pathogen that causes smut disease in maize. In this pathogen a conserved mitogen-activated-protein-kinase (MAPK) module regulates important steps of fungal development. We are interested in the identification of direct phosphorylation targets of the related MAP kinases Kpp2 and Kpp6 which control mating and plant colonisation. Currently we are using a phospho-proteomics approach for detecting the phosphorylated targets of these MAP kinases. For this approach we generated strains in which MAP kinase signaling can be induced by expressing a constitutively active version of the MAPKK Fuz7 (Fuz7DD) under an inducible promoter in the presence or absence of *kpp2* and *kpp6*. These strains were phenotypically characterized with respect to formation of conjugation tubes and time-course of activation of remaining MAP kinases. Detection of phosphorylated proteins within complex mixtures was done after Fuz7DD induction by separating proteins by SDS-PAGE and visualizing phosphorylated proteins directly by using the phospho-specific dye Pro-Q Diamond. The current state of these experiments will be described. Differentially phosphorylated proteins, i.e. likely targets of Kpp2 and Kpp6, will be identified by mass spectrometry after enrichment. The role of these proteins in signaling and pathogenicity will then be studied by generating mutants and characterisation of their phenotype. Once these novel targets are characterized we will determine whether related targets are of relevance for disease in the other fungi studied in the ARIADNE network.

PR7.26

Molecular responses of *Trichophyton rubrum* to ambruticin and fludioxonil.

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The incidence of fungal infections is increasing worldwide, mainly due to the high prevalence of immunocompromised patients, promoting the search for new antifungal drugs essential to expand the options of viable therapies. Dermatophytes are one of the most prevalent fungal pathogens that cause cutaneous infections in both healthy and immunocompromised patients. Fludioxonil and ambruticin are antifungals that interfere with the osmoregulation system, leading to hyphal-tip swelling, cell wall alterations, and subsequent cell death. In this work, we present evidence on the efficacy of these drugs on the anthropophilic dermatophyte *Trichophyton rubrum*. The results showed that low concentrations were able to inhibit fungal growth in all evaluated infection models. Moreover, according to the infection site, ambruticin differently modulates the expression of *T. rubrum* genes, showing a site-specific response to antifungal drugs that may reflect the adaptive strategies used by *T. rubrum* to overcome the cytotoxic effects caused by the drug.

PR7.27

Transcriptional Profile of the Dermatophyte *Trichophyton rubrum* in Response to Acriflavin

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The dermatophyte *Trichophyton rubrum* is an anthropophilic fungus that is the most common etiologic agent isolated in cases of human dermatophytoses. Recently, it has become the cause of deep and widespread infections in immunocompromised patients. The therapeutic strategies to control this kind of infection have several limitations such as the appearance of resistant strains and the restricted number of cellular targets. New therapeutic strategies are necessary, being the focus of many investigations. Acriflavin is an acridine derivative compound with antifungal activity involved in topoisomerase inhibition. Although it presents DNA intercalating properties, has been reported the over-expression of genes coding for enzymes involved in mitochondrial respiratory-electron transport and in iron transport, suggesting a broad spectra of cellular effects. In order to better understand its molecular effects we evaluated *T. rubrum* transcriptome in response to Acriflavin in a time-course assay using the next generation sequencing technology SOLiD System. RNAseq was performed comparing *T. rubrum* growth in malt extract medium and the three periods of drug exposure, 3h, 12h, and 24h. The transcriptional analysis revealed 490 modulated genes in response to Acriflavin in the conditions analyzed. These genes are involved in various processes including pathogenicity, transmembrane transport, metal ion binding and fatty acids biosynthesis. Furthermore, several genes involved in glyoxylate cycle, such as malate synthase and isocitrate lyase, considered virulence factors, were repressed by the drug. These genes constitute potential candidate targets for the development of antifungal drugs and reinforce the broad spectrum of effects caused by sub-inhibitory concentrations of acriflavin.

PR7.28

Temperature adaptation in *Rhynchosporium commune*

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Rhynchosporium commune is a haploid ascomycete and a major pathogen of barley (*Hordeum vulgare*). Despite the agricultural and economic importance of *R. commune*, little is known about its evolutionary ecology, including adaptation to different temperatures across populations from climatically diverse locations. We conducted common garden experiments with 126 genetically distinct isolates from 9 field populations, measuring phenotypic variation in growth rates at 12°, 18°C and 22°C.

Populations from colder climates with higher temperature variation grew faster at all three temperatures compared to populations from warmer climates, indicating that the former populations contained more thermal generalists. Across the three temperatures genotype-by-environment interactions (GxE) accounted for 1.3 times more of the phenotypic variance than the genetic variance. Population differentiation for growth rates (Q_{ST}) was significantly lower at 18°C than population differentiation at neutral microsatellite loci (G_{ST}) and not significantly different at 12°C but at 22°C the Q_{ST} was significantly higher, consistent with local adaptation for growth at higher temperatures.

We found that *R. commune* has a high potential to rapidly adapt its growth rate because of the high ratio of phenotypic plasticity to environmentally independent genetic variance. We found that this pathogen with a worldwide distribution has indeed adapted locally to climatic conditions, though not through a shift in temperature optimum but rather by acquiring generally fast growth (in cooler/variable climates) or slow growth (in warmer/constant climates). This latter result implies that there may be costs associated with fast growth under warm/constant climates.

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PR7.29

The SOFT homolog PRO40 is part of the cell wall integrity pathway in *Sordaria macrospora*

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The homothallic ascomycet *Sordaria macrospora* has been studied as a model organism for fungal sexual development since the 1950s. In forward genetic approaches several mutants were generated which showed the same sterile phenotype. From this set of mutants it was possible to characterize the PRO-proteins involved in sexual development. One of these proteins is PRO40, a homologue of Soft from *Neurospora crassa*, both are characterized by a WW domain. Several interaction studies revealed MEK1 as an interaction partner of PRO40. MEK1 belongs to one of three MAP-kinase (MAPK) cascades of *Sordaria macrospora*. These cascades are involved in processes like the adjustment of gene expression to changing environmental conditions. They consist of three kinases, which are activated consecutively through phosphorylation. Homology-based analysis puts the MAPKK MEK1 into cell wall integrity pathway. Here, we demonstrate that Δmek1 shows similar growth defects as Δpro40. Beside the interaction of PRO40 and MEK1, there are also hints for a putative function of PRO40 as scaffold protein for the cell wall integrity pathway. Yeast-two-hybrid studies show no direct interaction between MEK1 and the MAPKKK MIK1. In contrast we provide evidence for an interaction between PRO40 and MIK1. To deduce the function of PRO40 in this context further, experiments like co-immunoprecipitation with tagged proteins and generation of constitutively active MAK1 derivatives will be performed.

PR7.30

XlnR-independent Pathway Regulates both Cellulase and Xylanase Genes in *Aspergillus aculeatus*

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To help understand substrate-induced gene expression mechanisms of cellulose- and xylan-degrading enzymes in *A. aculeatus*, factors regulating the transcription of these genes were examined. A comparison of gene expression profiles between the host strain and an *xlnR* disruptant demonstrated that *A. aculeatus* possesses at least two signaling pathways for these genes induction: the XlnR-dependent and -independent pathways. The expression of FI-carboxymethyl cellulase (*cmc1*) and FIb-xylanase (*xynIb*) genes was controlled by XlnR; in contrast, the expression of the FIII-avicelase (*cbhI*), FII-carboxymethyl cellulase (*cmc2*), and FIa-xylanase (*xynIa*) genes was controlled by an XlnR-independent pathway. To gain deeper insight into the XlnR-independent pathway, the expression profile of *cbhI* was analyzed as a representative target gene. Cellobiose together with 1-deoxynojirimycin (DNJ), a glucosidase inhibitor, induced *cbhI* the most efficiently among disaccharides composed of β-glucosidic bonds. Furthermore, cellobiose with DNJ induced the transcription of all genes under the control of the XlnR-independent pathway, whereas genes under the control of XlnR were not induced. GUS reporter fusion analyses of truncated and mutated *cbhI* promoters revealed that three regions were necessary for effective cellulose-induced transcription, all of which contained the conserved sequence 5'-CCGN_2CCN_7G(C/A)-3' within the CeRE, which has been identified as the upstream activating element essential for expression of *eglA* in *A. nidulans*. The data therefore delineate a pathway in which *A. aculeatus* perceives the presence of cellobiose, thereby activating a signaling pathway that drives cellulase and hemicellulase gene expression under the control of the XlnR-independent regulation through CeRE.

PR7.31

Insights into the mechanism for integration of nutrient and light signals in *Trichoderma reesei* (*Hypocrea jecorina*)

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Although the biotechnological workhorse *Trichoderma reesei* (*Hypocrea jecorina*) is predominantly known for its capability of efficient plant cell wall degradation, recent studies show that it has not lost its evolutionary heritage. Adjustment of nutrient utilization and response to light and darkness are achieved by interacting pathways, also under laboratory conditions.

Transmission of nutrient signals via the heterotrimeric G-protein pathway has been shown to be influenced by light. We show that this interconnection is mainly established by the light regulatory protein ENV1 and the phosducin like protein PhLP1, via mutual transcriptional regulation and presumably by influence on GNB1 (G-protein beta subunit) function. ENV1 thereby exerts a more severe effect on gene transcription than BLR1 or BLR2. Lack of either one of the photoreceptors or PhLP1, GNB1 or GNG1 leads to a partial shut down of processes up-regulated in light, indicating that heterotrimeric G-protein signaling exerts its major function in light and is a target of the light response machinery. Consequently, signals transmitted via the G-protein pathway are of different relevance in light and darkness. Investigation of regulation of glycoside hydrolases as one of the major output pathways of this mechanism revealed that 79 % of all genes belonging to this group, representing all GH-families available in *T. reesei*, are potentially responsive to light. We conclude that ENV1 is a key factor in connecting nutrient signaling with light response and establishes a signaling output pathway independent of BLR1 and BLR2.

PR7.32

Signalling the induction of sporulation involves the interaction of two secondary metabolites in *Aspergillus nidulans*

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

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PR7.33

The RGS-PX domain containing protein Rgs3 is a novel regulator of the pheromone MAPK signaling pathway in Ustilago maydis

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In the phytopathogenic fungus Ustilago maydis cell fusion of compatible haploid cells is controlled by a pheromone/receptor system. Pheromone triggers the activation of cAMP signaling as well as MAP kinase signaling. We have identified a RGS and PX domain-containing protein termed Rgs3 (Regulator of G protein Signaling), regulating the mating MAPK signaling pathway. Microscopic observation showed that Rgs3 resides in the endoplasmic reticulum. The rgs3 deletion strain showed reduced conjugation tube formation in response to pheromone stimulation, was reduced in cell fusion and in virulence. The activation of the MAP kinase Kpp2 in the rgs3 deletion strain was dramatically attenuated and the induction of b gene expression was also severely reduced upon pheromone stimulation. Moreover, the overexpression of pheromone receptor pra1 in rgs3 deletion strains rescued the defects in Kpp2 activation and conjugation tube formation after pheromone induction. This indicates that there may be a link between Rgs3 and Pra1. Current studies will be presented that at which level Rgs3 regulates the pheromone receptor Pra1.

PR7.34

Functional characterization of light-regulated transcription factors in *Penicillium chrysogenum*

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Many responses to external and internal stimuli have been identified to regulate gene expression in the industrial penicillin producer *Penicillium chrysogenum*. Light for instance acts as a major carrier of information, but in case of *P. chrysogenum* little is known about the effect of illumination on regulatory networks. It has been shown, that light has an effect on morphology and secondary metabolite production, although only few regulators have been found so far at the molecular level. To identify light induced regulatory responses, and the proteins involved, we used microarray-analysis for our experimental approach. We compared expression levels of cultures grown in light with those grown in darkness, to identify differently regulated genes. The putative light regulated genes were compared with sets of genes from previous microarray experiments where expression levels were analysed of disruption strains with deleted genes encoding core elements of the light dependent *velvet* complex [1]. To identify regulatory factors, we screened candidate genes for putative transcription factors by BLAST and PFAM analysis. Four transcription factors, namely PcrpnD, PchoxM, PcatfA and Pc04780 have been selected for functional characterization. Subsequently we have generated deletion strains using the FLP/*FRT* recombination system [2]. Further functional characterisation showed an effect of gene disruption on morphology, conidiation and secondary metabolite production.

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PR7.35

***CoPacC*, pH-responsive transcriptional factor, is involved in the entry mode selection of *Colletotrichum orbiculare* at wounded sites of *Arabidopsis* leaves.**

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Colletotrichum orbiculare (*Co*) is the causal agent of cucumber anthracnose disease. Normally, *Co* forms melanized appressoria which enable the fungus to invade the plant. However, we have recently found that *Co* exhibits hyphal tip-based entry (HTE), uncoupled with melanized appressoria, at wounded sites of *Arabidopsis* leaves. To investigate what kinds of signals at wounded sites induce HTE, we looked for the in vitro conditions that can trigger HTE. As a result, we found that appressorium development of *Co* on hydrophobic surface was suppressed when ambient pH was shifted to alkaline condition. PacC is known to pH-responsive transcriptional factor in several filamentous fungi. To assess the potential involvement of PacC in switching to HTE, we identified the *PacC* homolog of *Co* and generated *CopacC* null mutants. Remarkably, at wounded sites of *Arabidopsis* leaves, the ratio of melanized appressorium formation of the *CopacC* mutants significantly increased in comparison with that of the wild type. Furthermore, the transudate collected from wounded sites of *Arabidopsis* leaves induces HTE-like morphogenesis of *Co* in vitro in the CoPacC-dependent manner, and its activity severely decreased when the transudate pH was altered. It was reported that PalC is the activator of PacC and localizes to cortical structures when pH signalling is active in *Aspergillus nidulans*. To assess the activation of pH signalling during HTE-like morphogenesis and appressorium development of *Co*, we have generated a PalC GFP-tagged mutant. Our results suggest that the PacC-mediated pH sensing and regulation is involved in the entry mode selection of *Co*.

PR7.36

Early Infection of *Ustilago maydis*: Hdp2 and Biz1 Control Appressoria Formation and Plant Surface Penetration

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In the corn smut fungus *Ustilago maydis*, sexual development is initiated by fusion of two haploid sporidia, resulting in a filamentous dikaryon that is capable to infect the plant. This process is orchestrated by the *a* and *b* mating-type loci. The bE/bW transcription factor encoded by the *b*-mating type triggers a regulatory network consisting of different transcription factors e.g. Rbf1, as a master regulator, which is required for the expression of most *b*-regulated genes [1]. To get insight into the processes that precede plant infection, we performed microarray analysis of *U. maydis* cells grown on the plant surface. Two of the genes specifically induced in a pathogenic strain are a C_2H_2 zinc finger transcription factor and a homeodomain transcription factor named *biz1* [2] and *hdp2*, respectively. We show that Δ*hpd2* strains are completely blocked in appressoria formation, whereas Δ*biz1* cells are severely reduced in their ability to form appressoria and to penetrate the plant [2]. Furthermore, Hdp2 appears to be required for the expression of about 30% of all genes induced on the plant surface, while Biz1 can induce about 30% of all genes up-regulated on the plant surface. The loss of regulation of these genes may explain the severe phenotypes observed in Δ*hpd2* and Δ*biz1* strains.

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PR7.37

The global transcription regulator VTA2 of the plant pathogen *Verticillium* spp. mediates biofilm formation and virulence and reprograms yeast for adhesion

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The hybrid phytopathogen *Verticillium longisporum* infects oilseed rape resulting in a servere reduction of oil yield. Little is known about the molecular mechanism of plant host infection, especially the first step when the pathogen interacts and attaches to the host surface before penetration. We have identified genes that are involved in adhesion and fungal biofilm formation by screening a *V. longisporum* cDNA library expressed in non-adherent *S. cerevisiae*. Twenty-four different genes from the cDNA library rescue adhesion in non-adherent yeast. They include two similar cDNAs representing isogenes for the same transcription regulator VTA2 (**V**erticillium **T**ranscription **A**ctivator) which is localized in the fungal nucleus. The genomic loci revealed that the two *V. longisporum* isogenes *VTA2-1* and *VTA2-2* share 96% identity. Both isogenes can be distinguished by the first exon and the third intron of together five exons separated by four introns. *VTA2-1* of *V. longisporum* is identical to *VTA2* of *V. dahliae*, whereas *VTA2-2* is very closely related to *VTA2* of *V. albo-atrum*. Disruption of *VTA2* in *V. dahliae* resulted in loss of biofilm formation and surface hydrophobicity, reduction of virulence on its host combined with defects in morphological differentiation, reduction in growth rate, early maturation of the resting structures (microsclerotia) and sensitivity to oxidative stress. We propose yeast as a model to identify regulator genes of pathogenicity like *VTA2* which is conserved in the genomes of other phytopathogens including *Fusarium*, *Magnaporthe* and *Botrytis.*

PR7.38

Nitrogen source utilization modulates invasive growth of *Fusarium oxysporum* through changes in extracellular pH

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During infection, fungal pathogens activate virulence mechanisms such as host adhesion, penetration and invasive growth. In the vascular wilt fungus *Fusarium oxysporum* the mitogen-activated protein kinase (MAPK) Fmk1 is required for efficient root adhesion, penetration of cellophane membranes and plant infection. Previous work established that MAPK-dependent virulence functions are repressed in the presence of the preferred nitrogen source ammonium, and that this repression can be reversed by rapamycin, a specific inhibitor of the TOR kinase. Here we studied the role of the ammonium permease MepB in ammonium uptake and utilization by *F. oxysporum*, as well as its role in repression of MAPK-regulated virulence function. Mutants lacking MepB or carrying point mutations that affect ammonium transport were still able to perform root adhesion and cellophane penetration in the presence of ammonium. Similarly, deletion mutants in MeaB, a bZIP regulatory protein required for ammonium utilization, are insensitive to ammonium repression of MAPK-dependent virulence functions. In the *F. oxysporum* wild type strain, ammonium uptake from the medium resulted in a rapid decrease in extracellular pH, while this pH shift was abolished in the *meaB* and *mepB* null mutants. Addition of rapamycin did not affect ammonium uptake or the pH shift, suggesting that TOR represses virulence functions through a distinct mechanism. These studies reveal a functional link between ammonium uptake, extracellular pH and MAPK-dependent infectious growth.

PR7.39

The role of the RNA-binding protein Whi3 in control of cell division and development

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In *S. cerevisiae*, the RNA-binding protein Whi3 is required for several processes including cell division and cellular development. Initial studies provided evidence that during cell cycle progression Whi3 functions as a cytoplasmic retention factor for the CDK Cdc28-Cln3 complex. Moreover, Whi3 has been shown to bind a large set of mRNAs *in vivo* indicating that it might be a more global regulator. We have previously shown that Whi3 regulates cellular adhesion by posttranscriptional control of the dual-specificity kinase Yak1, which lies at the center of a signaling pathway for adhesion and stress response. In addition, our current data suggest that Whi3 is able to control adhesion by Yak1-independent mechanisms. We demonstrate that Whi3 controls further regulators of cell adhesion and development at the posttranscriptional level, e.g. the genes for the transcriptional regulators Flo8 and Tec1 and for the G1-specific cyclin Cln1. These data support the view that Whi3 is a more global posttranscriptional regulator for cell growth and development than previously anticipated.

PR7.40

Dynamics of MAPK signaling in *Saccharomyces cerevisiae*

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Integration of multiple signals and execution of the appropriate response are crucial properties of many signal transduction pathways. In *Saccharomyces cerevisiae*, the Fus3/Kss1 mitogen-activated protein kinase (MAPK) module differentially controls the two developmental programs of mating and biofilm formation. For induction of the mating program, a strong pheromone signal is required that strongly activates the MAPK module and leads to the transcription of mating-specific genes. In contrast, activation of the biofilm program and corresponding genes is achieved by stimulation of the MAPK module through a weak signal of unknown nature. Importantly, weak MAPK stimulation does not activate mating and strong stimulation switches off biofilm formation. Previous studies have revealed detailed insights into the topology of the MAPK module. How the dynamic behavior of module components affects the correct processing of different signals is largely unknown. In this study, we aim at understanding the dynamics of the central signaling units and the genetic circuits of the Fus3/Kss1 module. For this purpose, we have established a fluorescence microscopy-based system for quantitative measurement of MAPK signaling *in vivo* at the single cell level. In addition, we are developing mathematical models to identify critical reaction rates and threshold concentrations. These approaches should enable us to identify general regulatory principles of MAPK modules and exploit these insights for the design of synthetic signaling pathways with tailored properties.

PR7.41

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

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

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PR7.42

The major changes in the transcriptome during germination occur before isotropic growth and are not affected by the antifungal compound natamycin

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The transcriptome of *Aspergillus niger* was analysed during germination of conidia in the absence or presence of the antifungal compound natamycin. Early germination was accompanied by a reduction in cellular microviscosity and a drop in mannitol and trehalose levels. During 8 hours, normal germlings grew isotropically, followed by polarized growth (germ tube formation) and performed 1 cycle of mitosis. With 3 µM natamycin, germination was arrested at the stage of mitosis and germ tube formation and at 10 µM natamycin conidia even did not swell and accumulated some mannitol.

After 2 h of germination, upregulation in the transcriptome of natamycin treated conidia was very similar to untreated conidia, especially protein synthesis, energy and rRNA translation. Correlation of the RNA profiles between 2- and 8 h indicated that all conidia change extensively, but highest in 10 µM natamycin, despite the lack of morphological change. The RNA profiles of these cells were slightly more similar to dormant conidia then all other samples. After 2- and 8 h, natamycin-treated cells contained increased numbers of expressed genes compared to the controls in a dose-dependent manner. Transcripts of a number of protective compounds specific for dormant conidia were highly accumulated in 8 hour old treated spores. These habits have developed secondary after a large shift in the transcriptome.

All these cues point into the direction that germinating conidia that are confronted with adverse conditions have no focus on strong vegetative development, but regain relatively high stress resistance, absence of growth and prolonged survival.

PR7.43

Analysis of a putative α-carbonic anhydrase from the filamentous ascomycete *Sordaria macrospora*

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Carbonic anhydrases (CA) are ubiquitous enzymes, which catalyze the reversible hydration of carbon and protons. CAs have evolved in all three domains of life. Based on their amino acid sequence and structure, they can be divided into five distinct groups (α,β,γ,δ,ξ) which share no sequence similarity and have supposable evolved independently. All known fungal CAs belong either to the α- or to the β-class (Elleuche and Pöggeler 2010, Microbiology 156: 23-29). The filamentous ascomycete *Sordaria macrospora* encodes four carbonic anhydrases, three of the β-type, termed CAS1, CAS2 and CAS3 and one of the α-type. CAS1, CAs2 and CAS3 have been previously characterized and have been shown to be involved in spore germination, hyphal growth and fruiting-body development (Elleuche and Pöggeler 2009, PloS One: 4:e5177). Here, we show the analysis of the α-CA CAS4. We analyzed the enzyme activity of all four CAs by a yeast complementation experiment.

CAS4 exhibit a signal peptide for secretion. Using Western-Blot analysis we were able to demonstrate secretion of a Flag-tagged version of CAS4. Moreover, using fluorescence microscopy we localized a GFP-tagged-CAS4 in a net-like structure resembling the endoplasmic reticulum. To better understand the role of the *S. macrospora* CAS4 and its interplay with the three β-CAs, we generate a Δcas4 single

PR7.44

Drugs transporters in the fungus *Botrytis cinerea* during infection of treated and non-treated plants

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The grey mould fungus *Botrytis cinerea* causes losses of commercially important fruits, vegetables and ornamentals worldwide. Various fungicides with different modes of action are effective against this pathogen, but isolates with multiple fungicide resistance phenotypes have been observed with increasing frequencies.

In fungi, two major types of membrane proteins participate to drug efflux: ATP binding cassette (ABC) and major facilitator superfamily (MFS) transporters. The activity of these proteins has often been correlated with their gene transcription level, and mutations leading to over-expression of individual genes has been shown to increase export and thereby reduce sensitivity to drugs.

The objective of our work is to identify transporters involved in detoxication during infection of fungicide-treated plants. Gene expression profiling of the *Botrytis cinerea* ABC and MFS transporters genes has been investigated on non-treated and fungicide-treated plants using the Nimblegen microarray technology. First results show that at least 4 ABC transporters and 4 MFS transporters genes are differentially up-regulated, and this is confirmed by PCR analysis. This study aims at a better understanding of the putative role of the fungal efflux system in plant infection as well as in infection of treated plants.

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Poster Category 8: Biotechnology

PR8.1

Establishing a novel protein expression system in *Ustilago maydis*

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In industrial biotechnology some heterologous proteins cannot be produce with established expression systems. Thus, there is a great interest to develop novel protein expression platforms. In this project we establish a new expression system using the basidiomycete fungus *Ustilago maydis*. This eukaryotic microorganism is very well suited for genetic, biochemical, genome-wide as well as proteome-wide approaches. During filamentous growth microtubule-dependent mRNA transport plays a crucial role. The RNA-binding protein Rrm4 is responsible for long-distance mRNA transport and essential for the efficient secretion of the endochitinase Cts1. With fusions of β-glucuronidase and Cts1 we demonstrated, that Cts1 can mediate the export of active heterologous proteins. β-glucuronidase is inactivated by N-glycosylation during conventional secretion. Using this enzyme as a reporter our data reveal that Cts1 is secreted via an unconventional secretion process avoiding N-glycosylation. In addition, the lack of a canonical N-terminal secretory signal peptide in Cts1 was confirmed by analysis of truncated Cts1 variants. Circumventing N-glycosylation can be beneficial in various applications, for example to prevent undesirable immune responses in medical applications. As a proof-of-principle we successfully expressed codon optimized lipase CalB in *U. maydis* and confirmed its activity by Tributyrin plate assays. Next, we will express difficult-to produce lipases and characterize them by liquid assays.

PR8.2

Approaches for directed strain improvement targeting enhanced biosynthesis of gibberellic acid in *Fusarium fujikuroi*

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The filamentous fungus *Fusarium fujikuroi* is famous for producing high amounts of gibberellic acids (GAs). These phytohormones exhibit a great biotechnological impact as application of GAs in higher plants induces early flower bud formation and shoot elongation as well as an increased fruit size. Each year about ten tons of gibberellins are used as plant growth regulators by the agricultural and plant breeding industry.

Therefore, we developed strategies to increase GA yields by directed genetic modifications of genes involved in primary and secondary metabolism. Thus, overexpression of the first GA specific gene *Ggs2* (geranylgeranyl-pyrophosphate synthase 2), was performed. In addition, the negative feedback regulation of the key enzyme of the mevalonate pathway, *HmgR* (Hydroxy-methyl-glutaryl-CoA reductase), has been circumvented by deleting the regulatory domains. Overexpression of the truncated gene resulted in higher GA yields. Furthermore, regulation on transcriptional and protein level should be further investigated. This shall be amongst others elucidated by identification of positively or negatively acting transcription factors.

Another approach for enhancing GA-biosynthesis is to knock down a whole set of secondary metabolite pathways competing for the same precursors by deletion of the 4'-phosphopantetheinyl transferase Ppt1. This led to loss of function of all polyketide synthases (PKSs) and non-ribosomal peptide synthases (NRPSs) that are essential for many secondary metabolite syntheses. This modification altered the GA spectrum and resulted in higher GA amounts.

PR8.3

Analysis of the White-Rot Model Pleurotus *ostreatus* Secretome

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The utilization of low-value substrates such as lignocellulosic wastes offers a great potential to reduce the production costs of bioethanol. The biological process of bioethanol production using lignocellulose as feedstock requires its delignification to liberate the cellulose and hemicellulose from their complex with lignin. *P. ostreatus* is a model white rot basidiomycete that produces various ligninolytic enzymes useful in this process.

With the aim to expand knowledge about proteins involved, and through the availability of the whole genome sequence of the two monokaryotic strains PC9 and PC15 that compose the dikaroytic strain N001 obtained in the DOE JGI, we have carried out a study of the secretome of the fungus cultured on three different liquid media using wood, glucose or both as carbon source.

We have used the 2D electrophoresis technique prior to mass spectrometry analyses of the spots in order to make a first approach to the proteins secreted by the fungus. Afterwards, the use of a shot gun technique to deepen in the analysis of the proteins involved, in addition to the analysis of the computationally predicted secretome, have enabled us to compare the enzymatic profile between the monokaryons PC9 and PC15 and the dikaryon N001 and provide valuable insight into how white rot fungi degrade lignocellulosic biomass.

PR8.4

Investigating the yet-unknown biomass degrading and modifying enzymes of Aspergillus *oryzae*

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The associations of plant cell wall components with *A. oryzae*'s extracellular enzyme machinery was investigated from a chemical perspective using an integrated analysis of the transcriptome profile.

*S*train RIB40 of *A. oryzae* was cultured on various carbon sources, namely cellohexaose, mannohexaose, xylopentaose, arabinoheptaose, glucohexaose, glucosyl maltotriosyl maltotriose, galactosyl mannotriose, turanose and sophorose, and the transcribed genes were determined with DNA microarrays. The statistically significant genes were selected and novel hydrolases were identified, which would be further heterologously expressed for subsequent characterization. This work represents a novel way of integrating computational chemical biology and classical enzyme research for improving lignocellulose bioconversion.

More specifically, we aim at the heterologous expression and characterization of seven different hypothetical and non classified proteins of *A. oryzae*, which could prove to be useful tools in the wood biomass separation and modification process.

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PR8.5

Transcriptomics-based genome-scale prediction of secondary metabolite gene cluster members in *Aspergillus niger*

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The biosynthetic pathways for fungal secondary metabolites (SMs) are currently the focus of a large amount of efforts to elucidate the genetic basis of the biosynthesis. Major drivers for this effort are the large potential of fungal metabolites as bioactive compound, as well as an interest in utilizing the enzymes for synthetic biochemistry.

In an effort to alleviate the large amount of work required to identify the biosynthetic genes associated with a given SM synthase, we have previously developed a method for predicting SM clusters size from transcriptomic data, and showed it to be accurate in *A. nidulans*. In this study, we developed a new DNA microarray for *A. niger*, and employed it to build a microarray compendium of 73 samples from a diverse set of growth conditions.

Using the SMURF algorithm (Khaldi et al. 2010), we identified putative NRPS's, PKS's, hybrids and DMATs from the *Aspergillus niger* ATCC 1015 genome. This analysis yielded 81 putative SM synthases. Of those, 75 synthase genes are active in our gene expression catalog. This allowed the assignment of cluster genes for all 75 active synthases. Cluster sizes range from 15 genes (Gene ID: 118581, apparently absent in *A. niger* CBS 513.88) to one gene.

We have further employed the data set to predict cross-chemistry between physically separated gene clusters.

PR8.6

Characterization of the cyp684 gene involved in fenhexamid resistance in the species *Botrytis pseudocinerea*

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The Botrytis species complex responsible for the grey mold disease on multiple crops is composed of several species. The major one is Botrytis cinerea, the second one was called Botrytis pseudocinera. Despite their genetic polymorphism both species cannot be morphologically distinguished. However they differ for their sensitivity to several fungicides, especially to the sterol biosynthesis inhibitor fenhexamid. While B. Cinerea is sensitive to this hydroxyanilide, but can acquire resistance through target site mutations, B. Pseudocinerea is a naturally resistant species. We found a strong synergism between fenhexamid and sterol 14α-demethylation inhibitors (DMIs), especially on B. Pseudocinerea. Since DMIs inhibit Cyp51, a cytochrome P450 protein, we supposed detoxification of fenhexamid by a cytochrome P450 similar to Cyp51 to be involved in B. Pseudocinerea's resistance. The gene with the highest similarity to cyp51, named cyp684, was deleted in a B. Pseudocinerea strain. Cyp684 knock out mutants exhibit increased fenhexamid sensitivity and decreased fenhexamid metabolisation, showing that the Cyp684 cytochrompe P450 is responsible for B. Pseudocinerea's (HydR1) natural resistance to fenhexamid. Although cyp684 is also present in B. Cinerea sensitive to fenhexamid, we observed several polymorphisms: i/ in B. Pseudocinerea the cyp684 promoter shows a deletion of 25 bp, ii/ the peptide sequence varies by 4 amino acid residues between the species. We are currently establishing the cyp684 expression profiles in both species in order to analyze the impact of the promoter deletion on its expression. We will then study which part of the gene is/are responsible for fenhexamid resistance in B. Speudocinerea prior to establish its physiological and enzymatic functions.

PR8.7

Transcriptional and enzymatic profile of *Pleurotus ostreatus* multigene family in submerged and solid state fermentation

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The functional differences among the members of multigene families can be analyzed by studying their transcriptional profiles under different environmental conditions and different genotypes. The white rot fungus *Pleurotus ostreatus* can be a suitable model for genomics and transcriptomics studies in basidiomycetes due to the information available after the sequencing of the two haploid genomes composing the N001 strain. In this work, we have studied the differential regulation of the laccase gene family transcription using a RT-qPCR approach. The study has been made using different monokaryotic and dikaryotic (isogenic and non-isogenic for the growth-rate containing QTL chromosome VIII) strains, cultured in submerged cultures in the presence or in the absence of a laccase inducer, and in solid fermentation. Our results revealed (1) the importance of measuring the amplification efficiency and of carefully selecting the internal standards for the relative quantification of gene expression, (2) that the *Lacc2* and *Lacc10* genes are the responsible of laccase induction in submerged cultures, (3) that these two genes displayed opposite transcriptional response in PC9 type and PC15 type full-sibs strains, suggesting that laccase induction in submerged fermentation is linked to vegetative growth rate, (4) that the expression of these two genes increased in solid fermentation with increased water availability in the culture, (5) that the enzymatic activities and intracellular/extracellular isozyme patterns confirmed the differential behaviour of fast growing and slow growing strains, and characterized the intracellular and extracellular laccase fractions in solid and submerged cultures.

PR8.8

Real-Time Viability Assay for Fungal cells

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Detection of live microorganisms, including fungi, is currently performed by enumeration of colony forming units (CFUs) on agar plates. Although reliable and widely accepted, this method has disadvantages. Determination of CFUs is timely, laborious and limited to readily culturable microorganisms. Cultivation-independent methods are available nowadays, but they mostly involve multiple incubation steps for assessment of the number of viable cells and do not discriminate between dead or live microorganisms. Here a novel method is presented, capable of specifically monitoring living fungal and yeast cells (and bacteria too) in a real-time manner.

The assay is based on a novel viability criterium, the ability of cells to maintain a neutral pH in an acidic environment. Therefore, we searched for probes that show a fluorescent signal in a neutral environment, and not in an acidic environment. Since fluorescence is only produced in cells with a neutral pH (*i.e.* living cells), the fluorescent intensity is a measure for the amount of viable cells.

We have identified a number of probes that allow real-time viability (RTV) assays of fungal samples. These probes have succesfully been tested on *Aspergillus niger* and *A. fumigatus*, *Saccharomyces cerevisiae*, and *Candida albicans*. Based on these results it is expected that the RTV assay will work for other fungal species too. The RTV assay for fungi opens ways to assess in a fast, automated manner the viability in fungal samples such as spore batches, anti-fungal treated fungal cells and all other applications where viability of fungal cells are of interest.

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PR8.9

Hydrophobin fusions for high level intracellular protein production and purification in *Trichoderma reesei*

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Insufficient accumulation levels and the lack of efficient purification methods constitute two major bottlenecks hindering the recombinant protein production. Low yields are often seen in the cases where the host is distantly related to the organism from which the product is derived. Microbial production systems where the product is secreted to the medium are hampered by host proteases, which can destroy the target protein. Secretion in eukaryotic cells is finely tuned, and production is frequently limited by inefficient secretion of exogenous proteins. Microbial intracellular protein production, on the other hand, often results in aggregation of the denatured product in inclusion bodies, from which the active conformation is difficult to recover. Hydrophobins are small amphipathic proteins ubiquitously expressed in filamentous fungi. Hydrophobins are capable of altering the hydrophobicity of their respective fusion partner to enable purification by surfactant-based aqueous two-phase separation (ATPS). We have demonstrated that hydrophobin fusions targeted to endoplasmic reticulum (ER) induces formation of large intracellular protein bodies in *Trichoderma reesei*. The fusion protein remains soluble in the protein bodies surrounded by the ER-membrane and can be easily recovered from the cell lysate by ATPS. It is hypothesized that packing of hydrophobin fusions into these protein bodies may exclude the recombinant protein from the host proteolysis, simultaneously protecting the host cell from toxic effects of massive intracellular accumulation of the target protein. The implications of these results in development of novel strategies for production of recombinant proteins will be discussed.

PR8.10

Application of an optimized FLP/*FRT* recombination system in diverse filamentous fungi

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A molecular tool for marker recycling was established to overcome the limited availability of resistance markers in filamentous fungi. For this purpose the FLP/*FRT* recombination system from *Saccharomyces cerevisiae* was optimized for the penicillin producer *Penicillium chrysogenum*. In a first approach, we used a two-step strategy to test the functionality of the system. Therefore we generated a nourseothricin resistance cassette flanked by *FRT* sequences in direct repeat orientation (*FRTnat1* cassette) and ectopically integrated this construct into a *P. chrysogenum* recipient strain. In a second step a codon-optimized *Pcflp* recombinase gene were transferred into the *P. chrysogenum* strain, carrying the *FRTnat1* cassette. We observed in several tested transformants the successful recombination event due to the use of a codon-optimized recombinase. To further extend the application of the FLP/*FRT* recombination system, we generated a marker-free ΔPcku70FRT2 strain which enables the production of multiple deletion strains by highly efficient homologous recombination. Moreover a *nat1* flipper was generated to establish a one-step marker recycling. Therefore the FLP/*FRT* system and the *nat1* marker gene were combined in a single construct. For induction of the recombinase gene expression we used the *xyl* promoter. In further experiments we will use different flipper cassettes together with the ΔPcku70FRT2 strain to construct marker-free double and triple mutants.

Moreover the applicability of the developed tool was demonstrated by marker recycling in the ascomycetes *Sordaria macrospora* and *Acremonium chrysogenum* indicating, that the optimized FLP/*FRT* recombination system is suitable to a broad range of filamentous fungi.

PR8.11

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

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Fungi secrete enzymes to convert organic matter into small molecules that can serve as nutrients. Hyphae at the periphery of the colony are exposed to unexplored organic material, whereas the center of the colony experiences a utilized substrate. This suggests that the enzymes that are secreted by different zones in the colony are different. *Aspergillus niger* is an important cell factory for the industrial production of enzymes. Here, we determined with stable isotope dimethyl labeling the secretome of 5 concentric zones of 7-day-old xylose-grown colonies of *A. niger* that had either or not been treated with cycloheximide. As expected, cycloheximide blocked secretion of proteins at the periphery of the colony. Unexpectedly, protein release was increased by cycloheximide in the intermediate and central zones of the mycelium when compared to non-treated colonies. Electron microscopy indicated that this is due to partial degradation of the cell wall. A total of 124 and 59 proteins were detected in the medium of xylose grown colonies that had or had not been treated with cycloheximide. Apparently, a major part of the proteins are associated with the cell walls of *A. niger*. Taken together, cycloheximide can be used to obtain a (near) complete secretome of *A. niger*. Moreover, the total amount of protein is increased upon treatment with this antibiotic. The composition of the secretome in each of the 5 concentric zones differed. This study thus describes spatial release of proteins in *A. niger*, which is instrumental in understanding how fungi degrade complex substrates in nature.

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PR8.12

D-xylose Concentration-dependent Hydrolase Expression Profiles and the According Role of CreA and XlnR in *A. niger*

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Aspergillus niger is an industrially important organism for the production of industrial enzymes like hemicellulases and pectinases. The xylan-backbone monomer D-xylose is known as an inducing substance for the coordinate expression of a high number of polysaccharide-degrading enzymes. In this study a total number of 22 genes, which encode enzymes that function as xylan backbone-degrading enzymes, accessory enzymes, cellulose-degrading enzymes, or enzymes involved in the pentose catabolic pathway in *A. niger* have been investigated concerning their response to low (1 mM) and high (50 mM) D-xylose concentrations. Notably, genes encoding enzymes that have similar function (*e.g.* xylan backbone-degradation) respond in a similar way to different amounts of D-xylose. Although low D-xylose concentrations provoke - in particular for hemicellulase-encoding genes - highest transcription response, transcript formation in presence of high amounts of D-xylose was also observed. It even turned out that a high D-xylose concentration is favourable for certain groups of genes. Furthermore, the repressing influence of CreA on the transcription of a selection of these genes was observed on D-xylose, regardless whether low or high amount of D-xylose is used. Interestingly, the decrease in transcription of certain genes on high D-xylose concentrations is not reflected by transcription of their activator XlnR. Regardless of the D-xylose concentration applied and whether CreA was functional or not, *xlnR* was constitutively expressed at a low level.

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PR8.13

The *de novo* designed antifungal hexapeptide PAF26 is internalized by endocytosis prior to killing fungal cells

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Natural and synthetic antimicrobial peptides (AMPs) provide promising alternatives for the control of microbial pathogens. PAF26 is a *de novo*-designed hexapeptide, cationic tryptophan-rich that has been shown to cross the plasma membrane and be fungicidal against pathogenic fungi. In the present work, the mechanism of internalization of PAF26 has been characterized in detail using the model fungus *Neurospora crassa*. PAF26 possesses two well-defined motifs: a N-terminal cationic and a C-terminal hydrophobic regions. We have shown how these motifs are independently responsible during the three steps of the PAF26 action involving its: (a) electrostatic interaction with cells, (b) cellular internalization; and (c) intracellular toxicity. Live-cell imaging of fluorescently labelled PAF26 and organelle probes, and mutant analyses indicate that it is endocytically internalized at low fungicidal concentrations. PAF26 initially accumulated in vacuoles that expanded, and then was actively transported into the cytoplasm, which coincided with cell death. Deletion mutants of the endocytic proteins RVS-161, RVS-167 and RAB-5 exhibited reduced rates of PAF26 internalization and fungicidal activity. Pharmacological experiments with live-cell probes showed that PAF26 internalization and antifungal action were energy-dependent, primarily actin-mediated, disrupted intracellular calcium homeostasis, and also induced rapid plasma membrane depolarization. PAF26 antifungal activity at low concentrations was shown to rely on its endocytic internalization. However at high fungicidal concentrations, PAF26 internalization was energy-independent and involved passive translocation. Our results provide new mechanistic insights into the mode-of-action of cell penetrating AMPs and for the rational design of more effective PAF26-based AMPs.

PR8.14

Molecular and chemical characterization of secondary metabolite gene clusters in *Fusarium fujikuroi*: the fusarin gene cluster

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The filamentous fungus *F. fujikuroi* is known to produce a variety of structurally diverse secondary metabolites such as the plant hormones gibberellins which cause enormous economical losses in trade of crops. In order to reduce the health risk of mycotoxins in food, feed and biotechnologically produced gibberellin preparations, identification of mycotoxin biosynthesis genes is of great importance. The recently sequenced genome of *F. fujikuroi* contains 16 polyketide synthases (PKS). So far only four of them can be linked to specific products: bikaverin, fusarin C, fumonisin and fusarubin. The focus of this work is studying the biosynthesis and regulation of the mutagenic mycotoxin fusarin C.

Since now only the hybrid polyketide synthase/nonribosomal peptide synthetase (PKS/NRPS) gene from the fusarin C cluster in *F. venenatum* is known. Here we present the characterization of the entire fusarin C gene cluster in *F. fujikuroi* by generating deletion mutants of each single cluster gene including the PKS/NRPS-encoding gene. By using these mutants, we are identifying the intermediates to finally unravel the entire biosynthetic pathway. In addition, we have created a deletion mutant missing all cluster genes except for the PKS/NRPS key enzyme gene to identify the first intermediate in the fusarin C pathway.

Besides, we study the regulation of gene expression for fusarin pathway genes by external signals, such as nitrogen availability and pH and the involvement of potential transcription factors and global regulators such as AreA, AreB, PacC and Velvet.

PR8.15

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

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

PR8.16

Analysis of a New Secondary Metabolite Gene Cluster in *Fusarium fujikuroi*

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The filamentous fungus *Fusarium fujikuroi* is best known for its production of gibberellins (GAs), natural phytohormones that lead to hyperelongation and chlorosis in rice plants. Besides the production of GAs, *F. fujikuroi* produces a wide range of other secondary metabolites, such as the polyketide-derived mycotoxin fusarin C or bikaverin.

The recently sequenced genome of *F. fujikuroi* revealed 16 polyketide synthases (PKSs) of which so far only a few can be assigned to their respective product. Here we present the discovery and subsequent investigation of an almost forgotten group of polyketides belonging to the fusarubin (FSR) family in *F. fujikuroi*. The corresponding gene cluster was identified and the regulatory network that governs FSR production has been studied. Our results so far indicate a rather complex regulation, including the importance of the initial pH, the nitrogen availability and the controverse involvement of Velvet and G-Protein mediated signaling. This complex regulatory network leads to an even more complex accumulation of the various FSR derivatives, of which the predominant products were determined using different chemical approaches. In addition, single deletion mutants of all *fsr* cluster genes revealed the biosynthetical pathway leading to the formation of the FSR derivatives. Phylogenetic analyses showed close homologies of the PKS Fsr1 to Pgl1 from *F. graminearum* and *F. verticillioides*, indicating the involvement of these pigments in the coloration of the fruiting bodies. Sexual crosses confirmed that the FSR pigments are the so far unknown perithecial pigments in *F. fujikuroi*.

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PR8.17

ChemoGenomics: Discovery of novel fungicides and their targets in the phytopathogen *Fusarium graminearum*

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Chemical genetics screen is based on the ability of small chemical molecules to bind to biological molecules and alter their function. Screening of pharmaceutical libraries has revealed novel molecules effective against cancer and other diseases. We have adopted similar approach and identify bio-active compounds that will block the growth and development of *F. graminearum*. We have developed a 96-well format to monitor the growth *of F. graminearum* in liquid media. The fungus is tagged with a green fluorescent protein (GFP) and the growth is monitored by the measurement of fluorescence of the GFP. This format facilitates high throughput screening for small molecules that could potentially disrupt the growth of the fungus. As proof of concept, we screened ~560 compounds from theTimTec NDL-3000 natural product collection (TimTec LLC,Newark,DE,USA) and identified several compounds with anti-Fusarium properties.

One compound identified form our screen, “Antofine” was purified from *Vincetoxicum rossicum* and was used in subsequent studies, to identify targets in the fungus. We used the gene deletion library of the budding yeast *Sacchromyces cerevisiae* to identify targets for Antofine. Twenty two potential targets of Antofine were identified and GeneMANIA (http://www.genemania.org), an online multiple association network integration algorithm was used to uncover information pertaining to genetic and physical interactions of these targets. Our efforts to identify targets in Fusarium against Antofine will be discussed

PR8.18

Efficient plant biomass degradation by thermophilic fungus *Myceliophthora heterothallica*

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Rapid and efficient enzymatic degradation of plant biomass into monomeric sugars is currently a major challenge for sustainable production of biochemicals and biofuels. The best studied and most widely used plant-degrading enzymes are produced by *Trichoderma* and *Aspergillus* species, and they are most effective over a temperature range of 40 – 50°C. As a consequence, these moderate temperatures have long reaction times for complete saccharification of plant biomass. It would therefore be desirable to have elevated hydrolysis temperatures using thermostable enzymes. The collection at the CBS Fungal Biodiversity Centre contains several thermophilic fungi, which produce thermostable enzymes up to 70-80°C.

A screening of 32 thermophilic species resulted in several candidates with interesting plant-degrading enzymes. Particularly the genus *Myceliophthora* contains isolates with rapid growth on complex polysaccharides. We elucidated the phylogeny of *Myceliophthora* isolates and distinguished 10 different species, of which four are thermophilic. The isolates with the fastest growth on crude plant material were divided in two species: *M. thermophila* and *M. heterothallica*. The new phylogenetic classification of *M. heterothallica* isolates was further supported by physiological differences between the two species. Also, in contrast to *M. thermophila* isolates, *M. heterothallica* has a functional sexual cycle. *M. heterothallica* isolates were studied in detail for their ability to release sugars from crude plant biomass. Furthermore, crossing experiments between *M. heterothallica* isolates resulted in offspring with an even higher potential in rapid and efficient enzymatic degradation of plant biomass.

PR8.19

Effective production of Itaconic Acid in A. *niger*

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Itaconic acid is an important building block for the chemical industry that can be produced from sugars in a fermentative process. Currently, *Aspergillus terreus* is most frequently used for the commercial production of itaconic acid. The itaconic acid production pathway in *A terreus* is similar to the citric acid pathway in *Aspergillus niger*. Citric acid is the precursor for *cis*-aconitic acid which can be converted to itaconic acid by decarboxylation. Itaconic acid in *A. terreus* is produced upto a concentration of 80 g/L while citric acid production in *A. niger* reaches concentrations over 200 g/L which show the enormous potential of *A. niger* as a production host for itaconic acid. However, the key-enzyme *cis*-aconitic acid decarboxylase (CadA) in the itaconic acid production pathway is lacking in *A. niger*. Within the genome of *A. terreus* the *cadA* gene is flanked by two putative transporters, a mitochondrial transporter and a plasmamembrane transporter. The expression of the *cadA* gene in an *A. niger* strain optimized for citrate production resulted in the production of itaconic acid. The amount of itaconic acid produced by *A. niger* is further improved by using a codon-optimized version of the *cadA* gene. Still, significant amounts of citrate were produced suggesting that the conversion to itaconic acid is not very efficient. To improve the efficiency and to increase the itaconic acid production both putative transporters are introduced. Introduction of the mitochondrial transporter strongly increased the itaconic acid production.

PR8.20

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

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

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PR8.21

Genetic And Molecular Characterization Of The *Penicillium chrysogenum* PcrsmA Gene, Encoding A Homologue Of The *Aspergillus nidulans* bZIP Transcription Factor RsmA

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Penicillium chrysogenum is the major industrial producer of the ß-lactam antibiotic pencillin. The regulation of cellular processes like secondary metabolism as well as morphogenesis and development in this filamentous fungus is controlled by components of the so called velvet-like complex which was first described in *Aspergillus nidulans*. Previous studies identified *rsmA* (remediator of secondary metabolism) in *A. nidulans* which after overexpression was shown to remediate secondary metabolism defects in knockout strains of the velvet components LaeA and VeA. Hence, it is believed that the putative bZIP transcription factor RsmA may act as a positive regulator of many secondary metabolite pathways. Bioinformatics confirmed the existence of many known and putative bZIP proteins from several fungi such as *P. chrysogenum* and higher eukaryotes which show high homology to RsmA from *A. nidulans*.

Until now, little is known about the biosynthetic regulation of the ß-lactam antibiotic penicillin. Thus, further investigation of putative regulators of secondary metabolism such as PcRsmA, the *P. chrysogenum* homologue to RsmA is necessary to extend the current knowledge of the regulatory network controlling both penicillin biosynthesis and morphogenesis in *P. chrysogenum*. A Δ*Pcku70* strain as recipient for homologous recombination together with the FLP/*FRT* recombination system were used to generate a marker-free Δ*PcrsmA* strain for functional and morphological characterization of PcRsmA. Our results will support deciphering of regulatory networks related to the velvet-like complex in *P. chrysogenum*.

PR8.22

Contamination of peripheral venous catheter associated fungal biofilms

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Introduction: In the hospital, the use of venous catheters for the administration of drugs exposes patients to increased risk for fungal contamination. Yeasts can form biofilms on the surface of catheters. These properties give them resistance to antifungal agents. In an attempt to integrate this clinical reality, we undertook a study in the neonatal unit of EHS-Tlemcen, for aim to isolate yeasts from venous catheters directly after excision of newborns hospitalized. The approach is to test the ability of yeasts isolated to form biofilms and to test their resistance against amphotericin B.

Materiels and Methods: These samples were taken from implanted venous catheters for 72 hours or more. They are removed directly from patients and placed in Sabouraud liquid medium.The tubes were then agitated in a vortex for 1 minute. Purified strains were identified by API Candida (Biomerieux,France)

Results: From 281 samples, 15 yeasts were isolated, colonizing venous catheters implanted in newborns. *Candida albicans, Candida parapsilosis, Cryptococcus néoformans , Candida famata, Trichosporon spp.* and *Saccharomyces cereviseae* were isolated. C*andida* and *Cryptococcus* species have the ability to form biofilms

Conclusion: Sessile *Candida* species and *Cryptococcus neoformans* isolates were less susceptible than the planktonic populations to AmB.

These results suggest contamination of venous catheters with strains isolates able to form biofilms may be associated with infection.

PR8.23

A *velvet*-like complex in *Penicillium chrysogenum*: the two faces of PcVelC

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The filamentous fungus *Penicillium chrysogenum* is the main industrial producer of the pharmaceutically relevant beta-lactam antibiotic penicillin. All three biosynthesis genes are found in a single cluster and the expression of these genes is known to be controlled by a complex network of global regulators. Recently, the velvet complex containing several global regulators of secondary metabolism was described for the model fungus *Aspergillus nidulans*. Next to the founding member VeA, several other velvet-like proteins were meanwhile identified in *A. nidulans* and many other filamentous fungi.

Here we provide a functional analysis of a *velvet*-like complex in *P. chrysogenum* with structurally conserved components that have distinct developmental roles, illustrating the functional plasticity of these regulators in genera other than *Aspergillus*. Data from penicillin bioassays, quantification of conidiospores as well as detailed microscopic investigations of these knockout mutants clearly show that all velvet-like proteins are involved in secondary metabolism and other distinct developmental processes. Interestingly, the velvet-like protein PcVelC seems to be a major regulator of penicillin biosynthesis and conidiation. By protein-protein interaction studies using bimolecular fluorescence complementation, tandem-affinity purification and yeast two-hybrid, we want to extend the analysis of the velvet-like complex in *P. chrysogenum*. These analyses will focus on the velvet-like protein PcVelC to elucidate its opposing roles in the regulation of penicillin biosynthesis and conidiation. Our results widen 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.

PR8.24

New hemicellulolytic enzymes for bioethanol production

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Hemicellulolytic enzymes such as endoxylanases from glycosyl hydrolysis family 11 (GH11) and 10 (GH10) have a major importance in several industrial application such as bioethanol production. They improve the yield of monosaccharides from plant biomass by depolymerization of hemicellulose. This will expand the set monosaccharides available for conversion to bioethanol by yeast.

Most commercially applied fungal endoxylanases are obtained from *Aspergillus* or *Trichoderma* species. However, comparison of fungal genomes identified several species that are much richer in genes encoding these enzymes. One of them is *Podospora anserina*, which has a saprobic life style, and is only found in dung of herbivores [1]

A growth profile of *P. anserina* on various substrates shows a high ability to growth in substrates that are rich in hemicellulose such as wheat straw and Arundo donax. Both substrates are commonly used in ethanol production. Using a combination of comparative genomics and phylogeny new GH10 and 11 enzymes were selected to increase the efficiency of ethanol production.

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PR8.25

Genetic and biochemical investigations of natural product formation in *Boletales*

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The release of an increasing number of basidiomycete genomic sequences provides valuable insight into their capacity to biosynthesize small molecule natural products. The *Boletales* are represented by *Serpula lacrymans* (dry rot fungus) and *Paxillus involutus* (roll rim mushroom). Assessed by the number of genes encoding polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPSs) in these representative genomes the *Boletales* include particularly prolific producers of secondary metabolites.

This poster presents our current work on genera within the *Boletales* focusing on NRPSs and quinone synthetases, i.e., NRPS-like enzymes. Methodically, we relied on a combined approach of genetic and biochemical methods, complemented by liquid chromatography.

PR8.26

***Aspergillus clavatus* as a potential enzyme source to use in biomass degradation to produce second-generation ethanol: Cloning and expression of hemicelulases after secretomic analysis using different pre-treatment sugar cane bagasse**

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Aspergillus clavatus is a cosmopolitan fungus that had its genome sequenced by The Institute for Genomic Research. A detailed comparative genomic analysis using a large number of fungi database showed that this microorganism is a potential enzymes producer. In addition, there are few studies about this fungus. The plant cell wall polysaccharides are being considered as source of renewable energy such as biofuels owing to the fact that they are the most abundant reserves of carbon. It can be conveniently divided into three groups namely cellulose, pectin and hemicellulose, with hemicellulose being the second most abundant biopolymer component of plant cell wall and composed by xylan, arabinan, mannan, glucomannan, galactomannan, and glucogalactomannan. Due to its complexity a large set of enzymes are necessary to degrade the plant cell wall. In order to study potential enzymes directly involved in degradation of the most abundant brazilian biomass we report in this work, the comparative analysis of the *A. clavatus* secreted in 5 different pre-treatment sugar cane bagasse using glucose as the control, cloning and expression of hemicellulases. The proteomic analyses have identified 135 different proteins where 2% of those are enzymes related with biomass degradation. The relative difference reflect the necessity to use specific enzymatic pool to degrade different pre-treated sugar cane bagasse, because of diference in the sugar compositions. In addition, to perform the heterologous protein expression of *A. clavatus* hemicellulase we used our *Aspergillus nidulans* expression system and the recombinant enzymes were highly expressed and secreted to the culture medium.

PR8.27

Molecular diversity of *Cercospora zeina* on maize in South Africa

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Grey Leaf Spot is a prevalent worldwide maize disease of global economic importance, and is specifically caused by *Cercospora zeina* in South Africa. In order to implement control measures, it is important to understand the population diversity of the fungus. As no sexual stage of *C. zeina* has been observed, we hypothesised that there would be greater variability among isolates from different populations rather than within a population in South Africa. In order to address this question, we analysed *C. zeina* isolates from GLS infected maize from three regions within South Africa viz. Machadodorp, Greytown and Cedara. We collected 40 GLS infected maize leaves from each region, and isolated and maintained cultures from 40 single *C. zeina* conidia from each area. DNA was isolated from fungal cultures, and a subset of 30 isolates from different regions was screened with 36 Simple Sequence Repeats (SSRs) designed from the genome sequence of a US *C. zeina* isolate. Twelve SSRs were found to display polymorphisms across isolates, and six were selected to score samples against. The amplified SSRs were analysed on agarose gels and scored for variability to assess the diversity amongst isolates within South Africa. From these results we could determine that there was variability within and between isolates from different regions. Thus, we observed greater diversity within *C. zeina* populations than originally anticipated.

PR8.28

Identification And Biochemical Characterization Of Putative miRNAs In The Penicillin Producer *Penicillium chrysogenum*

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Since the early 1990s small non-coding regulatory RNAs were identified in a variety of different eukaryotic organisms. In fungi, the transient inactivation of gene expression by homologous sequences was first observed in *Neurospora crassa*. The effect of short interfering RNAs (siRNAs) is mediated by the RNA-induced silencing complex (RISC). Besides the class of exogenous siRNAs a previous study shows that there is another class of regulatory small RNAs, which interact with RISC in filamentous fungi. These endogenous small RNAs are derived from RNA hairpin structures of RNA polymerase II transcripts and show typical characteristics of microRNAs (miRNAs), which play an important role in the regulation of gene expression in plants and metazoan.

The aim of this study is to investigate whether miRNA-like regulatory RNAs can be detected in the penicillin producer *Penicillium chrysogenum*. We performed an *in silico* analysis, based on RNA next-generation sequencing data, to predict putative miRNAs hairpin structures. By this approach, sequences with familiar characteristics of previously identified miRNA-precursors could be identified. To confirm the *in silico* predictions, transcript analysis were done *in vitro*. The statements of the predictions and the results from the transcript analysis confirmed the existence of the small RNAs and their precursors. To provide evidence for a regulatory activity of the putative miRNA-like sequences, mRNA-targets were chosen and inducible overexpression constructs of the miRNA-like sequences were generated. The results of this study suggest the existence of a miRNA based silencing mechanism in *P. chrysogenum*.

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PR8.29

Secretion of Client Proteins in Aspergillus

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Production of pure and high-yield client proteins is an important technology that attends the needs for industrial applications of enzymes as well as basic experiments such as protein crystallization. Client protein expression platforms are available in *Escherichia coli* and the methylotrophic *Pichia pastoris* that result in proteins released to the intracellular cell extract and extracellular medium, respectively. Fungi are utilized in industrial protein production because of their ability to secrete large quantities. In this study we engineer a high-expression-secretion vector, pEXPYR that directs proteins towards the extracellular medium in two *Aspergillii* host strains, examine the effect of maltose overexpression, production time and pH-dependent protein stability in the medium. We describe five client proteins that accumulated 50-100 mg of protein per liter and only one protein was secreted at low quantities. We also test a recyclable genetic marker that allowed secretion of multiple client proteins, enabling the design of an enzyme activity set.

PR8.30

Functional characterisation of Cytochrome P450 genes from the wheat leaf pathogen *Mycosphaerella graminicola*

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The Ascomycete fungus *Mycosaphaerella graminicola* (Anamorph *Septoria tritici*) causes the disease Septoria tritici blotch (STB) in wheat. This pathogen is typical of temperate, high-rainfall environments and is of major international importance owing to its ability to substantially reduce agricultural yields. *M. graminicola* infects both hexaploid bread wheat (*Triticum aestivum*) and tetraploid durum wheat (*T. turgidum*) and is largely controlled by fungicide applications. However *M. graminicola* has evolved resistance to several classes of fungicides highlighting the continued need to identify novel targets for disease intervention.

Little is known about fungal metabolism during plant infection. The cytochrome P450 (CYP450) gene superfamily represents an ideal target for further investigation. Members of this group are (1) known to be present and regulated together with clusters of other genes important for the synthesis of secondary metabolites including mycotoxins, pigments and defence compounds; (2) known to act directly upon potentially harmful xenobiotics such as plant defence compounds and potentially fungicides in order to detoxify them; and (3) known to operate in various cellular locations. As a preliminary to this project, Solexa next generation transcriptome sequence analyses were performed at different time points post inoculation of wheat leaves with the fungus. Analysis of expression of members of the CYP450 gene family has identified many which are specifically expressed early during plant infection and some which may reside within secondary metabolite clusters. These will be further investigated initially through the generation of fungal CYP450 gene deletion strains. It is anticipated that this project will provide new insights into the genetic basis underlying the metabolic changes occurring during *M. graminicola* infection of wheat.

PR8.31

Mutation of Genes *areA*, *wcoA*, *cryA* and *acyA* Affect The Regulation of Fusarin Production in *Fusarium fujikuroi*

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Fusarium fujikuroi is a rice pathogen that produces a diversity of secondary metabolites, that includes terpenoids with biotechnological applications, as carotenoids and gibberellins, and potentially harmful polyketides, as bikaverin and fusarins. In contrast to other compounds, very limited information is available on the biosynthesis and regulation of fusarins in this species. We formerly reported than fusarin production depends on nitrogen availability and is negatively affected by light. To learn more about the genetic basis of this regulation, we have studied the fusarin-producing pattern of targeted mutants on several regulatory genes affecting the synthesis of other metabolites. The loss of the global regulator of the nitrogen metabolism AreA affects fusarin production, but the effect varies depending on the nitrogen source. Our study was extended to two photoreceptor genes, encoding the White Collar protein WcoA and the DASH-cryptochrome CryA, and the gene for the adenylyl cyclase AcyA, that mediates the synthesis of the regulatory signal cAMP. The four classes of mutants investigated exhibit different alterations in the accumulation of fusarins, indicating that this pathway is under control of the regulatory network involved in nitrogen-regulated secondary metabolism. Despite the participation of two putative photoreceptors, the effect of light is mainly explained by light-induced instability of the secreted fusarins.

PR8.32

CefR* Acts As A Regulator Of β-Lactam Transporters In *Acremonium chrysogenum

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Acremonium chrysogenum is an ascomycete filamentous fungus which has been used for the industrial production of cephalosporin-like antibiotics. Our previous investigations about the cephalosporin C biosynthetic pathway led us to discover three genes, *cefT*, *cefP* and *cefM* involved in the secretion of intermediates and therefore in the compartmentalization of this pathway. The genes *cefP* and *cefM* codify membrane proteins that carry the intermediates isopenicillin N and penicillin N, respectively, through the peroxisomal membrane. *CefT* protein takes part in the secretion of hydrophilic β-lactams through the plasma membrane.

However, the regulation of the secretion of the intermediates and the final product of cephalosporin C biosynthetic pathway is still unknown. For this is a reason a search for regulator genes within the early biosynthetic cluster of cephalosporin C was made. A new ORF was found encoding a protein (CefR) which shows homology with other regulatory proteins and bears a “Fungal_trans” domain, characteristic of many fungal regulators. Targeted inactivation of *cefR* diminishes and delays the cephalosporin production but increases the penicillin N secretion. On other side, the overexpression of *cefR* decreases the secretion of penicillin N, preventing the loss of intermediates and then inducing the cephalosporin C production. Northern blot analysis revealed that CefR protein works as a repressor of *cefT* and *cefM* genes, making possible the use of these intermediates in the synthesis of cephalosporin C. In summary, CefR protein represents the first example of a regulator of β-lactam transporters described in *A. chrysogenum.*

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PR8.33

PenV Is A Vacuolar Membrane Protein Related To The Penicillin G Biosynthetic Pathway In Penicillim chrysogneum

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The penicillin G (PenG) biosynthetic pathway is compartmentalized in Penicillium chrysogenum, taking place in the cytosol, where δ-(L-α-aminoadipil-L-cysteinil-D-valine) (ACV) and isopenicillin N (IPN) are formed, and inside peroxisomes, where PenG is synthesized. Transport processes linked to this compartmentalization are largely unknown, but most of them would be mediated by transporter membrane proteins. During the search for this kind of transporters, was found the protein PenV showing a 42% of identical amino acids with the IPN transporter CefP, described in Acremonium chrysogneum. To elucidate the function of the protein PenV, the encoding gene, penV, was silenced by the mechanism of small interfering RNAs. As a consequence of the silencing process an alteration of the transcription levels of the β-lactam biosynthetic genes occurs, together with a drastic decrease of the yield of PenG and the intermediates ACV and IPN. The silencing process also causes alterations in several developmental aspects. The subcellular location of PenV was determined through the expression of the red fluorescent protein PenV-DsRed in the strain Wisconsin 54-1255. Microscopy analysis revealed the presence of PenV, not in peroxisomes but in the vacuolar membrane. Curiously, non ribosomal peptide synthetases appear to be linked to the cytosolic side of the vacuolar membrane. Given these results is purposed the involvement of PenV in the transport of amino acids from the vacuolar lumen to the cytosol, where they would be used as substrates for the synthesis of structural proteins and as precursors of the β-lactam biosynthesis.

PR8.34

The program of iterative fungal PKS-NRPSs

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Fungal hybrid polyketide synthase-non ribosomal peptide synthetases (PKS-NRPSs) are huge enzymes that carry out a large number of chemical reactions in the biosynthesis of the natural product. Examples of natural products are tenellin **1**, desmethylbassianin **2** and bassianin **3** produced by different Beauveria strains. Current knowledge about the programming of these enzymes is very limited. Important questions as how the chain length of a polyketide or the methylation pattern is encoded are as yet unanswered.

Tenellin synthase (TenS) and desmethylbassianin synthase (DMBS) produce together with their tailoring enzymes the similar yet not identical natural products **1** and **2**. These natural products differ in chain length and in methylation pattern. The domain architecture of both enzymes is identical, i.e. KS-AT-DH-CMet- ER^0-KR-ACP-C-A-T and homology is over 85%. Domain swaps between different PKS-NRPSs often fails presumably to incompatibility of protein structures. Here we present the successful swap of domains from two highly similar enzymes.

The poster covers domain swap strategy and outcome. It will also outline the use of the domain swap technology to resurrect the extinct compound **3**

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PR8.35

Three acidic residues Glu31, Asp142 and Asp171 of *Aspergillus oryzae* cutinase CutL1 are required for both interaction with hydrophobin RolA and consequent stimulation of polyester-degradation.

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Hydrophobins are amphipathic proteins, and are ubiquitous among filamentous fungi. When the industrial fungus *Aspergillus oryzae* is grown in a submerged medium containing a biodegradable polyester polybutylene succinate-coadipate (PBSA) as a sole carbon source, cutinase CutL1 and hydrophobin RolA are simultaneously secreted into the medium. RolA attached to the surface of PBSA particles specifically recruits CutL1, resulting in stimulation of PBSA hydrolysis (1). In our previous study, we identified amino acid residues involved in the RolA-CutL1 interaction by means of chemical modification and site-directed mutagenesis of RolA and CutL1. As a result, we found that His32 and Lys34 of RolA and Glu31, Asp142, Asp171 of CutL1 are involved in the RolA-CutL1 interaction. In the present study, to quantitatively elucidate the role of the three acidic amino acid residues of CutL1 in the RolA-CutL1 interaction, we characterized kinetics of the interaction between CutL1 variants of the three residues and wild type RolA by using Quartz crystal microbalance (QCM). The QCM analysis revealed that replacement of the three acidic amino acid residues of CutL1 to serine caused increases in K_D values for interaction with RolA. In conclusion, Glu31, Asp142 and Asp171 of CutL1 are critically required for the RolA-CutL1 interaction by multivalent effect.

(1) Takahashi et al. Mol Microbiol. 57:1780 (2005)

PR8.36

A New Method of Increasing the Hydrolytic Activity of *T.atroviride*

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Trichoderma species are widely used as biological control agents due to their strong antagonistic activity against phytopathogenic fungi and effectiveness in simultaneously promoting plant growth and defense mechanisms. Biocontrol efficiency may result from a direct interaction between the pathogen and *Trichoderma* when lytic enzymes secreted by the latter degrade the cell wall of the pathogen, causing damage of its cells.

It was observed that many, if not all, lytic enzymes secreted by *Trichoderma* were glycosylated. It was postulated that O-glycosylation of these enzymes was closely correlated with their secretion. Taking into account this correlation we decided to improve the biocontrol abilities of *T. atroviride* P1 against plant pathogens by activation of the mevalonate pathway; in this pathway dolichyl phosphate, a carrier of carbohydrate residues in the glycosylation processes, is produced together with many other biologically active molecules, such as sterols, terpenoids and quinones. The new strains of *T. atroviride* were characterized in terms of the activity of overexpressed enzymes, protein secretion, activity of secreted hydrolases and antifungal properties. Our results showed that activation of the mevalonate pathway could result in higher antifungal activity of the studied new strains.

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PR8.37

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

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Trichoderma reesei is a soft rot Ascomycete fungus able to secrete enzymes extremely efficiently. Availability of the complete genome sequence of *T. reesei* has made it possible to utilise genome wide methods to study protein production by the fungus and to utilise the information obtained to develop new strains with better enzyme production qualities. In order to study the co-expression of enzyme genes, a complete list of CAZymes of *T. reesei* needed to be obtained. Novel CAZymes were searched from the genome by mapping *T. reesei* proteome with Blast search to the protein sequences of the CAZY database. New annotation was given to several genes in order to gain more information on the possible function of novel candidate genes and to specify the annotation of previously identified genes. A phylogenetic approach was used to reveal the functional diversification of *T. reesei* enzyme genes within CAZY families and between the gene duplicates. Expression of the hydrolytic system of *T. reesei* Rut-C30 was studied by cultivating the fungus in the presence of different lignocellulose substrates. Cultures were subjected to transcriptional profiling using oligonucleotide microarrays. Differentially expressed genes were identified and expression profiles of genes encoding lignocellulose degrading enzymes were compared to identify co-regulated groups of genes and genes needed for the degradation of specific substrates. Transcriptional profiling revealed a group of genes co-regulated on all of the substrates and genes which expression profiles were more diverse. Also some examples were found from co-regulation of enzyme genes according to genomic localization.

PR8.38

Novel manganese peroxidases of the litter-decomposing fungus *Agrocybe praecox*

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Litter-decomposing fungi (LDF) are the primary decomposers of residual plant materials in forest soil. In spite of the ecological significance of LDF, relatively little is known about the molecular characteristics of their lignocellulose-degrading machinery. *Agrocybe praecox* is a litter-decomposing basidiomycete that is capable of mineralising synthetic lignin as well as decomposing various aromatic compounds. We have characterized the primary structure of two different MnP encoding genes of *A. praecox* which encode extracellular, short-type class II heme-containing peroxidases. *Mnp1* corresponds to previously characterized MnP1 enzyme and interestingly, both MnPs are deficient of one of the three conserved acidic amino acids involved in Mn^{2+} binding. Phylogenetically, the closest homologue to MnP1 is a class II peroxidase of the mycorrhizal, agaric basidiomycete *Laccaria bicolor* that lacks all conserved amino acids that bind Mn^{2+}. MnP2 resembles the hybrid-type of MnPs, as described in the wood-decaying, corticioid white-rot basidiomycete *Phlebia radiata*. When the fungus was grown on forest litter, laccase and MnP activities were detected. In birch leaf litter cultures, the transcript levels of expression of *mnp1* and *mnp2* were similar, whereas in cultures on conifer needle litter, transcription of the *mnp1* gene was up-regulated. Molecular characterization of the new MnP enzymes aims to understand better the physiology of litter and lignocellulose decay by *A. praecox*.

PR8.39

Characterization Of Two Redundant *Aspergillus flavus* Peptide Synthetase-like Enzymes

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LaeA, a regulator of secondary metabolism in fungi, controls expression of two genes encoding NRPS-like enzymes in Aspergillus flavus, termed LnaA and LnbA. Although these enzymes resemble α-aminoadipate reductases (i.e., primary metabolism enzymes), gene deletion and knockdown experiments pointed to a role for LnaA and LnbA in secondary metabolism. Specifically, the biosyntheses of heterocyclic L-tyrosine-derived natural products are dependent on these enzymes. Genetic data is also suggestive of functional redundancy of these two enzymes.

Hexahistidine-tagged LnaA and LnbA were heterologously produced, and assayed using the amino acid-dependent ATP-pyrophosphate exchange method. L-tyrosine was identified as clearly preferred substrate of both enzymes. Further biochemical characterization established divergent temperature and pH-optima, and differences regarding stereospecificity.

Our biochemical experiments prove that the LnaA and LnbA substrate spectrum is compatible with their tentative heterocyclic products. Further, participation of α-aminoadipate reductase-like NRPSs in secondary metabolism and functional redundancy of LnaA and LnbA, anticipated by genetic methods, is supported by the substrate spectrum of these enzymes.

PR8.40

Genome-wide transcriptome and proteome analysis of *Aspergillus oryzae* in the hypoxic stress condition

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

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PR8.41

Autonomously replicating plasmids as a transient expression tool in *Aspergillus niger*

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The possibility of having an autonomously replicating plasmid for filamentous fungi broadens the horizon of genetic engineering. Tools like recombinases or reporter genes can easily be inserted to cells and just as well be expelled when taking away the selective pressure. Such a system can be used as a transient expression tool for genetic engineering purposes in *Aspergillus niger*.

AMA1 is a 6.1 kb DNA fragment that allows extrachromosomal replication in filamentous fungi and strongly enhances the transformation efficiency. However, plasmid construction is hindered by the two palindromic, inverted sequences that flank the 0.3 kb central region of the AMA1 fragment. Hints in literature led to the assumption that the sequence can be shortened without loosing the positive influence on transformation efficiency.

In this study we characterized different plasmids carrying shortened fragments of AMA1. The transformation efficiencies as well as the plasmid stabilities were analyzed. The conducted experiments demonstrate that only one of the palindromic sequences together with the central region of AMA1 are necessary for autonomous replication in *Aspergillus niger*. Further shortening led to a drastic decline of transformation efficiency. Plasmids are lost at the latest in the 3rd generation when the spores are cultivated without antibiotic pressure. On the other hand, integration of the plasmids seems to be possible if antibiotic pressure is sustained.

PR8.42

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

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The filamentous fungus *Aspergillus oryzae* is widely used as a microbial cell factory for large scale heterologous protein production. *A. oryzae* is also known as a natural organic acid producer. However, the metabolism and regulation of organic acid production in *A. oryzae* are poorly characterized. Furthermore, the media composition has a major impact on the performance of this organism and the economic feasibility of an industrial fermentation process. We therefore evaluated the global expression response towards and the utilization of different nitrogen sources by *A. oryzae* in batch fermentations.

In this study, we aim for a deep investigation of the cellular mechanisms of the utilization of different nitrogen sources. Firstly, we performed batch cultivations with two strains (NRRL3488 and DSM1862) on defined and complex nitrogen sources using di-ammonium sulphate and peptone, respectively. In addition, transcriptome analysis was performed on samples from these fermentations to analyze the gene expression under exponential growth conditions (mid-exponential phase; 6h) and in nitrogen starvation (stationary phase; 30h) to further identify key-players in the metabolism and regulation of gene expression.

Cluster analysis revealed a conserved response of both strains and helped to identify regulatory sequences among the co-expressed genes. Furthermore the expression data pointed towards malic acid production as a respond to nitrogen starvation stress.

PR8.43

Proteins secrted by *Heterobasidion irregulare* during growth on spruce wood

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Heterobasidion irregulare is a severe conifer pathogen that causes root and butt rot. The white-rot fungus degrades simultaneously or selectively lignin. The genome of the fungus was established by the JGI (http://genome.jgi-psf.org/Hetan2/Hetan2.info.html) and the annotated genome can be used in proteomic studies. Here, *H. irregulare* was grown in liquid medium with and without *Picea abies* wood. Freely secreted and hyphal sheath associated proteins analyzed by 2D-gel electrophoresis revealed a high diversity between wood supplemented and control cultures. Protein identification by ESI-LC-MS/MS was either performed on single protein spots form 2D-gels or by application of a shot-gun method on complex protein mixtures. Using a MASCOT database with the proteome deduced from the *H. irregulare* genome, in total 118 different secreted proteins have been identified. 64 proteins were present under both culture conditions and only seven proteins were suppressed by wood supplementation. Addition of wood resulted in 47 new proteins secreted into the culture media. Redox-enzymes were represented by 23 proteins and most of them were induced by wood. Expression of laccases (except of one) and alcohol oxidases differed not between the two culture media. However, wood induced secretion of FAD-oxidoreductases and redox-enzymes with unknown function and furthermore secretion of specialized glycanases, lipases and proteases.

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PR8.44

Optimisation of vectors for transformations in Coprinopsis cinerea

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The genetic transformation of the model fungus Coprinopsis cinerea allows the genomic analysis and manipulation of this organism. Initially, transformations were used to study the structure; functions and regulation of expression of genes; in recent years usage for overexpression of industrially important enzymes are also emerging. For the transfer of genetic material, chromosomal integrative vectors are used. These vectors contain a selectable marker gene and/ or a gene of interest under the control of regulatory sequences such as promoter or terminator. Due to lack of systematic experimental data, little is known about the influence of vectors on transformation frequencies. This work targets at improvement of the transformation vector pCc1001 (1). This pUC9-based vector contains a 6.5 kb PstI genomic fragment of C. cinerea with the tryptophan synthetase gene (trp1) that can be used to complement trp1- defects. The vector however shows a surprising phenomenon. In single transformation it gives only low numbers of transformants whereas efficiencies in co-transformation raise by factors of >100%, yielding several hundreds of transformants per experiment. To investigate this phenomenon further, the vector was modified in length and fragments with the trp1 gene were subcloned into pBluescript KS-. The effects on the transformation efficiency were investigated by using several co-transformation experiments. (1) Binninger DM et al. (1987) DNA-mediated transformation of the basidiomycete Coprinus cinereus. EMBO J 6:835-840

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PR8.45

Genomics of *Aspergillus oryzae* and effective utilization of large scale genomic information.

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Recently, we have determined the genes responsible to the biosynthesis of kojic acid from *A. oryzae*, which is used as a cosmetic whitening agent. Transcriptome analysis and successive gene disruption approach has been successfully applied to the gene identification. We found three genes encoding oxidereductase, transporter and transcription factor, which are localized on the non-syntenic block. It took, however, more than one year for the identification because the target gene prediction was inaccurate and disruption of roughly 20 genes was required before finding the genes. To accelerate identification of the genes responsible to biosynthesis of novel metabolites, we have prepared our pipelines from genome sequencing to gene detection, which includes DNA sequencer, DNA microarray and LC/MS.

We have improved the performance of the *de novo* assembling pipeline for ABI SOLiD, and have optimized it for sequencing of microorganism genomes. Our pipeline generates scaffolds longer than 1 Mb, covering 99% of approximately 40 Mb genome with roughly 100 or less scaffolds in general. Resulted sequences are subjected to annotation, comparative analysis and the highly accurate gene prediction that we have developed. We have found that our pipeline detected the genes for secondary metabolites that have been already known. We are now evaluating the performance in accuracy by disruption and overexpression of some novel genes that have been predicted by the pipeline.

PR8.46

Oxalate decarboxylases of the white-rot fungus *Dichomitus squalens*: expression on wood and in acid-induced cultures

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Oxalate decarboxylase (ODC) is an oxalic-acid decomposing enzyme produced by certain bacteria and fungi with potential for diverse biotechnological applications. Uses of ODC range from assays of oxalate concentration and removal of oxalate salt deposits in industrial processes to transgenic *odc*-expressing crop plants.

In fungi, ODC has an essential role in regulation of toxic concentrations of intra- and extracellular oxalate. Oxalic acid is an important metabolite of basidiomycetous wood-rotting fungi as it assists in wood and lignin degradation by e.g. enhancing the reactions catalyzed by lignin-modifying oxidative enzymes. Moreover, ODC has been suggested to participate in energy production during the fungal vegetative growth.

We found previously that the selectively lignin-degrading white-rot fungus *Dichomitus squalens* secretes oxalic acid during growth in liquid cultures and on spruce wood[1]. The fungus demonstrated intracellular ODC activity after exposure to excess oxalic acid. Mycelial Ds-ODC protein was partially purified, and for the first time for a white-rot polypore species, we succeeded in complete cloning of ODC-encoding gene of *D. squalens*[2].

The whole genome sequence of D. squalens (www.jgi.doe.gov) reveals that the fungus harbours altogether five odc gene models. In this work, we studied the expression of the five Ds-odc genes by real-time RT-qPCR during the fungal growth on solid-state spruce wood and in acid-induced liquid cultures. These results support involvement of differently regulated, individual ODC isoenzymes in primary and secondary metabolism in wood-decaying fungi.

[1]Mäkelä M, Galkin S, Hatakka A, Lundell T (2002) Enzyme Microb Technol 30:542-549
[2]Mäkelä MR, Hildén K, Hatakka A, Lundell TK (2009) Microbiology 155:2726-2738

PR8.47

Transcriptomic And Genomic Approaches To Understand Cellulase Hyper-production In The Filamentous Fungus *Trichoderma reesei*

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The filamentous fungus *Trichoderma reesei* is well-known for its impressive capacity to secrete high amounts of cellulase and hemicellulase enzymes. These enzymes are key components of most cellulosic biomass to biofuel biological processes, and despite huge R&D efforts, their cost is still too high. Most industrial strains in use today have been obtained by random mutagenesis of the original QM6a isolate. Highest performances range from 40 to above 100 g/L proteins production, depending on the strain and process configuration used. While it is not known whether the already impressive performances of these strains can be further enhanced through targeted genetic engineering, there is still a high interest in understanding the genetic mechanisms leading to cellulase (hyper)secretion and to set up genomic tools that could be used to adapt strains to various industrial conditions. Toward this goal our group has been investigating the onset of cellulase production both on the single-gene level and on the whole transcriptome level using dedicated DNA oligonucleotide microarrays. We are currently completing our previous high-throughput sequencing of the NG14 and RUT C30 strain lineage with the sequencing of five other strains. These genome data are also being completed by transcriptome analysis with RNAseq technology. Our objective is to provide a whole picture of the mechanisms involved in cellulase production by *T. reesei* as well as potential new targets for genetic engineering of industrial strains.

PR8.48

Transposition of the miniature inverted-repeat transposable element mimp1 in the wheat pathogen Fusarium culmorum

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The genome of *Fusarium culmorum*, incitant of crown and foot rot on wheat and type B trichothecene producer, is now being sequenced. The number of predicted genes is estimated to exceed 10,000 and for many of them the function is still unknown. Consequently, there is a strong need for a high-throughput method for functional genomic analysis. Our aim was to test the efficacy of a double component system based on the ability of the *impala* transposase to transactivate the miniature inverted-repeat transposable element *mimp1* of *Fusarium oxysporum*. In this paper we report for the first time on the application of a tagging system based on an heterologous transposon and on the application of the splinkerette-PCR to identify *mimp1* flanking regions in the filamentous fungus *F. culmorum*. Similarly to what was previously observed in *Fusarium graminearum*, *mimp1* was shown to transpose in *F. culmorum* by a cut-and-paste mechanism into TA dinucleotides, which are duplicated upon insertion. Our results also show that *mimp1* reinserts in open reading frames in 16.4 % (i.e., 10 of 61) of the strains analysed, spanning throughout the entire genome of *F. culmorum*. Therefore the *mimp1/impala* double-component system is an efficient tool for gene tagging in *F. culmorum* as confirmed phenotypically for a putative aurofusarin gene. This system allowed also to identify two genes putatively involved in oxidative stress coping capabilities in *F.culmorum* as well as a sequence specific to this fungus, thus suggesting the valuable exploratory role of this tool.

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PR8.49

Bioinformatic prediction of *cis*-acting elements in *FUM* gene promoters putatively involved in transcriptional control of fumonisins biosynthesis

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Fumonisins are secondary metabolites produced by the maize pathogen *Fusarium verticillioides*, agent of pink ear rot; these mycotoxins cause agro-economical losses and detrimental health effects in animals and humans. The FUM genes needed for fumonisins biosynthesis are clustered and co-expressed in the fumonisins producers.

In eukaryotes, coordination of gene transcription is mostly attained through transcription factors shared by co-regulated genes, whose specificity relies on the recognition of *cis*-regulatory elements on the promoters of their targets. A bioinformatic analysis of FUM gene promoters in *F. verticillioides* identified a partially degenerated motif potentially involved in the regulation of FUM genes expression, and therefore in fumonisins biosynthesis. The same oligomer was found in the clustered FUM genes of the other fumonisins producers *Fusarium oxysporum* and *Aspergillus niger*; while it is not significantly over-represented in the scattered FUM homologs of the fumonisins non-producing euascomycetes *F. graminearum*, *A. nidulans*, *Magnaporthe grisea* and *Neurospora crassa*.

Comparison of the transcriptional strength of the intact *FUM1* promoter and of a synthetic version, where the motif discovered had been mutated, was carried out *in vivo* and *in planta* by quantifying GFP transcripts in *F. verticillioides* transformants, carrying either promoter upstream of the GFP reporter. Our results show that mutation of the main motif in *FUM1* promoter is sufficient to significantly impair its efficiency, thus validating our *in silico* approach as a discovery tool. The presence of the degenerated 6-mer in all clustered FUM genes suggests that this set of oligomers includes candidate regulatory sequences.

PR8.50

Biosynthesis of Natural Products Through a Fungal Molecular Genetics

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Analysis of fungal genome sequences to date has revealed that ascomycetes possess far greater numbers of gene clusters for biosynthesis of secondary metabolites, polyketides and peptides, than the numbers of natural products that have been isolated from the organisms. Therefore, such cryptic secondary metabolism gene clusters are anticipated to be a source of chemically diverse compounds. However, conventional methodologies in the field of natural products chemistry cannot be applied in hunting of natural products synthesized by enzymes encoded in the fungal genome as silent gene clusters. First, it is necessary to activate the gene cluster to induce biosynthesis of the corresponding compounds. Here, we expressed a gene encoding a transcriptional regulator associated with a target silent gene cluster to induce its natural product biosynthesis. This approach was used successfully to produce two polyketides from *Aspergillus oryzae* and three from *Chaetomium globosum*.

Second, once mRNA can be transcribed from the target gene cluster, cDNA can be synthesized to allow transfer of the cluster genes into budding yeast and achieve heterologous production of compounds. Here, we have developed an innovative method for biosynthesizing bioactive molecules using an engineered *Saccharomyces cerevisiae* strain as a host. We expressed five polyketide synthases and two nonribosomal peptide synthetases from *Aspergillus fumigatus*, *Chaetomium globosum* and *Coprinopsis cinerea*. Subsequent detailed chemical characterizations of the resulting natural products identified six polyketides and two peptides. The methodologies shown in this study can be applied in acquisition of numerous natural products biosynthesized by silent/unknown fungal secondary metabolism gene clusters.

PR8.51

Unravelling the MDR mechanism in new emergent phenotypes of *Mycosphaerella graminicola*

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Multidrug resistance (MDR) is a trait developed by many organisms to counteract the effect of chemicals and/or drugs for the control of these agents. The basic MDR mechanism relies on an overexpressed efflux transport system that actively expulses the compound outside the cell. MDR was observed in field populations of the wheat septoria tritici blotch fungus *Mycosphaerella graminicola* since 2007 in France, Ireland and the UK, which evidently threatens control options. Individual strains with MDR (MDR 6 and 7) were characterized on the basis of their high resistance levels to fungicides belonging to the DMI family and to their cross-resistance with QoIs and SDHIs. Two main strategies were adopted: (a) Investigating the relationship between MDR phenotypes and an efflux transport system: Reversal agents inhibiting ABC/MFS transport systems coupled to C14-radiolabeled Prochloraz shed light on the involvement of at least two different transporters. In addition, the Tolnaftate (thiocarbamate) phenotyping of 140 descendant from a cross of MDR6 x MDR7 confirmed allelism or close linkage of genes involved in the MDR phenotype; (b) Analysis of differentially expressed genes in sensitive and MDR isolates:

RNA sequencing profiling of the MDR6/7 strains *vs.* sensitive strains in untreated and Prochloraz treated conditions are ongoing and the latest data and analyses will be presented.

PR8.52

Correlation of gene expression and protein production rate - a system wide study

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Growth rate is a major determinant of intracellular function. However its effects can only be properly dissected with technically demanding chemostat cultivations in which it can be controlled. Recent work on *Saccharomyces cerevisia*e chemostat cultivations provided the first analysis on genome wide effects of growth rate. In this work we study the filamentous fungus *Trichoderma reesei (Hypocrea jecorina)* that is an industrial protein production host known for its exceptional protein secretion capability. Interestingly, it exhibits a low growth rate protein production phenotype. We have used transcriptomics and proteomics to study the effect of growth rate and cell density on protein production in chemostat cultivations of *T. reesei*. Use of chemostat allowed control of growth rate and exact estimation of the extracellular specific protein production rate (SPPR). We find that major biosynthetic activities are all negatively correlated with SPPR. We also find that expression of many genes of secreted proteins and secondary metabolism, as well as various lineage specific, mostly unknown genes are positively correlated with SPPR. Finally, we enumerate possible regulators and regulatory mechanisms, arising from the data, for this response. Based on these results it appears that in low growth rate protein production energy is very efficiently used primarly for protein production. Also, we propose that flux through early glycolysis or the TCA cycle is a more fundamental determining factor than growth rate for low growth rate protein production and we propose a novel eukaryotic response to this i.e. the lineage specific response (LSR).

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PR8.53

Enzyme production by Trichoderma reesei Rut C-30 followed by enzymatic hydrolysis of different lignocellulosic materials

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The filamentous fungus *Trichoderma reesei* is one of the main sources for cellulose degrading enzymes. We studied the enzyme profile produced during the fungal growth on cellulosic and lignocellulosic substrates and their capacity to hydrolyze cellulosic and lignocellulosic substrates with different chemical and physical properties. The results brought insight into the bottlenecks of enzymatic hydrolysis.

During the enzyme production study, we grew *T. reesei* strain Rut C-30 in submerged fermentations on Avicel PH-101, commercial cellulose, and industrial-like lignocellulosic substrates from spruce. These substrates were produced during the process of sodium hydroxide cooking, used in pulp and paper industry. Additionally we altered the chemical and physical properties of those substrates by drying and rewetting, treatment of sodium hydroxide and sodium chlorite in order to decrease or increase the surface area and delignify, respectively. We measured cellulolytic enzyme activity by enzymatic assays. Proteins were examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and two-dimensional gel electrophoresis.

The enzymes produced were subsequently used for enzymatic hydrolysis of lignocellulosic substrates and compared to enzymatic hydrolysis of model cellulosic substrates, namely, Avicel PH-101, nanocrystaline cellulose, phosphoric acid-swollen cellulose and cotton, which have defined characteristics. The structural properties of the substrates during the different times of hydrolysis were analyzed by solid-state nuclear magnetic resonance (NMR) technique. Dynamics of the hydrolysis was analyzed by quartz crystal microbalance with dissipation (QCM-D) technique. Hydrolysis products were verified by high performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD).

PR8.54

Structural and Functional analyses of Dehydrin-like proteins in the necrotrophic fungus *Alternaria brassicicola*

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Dehydrins (DHN) are a protein subclass of Late Embryogenesis Abundant proteins (LEA) which, in plants, are involved in the salt and hydric stress resistance. In fungi, dehydrin-like proteins have been identified in *Tuber borchii* and *Aspergillus fumigatus* and a signature pattern has been described for these proteins (1,2). Using this sequence motif, we have identified three DHN-encoding genes in the genome of the necrotrophic fungus *Alternaria brassicicola*. Sequence analysis confirmed that they all shared the characteristic features of dehydrins: high glycine, threonine and serine content, low cysteine and tryptophan content, high hydrophilicity, absence of secondary structures and a high proportion of disordered amino acids. Measures of the expression levels of the three DHN genes in conditions previously reported to induce fungal DHNs transcription (low temperature, salinity and oxidative stress) revealed that they were all up regulated in these conditions. To study their subcellular localization, dehydrin-like proteins were fused to eGfp at their carboxy-terminal end and expressed in *A. brassicicola* under control of their own promoters. Fluorescent protein fusions showed that at least one dehydrin was associated with peroxisomes. A functional analysis has been performed by the construction of knockout mutants deficient for each DHN. Although none of the dehydrin-like mutants were found more susceptible to NaCl than the WT strain, they were all characterized by a stronger susceptibility towards oxidative stress (menadione or H_2O_2). A double-mutant strain exhibited reduced virulence on host leaves and decreased seed transmission rates compared to the parental strain, indicating a role of DHNs in pathogenicity.

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PR8.55

Esterases of basidiomycetes as supporting enzymes in degradation of lignocellulosic material

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In contrast to fossil resources, lignocellulose as the main part of wooden plant material is available in almost unlimited amounts. Its main components - cellulose, lignin and hemicellulose – represent an important feedstock used in various industries, such as in paper manufacturing or bioethanol production. Unfortunately, lignocelluloses are recalcitrant materials, and harsh chemical conditions are needed to degrade them. In nature, fungi are capable of breaking up the wooden material by using a diverse set of extracellular enzymes, the so-called secretome. For degradation of the lignin, a number of oxidases including lignin peroxidases, manganese peroxidases, versatile peroxidases, and DyP-type peroxidases and laccases, are secreted which are able to oxidise aromatic and phenolic parts of the lignin structure. As a second part of the lignocellulosic fungal secretome, hydrolytic enzymes, such as cellulases and esterases, are needed for the extraction of sugars and are involved in the degradation of the lignocellulosic structures, respectively. The different cellulases are able to fracture the cellulose structure, whereas esterases (EC 3.1.1.x) are involved in the hydrolysis of several ester bonds. E.g., feruloyl-esterases (EC 3.1.1.73) participate in the breakup of linkages connecting hemi-cellulose (arabinoxylans) and lignin. Nevertheless, the knowledge on basidiomycete esterases is fragmentary and, thus, their biotechnological potential unknown.

In this work, 30 different basidiomycetes were screened for the ability to degrade several esterase substrates. Interesting candidates were cultured in minimal and complete liquid media with and without addition of lignocellulose. Esterase activities of up to 340 U/L were obtained when cultivating the fungi in shaken flasks at 24 °C. The supernatant of interesting fungal candidates was used together with already optimised cellulolytic enzyme cocktails to improve the degradation of lignocellulosic residues regarding the amount of reducing sugars gained by this bioconversion. Promising candidates will be purified, characterised and heterologously expressed in an ascomyceteous host.

PR8.56

Characterization of Cellobiohydrolase I (CBHI) of the White-Rot Fungus *Dichomitus squalens*

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In nature basidiomycetous white-rot fungi are the most efficient wood-decaying organisms. They express extracellular hydrolytic and oxidative enzymes that are needed to degrade all the wood polymers, i.e. cellulose, hemicellulose and lignin, but while their lignin modifying enzymes are well known their cellulolytic enzymes are much less studied. In a large screening of cellobiohydrolases (CBHs) of basidiomycetous fungi from the Fungal Biotechnology Culture Collection (FBCC, University of Helsinki) the white-rot fungus *Dichomitus squalens* strain FBCC312 appeared to be highly cellulolytic and a promising source of novel cellulases. In this work we purified and characterized native CBHI of *D. squalens*. The fungus produced extracellular CBH, endoglucanase, *β*-glucosidase, xylanase and laccase activities in liquid 1% (w/v) microcrystalline cellulose (Avicel) - peptone medium. Ds-CBHI was purified from this culture liquid after 6 to 10 days of cultivation, with anion exchange and size exclusion chromatography. Molecular mass of the purified Ds-CBHI was 45 kDa and p*I* 3.8-4.1, as determined by SDS-PAGE and IEF, respectively. Three internal peptides sequenced by LC-MS/MS were similar with the translated amino acid sequence of the cloned *D. squalens cbhI* gene. The putative polypeptide of Ds-CBHI lacks cellulose binding module and is similar to glycosyl hydrolase family 7 proteins. Ds-CBHI showed wide pH and temperature working ranges with artificial substrate 4-methylumbelliferyl-β-D-lactoside (MULac). Optimum temperature of Ds-CBHI for MULac reaction was +65°C and optimum pH 4.0. Purified Ds-CBHI resembled the known white-rot fungal CBHs by its acidic p*I* and catalytic optimum pH.

PR8.57

Associated biocontrol of cotton pest *Dysdercus peruvianus* by the fungus *Metarhizium anisopliae* and environmental isolates of *Pseudomonas fluorescens*

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Dysdercus peruvianus is an insect pest of cotton culture, which causes serious economic damages. The method currently used to control this plague is by the use of chemical pesticides. However, this practice is increasingly questioned by society, due environmental impact, high costs, low specificity and risk in its handling. *Metarhizium anisopliae* is a filamentous fungus used in biological control with attested effect on several arthropod pests, including *D. peruvianus*. The main barrier to apply this fungus, or any other biocontrol agent, is the time of death of target pest; which is generally greater than the corresponding chemical pesticide. In this work, the isolation, identification and evaluation of environmental bacteria in association with *M. anisopliae* were performed to optimize the biocontrol of the *D. peruvianus*. Four bacteria isolated from soil effectively accelerate the biocontrol of *D. peruvianus*, when associated with the fungus, and the two best bacteria were identified as *Pseudomonas fluorescens*. The formulation containing *M. anisopliae* conidia and bacterial culture of *P. fluorescens* showed efficiency up to 96% in reducing the time of the death of *D. peruvianus*. Besides molecular aspects of this interaction, including the expression of enzymatic arsenal of *M. anisopliae* in association of bacterial isolates were also evaluated. This work attests the efficiency of associated biocontrol applying the fungus *M. anisopliae* and bacterial isolates collected from environment using a specific strategy. Also, this alternative represents a significant increase in efficiency of biological control, increasing the interest in application of this environmentally safe way of pest control.

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PR8.58

Morphological and molecular characterization of Hyphodermella rosae the causal agent of dry fruit rot on plum and peach in Iran

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In June 2011, a new symptoms of dry fruit rot on plum (Prunus domestica) and peach (P.persica) was observed in Mazandaran province of Iran. Initial symptoms, appeared as dark brown, circular necrotic spots on fruits. The fungus Hyphodermella rosae isolated and identified on the basis of morphological characteristics on PDA. Basidiomata were effuse, resupinate, 15 × 10 mm, tubercules small with apical bristles, light orange to greyish orange. Subhymenium composed of vertically arranged, short-celled, non-agglutinated hyphae; subhymenial hyphae were 3-4 µm in diam. Basidiospores were ellipsoid, 7.5× 5.5 µm and their cell walls were thin, hyaline and smooth (1). A CTAB DNA extraction protocol was used to acquire DNA from mycelium culture. The primer pair ITS4 (5-TCCTCCGCTTATTGATATGC-3) and ITS5 (5-GGAAGTAAAAGTCGTAACAA-3) was used to amplify the Internal Transcribed Spacer (ITS) regions including 5.8S from ribosomal DNA (4) and the PCR product was sequenced. The 627-bp and 604bp fragments of Plum and peach isolates was amplified, respectively. After multiple sequence alignment with CLUSTALW software the obtained sequences were compared with the other related sequences of Hyphodermella genus deposited in GenBank. Blast analysis of the MA4099 (plum isolate) and VA1345 (peach isolate) sequences confirmed a 99 and 100% similarity with the sequences of H. rosae (GenBank accession no FN600386.1, FN600385.1) respectively. The pathogenicity of the isolates has been proven. The genus Hyphodermella has been reported causing wood rot on apricot (2), sweet and sour cherry (3). To our knowledge, this is the first report of H. rosae on stone fruit species in the world.

PR8.59

Screening the secondary metabolome of an unidentified basidiomycete for antifungal compounds

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The isolate BY represents an unidentified anamorphic fungus. Based on the analysis of ITS/IGS-sequence data it was tentatively assigned to the russuloid clade of the basidiomycetes. However, its genus remains obscure. As cultures of this isolate exert strong antifungal effects further research into its secondary metabolism was warranted. A series of natural products has been isolated, structurally elucidated, and tested for antifungal activity. Our results suggest that the bioactivity of BY is not related to a single agent but due to several structurally dissimilar compounds.

They possibly originate from different metabolic pathways, as polyene and phenylpropanoid core structures were found, alongside polyketidic scaffolds. To clarify the respective biosynthetic routes, feeding experiments with 1-13C labeled acetate have been initiated, accompanied by genetic screens for natural product biosynthesis genes.

PR8.60

Heterologous expression improvement in filamentous fungi by using RNA interference tool

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Fungi are industrial workhorses of secretion, where commercially useful proteins are targeted for secretion and high-yield accumulation. High-yield protein secretion and accumulation are amino-acid sequence dependent and in many cases, more often than not, high-yield of secretion is not observed. *Aspergillus nidulans* has been recognized and utilized as an excellent host to homologous and heterologous protein production. Researches have studied the protein production, because it shows an excellent extracellular secretion capability for a large amount of proteins compared to described in other microbial secretion systems such as *E. coli* and *S. cerevisiae*. Some post-translational modifications including glycosylation and folding are expected to take place in *A. nidulans*. For these reasons, it is considered one of the most adequate hosts to produce higher eukaryotic proteins. To enhance the protein production ability, it is important to construct the host applicable to multiple rounds of genetic manipulation. Recently, in order to carry out multiple gene silence and expression rapidly and efficiently, we developed *pyrG* marker recycling system. Using this system, successive rounds including concatenated gene silence for proteases respectively in *A. nidulans* were successfully achieved with gene-targeting frequency. Based in proteomics results, the genes encoding proteases, we concatenated five proteases in only one construction to analyze the effect of RNA interference on heterologous protein production by *A. nidulans*. Moreover, based on these data, we also confirmed the improvement of cellobiohydrolase productivity with the protease deficient strain. As a result, we constructed a quintuple protease genes disruptant having enhanced levels of cellobiohydrolase protein productivity.

PR8.61

Heterologous expression of the human peptide hormone obestatin in *Trichoderma reesei*

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The filamentous fungus *Trichoderma reesei* is an efficient expression host widely exploited as an industrial workhorse. However despite being regularly used for proteins and enzyme of larger size its capability of expressing small peptides of (T. reesei as an expression host for peptide production by engineering a strain for the expression of obestatin, a small peptide hormone of 23 amino acids in size that suppresses the appetite and regulates body weight gain in human and other mammals. Thus providing an alternative source for this otherwise naturally scarce and expensive to chemically synthesize peptide hormone. Preliminary expression of obestatin, with HIS tag fused at either N- or C- termini of the peptide, was carried out in *Escherichia coli* and the expression level was evaluated by enzyme immunoassay (EIA). The expression of obestatin was subsequently evaluated in *T. reesei* with a C-terminal purification tag, Hydrophobin I tag (HFBI) which is native to the host. Following the successful expression of obestatin in *T. reesei,* modifications of growth conditions were made to optimize the production of the peptide. Results indicated that it was possible to express the small peptide hormone obestatin in *T. reesei* at a higher concentration than it is in the *E. coli* expression system. It was also possible through strain selection and modification of the culture medium to achieve yield of up to 7µg/ml of obestatin in *T. reesei*.

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PR8.62

Extracellular enzymes from *Trichoderma harzianum*, *Aspergillus terreus* and AM fungus

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Cellulotic enzymes consistitute form the group well described biologically important enzymes,The enzyme cellulase, a multi enzyme complex made up of several proteins, catalyses theconversion of cellulose to glucose in an enzymatic hydrolysis. Which catalyze the transfer of the glycosyl group between oxygen nucleophiles. important role this enzymes in the biology is include degradation celluloses of the biomass fungi and bacteria, degradation of glycolipids in mammalian lysosomes, and the cleavage of glycosylated flavonoids in plants. Fungus Trichoderma and Aspergillus have biotechnologicaly importance, since they are a producer extracellular enzyme. We studies from the new strains selection high cellulotic activities and using them in the biotechnical studies. Studing extracellular enzymes from 3-fungi, Aspergillus terreus, Trichoderma harzianum and Mucorrhiza conducted in minimum ambience, containing wheat bran as single source of the carbon. Enzyme activities were assayed spectrophotometrically by using Samogy-Nelson. Temperature and pH optimum of some purified enzymes were determined also. The isolation and purification cellulotic enzymes we are used the ion exchange chromatography on DEAE TOYOPEARL 650 M gel in the gradient 0,5M NaCl. The test strains of each species ., Aspergillus terreus, Trichoderma harzianum and AM fungi were analyzed on the basis of extent of hydrolyzing ability. Cellulose hydrolysis of all three strains was immensely affected by varying pH and medium. The results indicated statistically significant interaction in all correlating factors of strain, growth medium and pH level

PR8.63

Finding the conserved mushroom developmental pathway

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Mushrooms are important sources of food, medicinal compounds, for industrial applications, waste recycling and environmental health. However, cultivation of most mushrooms is difficult or yet impossible. A major obstacle for improvement of mushroom cultivation technology is the lack of knowledge on molecular genetic mechanisms that underlie mushroom development. Available information is highly scattered, describing specific, unrelated studies in model mushrooms and more recently including individual studies on major cultivated mushrooms.

Despite the high variations in shape, color, composition, substrate and triggers for fruiting induction, mushroom life cycles follow a general course. Within the developmental stages of the mushroom, especially the formation of hyphal knots and primordia seem highly similar between species. We decided to 'access' the general developmental pathway through interspecies comparison of primordium specific gene expression patterns, including model as well as major cultivated species. Promising universal, primordium specific genes will be studied in detail in the model organisms *Coprinopsis cinerea* and *Schizophyllum commune* by means of gene deletion, fusion to fluorescent markers and quantitative PCR. Once confirmed to play a role in mushroom development, these genes will serve as foundation for compilation of a general mushroom development model in two directions; stages preceding, and stages following primordium formation.

At the moment, superSAGE datasets are being assembled and most mushroom species are still under cultivation. We just started comparison of our first four datasets (dikaryotic mycelium versus primordia) in *C. cinerea* and *Polyporus brumalis*. Considering the effectiveness of other expression-based studies for identification of stage specific developmental genes in mushrooms, we expect to reveal several universal genes to start our model in the near future.

PR8.64

Laccase functions in *trichoderma virens*

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Fungal laccases are involved in multiple functions, such as lignin degradation, pigments synthesis and degradation, detoxification and pathogenesis. Furthermore, they are useful biocatalysts for several biotechnological applications. Six laccase genes were previously identified in *Trichoderma virens*, an effective biocontrol agent, and one of them was deleted and proved to be involved in the mycoparasitic activity against *Botrytis cinerea* sclerotia. Laccase activity in some *Trichoderma spp.* is also associated with the production of green pigment in conidial spores. Further investigations on the laccase gene family in *T. virens* were performed in order to explore substrate specificity and mechanisms putatively involved in ligninolysis, conidiogenesis and industrial dyes decolorization. Laccase functions in lignocellulosic process and sporulation mechanisms were studied by growing *T. virens* on two different substrates: wheat straw liquid medium, containing lignocellulose as the only carbon source, or solid Hölker medium, formulated to induce spore formation. Laccase expression analysis induced by multiple substrates is in progress to identify the more effective molecules or the pathways involved in *T. virens*. Further biochemical analyses are going on to search laccase isoforms when fungal cultures are grown on specific substrates. Possible variations of intra/extra-cellular enzymatic levels is also under study. In addition liquid cultures containing twelve commercial textile dyes were set up and *T. virens* efficiently decolorized three of them. In conclusion information is gained about the *T. virens* laccase gene family, involved in physiological processes important for fitness or antagonistic attitude and exploitable in biotechnological applications related to textile dyes decolorization or ligninolysis.

PR8.65

Development of a homologous protein carrier system for heterologous protein production in *Myceliophthora thermophila.*

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Filamentous fungi have proven to produce and secrete large quantities of extracellular enzymes. Species such as *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei* and *Myceliophthora thermophila* C1 are used in industry as work horses for enzyme production. High yields of homologous enzymes can be readily obtained. On the contrary, heterologous proteins are often produced at low levels. One of the main reasons for this is the presence of host proteases that partially or fully degrade the heterologous protein. In addition at the level of transcription, translation and transport and processing through the secretion pathway problems may be encountered. In order to increase the chances of success, protein carriers have been used for improved heterologous protein production in filamentous fungi[1]. It is believed that the carrier protein will "drag/guide" the heterologous protein through the (initial stages) of the secretion pathway protecting it from mis-folding and proteolysis. Previously, we have used this technology successfully in the expression of human antibodies in C1[2]. The carrier protein used in that study was the catalytic domain of *Aspergillus niger* glucoamylase A, which by itselfis a heterologous protein to C1. In the present study we investigated whether the homologous C1 glucoamylase yields higher levels of a heterologous xylanase sensitive to proteolysis. [1] Gouka *et al*. (1997) Efficient production of secreted proteins by *Aspergillus*: progress, limitations and prospects. Appl. Microbiol. Biotechnol. 47: 1-11. [2] Visser *et al*. (2011) Development of a mature fungal technology and production platform for industrial enzymes based on a *Myceliophthora thermophila* isolate, previously known as *Chrysosporium lucknowense* C1. Ind. Biotechnol. 7(3): 214-223.

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PR8.66

Closely related fungi employ diverse enzymatic strategies to degrade plant biomass

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Fungi can grow in many biotopes and on many different carbon sources. In natural biotopes, plant biomass is the predominant carbon source for most fungi. Plant biomass consists largely of polymeric compounds of which polysaccharides are the main components. Fungi cannot take up the intact polysaccharides, but need to degrade them extracellularly to monomeric and small oligomeric compounds. To achieve this, fungi produces diverse enzymatic mixtures that are tailored specifically to the available polysaccharides.

A recent study demonstrated significant differences in the polysaccharide degrading ability of three Aspergilli, while only small differences were detected in their growth on various plant polysaccharides (Coutinho et al, 2009). This suggests that related fungal species may have developed different approaches to plant biomass degradation, employing different enzyme sets. A better understanding of these strategies will not only increase our insight in fungal biodiversity, but will also help in designing more efficient industrial processes for plant biomass degradation.

In this study we have compared the plant biomass degrading potential and strategy of 8 Aspergilli and demonstrate that they have developed a highly diverse approach to using these complex carbon sources. Although all eight species contain the main transcriptional activators involved in plant polysaccharide degradation (AmyR, XlnR, AraR, InuR) the enzymatic sets produced by them differs hugely, suggesting a species specific fine-tuning of plant biomass degradation.

PR8.67

Induction of lignocellulose degrading enzymes in Neurospora crassa by cellodextrins

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Neurospora crassa colonizes burnt grasslands in the wild and metabolizes both cellulose and hemicellulose from plant cell walls. When switched from a favored carbon source such as sucrose to cellulose, N. crassa dramatically upregulates expression and secretion of a wide variety of genes encoding lignocellulolytic enzymes. However, the means by which N. crassa and other filamentous fungi sense the presence of cellulose in the environment remains unclear. Here, we show that a N. crassa mutant carrying deletions of two genes encoding predicted extracellular β-glucosidase enzymes and one intracellular β-glucosidase enzyme (Δ3βG) lacks β-glucosidase activity, but efficiently induces cellulase gene expression and cellulolytic activity in the presence of cellobiose as the sole carbon source. These data indicate that cellobiose, or a modified version of cellobiose, functions as an inducer of lignocellulolytic gene expression and activity in N. crassa. In addition, we have identified two cellodextrin transporters involved in sensing cellulose. A N. crassa mutant carrying deletions for both transporters is unable to induce cellulase gene expression in response to crystalline cellulose. Furthermore, a mutant lacking β-glucosidase enzymes and transporters (Δ3βGΔT) does not induce cellulase gene expression in response to cellobiose. We are currently in the process of characterizing the transport kinetics of each individual transporter in the Δ3βG background with the goal of understanding how the transport of cellodextrins influences cellulose sensing and induction of cellulase gene expression.

PR8.68

A novel group of class II hydrophobins from *Trichoderma* with biased occurrence

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Hydrophobins are small secreted proteins containing eight positionally conserved cysteine residues, are unique to Pezizomycotina (Ascomycota). These proteins assemble in amphiphilic layer on the outer fungal cell wall, where they mediate interactions between the fungus and its environment. The amphiphilic properties have also raised considerable industrial interest in the application of hydrophobins for the processes requiring surface modification. *Trichoderma* spp. possess an amplified arsenal of class II hydrophobins compared to other fungi. In order to exploit this richness for biotechnology, we performed a genus-wide screening for novel *Trichoderma* hydrophobins. Thereby we discovered a group of class II hydrophobins which is restricted to *T. virens* (teleomorph *Hypocrea virens*) and taxa from closely related Harzianum Clade (both section Pachybasium). The intraspecific nucleotide diversity π is in the range <0.062, which is similar to the interspecific diversity of other *Trichoderma* class II hydrophobins, indicating a high rate of evolution. Fishers exact test showed that the gene is under purifying selection and exhibits a high relative synonymous codon usage (>0.8). The expression of these new hydrophobins under different conditions and the characterization of their amphiphilic properties will be reported.

PR8.69

The HFB4 family: novel class II hydrophobins of *Trichoderma* with universal infrageneric distribution and potential for industrial applications

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Hydrophobins are small secreted proteins containing eight positionally conserved cysteine residues, are unique to Ascomycota. They usually assemble in amphiphilic structures on the outer fungal cell wall, thus mediating interactions between the fungus and its environment. These amphiphilic properties have also raised considerable industrial interest in the application of hydrophobins for the modification of surfaces. We have previously shown (Kubicek *et al.,* 2008. BMC Evol Biol) that *Trichoderma* spp. possess an highest diversity of class II hydrophobins compared to other fungi. This variability likely arose by iterating patterns of gene duplications and gene loss processes (”birth and death evolution”). Here we studied the HFB4 family, which comprises the most conserved clade of class II hydrophobins in Trichoderma. HFB4 orthologues occur almost in all infrageneric groups of *Trichoderma*, with the exception of the the *Hypocreanum, Psychrophila* and *Lutea* clades. HFB4 sequences from some species exhibited a significant amino acid sequence variation (e.g. *HFB4s in Trichoderma* section Trichoderma), whereas that of other taxa was identical (e.g. section *Longibrachiatum*). The K_a/K_s ratio of 1.93, as well as Tajima’s test (significantly positive) and Fishers exact test (< 0.3; see ref. above) showed that *T. atroviride (*teleomorph *Hypocrea atroviridis*) HFB4 is apparently under positive selection pressure, whereas the other confirmed the birth and death mechanism. The expression of *hfb4* gene accompanied the conidia formation of *T. atroviride*, *T. reesei* (teleomorph *H. jecorina*) and *T. virens* (teleomorph *H. virens*). The amphiphilic properties of HFB4 were confirmed by overexpression of the protein from the three above mentioned species in *E. coli,* purification and analysis by contact angle measurement after dropping water on the surface of hydrophilic and hydrophobic surfaces, respectively. HFB4 may comprise a new group of class II hydrophobins with potential industrial properties.

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***In Vitro* Assessment of Chitosan on *Ganoderma boninense*, Pathogen of Basal Stem Rot Disease in Oil Palm**

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Chitosan is a natural by-product polymer derived from chitin component of crustaceans, insects and fungi which exhibits antimicrobial properties against various pathogens. This study aims to evaluate the *in vitro* antifungal poperties of chitosan on *Ganoderma boninense*, the causal agent for basal stem rot disease in oil palm. Five concentrations of chitosan (1.0, 1.5, 2.0, 2.5 and 3.0 % w/v) in Potato Dextrose Agar (PDA) media were tested for their efficacy to control the *in vitro* growth of *G. boninense* mycelium during culture for 21 days. All of the concentrations tested significantly reduced mycelial growth compared with the control treatment. Chitosan exhibited a fungistatic effect on mycelial growth of *G. boninense* and markedly reduced radial growth via dose-dependent manner. The highest inhibition of radial growth (PIRG) of 90.09 % was observed with chitosan at 3.0 % (w/v). Chitosan also caused morphological changes in *G. boninense* mycelium including the occurrence of small vesicles due to coagulation of fungal cytoplasm and formation of excessive abnormal hyphal branching at higher concentrations. Spores of *G. boninense* treated with the same five concentrations of chitosan during the 21 days incubation period were unable to germinate.

PR8.71

Post-genomic analyses of fungal lignocellulosic biomass degradation reveal the unexpected potential of the plant pathogen *Ustilago maydis*

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Filamentous fungi are potent biomass degraders due to their ability to thrive in ligno(hemi)cellulose-rich environments. During the last decade, fungal genome sequencing initiatives have yielded abundant information on the genes that are putatively involved in lignocellulose degradation. At present, additional experimental studies are essential to provide insights into the fungal secreted enzymatic pools involved in lignocellulose degradation.

In this study, we performed a wide analysis of 20 filamentous fungi for which genomic data are available to investigate their biomass-hydrolysis potential. A comparison of fungal genomes and secretomes using enzyme activity profiling revealed discrepancies in carbohydrate active enzymes (CAZymes) sets dedicated to plant cell wall. Investigation of the contribution made by each secretome to the saccharification of wheat straw demonstrated that most of them individually supplemented the industrial *Trichoderma reesei* CL847 enzymatic cocktail. Unexpectedly, the most striking effect was obtained with the phytopathogen *Ustilago maydis* that improved the release of total sugars by 57% and of glucose by 22%. Proteomic analyses of the best-performing secretomes indicated a specific enzymatic mechanism of *U. maydis* that is likely to involve oxido-reductases and hemicellulases.

This study provides insight into the lignocellulose-degradation mechanisms by filamentous fungi and allows for the identification of a number of enzymes that are potentially useful to further improve the industrial lignocellulose bioconversion process.

PR8.72

Expression profile of beta-galactosidases in *Penicillium chrysogenum*

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Penicillium chrysogenum is used as industrial producer of penicillin. We investigated the catabolism of lactose, an abundant component of whey that has been used extensively in pencillin fermentation, comparing NRRL 1951 as a wild-type reference with the industrial penicillin-producer ASP-78.

Both strains grew similarly on lactose under batch conditions. The time-profile of sugar depletion concurred with the presence of intra- and extracellular beta-1,4-D-galactosidase (bGal) activities. Upon growth on D-glucose, D-fructose, D-xylose, D-galactose and glycerol, neither extra- nor intracellular bGal could be detected. However, L-arabinose induced activity to about half the values measured on lactose. The measured bGal activities were similar for the two investigated strains.

In silico analysis revealed that P. chrysogenum features at least five putative bGal-encoding genes at the annotated loci Pc22g14540, Pc12g11750, Pc16g12750, Pc14g01510 and Pc06g00600. The first two proteins appear to be orthologs of the Aspergillus nidulans intracellular family 2 glycosyl hydrolases AN3201 and AN3200. The latter three P. chrysogenum proteins feature an N-terminal secretion signal and appear distinct paralogs to the extracellular bGal from Aspergillus niger, LacA.

Transcript analysis of Pc22g14540 and Pc12g11750 showed that they were expressed exclusively in response to lactose but completely repressed on the mixed growth substrate glucose/lactose. Pc16g12750 was seemingly co-expressed with the two putative intracellular bGal genes, while its two paralog genes were apparently not transcribed under any condition tested. This expression profile is distinct from those in other ascomycetes, like Trichoderma reesei or A. nidulans, where bGal genes are induced by the monosaccharides D-galactose and/or L-arabinose.

PR8.73

D-galactose uptake of Aspergillus niger

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The majority of Black Aspergilli (Aspergillus Section Nigri), including *Aspergillus niger*, as well as many other Ascomycetes fail to germinate on D-galactose as a sole carbon source. Here, we provide evidence that the ability of *A. niger* to transport D-galactose is growth stage dependent, being absent in the conidiospores but partially present in the mycelia. Despite earlier claims, we could identify galactokinase activity in growing cells and all genes of the Leloir-pathway (responsible for channeling D-galactose into the EMP-pathway) are well induced on D-galactose (and also on lactose, D-xylose and L-arabinose) in the mycelial stage. Expression of all Leloir pathway genes was also detectable in conidiospores, though *galE* (encoding a galactokinase) and *galD* (encoding a UTP-galactose-1-phosphate uridylyl transferase) were expressed very poorly. These results suggest that the D-galactose-negative phenotype of *A. niger* conidiospores is due to the lack of inducer uptake.

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PR8.74

Approaches for evaluating the performance of lignocellulosic biomass hydrolysates obtained by using fungal enzyme cocktails

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The feedstock for 2nd generation biofuel is lignocellulosic biomass, such as wheat straw, bagasse and corn stover. For microorganisms to utilize the biomass, a pretreatment and a hydrolysis step are needed to release fermentable sugars into the lignocellulosic biomass hydrolysate. Fungal enzyme cocktails are used in the hydrolysis step. The hydrolysates differ in their compositions and effects on growths of yeasts and fungi, due to (i) the biomass type (ii) the pretreatment and hydrolysis method (iii) strain characteristics. To select the best combination of these factors is a key step in conducting 2nd generation biofuel research.

In this study, methods were developed to determine the composition of various hydrolysates, generated from diverse biomass and by different pretreatment and hydrolysis methods. In particular, a HPAEC-MS method was found suitable to identify the limiting and interfering factors to the activities of fungal enzyme cocktails. The results of this analysis allow targeted optimization of these cocktails. The performance of these hydrolysates was screened in Bioscreen C Analyzer using *Saccharomyces cerevisiae* CEN.PK 113-7D as model strain.

The results show that our approaches are effective for evaluating and selecting the most suitable hydrolysate for a specific production purpose. The developed analysis methods have potential to enhance the enzymatic hydrolysis efficiency in the hydrolysates, and enable the systematic study on the hydrolysate inhibitory effects.

PR8.75

Enhance itaconic acid production in Aspergillus via cultivation condition optimization

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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. The transcriptomics analysis from A. terreus identified the most relevant itaconic acid related genes. Subsequently, expressing the specific cis-aconitate decarboxylase (CAD) encoding gene in A niger lead to itaconic acid production in the fermentation medium. To enhance itaconic acid production level via medium improvement, 20 different media were designed based on a reference medium from A. terreus and a 96-well micro-titer plate screening assay was applied for screening . The best medium increased the production level in controlled batch fermentation. This was confirmed showing that various levels of one of the trace elements correlated with itaconic acid production under these conditions. In addition, several other parameters such as pH, temperature and dissolved oxygen tension (D.O.) in controlled batch fermentations were shown to be important for itaconic acid production .

PR8.76

Brazilian mangrove fungi in biological synthesis of silver nanoparticles

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Mangrove is an ecosystem in which there is a high competition for nutrients among microorganisms and where lack of oxygen supplies enables many oxido-reduction reactions to occur. In this context our study has been focused on the biological synthesis of silver nanoparticles (Ag NP) by some fungi isolated from Sao Paulo State's (Brazil) mangrove and evaluation of Ag NP antimicrobial activities. Fourteen fungi were cultivated in Potato Dextrose Broth at 25°C and 150 rpm for 72 h. The biomass was filtered and incubated at the same conditions with water (0.1 g mL^{-1}). The biosynthesis of Ag NP was performed adding $AgNO_3$ (1 mM) into fungal filtrate. The Ag NP formation was confirmed by Plasmon resonance band (λ = 440 nm). The nanoparticles were characterized applying TEM, size, zeta potential, and protein portion adhered to Ag NP was analyzed by SDS-PAGE electrophoresis. Antimicrobial activities were tested against some Gram-negative and Gram-positive bacteria and *Candida* species. The results showed that the fungi coded as L-2-2, R-2BI-4, MGE-201, MGE-202 and R-3BI-10 were able to produce Ag NP in satisfactory yields, pronounced antimicrobial activities, spherical morphology, and size in a range of 10-30 nm. TEM and SDS-PAGE revealed the presence of proteins around the Ag NP with molecular weight in the range of 75 to 328 kDa and further investigations are being performed to characterize these proteins. Fungi were taxonomically identified as *Bionectria ochroleuca* (L-2-2), *Cladosporium* spp (R-2BI-4), *Aspergillus tubingensis* (MGE-201), *A. niger* (MGE-202) and *Fusarium proliferatum* (R-3BI-10), respectively.

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PR8.77

Identification and characterisation of novel antifungal compounds against fungal human pathogens

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Fungal infections represent a serious health problem for immune suppressed patients who can be highly susceptible to life-threatening systemic infections. The increasing number of fungal infections and the development of resistance as well as the significant side effects result in the need for the identification of novel antifungal drugs. To identify, evaluate and optimize new tolerable and potent compounds with antifungal activity we have developed an *in vitro* High-Throughput-Screening Activity-Selectivity Assay (AS-HTS-Assay). This assay mimics the smallest unit of a natural infection by incubating host cells with the pathogen, e.g. *Candida species*, in the presence or absence of antimicrobial compounds. Thereby, it covers all potential targets of pathogen and host simultaneously in one assay and provides the minimal inhibitory concentration of active compounds in a host context and the tolerability of these compounds by the host cells. Using this assay we screened more than 100,000 compounds for antimycotic activity. One hit, a benzimidazole derivative, showed high antifungal activity against Candida spp. and good compatibility with human cells. This compound showed a good tissue penetration, tolerability and efficiency in complex 3D-epithelial tissue models and in multicellular organisms as demonstrated in first nematode models. The results of transcriptional profiling of *Candida albicans* indicated that the compound is a potential inhibitor of the ergosterol pathway. This is in contrast to other benzimidazole-derivatives which target microtubules. To further verify the specific target enzyme in the ergosterol pathway sterol pattern of different Candida spp. were carried out by GLC/MS analysis.

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PR8.78

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

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

PR 8.79

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

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Peptides are widely used in the pharmaceutical industry and are also gaining importance as food ingredients. However, the current state-of-the-art production of oligopeptides is via chemical synthesis, which is expensive especially for long peptides. In addition, peptides can be produced by means of fermentation, wherein multimeric genes encoding tandem repeats of the peptide are fused to a carrier protein. After production of the fusion protein peptides are cleaved from the carrier protein. However the yield of the process is generally low and the fusion protein generally accumulates in inclusion bodies.

We developed a new approach in which peptides are inserted in the variable loops of ankyrin repeats. Ankyrin repeat domains are conserved structures and consists of tandem repeats of a 33 amino acid ankyrin repeat unit. Especially interactions between units are important for stability and folding. Therefore, ankyrin repeat domains usually consist of 4 or 6 units. Interestingly, in between two adjacent ankyrin units inserts up to 47 amino acids has been observed in nature.

We have shown in E.coli and Aspergillus niger that peptides can be inserted in between two adjacent ankyrin repeat units and peptides can be isolated from the protein. In Aspergillus niger, ankyrin repeat proteins comprising peptides were fused to truncated glucoamylase. The chimeric repeat protein comprising peptide was successfully expressed and secreted by Aspergillus niger.

In conclusion, production of peptides by inserting peptides in ankyrin repeat proteins is a successful new approach for fermentative peptide production.

PR8.80

The transcriptional regulator RhaR of *Aspergillus niger* is involved in L-rhamnose catabolism and in degradation of Rhamnogalacturonan-I

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

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

RhaR also appears to regulate L-rhamnose catabolism as growth of *rhaR* disruptant strains on L-rhamnose was abolished and two genes encoding putative L-rhamnose catabolic enzymes were down-regulated in the Δ*rhaR* strain.

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Poster Category 9: The Fungal Cell Wall

PR9.1

***Magnaporthe oryzae* evades MAMP (microbe-associated molecular pattern)-triggered immunity of the host rice with surface α-1,3-glucan on the cell wall**

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Plants evoke innate immune defenses against fungal challenges upon recognition of MAMPs such as chitin, a major cell wall component of fungi. Nevertheless, fungal pathogens somehow circumvent the innate immunity of host plants. We previously reported that the rice blast fungus *Magnaporthe oryzae* masks cell wall surface with α-1,3-glucan, an undegradable polysaccharides for many plants, in response to a plant wax component via activation of Mps1 MAPK signaling (Fujikawa et al., 2009). We further studied role of α-1,3-glucan in *M. oryzae*-rice interactions. A *M. oryzae* mutant lacking α-1,3-glucan normally produced infectious structures. However, the inoculation of the mutant rapidly induced defense responses of susceptible rice plants and, as a result, the fungal infection was completely blocked. Moreover, a transgenic rice expressing a bacterial α-1,3-glucanase rapidly responded to the *M. oryzae* and showed strong resistance to the fungal infection. Overall, our results suggest that the surface α-1,3-glucan plays indispensable roles in escaping the host innate immunity and consequently in establishing the infection in *M. oryzae*.

PR9.2

Role of hydrolases produced by yeast antagonists in the biocontrol of postharvest pathogens of apples and peaches

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Biocontrol of postharvest pathogens by antagonistic yeasts involves several modes of action. Different yeast – including strains of Pichia guilliermondii, Metschnikowia pulcherrima, M. fructicola and Aureobasidium pullulans – have been studied for their biocontrol mechanism. Production of hydrolases is one of the components of the mechanism of action: enzymatic assays permitted to evidence exo-1,3-beta-glucanase, chitinase and alkaline protease activities. An exo-1,3-beta-glucanase gene of 1,224 bp without introns (PgExg1 gene) was amplified from the genomic DNA of P. guilliermondii M8. The gene belongs to the cellulose superfamily. Similarly, two chitinase genes (MpChi1 and MfChi1 genes) were amplified from the genomic DNA of M. pulcherrima strain MACH1 and M. fructicola strain AP47. Both genes lack introns and belong to GH18-chitinase-like superfamily. An alkaline protease gene of 1,351 bp (ALP5) was amplified respectively from the genomic DNA of A. pullulans PL5. The cDNAALP5 gene had a 18-amino acid signal peptide, two introns, one N-gylcosylation, one histidine active site, one serine active site. The protein encoded had 100% homology with the protease enzyme (ALP2) of the sea yeast A. pullulans. Expression in Escherichia coli, followed by identification with Western-blotting, purification with Ni-NTA and analysis with enzyme assay, yielded a homogeneous recombinant cDNAALP5 which hydrolyzed the substrate casein and inhibited pathogen mycelia growth. Production of hydrolases may can greatly contribute to the biocontrol effectiveness.

PR9.3

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

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The identification of suitable targets is crucial for the discovery and development of new antifungals. Since the fungal cell wall is an essential organelle, the identification of genes involved in cell wall biosynthesis is expected to help discover new antifungal targets. From our collection of cell wall mutants, we selected a thermosensitive, osmotic-remediable mutant with decreased resistance to SDS for complementation analysis. The phenotypes of this mutant were complemented by a gene encoding a protein with high sequence similarity to subunit D of the eukaryotic Vacuolar-H+-ATPase (VmaD). Genetic analysis revealed that the mutant allele encodes a protein that lacks 12 amino acids at the C-terminus. Deletion of the entire gene resulted in very poor growth. The conditional mutant displayed several phenotypes that are typical to V-ATPase mutants, including increased sensitivity to zinc ions and reduced acidification of the vacuole as observed by quinacrine staining. Treatment of A. niger germlings with the V-ATPase inhibitor bafilomycinB1 also induced the expression of the agsA gene. Furthermore genes involved in cell wall reassembly like fksA, agsA and phiA are clearly up-regulated in the conditional mutant. Moreover, expression of cell wall related genes can be induced by treatment with the V-ATPase inhibitor bafilomycinB1. Our results indicate that the ATP-driven transport of protons and acidification of the vacuole is crucial for the strength of the fungal cell wall and that reduced activity of the V-ATPase induces the cell wall stress response pathway.

PR9.4

Identification Of Chitin Synthase Genes (*CHS*) In The Postharvest Pathogen *Pencillium digitatum*. Changes Of Expression During Growth And Citrus Fruit Infection.

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The main citrus postharvest pathogen is *Penicillium digitatum*, a necrotrophic fungus with a narrow host range that penetrates citrus fruit through injured peel. Fungal cell wall (CW) is composed of chitin, glucans, mannans and glycoproteins, and is considered an excellent potential target for the development of novel antifungals. Chitin is synthesized by a complex set of chitin synthase genes (*Chs*) that belong up to seven distinct gene families in filamentous fungi. We have carried out the isolation and characterization of chitin synthase genes (*Chs*) of *P. digitatum*. Using distinct sets of degenerate primers designed from conserved regions of *Chs* genes of yeast and filamentous fungi, PCR methods and a DNA genomic library, five complete *Chs* genes (*PdigChsI*, *PdigChsII, PdigChsIII*, *PdigChsV* and *PdigChsVII*) were identified, isolated, sequenced and characterized. A very high sequence identity and strong synteny was found with corresponding regions from the genome of *Penicillium chrysogenum.* Gene expression of *P. digitatum Chs* genes during mycelium axenic growth under different conditions and infection of citrus fruit was quantified using qRT-PCR. *PdigChsIII* had the highest expression among the five genes by one order of magnitude, while *PdigChsII* had the lowest. Results suggest that *PdigChsI*, *PdigChsV* and *PdigChsVII* could have a specific role during the interaction with citrus, since their expression was up-regulated at late times of infection. *PdigChsV* and *PdigChsVII* co-expressed in all the experiments carried out and the analysis of their genomic sequences revealed that they are separated by a 1.77 kb intergenic region that contains several conserved regulatory motifs.

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PR9.5

Development of a screening method for (synthetic) anti-fungal peptides

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Drug resistant fungi are a rapidly upcoming problem and a threat to public health. Triazole agents, the most used group of antifungal drugs, become less effective to mold infections. Successful treatment of e.g. aspergillosis requires active drug therapy. The demand for new and effective anti-microbial agents is rising. Natural antimicrobial compounds known as host defense peptides or antimicrobial peptides (AMPs) are involved in the direct destruction of various microorganisms. AMPs form a promising new source for drug therapy towards fungi. Fungal cell wall biosynthesis is a potential target for drug development. The cell wall integrity pathway plays an important role in cell wall synthesis. *Aspergillus niger* alpha-1,3-glucan synthase (*AgsA*), is strongly and specifically up-regulated in response to cell wall stress (Damveld et.al., 2005b; Meyer et.al., 2007). This gene is used for the construction of a cell wall stress *A. niger* reporter strain.The goal of this research is to develop a fast and hightroughput system for anti-fungal peptide screening.

PR9.6

Fungal a-arabinofuranosidases of glycosyl hydrolase families 51 and 54 show arabinofuranosyl- and galactofuranosyl hydrolyzing activity

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The filamentous fungus *Aspergillus niger* secretes two α-L-arabinofuranosidases that are encoded by the *abfA* and *abfB* genes. Expression of these genes in *Pichia pastoris* and enzymatic characterization of the purified recombinant AbfA and AbfB proteins revealed that both enzymes do not only hydrolyze p-Nitrophenyl-α-L-arabinofuranoside (pNp-α-Ara*f*), but are also capable of hydrolyzing p-Nitrophenyl-β-D-galactofuranoside (pNp-β-Gal*f*). Both AbfA and AbfB showed a higher specific activity towards pNp-α-Ara*f* than towards pNp-β-Gal*f*. Molecular modeling of the AbfB protein with pNp-α-Ara*f* or pNp-β-Gal*f* confirmed the possibility for AbfB to interact with both substrates in a similar manner. The *P. pastoris* expressed AbfA and AbfB proteins did not show hydrolyzing activity towards galactomannan isolated from *A. niger*, despite the presence of β-linked terminal and internal galactofuranosyl moieties within this compound. These data suggest that the AbfA and AbfB proteins are not responsible for the β-galactofuranosidase activity detected in the culture medium of *A. niger*. In addition, culture medium from an AbfA knockout *A. niger* strain showed a similar β-galactofuranosidase activity compared to the medium of a similarly grown parental *A. niger* strain, which does not support a role for AbfA in Gal*f* hydrolysis. In summary our studies show that both AbfA and AbfB contain a Gal*f*-hydrolyzing activity which may have a biological function by hydrolyzing natural Gal*f*-containing substrates from other organisms, or may be a side activity of the Ara*f*-hydrolyzing capacity.

PR9.7

Lectin-like proteins in *Piriformospora indica*

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The identification of microbial cell wall components by the plant host is an effective strategy to seek for potential threads. In order to avoid identification by the host, which may lead to oligosaccharide-triggered immunity, microbes have evolved different strategies, like cell wall masking. Recent findings show that lectin-like proteins play an important role in the suppression of oligosaccharide-triggered immunity. The symbiotic root endohyte *Piriformospora indica* can evade detection and suppresses immunity triggered by various microbe-associated molecular patterns, but the underlying mechanisms remain unclear. Our hypothesis is that the ability of *P. indica* to colonize roots from a wide range of unrelated plants depends on the evolution of strategies for broad immune system evasion and suppression. Comparative genomics revealed a significant expansion of lectin-like proteins containing either one or a combination of the carbohydrate binding domains LysM (chitin-binding); WSC (glucan-binding) and CBM1 (cellulose-binding) in the genome of *P. indica*. These putatively secreted proteins are also induced at the pre-penetration stage and early biotrophic phase and we therefore speculate that they are involved in modulating recognition by masking microbe-associated molecular patterns (MAMPs) and thus avoiding recognition by the host plant. Based on expression profiles data *in planta* we are functionally characterizing selected candidate genes within these 3 different categories.

PR9.8

A Gly579Arg mutation in the *Aspergillus fumigatus pkcA* encoding gene leads to defects in the cell wall integrity maintenance

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Aspergillus fumigatus is a ubiquitous mold that causes a number of clinical diseases in humans including invasive pulmonary aspergillosis, the life-threatening form of infection. The CWIP (cell wall integrity pathway) signaling cascade is activated in fungal cells under stressing conditions and plays a role in the adaptation of several fungal pathogen to the human host. In many fungi, CWIP is launched via the activation of protein kinase C which is ultimately associated to the transcription of a number of genes related to cell wall reinforcement and remodeling. We have recently observed that *pkcA* is an essential gene in *A. fumigatus*. Here we constructed a Gly579Arg mutant through DNA-mediated transformation of a engineered cassette which comprises a G2044C transversion located in the cysteine-rich C1B regulatory domain. To test the involvement of *pkcA*^{G579R} in the CWIP, different concentrations of conidia from the wild type and *pkcA*^{G579R} strains were spotted on complete and minimal agar plates supplemented with different concentrations of substances disturbing/interfering CWI (congo red, calcofluor white, caffeine, glucanex, anidulafungin, SDS). The sensitivity of the *pkcA*^{G579R} to these drugs were increased and could be partially restored by D-sorbitol. Polar and vegetative growth of the *pkcA*^{G579R} mutant strain were also considerably affected mainly at 30°C and 45°C. No defects were observed in the hyphal morphology or in the asexual reproductive structures. These data reinforces the role of *pkcA* signaling cascade in cell wall maintenance in *A. fumigatus*. Virulence assays for the *pkcA*^{G579R} mutant are in progress.

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PR9.9

Genes Involved in Protein Glycosylation Determine the Sensitivity of Saccharomyces cerevisiae to the Cell-Penetrating Antifungal Peptide PAF26

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Protein glycosylation is a complex process that occurs through the N- or O-glycosylation pathways by the coordinated regulation of numerous genes and enzymes. Several glycosylation genes have been previously involved in the sensitivity of fungi to antifungal peptides and proteins. We have characterized the synthetic hexapeptide PAF26 as a cell-penetrating and non-lytic antifungal peptide that inhibits Saccharomyces cerevisiae and filamentous fungi. We searched the S. cerevisiae public collection of deletion mutants for glycosylation genes whose deletion altered sensitivity to PAF26. We observed that deletion of genes coding for the conserved protein O-mannosyltransferases (PMT) responsible for the addition of the first mannosyl residue of O-linked carbohydrates and for the EOS1 enzyme involved in N-glycosylation of cellular proteins, among other genes, resulted in specific increased resistance to diverse antifungal peptides including PAF26. Many cell wall (CW) proteins in fungi are glycosylated and/or anchored to the CW by diverse glycan structures. Microscopic visualization of S. cerevisiae cells exposed to fluorescently labelled peptide has shown that PAF26 firstly interacts with the cell envelope, prior to cell internalization, and subsequent causes intracellular cell death. Protoplasts lacking CWs interacted poorly with the peptide, and were more resistant to peptide killing than cells possessing CWs. Microscopic studies on the S. cerevisiae Δeos1 deletion mutant demonstrated a blockage of peptide internalization into cells. Interestingly, protoplasts obtained from this mutant behaved similarly to the parental strain. Collectively, these observations indicate that EOS1p exerts its activity through the glycosylation of CW protein(s) involved in the internalization of antifungal peptides.

PR9.10

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

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Galactofuranose-containing glycoconjugates are present in numerous microbes, many of which are pathogenic for humans. Metabolic aspects of the monosaccharide have proven difficult to elucidate, because galactofuranose metabolites and glycoconjugates are relatively unstable during analyses. Recent advances with genetic approaches have facilitated a better understanding of galactofuranose metabolism. Galactofuranose (Gal*f*) the five-ring isomer of galactopyranose (Gal*p*), is an essential component of the cell wall and required for a structural integrity [1-2]. Recently it has been postulated that Gal*p* bound to UDP, is converted to Gal*f* by a UDP-galactopyranose mutase (UGMA) and subsequently transported into the Golgi by a putative gal*f*-transporter namely GlfB [3] for the further biosynthesis of e.g. galactomannan, galactoaminogalactan and cell wall glycoproteins(galactomanno-proteins) [4-6]. The sugar units can be cross-linked with each other via covalent or by hydrogen bonds but the actual composition is changing depending on the respective fungal species and environmental factors [5, 6, 8].

Based on homology search we have identified two putative Gal*f*-transporters in *A. niger* (GlfB homologue) UGTA/UGTB and investigated their role in the biosynthesis of Galf-containing glycoconjugates and their role in Galf containing cell wall compartments. Moreover, we evaluated growth and morphological effects of the deletion of the corresponding genes in relation to fungal biology.

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PR9.11

The product of NRPS 4 from *Fusarium graminearum* - A fungal raincoat?

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Nonribosomal peptide synthetases (NRPSs) are large multi-modular enzyme-complexes that produce small peptides independently of the ribosomal machinery. *Fusarium graminearum* has 19 putative NRPS genes for which the products of three are known. These are all iron chelating siderophores, whereas the products of the remaining predicted NRPSs are unknown.

The *NRPS4* of *F. graminearum* encodes an 844 kDa enzyme consisting of five modules and is conserved throughout all sequenced *Fusarium* species and across a range of other plant pathogenic filamentous fungi. Deletion of *NRPS4* in *Cochliobolus heterostrophus* and *NRPS 2* in *Alternaria brassicicola* (48% and 49% consensus identity to *FgNRPS4,* respectively) resulted in phenotypes where surface hydrophobicity was significantly reduced. In the present study we have generated deletion and over expression mutants of *NRPS4* in *F. graminearum.* Both mutants show a clear phenotype as the deletion mutant displays a reduced surface hydrophobicity whereas the over expression mutant is completely water resistant.

PR9.12

Characterisation of *Aspergillus niger* chitinases involved in aging identifies a novel activity in fungal GH18 chitinases

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The filamentous fungus *Aspergillus niger* has significant industrial importance as a production host for metabolites and extracellular enzymes. Fungal aging and stress conditions during industrial fermentations may lead to the initiation of sporulation and autolysis, during which glycoside hydrolases (GH) can modify the cell wall structure. A full understanding of processes taking place during aging and nutrient starvation contributes to the identification of strategies to increase fermentation efficiency.

Using transcriptome analysis, we identified glycoside hydrolases of *A. niger* produced by the aging and nutrient starved mycelium. Two enzymes were heterologously expressed, purified and characterised to gain more insight in their physiological and metabolic function. Both CfcA and CfcI belong to GH family 18, which fungal members consist mainly of (putative) chitinases. CfcA releases mainly chitobiose from the non-reducing end of chitin oligosaccharides and from chitin present in the fungal cell wall.

CfcI is capable of hydrolysing chitotriose and longer chitin oligosaccharides. CfcI by itself is not capable of releasing products from the fungal cell wall. However, *cfcI* is expressed together with *ctcB,* which encodes a putative endochitinase. The oligosaccharides generated by CtcB activity on cell walls may act as substrates for CfcI. CfcI functions by cleaving off monomers, possibly in a processive mode, acting on the reducing end of the oligosaccharide substrates. To the best of our knowledge, this activity has not been reported before for fungal chitinases of glycoside hydrolase family 18.

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PR9.13

Structural and functional characterization of *Candida glabrata* epithelial adhesins

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Candida glabrata is an emerging human pathogen that is responsible for about 15 % of mucosal and systemic fungal infections. The genome of *C. glabrata* encodes a set of more than twenty surface-exposed, epithelial adhesins (Epa) for host cell adhesion and colonization. Host glycan recognition by the Epa protein family is governed by their adhesin (A) domains and hence crucial for discrimination of various target tissues. In this study, we focus on the structural and biochemical characterization of different Epa A domains to obtain detailed insights into ligand specificity. The crystal structure of the Epa1A domain reveals how core1 and core2 mucin-type O-glycans are recognized by the major Epa1 subtype. Structural and functional characterization of subtype-switched Epa variants shows that specificity is governed by two loops, CBL1 and CBL2, involved in calcium binding. Together with a conserved tryptophan, these loops organize the recognition site into an inner subsite for general, calcium-dependent galactose binding and an outer subsite for specific interactions with different types of host glycans. These structural insights show that the Epa family consists of at least four different subtypes that are discriminated from each other by conserved sequences within the CBL2 region. Overall, our study demonstrates how *C. glabrata* colonizes host tissues and provides a promising structural basis for the development of tailored antimycotics.

PR9.14

Structural and functional analysis of *Saccharomyces cerevisiae* cell surface adhesins

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Saccharomyces cerevisiae contains a set of cell wall-associated proteins, the flocculins, which confer adhesion. The highly related Flo1, Flo5 and Flo9 proteins confer cell-cell adhesion by lectin-like and calcium-dependent binding of mannoproteins on neighboring cells. This type of adhesion is known as flocculation and allows *S. cerevisiae* to form protective multicellular flocs. A further structurally unrelated flocculin, Flo11, enables yeast cells to adhere to abiotic surfaces such as agar and plastic. Finally, Flo10 represents a flocculin, whose function is discussed controversially, because it has been described to confer both flocculation and agar adhesion when overproduced. In this project, we have initiated a structural and functional analysis of the Flo10 adhesion domain (= A domain). Modeling of Flo10A reveals a high structural similarity to Flo5A and shows that Flo10A is a PA14-related protein. However, Flo10A differs from Flo5A by its subdomain, which in case of Flo5A has been suspected to confer ligand binding specificity. By using a *FLO11*-based expression system we find that Flo10A, in contrast to Flo5A, does not confer flocculation. Furthermore, deletion of the Flo5A subdomain results in a loss of flocculation, while mutual exchange of the Flo10 and Flo5 subdomains is sufficient to swap functionality. Our results highlight the crucial role of adhesin subdomains in conferring ligand binding specificity to PA14-related proteins.

PR9.15

gcsA*, an ARF-GAP-ENCODING GENE IN *Aspergillus fumigatus.

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Aspergillus fumigatus is one of the most important airborne pathogenic fungi, which has the potential to cause Invasive Pulmonary Aspergillose (IPA). Sphingolipids are the major component of the eukaryotic plasma membrane and are involved in the cell wall integrity and virulence. The ADP-ribosilation factor (ARF) family of proteins belongs to the Ras superfamily of small GTPases. The hydrolysis of ARF GTP-bound is mediated by GTPase-activating proteins (GAPs). ARF-GAPs are required for vesicular coat formation in endocytic pathway and have been related to hyphal growth, drug resistance and virulence in *C. albicans*. In this work we identified *gcsA*, the *C. albicans age3* ortholog gene in *A. fumigatus. gcsA* null mutant has normal hyphal growth, exhibit no differences in sensitivity to antifungal agents, was not able to form biofilm, and is virulent in an IPA mouse model as the wild type. Thus, despite the *gcsA* influence on the sphingolipids biosynthesis, here shown by the decreased cell polarization in the presence of Myriocin, *gcsA* null mutant has no change on antifungal sensitivity, virulence, and hyphal growth profile, suggesting a probable distinct function of this gene in the filamentous fungus *A. fumigatus*.

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PR9.16

Functional analysis of the Mps1 MAP kinase pathway in the rice blast fungus *Magnaporthe oryzae*.

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Magnaporthe oryzae is a fungal rice pathogen responsible for large yield losses worldwide. Our project concerns the study of the Mps1 MAP kinase pathway of *M. oryzae,* involved in cell wall integrity, sporulation and pathogenicity. Mps1 is orthologue of yeast SLT2 and activates the transcription factors Rlm1, Swi4 and Swi6, while calcineurin activates Crz1. *M. oryzae* genes orthologous to yeast *CRZ1*, *MPS1*, *RLM1, SWI4,* and *SWI6* genes were identified. Swi4 and Swi6 interact with Mps1 in yeast two hybrid experiments (a) as well as Mps1 and Rlm1 (b). The main task focuses on the analysis of the Mps1 downstream elements Rlm1, Swi4 and Swi6. *Δmps1* mutants displayed an abnormal mycelial growth (no aerial hyphae), did not sporulate, and were non-pathogenic on plants as reported (c). *Δswi4* displayed phenotypes similar to *Δmps1* with milder growth and sporulation defects, while it was as non-pathogenic as *Δmps1*. *Δcrz1* and *Δswi6* mutants have a normal mycelial growth and sporulation rates, while *Δrlm1* has a reduced sporulation rate. *Δcrz1* and *Δrlm1* were non pathogenic on barley and rice, while *Δswi6* was pathogenic. These studies suggest Swi4 is the major target of Mps1 during mycelial growth, while both Rlm1 and Swi4 are the targets of Mps1 during sporulation and infection. *Δmps1, Δrlm1, Δswi4* and *Δswi6* null mutants are currently tested for their sensitivity to cell wall degrading enzymes and inhibitors of cell wall biosynthesis.

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PR9.17

Cell wall stress modulates the expression of the ram signaling network components in *Trichophyton rubrum*.

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Trichophyton rubrum is a pathogenic, cosmopolitan and anthropophilic fungi that infect keratinized tissues mainly skin and nails. The genome of several dermatophytes, including *T. rubrum*, was sequenced by the Broad Institute/NIH, enabling studies on the expression regulation of genes related to diverse cellular processes. The transcription factor (TF) Ace2 participates in a network of genes called RAM (Regulation of Ace2 activity and cellular morphogenesis), involved in the regulation of morphogenesis, cell division, and development of conidiophores. In dermatophytes this network has not yet been characterized. Therefore, the aim of this study was to identify these genes in *T. rubrum* genome, as well as their possible regulation by Ace2 through *in silico* analysis, and to evaluate the transcriptional profile of these genes in response to various cell wall disturbing agents, and the osmotic and oxidative stresses. *In silico* analysis suggested their possible regulation by this TF in *T. rubrum*. To analyze the expression of the RAM network genes, *T. rubrum* was exposed to several cell wall stressor agents. The transcriptional profile of the RAM network genes in response to disturbances in cell wall assists a better comprehension of the involvement of this pathway in regulating a variety of processes that enable cell viability during environmental stress, once the RAM signaling network components are highly conserved in eukaryotes.

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Poster Category 10: Other Fungal Features and Oddities

PR10.1

Effect of fungicide application on the occurrence of *Fusarium culmorum* and mycotoxin production in wheat grain determined using Real-Time PCR

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Numerous analyses show that the presence of fungi of *Fusarium* genus in cereal grain is associated not only with decreases in the yield and technological quality, but also poses a threat to human and animal health because of the mycotoxins produced by these fungi. The amount of mycotoxins is related to the degree of grain contamination by fungi. Fungicides significantly reduce *Fusarium* species, but their application in some conditions may cause the higher incidence of toxic metabolites in grain.

The aim of the study carried out at experimental field in Lisewo Malborskie in Poland was to determine if azoxystrobin, metconazole and prothioconazole with t*ebuconazole* used for the control of wheat FHB at half, full, and quarter more the recommended dose rate may affect in differentiated way on the occurrence of *Fusarium* spp. and their ability to mycotoxin production in harvested grain, in wheat ears artificially inoculated with two DON-producing isolates of *F. culmorum*. After DNA isolation from harvested grain the presence of *F. culmorum* was determined using traditional SCAR-PCR with species specific primers and with Real-Time PCR technique using a LightCycler 480II (Roche) and SYBR Green I dye. Also the deoxynivalenol (DON) content was determined by GC-ECD. We revealed that there is correlation of gene copy number with actual concentration of mycotoxins and that improper use of fungicides may increase the concentration of toxins in the grain.

PR10.2

Fusarium Ear Rot Pathogens And Their Mycotoxins Associated With South African Maize

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Maize is the most important agricultural crop produced in Southern Africa, and is consumed daily by millions of Africans as a staple food. In South Africa, the crop is often affected by ear rot pathogens belonging to the genus *Fusarium* and their mycotoxins. To determine the prevalence of *Fusarium* species and their toxins, samples were collected from two susceptible maize cultivars at 14 localities in South Africa during 2008 and 2009. *Fusarium* species was quantified by real-time PCR and their mycotoxins by multi-toxin analysis using HPLC-MS. In 2008, *F. graminearum* was the predominant species in the eastern Free State, Mpumalanga and KwaZulu-Natal provinces, while *F. verticillioides* was predominant in the Northwest, the western Free State and the Northern Cape provinces. In 2009, maize ear rot infection was higher and *F. graminearum* became the predominant species found in the Northwest Province. *Fusarium subglutinans* was associated with maize ear rot in both years at most of the localities, while *F. proliferatum* was not detected from any of the localities. Deoxynivalenol and zearalenone correlated well with the amount of *F. graminearum* found in maize grain, fumonisins with *F. verticillioides*, and moniliformin and beauvericin with *F. subglutinans*. Our findings suggest a shift in the occurrence of *Fusarium* species and their mycotoxins in South African maize, which could be contributed to changing agricultural practices and climatic changes in production areas.

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PR10.3

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

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Current options for the treatment of Aspergillus infections are limited and suffer from a variety of shortcomings. Despite the discovery of numerous promising drug targets, few lead compounds have been discovered by target based approaches. This can be explained, in part, by the 'druggability' of a target as some compounds which demonstrate promising activity against an enzyme are not active against the whole cell or are toxic. A solution to this problem is to employ techniques to identify gene targets from compounds that already show antifungal activity and have clean toxicity profiles.

Chemical genetic profiling aids identification of drug mechanism of action as diploid strains lacking a single copy of a drug's target are hypersensitive to that drug. Heterozygote *S. cerevisiae* and *C. albicans* libraries have been used to identify the mechanism of action of several promising compounds; however, this has been hindered in *A. fumigatus* by the complexity in generating an adequate set of heterozygous strains. A high-throughput targeted gene KO method for *A. fumigatus* has been established by employing fusion-PCR to generate targeted gene disruption cassettes, optimizing the common transformation protocol for *A. fumigatus* high-throughput gene disruption, and utilising a diploid *Ku80*$^{-}$*/Ku80*$^{-}$mutant to facilitate more reliable homologous recombination. Preliminary efforts have produced 46 heterozygous KO strains and subsequently, the feasibility of chemical genetic haploinsufficiency studies in filamentous fungi has been demonstrated. This enables high-throughput methods for surveying the genome of *A. fumigatus* for new drug targets and supports unveiling the mechanisms of action of antifungal drugs.

PR10.4

Intracellular Proteome Response of *P. Chrysogenum* To The Addition Of Polyamines 1,3 Diaminopropane And Spermidine

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Penicillium chrysogenum is a filamentous fungus industrially used for the production of the beta-lactam antibiotic penicillin. It has been recently reported that the addition of polyamines 1,3-diaminopropane and spermidine induces penicillin gene expression and leads to an increase of the penicillin titers. In order to characterise the metabolic processes that have been modified by these polyamines, the intracellular proteome of *P. chrysogenum* Wisconsin 54-1255 grown in the presence of either of these two polyamines was analysed by means of two-dimensional electrophoresis followed by protein identification by MALDI TOF-TOF.

Protein changes related to some mechanisms that have a positive effect on penicillin production were found after the addition of 1,3-diaminopropane or spermidine. One of them is the visualization of a novel isoform of the isopenicillin N acyltransferase (the last enzyme of the penicillin pathway). Another modification has a positive effect on the biosynthesis of beta-alanine. This aminoacid is an intermediate in the biosynthesis of panthotenic acid, which is converted to 4´-phosphopantetheine. The latter is an essential prosthetic group for several enzymes, including the alpha-aminoadipyl-L-cysteinyl-D-valine synthetase, which is the first enzyme of the penicillin biosynthetic pathway. A decrease of the enzymes for the catabolism of phenylacetic acid (the side chain precursor of benzylpenicillin) is induced by those polyamines. This mechanism may also explain the increase in penicillin titers since more amounts of the side chain precursor would be available during penicillin biosynthesis.

These proteomics studies offer us a global vision of the effects that the polyamines 1,3-diaminopropane and spermidine have on *P. chrysogenum* primary and secondary metabolism.

PR10.5

The Regulatory Factor PcRFX1 (CPCR1 Ortholog) Controls Penicillin Biosynthesis And Sporulation In *Penicillium Chrysogenum*

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Penicillium chrysogenum is a filamentous fungus mainly known for being the industrial producer of the β-lactam antibiotic penicillin. No penicillin pathway-specific regulators have been found in the amplified region containing the penicillin gene cluster so far and therefore, penicillin biosynthesis seems to be controlled directly by global regulatory factors.

The transcriptional factor CPCR1 has been recently identified in another β-lactam producer, *Acremonium chrysogenum,* where it acts as a positive regulator of the cephalosporin C biosynthesis and it seems to be involved in morphological development

The CPCR1 ortholog in *P. chrysogenum* has been characterized in our group. The gene encoding this transcription factor (*Pcrfx*1) was identified in the *P. chrysogenum* genome (Pc20g01690).

The promoter region of the penicillin biosynthetic genes was analysed to search for putative PcRFX1 DNA binding sites. Two binding sites in *pcbAB*, one in *pcbC* and another in the promoter region of *penDE* were found. The putative PcRFX1 DNA binding sequences located on the penicillin biosynthetic gene promoters were proven to be functional.

Gene silencing of *Pcrfx*1 decreased 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 reduced.

Finally, the effect of gene silencing on hyphae morphology and sporulation was analysed both in the wild-type and in the knock-down mutant strains. A more abundant sporulation has found in the wild-type regarding the knock-down mutant. These results indicate that PcRFX1 acts as a global regulator in *P. chrysogenum*.

PR10.6

Phylogeny of *Penicillium* species based on ß-tubulin gene sequences.

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AAFC

Many *Penicillium spp*., including *Penicillium expansum,* cause blue mold, an important postharvest disease of apples world wide. To determine the identity of the species*,* 33 *Penicillium* isolates collected from floatation tanks in three apple packinghouses in Ontario, Canada and six reference isolates were selected for DNA sequencing. Sequencing was performed using forward primer Bt-T2M-Up (5'-CAACTGGGCTAAGGGTCATT-3') and reverse primer Bt-LEV-Lo1 (5'-GTGAACTCCATCTCGTCCATA-3') from the ß-tubulin gene. The partial ß -tubulin gene sequences of the test isolates were compared with known reference isolates. The phylogenetic analysis of ß -tubulin gene sequence data of 33 isolates, led to the identification of 3 isolates as *P. solitum*, and the remaining 30 isolates as *P. expansum*. The fungal colony morphology and pathogenicity were correlated with the species. A very low genetic diversity was observed.

Some isolates of *Penicillium spp*. have developed resistance to thiabendazole (TBZ, Mertect™), a fungicide registered for the control of blue mold. The DNA sequence at codon 198 of the ß -tubulin gene corresponds to thiabendazole resistance if the GAG sequence had a substitution. Based on the analysis of DNA sequence of codon 198, six *P. expansum* isolates and three *P. solitum* isolates were found to be TBZ-resistant. The remaining 18 *P. expansum* isolates were sensitive to TBZ. The TBZ-amended media studies correlated with the sequence data of the isolates identified either as TBZ-resistant or -sensitive. This information, the identification of *Penicillium* species and their resistance to TBZ, is important in developing postharvest disease management strategies for stored apples.

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PR10.7

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

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

PR10.8

Sodiomyces alkalinus – a New Holomorphic Alkaliphilic Ascomycete from Soda Soils Is a Member of Plectosphaerellaceae

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The majority of fungi are considered to have optimal growth around slightly acidic or neutral pH. Fungi optimally growing at high alkaline pH values (>9) are rare and have been poorly described. Among probable reasons for that are relative remoteness and low abundance of potential habitats where these fungi could be possibly isolated. Only few filamentous alkaline fungi have been reported to date. In the present study, we characterize a new alkaliphilic holomorphic ascomycete isolated from soda soils. Growth experiments in a wide pH range have confirmed an alkaline nature of this new fungus. Scanning electron microscopy images have revealed morphological features which possess adaptive defensive properties in order to cope with harsh external environments. Originally this fungus was assigned to the genus Heleococcum (order Hypocreales) based on morphology but molecular taxonomy shows that our fungus represents a new genus in a Plectosphaerellaceae family clade. Sequences of four genes (RPB2, nSSU rRNA, nLSU rRNA and 5.8S rRNA) were used in a Bayesian approach in order to pinpoint the taxonomic position of this alkalophilic fungus. Representing an extreme case of adaptive evolution to alkaline conditions, this species offers a great potential for studying exocellular alkaline enzymes which may also be of interest to industry.

PR10.9

Discovery of novel basic helix-loop-helix (bHLH) transcription factors regulating development in *Aspergillus oryzae*

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The basic helix-loop-helix (bHLH) family of proteins comprises a group of transcriptional factors that are often important in development and differentiation. In our previous report, we identified a *sclR* gene encoding a bHLH transcription factor. We constructed *sclR*-disruptant and *sclR*-overexpressing strains, finding that there was hardly any sclerotium-like body to be observed in the *sclR*-disruptant strain, whereas *sclR*-overexpressing strain produced less conidia and more sclerotium-like body on the malt agar medium.

In this study, we identified another bHLH transcription factor-encoding gene, *ecdR*. The *ecdR* gene disruptant hardly produced conidia. Conversely, the overexpression of *ecdR* resulted in the formation of a large number of conidia at an early stage. Additionally, when serially diluted conidia were spread-cultivated onto malt agar medium, we found that conidial number of the control strain depended on the cultivated conidium density, while the *ecdR*-overexpressing strain showed no significant change in conidiation. These phenotypes of the *ecdR*-disruptant and *ecdR*-overexpressing strains are partially similar to those of the *sclR*-overexpressing strain and *sclR*-disruptant, respectively. Yeast two-hybrid assays indicated that EcdR interacted with SclR to form heterodimer and simultaneously they could also form homodimer. Interestingly, although EcdR interacted with SclR, their expression patterns were completely different. From these results, we concluded that EcdR and SclR have opposite roles in development. By competitively interacting with each other according to culture conditions, they form heterodimer and may result in a mutual inhibition of function.

PR10.10

Investigation of Antimicrobial Effect of *Neosartorya fischeri* Antifungal Protein (NFAP)

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Objectives: Small molecular mass, cysteine-rich antifungal protein was isolated and characterized from an ascomycetous fungus *Neosartorya fischeri*. *Neosartorya fischeri* antifungal protein (NFAP) shows remarkable antimicrobial effect against other filamentous fungi. In this study, we investigated the antimicrobial effect of NFAP with heterologous expression of the *nfap* gene in an NFAP-sensitive fungus, *Aspergillus nidulans*.

Methods: Heterologous expression of nfap gene was carried out in *A. nidulans* (pyrG89) using a pAMA-based autonomously replicating vector construction. Effect of the produced NFAP on the germination of *A. nidulans* conidia was investigated with 4'-6-Diamidino-2-phenylindole- (DAPI, Serva) and calcofluor white-staining (CFW, Sigma-Aldrich). Annexin V-FITC Apoptosis Detection Kit (Sigma-Aldrich) was used for revealing the possible apoptotic effect.

Results: The *nfap* gene was expressed in the *A. nidulans* transformant strains. Macroscopic observations revealed the reduction of hyphal growth in case of the transformants expressing the nfap gene compared to the untransformed *A. nidulans* strain. Transformants displayed abnormal and delayed germination: conidiospores formed very short, swelled hyphae with multiple branches. The germination tubes were destructed after 8 hours of cultivation. Later, membrane damage and accumulation of nuclei in the broken hyphal tips were detected by DAPI- and CFW-staining. Apoptotic events were also detected in case of NFAP-producing A. nidulans strains.

Conclusion: Manifestation of antifungal effect of NFAP on a sensitive fungus is similar to those described previously for the related peptides of *Aspergillus giganteus* (AFP) and *Penicillium chrysogenum* (PAF).

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PR10.11

Cloning and Functional Analysis of Genes Coding for Some Enzymes of the Mevalonate Pathway in Trichoderma

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The mevalonate pathway leads to synthesis of many biologically active molecules such as terpenes or quinines. Furthermore, dolichyl phosphate, a carrier of carbohydrate residues in glycosylation processes, is also produced in this pathway. In this study we decided to clone and analyze the function of rer2Tr and erg20Tr genes coding, respectively, for cis-prenyltransferase and farnesyl diphosphate synthase (FPPS) in Trichoderma. We cloned and expressed both genes in the appropriate S. cerevisiae mutants. Expression of the rer2Tr gene in the S.cerevisiae SRT1/Δrer2 mutant resulted in a very high amount of the rer2Tr transcript and only 19% higher activity of cis-prenyltransferase. Since dolichols isolated from the S.cerevisiae SRT1/Δrer2/rer2Tr mutant were synthesized by yeast Srt1p and the RERII protein from Trichoderma, we expected a mixture of dolichols characteristic for both organisms. HPLC analysis of dolichols isolated from the strain revealed only the yeast type. This result suggests that either the Trichoderma enzyme is not active or it produces the yeast type of dolichols. To elucidate this problem we expressed the rer2Tr gene in the Δrer2/Δsrt1 double mutant. Cloning and analysis of the Trichoderma erg20Tr gene in the S. cerevisiae Δerg20 mutant showed that the yeast gene could not be suppressed by the Trichoderma one. To analyze erg20Tr gene function we overexpressed it in T. reesei. The transformants exhibited higher activity of FPPS.

PR10.12

Antifungal susceptibility of *Aspergillus* spp. under hypoxic growth conditions

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Invasive aspergillosis is a major life-threatening disease in immunocompromised patients, with mortality rates up to 90 %. The most common species is *Aspergillus fumigatus* (90 % of infections), followed by *A. flavus,* and *A. terreus*.

During infection, fungal pathogens must adapt to microenvironmental stresses, including hypoxia as well as high CO_2 levels. Such oxystress conditions are usually not taken into account in current models of infection and assessment of antifungal sensitivities. As antifungal test systems, such as Etests, we compared the *in vitro* activity of amphotericin B, various azoles, and echinocandines in hypoxic conditions (1 % O_2, 5 % CO_2) to their activity in normoxic conditions against 47 isolates of *Aspergillus* spp. belonging to *A. flavus (n=9)*, *A. terreus (n=16),* and *A. fumigatus (n=22)*. We found that in hypoxic conditions similar to those that might occur in *aspergillus*-infected tissue, a reduction in the *in vitro* MIC of amphotericin B for all three species occurred. Similar MIC reduction effects were found for azoles, especially for *A. flavus* species, while for echinocandines differences where less significant and the phenomenon of trailing was also persistent in hypoxic conditions, which makes determination of MIC rather difficult. Further tests are currently in progress to find out if similar results can be obtained with microbroth dilution assays, where not only gas concentrations are regulated to mimic host environments, but also other parameters such as pH, iron limitation or the provision of host cell components can be manipulated.

PR10.13

Evolution of necrotrophic effectors within *Phaeosphaeria nodorum* and close relatives

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The genetic relationships among *Phaeosphaeria* species that infect wheat are not well understood. This study expanded on earlier work using sequence data to define the species infecting wheat in a global sample. We sequenced 1,542 bp across 3 loci in 355 isolates from around the world, including many isolates from Iran, near the host center of origin. We were able to differentiate *P. nodorum* and two previously defined taxonomic groups of *Phaeosphaeria avenaria tritici* (Pat), called Pat1 and Pat3. We identified three new closely related *Pat* groups from grass hosts, named *Pat4, Pat5* and *Pat6.* We present two new species from Iranian wheat, tentatively named P1 and P2. We found evidence of incomplete lineage sorting between *P. nodorum* and Pat1. We propose these 9 groups as distinct phylogenetic clades.

The main virulence mechanisms identified in *P. nodorum* are host selective toxins (HSTs), which interact in a gene-for-gene manner with toxin sensitivity genes in wheat to cause lesion formation. We found that global populations of wheat-infecting *P. nodorum* carried *SnTox3, SnToxA* and *SnTox1* with widely varying frequencies. In a global sample of over 1000 isolates, the multi-toxin genotypes did not differ significantly from frequencies expected under random mating. Furthermore, the distribution of toxin sequence diversity did not coincide with the distribution observed for neutral markers in *P. nodorum.* By combining the species phylogeny with data on toxin distribution, we could elucidate the evolutionary timescales over which host selective toxins evolved to become major contributors to this disease complex in the wheat agro-ecosystem.

PR10.14

Molecular identification of clinically important *Bipolaris* species

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The anamorphic ascomycetous genus, *Bipolaris* includes several plant pathogenic species. Three of them, namely *Bipolaris australiensis*, *B. hawaiiensis* and *B. spicifera*, are also able to cause infections in both immunocompromised and immunocompetent humans. Using the conventional morphology-based identification methods, which involves the examination of the septation, shape and size of the conidia, their differentiation is unreliable and time consuming.

In the present study, we examined the phylogentic relationships, among the three human pathogenic *Bipolaris* species, based on this analysis, we tried to establish useful markers for molecular identification of species and strains. For this reason, 35 isolates of *B. australiensis*, *B. hawaiiensis* and *B. spicifera* were obtained from human keratomycosis and from international strain collections and the internal transcribed spacer (ITS) and the intergenic spacer (IGS) regions of the nuclear ribosomal RNA gene cluster, the β-tubulin and the translational elongation factor EF-1 alpha genes were sequenced and analysed. The sequences were investigated in a phylogenetic context also. Earlier molecular phylogenetic studies involving determined the taxonomic position of the *Bipolaris* and related genera, but within the genus, the position of the clinically important species remained ambiguous. The joint analysis of the above mentioned four sequences with Bayesian method completed with the morphological data of the studied isolates resolved the taxonomic questions in connection with the clinically important *Bipolaris* species. Based on the determined sequences, a rapid molecular identification method could be.

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PR10.15

Post-transcriptional suppression against potential transposable elements by cryptic splicing and premature polyadenylation in *Aspergillus oryzae*

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

PR10.16

***Aspergillus fumigatus* mycovirus infection is not dependent on the genetic up-make of the host**

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Introduction: Mycoviruses are viruses that selectively infect fungi and are ubiquitous in all major groups of filamentous fungi. Most mycoviruses have dsRNA genomes and replicate cytoplasmatically. Although most of the mycoviruses cause cryptic infections, mycoviruses are of scientific interest since some of these viruses can cause fungal hypovirulence such as reduced growth rate, altered pigmentation and sporulation. Viruses are considered to be host-specific, but within each host some individuals are more prone to develop a viral infection than others. Therefore, we determined if the genetic make-up of *Aspergillus fumigatus* was correlated to the presence of a mycovirus.

Materials & Methods: A collection of 112 clinical *A. fumigatus* isolates from the Erasmus MC, Rotterdam, The Netherlands was screened for mycovirus presence by isolating dsRNA from fresh lyophilized mycelial cultures using a Trizol/chloroform method. To determine genetic relatedness of *A.fumigatus* the cell surface protein (CSP) gene was typed by sequencing.

Results: Of the 112 clinical *A.fumigatus* isolates 16 (14.3%) contained dsRNAs. The *A.fumigatus* collection could be divided into 12 different CSP types, indicating that the collection used was of heterogenous origin. *A.fumigatus* isolates which contained dsRNA mycoviruses had similar CSP types as non-infected isolates. In both cases, the CSP types 1, 2, 3 and 4 were the most prevalent which was comparable to the CSP types observed in other Dutch collections.

Conclusion: Mycovirus infection is not related to a specific genetic *A. fumigatus* lineage

PR10.17

The genetic basis of conidial pigmentation in *Aspergillus niger*

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

PR10.18

Genetic diversity of *Rhizoctonia solani* isolates from UK potato crops

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Rhizoctonia solani is an important pathogen of potatoes, causing infection of stems and roots and tuber blemish diseases. The fungus is a species complex consisting of 13 related but genetically distinct anastomosis groups (AGs). Isolates are assigned to AGs on the basis of hyphal interaction between isolates. In the UK, AG3 predominates accounting for over 90% of findings but isolates of AG2-1 and AG5 have also been found in potatoes. Although knowledge of the diversity of AGs present is known at a national level in the UK, little is known about the diversity of isolates at the field scale and no studies have been undertaken to investigate the genetic diversity of UK AG3 isolates. To address this, mulitple isolates were taken from three different fields in the UK and compared with other UK isolates. All isolates were determined to be AG3 by using an AG3 specific TaqMan assay. The genotypes present within the AG3 isolates were determined using a range of previously published polymorphic co-dominant single locus PCR-RFLP markers or by sequencing the ITS region and part of the translation elongation factor and cytochrome oxidase genes. In addition, hyphal fusion tests were done between pairs of isolates to determine vegetative compatability group (VCG) for each. Analysis revealed that the population of AG3 isolates within a field is diverse with multiple genotypes and VCGs recovered from the same $30m^2$sampling area. This suggests that AG3 isolates from UK potatoes are not a clonal population.

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PR10.19

The role of conidial anastomosis tubes in *Colletotrichum lindemuthianum*

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Conidial anastomosis tubes (CATs) is a common phenomenon in bean pathogen *Colletotrichum lindemuthianum.* We used different strains to perform a microscopical analyse of the fusion between germlings and within mature colonies. In mature colonies, vegetative hyphal fusion between two incompatible strains caused rapid disappearance of nuclei and cell death. In marked contrast, CAT fusion between incompatible strains suppressed the heterokaryon incompatibility response. Heterokaryotic mycelium within the same colonies were produced, and dissected, resulting in different phenotypes. Vegetative incompatibility occurs after fusion and is triggered very fast in mature colonies, but is repressed and delayed during colony initiation. The heterokaryon mycelium originated from fused germlings survives long enough, to allow cytological and nuclear mixing, with important consequence for the cells involved. Through CAT fusion is possible to generate fungal genetic diversity.

PR10.20

Morphological and Molecular Identification of *Trichoderma* Species from West of Iran

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Some 500 *Trichoderma* isolates were obtained from soil samples and bark of trees collected from west parts of Kurdistan province. Cultures were grown on PDA and purified on 2% water agar by hyphal tip method prior to identification. The isolates were identified using morphological features, including colony characters (pigmentation and growth rate on PDA) and microscopic characters, including shape of conidiophores, shape and size of conidia and phialides. The microscopic features were studied and recorded 3-5 days after inoculation on cultures grown on CMD at 25°C under ambient laboratory condition. Nine species identified, including *T. citrinoviride, T. longibrachiatum, T. saturnisporum, T. asperellum, T. atroviride, T. harzianum, T.arundinaceum, T. brevicompactum* and *T.virens*, in addition six isolates possess conidiophor similar to species in section *Longibrachiatum* were morphologicaly different from others and introduced *Trichoderma* species until now, therefore these isolates can be a new species of *Trichoderma*. In order to accurate identification and support morphological studies internal transcribed spacers of the rDNA, translation elongation factor 1-alpha (*tef-1alpha*) and a fragment of the gene coding for endochitinase 42 (*ech42*) these isolated were nucleotide sequenced and compared by using blast search with introduce *Trichoderma* species. Result of molecular study supported morphological studies and these isolates could be a new species of *Trichoderma*, belonging to *Longibrachiatum* section.

PR10.21

The use of whole genome microarrays to study viral interactions with *Agaricus bisporus*

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Fungal viruses were first identified in the cultivated mushroom *Agaricus bisporus* in1962. Viral diseases can cause widespread devastation and are difficult to eradicate in mushroom crops which are vegetatively propagated. The latest viral disease known as Mushroom Virus X (MVX) has become a serious economic problem and is associated with changes in agronomic practices, in particular bulk substrate colonisation. Recent research has provided strong evidence that this disease is caused by a number of double-stranded RNA (ds-RNA) viruses, for example transcripts which are not of *A. bisporus* origin but hybridise to ds-RNAs, and are found at very high levels in tissues exhibiting strong disease symptoms.

Statistical analysis of microarray data has identified host transcripts differentially expressed (at the 0.05% level) between MVX infected and non-infected samples. *Agaricus* mycelium in casing had 755 genes up-regulated upon infection while infected mycelium in compost displayed an up-regulation of 2,173 genes. Surprisingly small numbers of genes were identified as up-regulated in fruitbodies where the symptoms are visible, or up-regulated in mycelium growing on defined (agar) culture. This suggests an interaction between viral action and tissue type/differentiation.
Oxidoreductases identified as potentially involved in the degradation of nutrients related to the mushrooms' ecological niche (e.g. laccases and aromatic peroxidises) were found to be routinely down regulated in MVX infected mycelium grown in compost.

Although the infected fruitbodies exhibited symptoms of brown colouration the transcriptomic data reveals that two of the tyrosinase genes (often associated with tissue browning) were down-regulated in infected fruitbodies by more than 6-fold.

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