Karlsruhe Institute of Technology

XI International Fungal Biology Conference

Karlsruhe, Germany
29th September – 3rd October 2013

KARLSRUHE INSTITUTE OF TECHNOLOGY

KIT – University of the State of Baden-Wuerttemberg and
National Research Center of the Helmholtz Association

www.kit.edu

SCIENTIFIC PROGRAM

SUNDAY, 29.09.2013

16:00 – 16:15 **Welcome Addresses**

16:15 – 17:00 **OPENING LECTURE**
Raymond J. St. Leger,
College Park, MD, USA
"How to use a fungus to combat malaria"

PLENARY SESSION: HOST-PATHOGEN INTERACTIONS

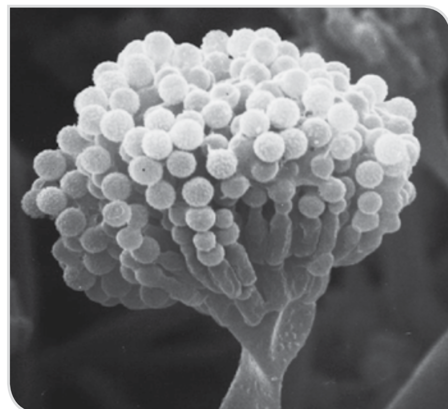
Chairs: **A. Brakhage**
Jena, Germany

17:30 – 18:00 **Clarissa Nobile**
San Francisco, USA
"A sticky situation: unraveling how *Candida albicans* forms biofilms"

18:00 – 18:30 **Robin May**
Birmingham, UK
„Hijacking of the immune system by a fatal fungal pathogen"

18:30 – 19:00 **Markus Künzler**
Zürich, Switzerland
„Fungi as hosts of ‚pathogens': Defense mechanisms of multicellular fungi against fungivorous nematodes"

19:00 **Welcome Reception**



MONDAY, 30.09.2013

08:30 – 10:30 **Poster session talks: Pathogenic fungi**

PLENARY SESSION: PLANT FUNGAL INTERACTIONS

Chairs: **Regine Kahmann**
Marburg, Germany
Nick Talbot
Exeter, UK

11:00 – 11:30 **Martijn Rep**
Amsterdam, The Netherlands
"Speculations on the evolution of host-specific pathogenicity in the *Fusarium oxysporum* species complex"

11:30 – 12:00 **Richard O'Connell**
Cologne, Germany
"Insights into *Colletotrichum* hemibiotrophy from genome and transcriptome sequencing"

12:00 – 12:30 **Ane Sesma**
Madrid, Spain
"Post-transcriptional regulation of *Magnaporthe oryzae* pathogenicity genes"

PLENARY SESSION: CELL BIOLOGY AND TROPIC GROWTH

Chairs: **Mertixell Riquelme**
Ensenada, Mexico

14:00 – 14:30 **Alexandra Brand**
Aberdeen, UK
"Understanding directional growth in fungal hyphae"

14:30 – 15:00 **Andre Fleissner**
Braunschweig, Germany
"Cell-cell communication and fusion in *Neurospora crassa*"

15:00 – 15:30 **Andrea Genre**
Torino, Italy
"Fungal recognition and accommodation in arbuscular mycorrhizal plants"

17:30 – 19:30 **Poster session: Plant fungal interactions**

XI International Fungal Biology Conference 2013

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Welcome address

Reinhard Fischer **Natalia Requena** **Jörg Kämper**

It is our great pleasure to welcome you to the XI International Fungal Biology meeting at the Karlsruhe Institute of Technology (KIT). More than 200 abstracts from all fields of mycology have been submitted and promise a very interesting and fruitful meeting. Twenty-seven speakers were invited for the plenary sessions and 25 scientists were chosen from the submitted abstracts as further speakers. The work of 182 participants will be presented as posters.

The Fungal Biology Conference series was started in 1965 in Bristol, UK and was held since then in irregular intervals in Provo (Utah, USA 1973), Gwatt (Switzerland 1980), Stirling (Scotland 1987), Helen (Georgia, USA 1991), Konstanz (Germany 1996), Groningen (The Netherlands 1999), Guanajuato (Mexico 2002), Nancy (France 2006), Ensenada (Mexico 2009), and now in Karlsruhe (2013).

The aim of this Conference series is to especially focus on exciting new fields at the frontiers of fungal biology. These fields are often developed by young scientists, who were therefore especially be chosen as speakers in the poster session talks. One of the international policy committee agreements for this meeting series is that all participants shuld be able to attend every talk. In consequence, there will be no concurrent sessions. This will allow broad discussions of all subjects, but of course will reduce the number of oral presentations chosen from submitted abstracts. We therefore appologize if some of the submitted abstracts could not be selected for oral presentations! However, poster sessions are always very lively at these meetings and reach a similar audience as oral presentations in other meetings. The International Fungal Biology Conference will this year be combined with the biannual German Molecular Mycology meeting.

Karlsruhe

Karlsruhe is a city in South-West Germany in the Rhine valley near the French-German border. Karlsruhe is one of the last major city foundations that have been designed on the drawing board. According to the legend, Charles III William, Margrave of Baden-Durlach, dreamt after a hunting trip in the woods close to the present-days city about founding a new city, which he named Karlsruhe, which translates as “Charles’ repose”. The city was founded on June 17, 1715. Three years later the residence was moved from the close-by city Durlach, and Karlsruhe became the new capital of Baden-Durlach. Built in 1822, the "Ständehaus" was the first parliament building in a German State. In the aftermath of the democratic revolution of 1848, a republican government was elected here. In 1860, the first ever international professional convention, the Karlsruhe Congress, was held in the city.

Founding of the city is closely linked to the construction of the palace. The city was planned with the tower of the palace (Schloss) at the center and 32 streets radiating out from it like spokes on a wheel, or ribs on a folding fan, so that a nickname for Karlsruhe in German is the "fan city" (Fächerstadt).

The magnificent garden of the castle was founded in 1731, and originally planned in French Baroque style. Already in the 18th century it was rebuild in the style of English landscape gardening, and in 1967 the garden was further development in the same style. You can find numerous rare plant species in the garden, but also pieces of art, monuments and fountains from various eras from Baroque to Modern.

At the western edge of the garden is the Botanical Garden of Karlsruhe. Between the Botanical Garden and the main court of the castle resides the “Bundesverfassungsgericht”, the Federal Constitutional Court of Germany.

University of Karlsruhe (TH), Research Center and KIT

Karlsruhe University (TH)

The predecessor of the University of Karlsruhe, the “Polytechnicum” was founded in 1825 by Ludwig I, Grand Duke of Baden, from Tullas "Karlsruhe School of Engineering," Weinbrenner's “Architecture School” and other institutions. In 1885, the name was changed to “Technische Hochschule” (Technical University), and since 1899 it was possible to receive the doctoral degree. The Karlsruhe University was the first university in Germany to admit women to a degree program (1904). A number of Nobel laureates studied in Karlsruhe or worked here for some time. Among them is Karl Ferdinand Braun (inventor of the Cathode ray tube, Braun tube), Fritz Haber (synthesis of ammonia, Haber-Bosch process), Georg Karl von Hevesy (father of nuclear medicine by using radioactive tracers to study the metabolism in plants and animals), or Hermann Staudinger (discovery of ketene molecules that allowed the development of polymers).

William Barton Rogers, the founding director of the Massachusetts Institute of Technology (MIT), summed up his personal vision of the Polytechnic School of Karlsruhe in 1864 as follows: "The Polytechnic Institute at Carlsruhe, which is regarded as the model school of Germany and perhaps of Europe, is nearer what it is intended the Massachusetts Institute of Technology shall be than any other foreign institution."

In 2006, the University of Karlsruhe was selected along with the Ludwig-Maximilians-University Munich and the Technical University of Munich in the framework of the Excellence Initiative as one of the first three “Elite Universities” in Germany.

View of the University of Karlsruhe (Campus South)

Research Center Karlsruhe

Similar to the establishment of the Polytechnical School in Karlsruhe, the founders of "Kernforschungszentrum Karlsruhe (KfK)" (Nuclear Research Center) entered new territory. In 1962, on a remote site in Eggenstein-Leopoldshafen, north of Karlsruhe, the first research reactor (FR 2) that was constructed and build exclusively in Germany, started to operate.

Since the early nineties, the focus shifted to environmental, energy and - especially physical - basic research. Accordingly, the institution was renamed in 1995 to Karlsruhe Research Center for Technology and Environment. Other areas that became increasingly important over the last decades include "Health" (Biomedical Research, Medical Technology) or "Key technologies" (Microelectromechanical Systems, Nanotechnology, Scientific Computing)

The connection to the University of Karlsruhe reaches back to the founding days of the Research Center: the working group "Reactor Construction" around Karl Wirtz, from Werner Heisenberg's Max Planck Institute for Physics in Göttingen, moved to Karlsruhe; K. Wirtz himself was founding director of the Institute for Neutron Physics and Reactor Technology and at the same time professor at the University of Karlsruhe.

View of the Research Center Karlsruhe (Campus North)

The merge:

Karlsruhe Institute of Technology (KIT)
(http://www.kit.edu/kit/english/index.php)

Unique in German Research, the Karlsruhe Institute of Technology (KIT) was founded by a merger of the Research Center Karlsruhe and the Universität Karlsruhe on October 01, 2009. KIT bundles the missions of both precursory institutions: A university of the state of Baden-Württemberg with teaching and research tasks and a large-scale research center of the Helmholtz Association conducting program-oriented provident research. Within these missions, KIT is operating along the three strategic fields of action of research, teaching, and innovation. **Research**: KIT is among the leading engineering research institutions worldwide. As a member of the Helmholtz Association, the largest science organization in Germany, our institution makes major contributions to top national and international research. **Teaching**: In 60 study courses, KIT students acquire knowledge and skills for new scientific breakthroughs and the development of viable applications. Combination of the strengths of the University and Research Center is the basis of excellent education and advanced training. **Innovation**: Research results are not only intended to be of theoretical use, but to be applied in practice. The KIT Innovation Management Service Unit supports the direct transfer of new findings, innovative ideas, or know-how to industry and society.

With about 9.250 employees (360 professors), 24.000 students and an annual budget of approximately EUR 785 millions, the Karlsruhe Institute of Technology is one of the largest research and teaching institutions worldwide. It has the potential to assume a top position worldwide in selected fields of research. The objective: KIT is to become an institution of top research and excellent scientific education as well as a prominent location of academic life, life-long learning, comprehensive advanced training, unrestricted exchange of know-how, and sustainable innovation culture.

Max Rubner Institute

The Max Rubner Institute is a governmental research institute whose task is the analysis and assessment of health-promoting constituents of foods, as well as food quality and safety assurance. MRI was founded on 1 January 2008 as the successor to the Federal Research Centre for Nutrition and Food (BfEL).

Four of MRI's eight departments as well as the MRI Analysis Division use a cross-product approach, focusing their research on investigating the quality rating of foodstuffs in terms of nutritional physiology and health, food safety and bioprocess engineering. This approach traces the active chain of nutrition from the level of molecules and cells to the entire organism. One particularly important area is the study of nutritional behaviour. The results of this cross-product research are incorporated in recommendations for healthy nutrition.

The research work undertaken in the other four departments relates to specific categories of foodstuffs such as cereals, vegetables, milk and meat. In these departments the entire food chain is studied. MRI's research targets the safety and quality of foodstuffs as well as the sustainability of food production. Within the Department of Safety and Quality of Fruit and Vegetables, which focuses on metabolomics of nutritionally important plant secondary metabolites, as well as on microbiological safety of plant-derived products, the group of Prof. Dr. Rolf Geisen is working on the regulation of fungal mycotoxin production in foods.

View of the Max Rubner Institute

Molecular Mycology in Karlsruhe

In 1832, the first biological discipline, the school of Forest Science, was incorporated into the Polytechnical School. The chair of Botany, held by Alexander Braun (1833 – 1846), lectured topics in botany and zoology. In 1872, Pharmacy was added, and before the end of the 19th century, the Botany and Zoology departments were split into two teaching units. In 1890, Walter Migula, a pioneer in bacterial systematics, habilitated in botany and served as professor until 1904; he described the important bacterial genus *Pseudomonas* in 1895. Forestry was later moved to Freiburg and Pharmacy to Heidelberg. After World War II, teaching started again, and in 1956 H. Kühlwein was appointed as the Chair of Botany. Although a professor of botany, Kühlwein became internationally recognized for his work on Myxobacteria. One of his Ph.D. students, H. Reichenbach, continued research with these gliding bacteria at the GBF in Braunschweig. Kühlwein also studied the physiology of some wood-degrading fungi.

In 1967, the university was restructured and renamed *University of Karlsruhe*, and several new Chairs were appointed, among them a new Chair of Microbiology, W. Zumft. One of the charms of KIT is that within Campus South (the former University of Karlsruhe) microbiologists are not only members of the Faculty of Chemistry and Biosciences, but also of the Faculties of Chemical Engineering (Institute of Life Sciences Engineering), Civil Engineering, and Geo- and Environmental Sciences (Institute of Biology for Engineers and Biotechnology of Wastewater Treatment). They are also situated at Campus North, at the Institute for Functional Interfaces, and close to Campus South, at the Max-Rubner Institute. Other microbiology research groups are located in the Water Technology Center (TZW) Karlsruhe or at Geilweiler Hof. Since various groups employ microorganisms in a range of technical processes, there are many established collaborations with industry. This is also reflected in the fact that researchers from the BASF SE company are integrated into the teaching program of the University. These wide microbiological interests, ranging from basic research with bacteria and fungi, pathogenic and symbiotic interactions, to applied aspects and bioengineering, offer a broad education for the students and the possibility to transform ideas into products at KIT.

Prof. Dr. Reinhard Fischer, *Cell biology of filamentous fungi*
Institute for Applied Biosciences, Dept. of Microbiology, Faculty of Chemistry and Biosciences; www.iab.kit.edu/microbio/

The *Aspergillus* research group studies the cell biology underlying polarized growth, which is driven by the concerted action of microtubules, actin and the corresponding motor proteins that deliver enzymes for cell wall biosynthesis to the cortex. Recently, it was discovered that at least two different populations of microtubules exist in hyphae of *Aspergillus nidulans*.

A. nidulans is able to initiate different morphogenetic programs and develop either asexual or sexual spores. One environmental trigger for these processes is light. The group recently identified phytochrome as one of the important photoreceptors in this fungus, which shows that phytochrome also functions outside the plant kingdom.

Prof. Dr. Jörg Kämper, *Molecular Phytopathology*
Institute for Applied Biosciences, Dept. of Genetics, Faculty of Chemistry and Biosciences; http://genetics.iab.kit.edu/

The basidiomycete *Ustilago maydis* is a ubiquitous pathogen of maize and a well-established model organism for the study of plant-microbe interactions. This fungus belongs to the group of biotrophic parasites that depend on living tissue for proliferation and development. Pathogenic development in *U. maydis* is linked to a dimorphic switch from budding to filamentous growth. The main interest of the genetics department is to understand the regulatory networks that link pathogenic development and the morphological changes of the fungal cell. Expression profiling, in combination with reverse genetic approaches, has led to the identification of various novel pathogenicity factors. Another focus of the group is the metabolic reprogramming of the maize plant by *U. maydis*, and the utilization of carbon sources by the fungus during pathogenic development.

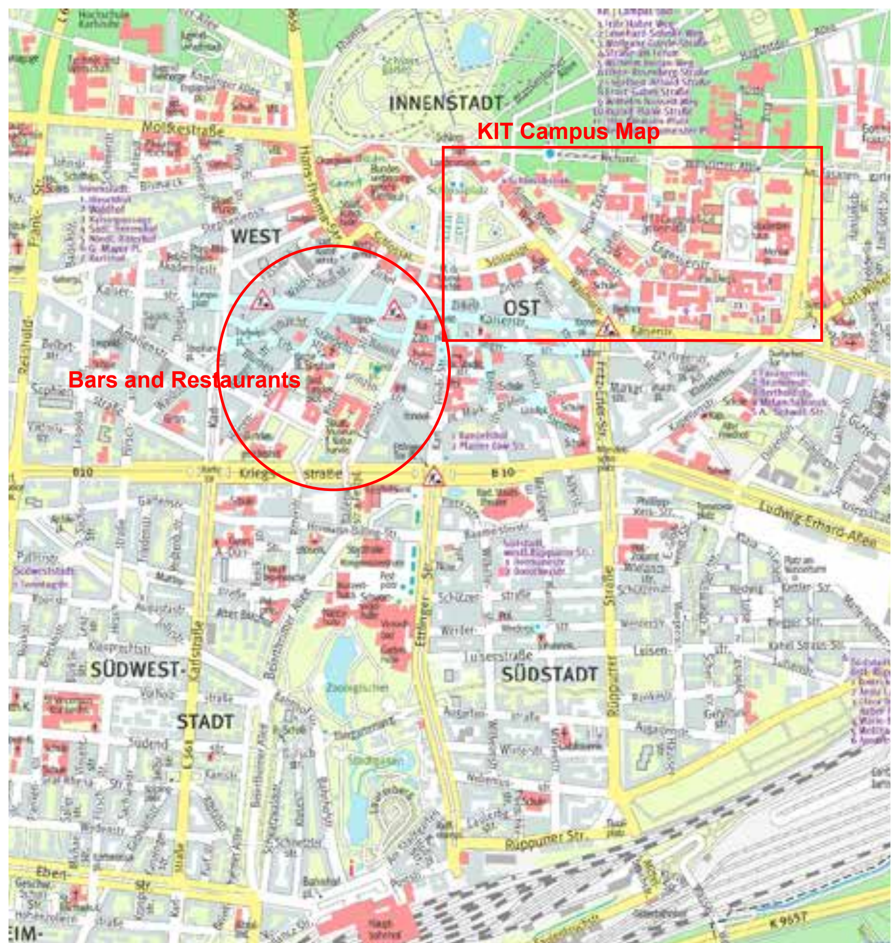
Prof. Dr. Natalia Requena. *Molecular biology of plant-fungal interactions*
Botanical Institute, Molecular Phytopathology Dept., Faculty of Chemistry and Biosciences; http://www.iab.kit.edu/heisenberg/

Microorganisms often live in association with plants either in mutualistic symbioses or as parasites. The focus of the plant-fungal interactions group is the arbuscular mycorrhizal symbiosis that involves the fungi of the Glomeromycota phylum and most plant roots. The colonization of a root by arbuscular mycorrhizal fungi involves a deep reorganization of the plant cell to accommodate the symbiont and to provide the fungus with photoassimilates. The group is interested in unraveling the recognition mechanisms and involved molecules that characterize this symbiosis. How have plants learned to distinguish between pathogenic and mutualistic fungi? How have some pathogens learned to escape the defense response of the plant? Studies employ mostly the arbuscular mycorrhizal fungus *Glomus intraradices* and the hemibiotrophic pathogenic fungus *Magnaporthe oryzae*.

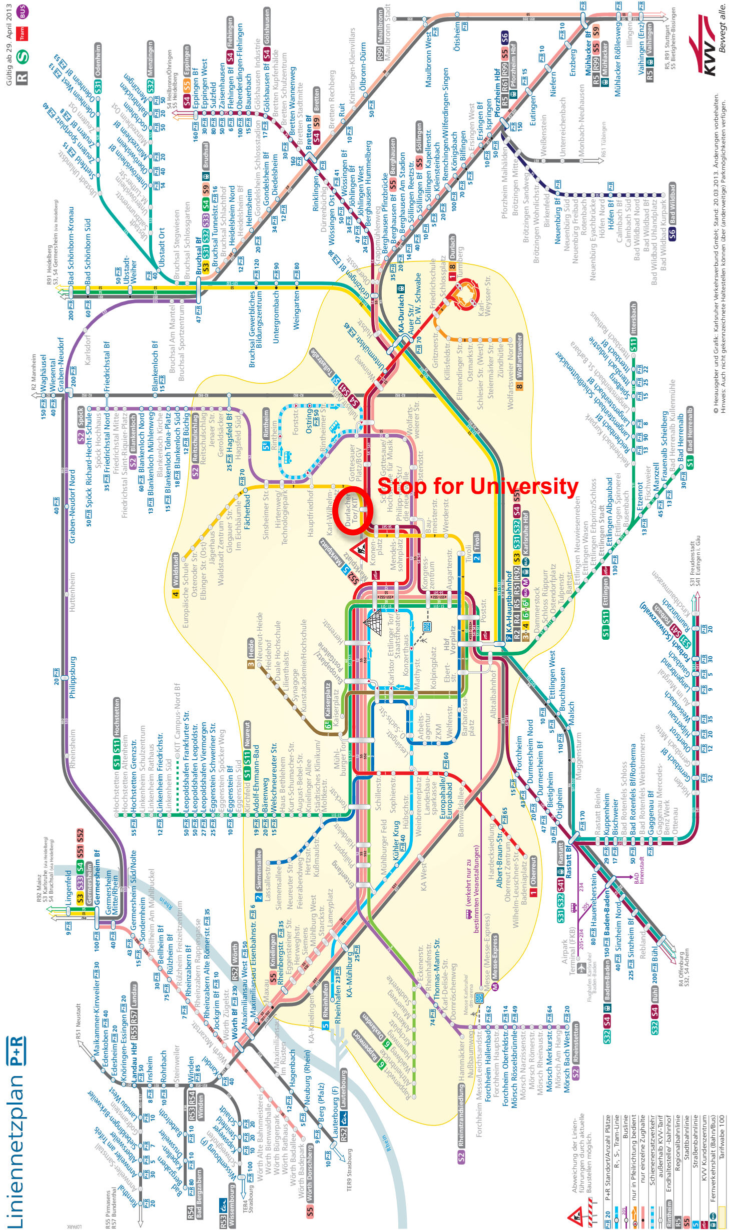
Prof. Dr. Rolf Geisen, *Molecular Food Mycology*
Max Rubner Institute; http://www.mri.bund.de/
The group of Prof. Dr. Rolf Geisen is working on the regulation of fungal mycotoxin production in foods. The role of food-relevant environmental conditions, and their transmittance via signal transduction pathways to regulate transcription of mycotoxin biosynthetic genes are being analyzed.

DFG Research Unit 1341, http://www.for1334.kit.edu/
This research unit was founded in 2010 and is a collaborative action among five groups from Mexico and nine groups from Germany. The topic of this new alliance is molecular analysis of the polarized growth of different filamentous fungi. Filamentous growth is adapted to different growth and developmental conditions, and is highly modulated by internal and external signals. These signaling processes, but also the basal principles to establish polarity are the focus of the research unit. Funding for the German groups is provided by the DFG and by CONACYT for the Mexican groups.

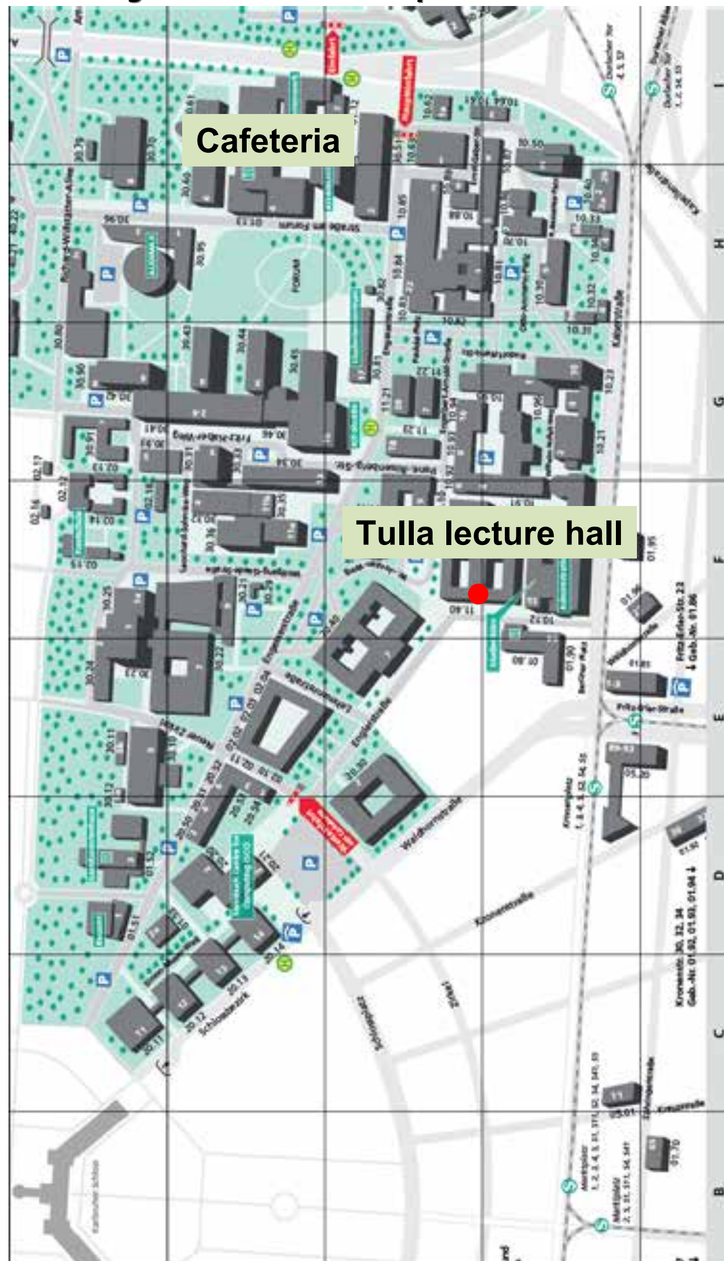
City Map



How to get around in Karlsruhe (http://www.kvv.de/)



How to get around on Campus



Sponsors

www.dfg.de

The DFG is the self-governing organisation for science and research in Germany. It serves all branches of science and the humanities. In organisational terms, the DFG is an association under private law. Its membership consists of German research universities, non-university research institutions, scientific associations and the Academies of Science and the Humanities. The DFG receives the large majority of its funds from the states and the Federal Government, which are represented in all Grants Committees. At the same time, the voting system and procedural regulations guarantee science-driven decisions.

www.vaam.de

Vereinigung für Allgemeine und Angewandte Mikrobiologie

Association for General and Applied Microbiology (VAAM)

Over 3.400 scientists working in the field of microbiology are members of the *Association for General and Applied Microbiology* (VAAM), which promotes the exchange of scientific information and co-operation of its members with a view to translating the results of microbiological research to the benefit of society and the environment. This is made possible through the traditional annual conference in spring where all the sectors of microbiology are represented, as well as through special conferences on individual microbiological topics and the members' journal BIO*spektrum* which is published seven times a year. Postgraduates are encouraged through Ph.D. prizes, travel subsidies and reduced fees at conferences. The VAAM itself is a member of the umbrella organisations VBIO, FEMS, and IUMS and can look back on a good co-operation with other related German scientific organisations.

www.nadicom.com

nadicom Gesellschaft für angewandte Mikrobiologie mbH is a leading international operating GMP-certified biotech company specialising in the identification and genotyping of bacteria and fungi in pure cultures, environmental samples and complex mixed cultures. The company was founded by the biologist Dr. Bernhard Nüßlein in 2002 who is managing partner of nadicom. Situated in Marburg and Karlsruhe our company is active for the pharmaceutical, food, cosmetics, chemical and agricultural industry. For the identification of micro-organisms the most modern molecular-biological methods are applied. Qualitative identification of bacteria, fungi and yeast from pure cultures is carried out via the latest PCR-based methods. We regard ourselves not as a standard laboratory but as trouble shooter in case of contaminations or for individual questions.

Scientific program

Sunday, 29.09.2013

14:00–18:30 Registration and mounting of the posters

16:15 – 17:00 OPENING LECTURE: Raymond J.St. Leger (Univ. of Maryland, USA)
"How to use a fungus to combat malaria"

17:00–17:30 Coffee break

I. PLENARY SESSION: HOST-PATHOGEN INTERACTIONS

Chair: A. Brakhage (HKI Jena, Germany)

17:30–18:00 Clarissa Nobile (University of California, SF, USA)
"A sticky situation: unraveling how *Candida albicans* forms biofilms"

18:00–18:30 Robin May (University of Brimingham, UK)
"Hijacking of the immune system by a fatal fungal pathogen"

18:30-19:00 Markus Künzler (ETH Zürich, Switzerland)
"Fungi as hosts of 'pathogens': Defense mechanisms of multicellular fungi against fungivorous nematodes"

19:30 GET TOGETHER with weJazz

Monday, 30.09.2013

08:30–10:30 Poster session talks: Pathogenic fungi

M. Brock (chair): Real-time visualization of fungal infections by non-invasive bioluminescence imaging

***Candida albicans* Stp2p, a transcription factor regulating amino acid permeases, is required for virulence and alkalinization of the phagosome**, S. Vylkova* and M.C. Lorenz

F-box protein 15 (Fbx15) links virulence of *Aspergillus fumigatus* to protein degradation and stress response, Bastian Jöhnk*, Özgür Bayram, Oliver Valerius, Thorsten Heinekamp, Ilse D. Jacobsen, Axel A. Brakhage and Gerhard H. Braus

The classical complement pathway induces phagocytosis of *Aspergillus fumigatus*, SGE Braem, JJPA de Cock, HAB Wösten, JAG van Strijp, PJA Haas

A transcriptomic and proteomic approach to dissect MAP kinase signaling in the mycoparasitic fungus *Trichoderma atroviride*, Albert Nemes, Sabine Gruber, Pawel Labaj, Martina Marchetti-Deschmann, David Kreil, and Susanne Zeilinger*

Septin-mediated plant tissue invasion by the rice blast fungus *Magnaporthe oryzae*, Yasin F. Dagdas, Lauren S. Ryder, Michael J. Kershaw, Yogesh Gupta, George Littlejohn and Nicholas J. Talbot*

10:30–11:00 Coffee break

II. PLENARY SESSION: PLANT FUNGAL INTERACTIONS

Chairs: Regine Kahmann (Marburg, Germany) and Nick Talbot (Exceter, UK)

11:00–11:30 Martijn Rep (University of Amsterdam, NL)
"Speculations on the evolution of host-specific pathogenicity in the *Fusarium oxysporum* species complex"

11:30–12:00 Richard O'Connell (MPI, Cologne, Germany)
"Insights into *Colletotrichum* hemibiotrophy from genome and transcriptome sequencing"

12:00–12:30 Ane Sesma (University of Madrid, Spain)
"Post-transcriptional regulation of *Magnaporthe oryzae* pathogenicity genes"

12:30–14:00 Lunch break ("Fungal diversity on Campus", Short excursion, Dr. Schöller, Museum of Natural History, Karlsruhe)

III. PLENARY SESSION: CELL BIOLOGY AND TROPIC GROWTH

Chair: Mertixell Riquelme (Ensenada, Mexico)

14:00–14:30 Alexandra Brand (University of Aberdeen, UK)
"Understanding directional growth in fungal hyphae"

14:30–15:00 André Fleißner (University of Braunschweig, Germany)
"The art of networking: Cell fusion in *Neurospora crassa*"

15:00–15:30 Andrea Genre (University of Torino, Italy)
"Fungal recognition and accommodation in arbuscular mycorrhizal plants"

15:30–17:30 Coffee break / Poster Session

17:30–19:30 Poster session talks: Plant fungal interactions

Antonio Di Pietro (chair): "On target: chemotropism of *Fusarium oxysporum* towards its host plant"

Laser microdissection and transcriptomics of infection cushions formed by *Fusarium graminearum*, Marike J. Boenisch, Stefan Scholten, Sebastian Piehler, Martin Münsterkötter, Ulrich Güldener, Wilhelm Schäfer

The secreted *Piriformospora indica* effector Dld1 is involved in penetration of plant cell wall appositions, Robin Nostadt, Magdalena Hilbert, Hannah Kleyer, Jörg Martin, Andrei Lupas, Alga Zuccaro

Anthocyanin induction via a secreted fungal effector promotes virulence through preventing lignifications, Shigeyuki Tanaka*, Thomas Brefort, Jörg Kahnt and Regine Kahmann

D-galacturonic acid utilization by *Botrytis cinerea*, Lisha Zhang*, Chenlei Hua, Joost Stassen, Sayantani Chatterjee, Maxim Cornelissen, Jan A.L. van Kan

***Colletotrichum orbiculare* CoPAG1, a component of RAM network in *Saccharomyces cerevisiae*, is involved in appressorium development triggered by plant-derived signals**, Sayo Kodama, Ayumu Sakaguchi and Yasuyuki Kubo*

Tuesday, 01.10.2013

08:30–10:30 Poster session talks: Cytskeleton and Polarity

M. Feldbrügge (chair): "Cytoskeleton, intracellular transport and hyphal tip growth"

Does endocytosis affect the dynamics of exocytic markers and cell wall assembly, Ramírez-Del Villar A., Echauri-Espinosa R., Bartnicki-García S. and Mouriño-Pérez R.R.*

The Rab GTPase YPT-1 coordinates vesicle traffic at the Golgi and at the Spitzenkörper in *Neurospora crassa,* Eddy Sanchez-Leon*, Barry Bowman and Meritxell Riquelme

Interaction of calmodulin with cytoskeletal proteins during colony initiation in *Neurospora crassa*, Chia-Chen Chang*, Thierry Le Bihan, and Nick D. Read

Woronin bodies: impact on stress resistance and virulence of *Aspergillus fumigatus* and anchoring at the septal pore of filamentous Ascomycota, Julia Beck and Frank Ebel*

Visualization of membrane domains, microtubule and actin cytoskeletons in *Aspergillus nidulans* by Photoactivated Localization Microscopy (PALM), Anna Bergs*, Yuji Ishitsuka, Yiming Li, Ulrich Nienhaus, Reinhard Fischer and Norio Takeshita

10:30–11:00 Coffee break

IV. PLENARY SESSION: ORGANELLES AND ORGANELLAR FUNCTIONS

Chairs: Michael Bölker (Marburg, Germany) and Jürgen Wendland (Copenhagen, DK)

11:00–12:30 Ida van der Klei (University of Groningen, NL)
"The biogenesis and function of fungal peroxisomes"

11:30–12:00 Gero Steinberg (University of Exeter, UK)
"Endosome motility in fungi: The how and why"

12:00–12:30 Benedikt Westermann (University of Bayreuth, Germany)
"Mitochondrial dynamics and inheritance in yeast"

12:30–14:00 Lunch break

V. PLENARY SESSION: BIOTECHNOLOGY

Chair: Jesus Aguirre (University of Mexico City) and Gerhard Braus (University of Göttingen, Germany)

14:00–15:30 Claus Bollschweiler (BASF SE, Ludwigshafen, Germany)
"Hydrophobins: Innovative performance proteins for industrial Biotechnology"

14:30–15:00 Ronald de Vries (CBS-KNAW Fungal Biodiversity Centre, Utrecht, NL)
"Fungal strategies for plant biomass degradation"

15:00–15:30 Katsuhiko Kitamoto (University of Tokyo, Japan)
"Developing *Aspergillus oryzae* as a host for heterologous protein production"

15:30–17:30 Coffee break / Poster Session

17:30–19:30 Poster session talks: Gene regulation

Y. Liu (chair): "Less is more, codon usage regulates protein expression, structure and function"

Signalling of the calcineurin responsive transcription factor CrzA in response to calcium and alkaline-pH stresses, Patricia Hernandez-Ortiz and Eduardo A Espeso*

Phytochrome and the white-collar proteins control light-induced genes in *Aspergillus nidulans* via chromatin remodeling, Stefan Rauscher*, Julian Röhrig, Maren Hedtke, Zhenzhong Yu and Reinhard Fischer

Import of peroxisomal matrix proteins in *Ustilago maydis*, Julia Ast*, Alina C. Stiebler, Johannes Freitag, Michael Bölker

Role of the small RNAs synthesis machinery on the antagonistic capacity of *Trichoderma atroviride*, Emma Beltrán-Hernández, Jorge Molina-Torres, Alfredo Herrera-Estrella*

Molecular basis of FLO11 mediated interactions in *Saccharomyces cerevisiae*, Timo Kraushaar*, Maik Veelders, Stefan Brückner, Hans-Ulrich Mösch and Lars-Oliver Essen

Wednesday, 2.10.2013

08:30–10:30 Poster session talks: Secondary metabolism

A. Calvo (chair): "Advances in the knowledge of fungal secondary metabolism"

The aflatoxin-like toxin dothistromin: virulence, regulation and evolution, R.E. Bradshaw*, M.S. Kabir, P. Chettri

Two histone deacetylases, FfHda1 and FfHda2, are important for secondary metabolism and virulence in *Fusarium fujikuroi*, L. Studt*, F.J. Schmidt, L. Jahn, C.M.K. Sieber, L.R. Connolly, E.-M. Niehaus, M. Freitag, H.-U. Humpf and B. Tudzynski

The expression of a secondary metabolite cluster is strongly repressed by the asexual development regulator FlbB in *Aspergillus nidulans*, Elixabet Oiartzabal-Arano*, Marc S. Cortese, Eduardo A. Espeso, Unai Ugalde and Oier Etxebeste

The role of histone acetyltransferases in the secondary metabolism of *Aspergillus fumigatus*, Derek Mattern*, Claudia König, Volker Schroeckh, Vito Valiante, and Axel A. Brakhage

Mono- and sesqui- terpene production in the biocidal endophytic fungus, Nodulisporium sp., Ross C. Mann*, Scott W. Mattner, Simone J. Rochfort, Ian J. Porter and German C. Spangenberg

10:30–11:00 Coffee break

VI. PLENARY SESSION: CELL SIGNALLING AND MORPHOGENESIS

Chairs: Michelle Momany (Athens, US) and Luis Corrochano (Sevilla, Spain)

11:00–12:30 Alex Idnurm (University of Missouri, USA)
“Light signaling in Phycomyces”

11:30–12:00 Alfredo Herrera Estrella (Langebio, Irapuato, Mexico)
“An injury-response mechanism conserved across kingdoms determines entry of *Trichoderma atroviride* into development”

12:00–12:30 Axel Diernfellner (University of Heidelberg, Germany)
“Molecular mechanisms of the *Neurospora crassa* circadian clock”

12:30–14:00 Lunch break

VII. PLENARY SESSION: SECONDARY METABOLISM

Chairs: Bettina Tudzynski (Münster, Germany) and Ulrich Kück (Bochum, Germany)

14:00–14:30 Joseph Strauss (University of Vienna, Austria)
"Heterochromatin influences fungal secondary metabolism and pathogenicity"

14:30–15:00 Marc Stadler (HZI, Braunschweig)
"Correlations between biodiversity and secondary metabolism in the Xylariaceae"

15:00–15:30 Sandra Bloemendal (University of Bochum, Germany)
"Strain development in fungal biotechnology: Regulation of beta-lactam antibiotic biosynthesis"

15:30-16:00 Poster Awards

16:15-17:00 Claudio Scazzocchio (Université Paris Sud, France and Imperial College, London, UK)

"Concluding remarks: Fungal Biology in the post-genomic era"

17:00–23:00 CONFERENCE DINNER at the Castle Schwetzingen
(not included in the registration fee)

Abstracts

Sunday, 29.09.2013

OPENING LECTURE: Raymond J.St. Leger (Univ. of Maryland, USA)

“How to use a fungus to combat malaria”

I. PLENARY SESSION: HOST-PATHOGEN INTERACTIONS

Clarissa Nobile (University of California, San Francisco, USA)
“A sticky situation: unraveling how Candida albicans forms biofilms”

Robin May (University of Brimingham, UK)
“Hijacking of the immune system by a fatal fungal pathogen”

Markus Künzler (ETH Zürich, Switzerland)
“Fungi as hosts of ‘pathogens’: Defense mechanisms of multicellular fungi against fungivorous nematodes"

How to use a fungus to combat malaria

Raymond St. Leger, University of Maryland, USA. Contact: stleger@umd.edu

Conservative estimates put the number of new malaria infections each year at 500 million, and every one of those infections started with a mosquito bite. Although millions die from malaria and other arthropod borne diseases, the ongoing development of pesticide resistance has reduced our control options. To develop a biological control alternative, we used the infectivity of the insect pathogen Metarhizium as a delivery system for expressing different combinations of anti-plasmodial and insecticidal proteins. We demonstrated that co-expression by Metarhizium of insect-selective spider and scorpion toxins targeting sodium, potassium or calcium nerve channels produced a fungal pathogen that has the potential to rapidly (< 2 days) block malaria transmission at very low spore doses relevant to field conditions. We also produced recombinant strains expressing molecules that target sporozoites as they travel through the hemolymph to the salivary glands. These fungi slashed the insect's parasite burden by 98%, even if their malaria infections were very advanced. This is a very specific treatment (a fungus that targets mosquitoes carrying weapons to combat a disease the mosquitoes carry), and as it does not kill insects until they are old it eliminates the issue of the insects evolving resistance. I will describe these different transgenic fungal approaches, and how we are tackling potential environmental impact issues that come with using transgenics (studies on environmental impact serendipitously led to the discovery that some strains of Metarhizium colonize plant roots, and are plant symbionts). Though applied by us to combat malaria, the transgenic fungal approach is a very flexible one that will allow design and delivery of gene products targeted to almost any disease-carrying arthropod.

A sticky situation: unraveling how *Candida albicans* forms biofilms

Clarissa J. Nobile*

Department of Microbiology & Immunology, University of California, San Francisco, USA. Contact: Clarissa.Nobile@ucsf.edu

The yeast *Candida albicans* is a normal resident of the human microbiome. It is also the most common fungal pathogen of humans, causing both mucosal and systemic infections, particularly in immune compromised individuals. This talk will discuss how *C. albicans* orchestrates the formation of biofilms – resilient, surface-associated, organized groups of cells. Biofilm formation is medically relevant because *C. albicans* biofilm growth is the cause of numerous mucosal infections in otherwise healthy individuals, and new *C. albicans* infections in the clinic are highly correlated with implanted medical devices, which provide efficient substrates for biofilm formation. Through a combination of genetic screens, genome-wide approaches, and animal modeling, we have identified the complex transcriptional network controlling biofilm formation in *C. albicans*. This network is composed of six transcription regulators that form a tightly woven network with ~1,000 target genes. The identification and validation of critical biofilm-specific target genes will set the groundwork for the development of novel diagnostic and therapeutic approaches to identifying and treating fungal biofilm-based infections.

Hijacking of the immune system by a fatal fungal pathogen

Robin C. May*

Institute of Microbiology and Infection & NIHR Surgical Reconstruction and Microbiology Research Centre, University of Birmingham. Contact: r.c.may@bham.ac.uk

My group is interested in host-pathogen interactions and, in particular, in understanding how some pathogens are able to subvert the innate immune system. Most of our work focuses on phagocytic cells, which some microorganisms are able to use as a 'safe house' within which to replicate.

In this talk, I will focus on cryptococcosis, a widespread and usually fatal fungal infection. This disease, caused by *Cryptococcus neoformans* or the related species *C. gattii*, is characterized by the ability of the fungus to proliferate within host phagocytic cells. We therefore study the events that lead both to phagosomal persistence of this organism and, in some cases, to a novel escape process termed 'vomocytosis'. We are also interested in the genetic changes that drive hypervirulent outbreaks of cryptococcosis and what the cellular consequences are of such changes. Finally, we have a growing interest in other fungal diseases that show similar behavior, in particular zygomycosis, which is often a fatal complication in patients with major trauma wounds.

Fungi as hosts of 'pathogens': Defense mechanisms of multicellular fungi against fungivorous nematodes

Markus Künzler[1]*, Silvia Bleuler[1], Therese Wohlschlager[1], Niels van der Velden[1], David F. Plaza[1], Stefanie S. Schmieder[1], Alex Butschi[2], Katrin Stutz[2], Orane Guillaume-Gentil[1], Claire E. Stanley[3], Julia Vorholt[1], Michael O. Hengartner[2], Andrew J. De Mello[3], Markus Aebi[1]

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Multicellular fungi are exposed to numerous antagonists including competitors, parasites and predators. In contrast to other multicellular eukaryotes, our knowledge about the defense mechanisms of multicellular fungi against their antagonists is scarce. We use a reductionistic and experimental approach to identify and characterize these mechanisms at molecular level. Special emphasis is put on fungal defense mechanisms against fungivorous nematodes since these predators are prevalent in many habitats of multicellular fungi.

Based on toxicity assays in which we employed model organisms from phyla containing fungivores, we hypothesize that the defense of multicellular fungi against predators is largely based on the production and accumulation of protein toxins in the fungal cytoplasm. We use the bacterivorous model nematode *Caenorhabditis elegans* to identify the molecular targets and to study the toxicity mechanism of these fungal protein toxins in nematodes. It turns out that many of these toxins are lectins that bind to specific glycoepitopes in the intestine of the nematode and kill the nematode by a yet unknown mechanism. Using cocultures of recombinant forms of the filamentous ascomycete *Ashbya gossypii* expressing protein toxins from other fungi and the fungivorous nematode *Aphelenchus avenae*, we were recently able to show that some of these protein toxins reduce the fitness of a fungivorous nematode and are thus likely to be effective in fungal defense against these predators.

Genome-wide gene expression studies by next generation RNA sequencing in the coprophile model mushroom *Coprinopsis cinerea* revealed that the genes coding for these protein toxins are regulated by both developmental and environmental cues. Challenge of *C. cinerea* vegetative mycelium with different types of antagonists resulted in the induction of non-overlapping sets of genes. Intriguingly, the set of genes induced by the fungivorous nematode *A. avenae* included some already characterized genes coding for nematotoxic proteins. These results suggest that the fungus is able to recognize its antagonist and mount an appropriate response. We are currently characterizing the fungal responses to different types of antagonists using microfluidics and fluidic force microscopy approaches. Preliminary results using a *C. cinerea* reporter strain expressing a red fluorescent protein under control of a *A. avenae*-inducible promoter show that the defense response can spread along the hyphae but is confined to sites of direct contact between the fungivorous nematode and the fungal mycelium. In addition to this local induction of toxin production in response to feeding by *A. avenae*, we observed a specific inhibition of *C. cinerea* hyphal growth in the absence of direct contact between the two organisms. This result suggests that there are other types of fungal defense responses to these predators. We are aiming at the identification of the signals and the cognate receptors that trigger these defense responses.

Monday, 30.09.2013

Poster session talks: Pathogenic fungi

M. Brock: Real-time visualization of fungal infections by non-invasive bioluminescence imaging

***Candida albicans* Stp2p, a transcription factor regulating amino acid permeases, is required for virulence and alkalinization of the phagosome**
S. Vylkova* and M.C. Lorenz

F-box protein 15 (Fbx15) links virulence of *Aspergillus fumigatus* to protein degradation and stress response
Bastian Jöhnk*, Özgür Bayram, Oliver Valerius, Thorsten Heinekamp, Ilse D. Jacobsen, Axel A. Brakhage and Gerhard H. Braus

The classical complement pathway induces phagocytosis of *Aspergillus fumigatus*
SGE Braem, JJPA de Cock, HAB Wösten, JAG van Strijp, PJA Haas

A transcriptomic and proteomic approach to dissect MAP kinase signaling in the mycoparasitic fungus *Trichoderma atroviride*
Albert Nemes, Sabine Gruber, Pawel Labaj, Martina Marchetti-Deschmann, David Kreil, and Susanne Zeilinger*

Septin-mediated plant tissue invasion by the rice blast fungus *Magnaporthe oryzae*
Yasin F. Dagdas, Lauren S. Ryder, Michael J. Kershaw, Yogesh Gupta, George Littlejohn and Nicholas J. Talbot*

II. PLENARY SESSION: PLANT FUNGAL INTERACTIONS

Martijn Rep (University of Amsterdam, The Netherlands)
"Speculations on the evolution of host-specific pathogenicity in the *Fusarium oxysporum* species complex"

Richard O'Connell (MPI, Cologne, Germany)
"Insights into *Colletotrichum* hemibiotrophy from genome and transcriptome sequencing"

Ane Sesma (University of Madrid, Spain)
"Post-transcriptional regulation of *Magnaporthe oryzae* pathogenicity genes"

Real-time visualisation of fungal infections by non-invasive bioluminescence imaging

Matthias Brock

Microbial Biochemistry and Physiology, Friedrich-Schiller University Jena and Leibniz Institute for Natural Product Research and Infection Biology, -Hans Knoell Institute-, Beutenbergstr. 11a, 07745 Jena, Germany. Contact: Matthias.brock@hki-jena.de

Investigation of fungal pathogenicity often requires the use of animal models to study disease manifestation and progression in dependence of the underlying immune status of the host or the genetic background of the pathogen. To assess the state of infection conventional animal models require the sacrifice of animals at pre-defined time-points and the investigation of tissues by histopathology. The drawbacks of this procedure are that (i) only a snapshot of the current status of infection can be obtained, (ii) an individual animal can only be monitored once and (iii) secondary or minor sites of infection are frequently overlooked. Thus, large numbers of animals are often required to get deeper insights into the infection process. To circumvent these drawbacks in vivo imaging provides an excellent alternative to study disease progression in living animals. Currently, the highest sensitivity is obtained by using bioluminescence imaging techniques, because background signals from infected animals are generally extremely low. To apply this technique luciferase producing reporter strains are required that are used for infection. Detection is performed by highly sensitive charge-coupled device cameras that allow tracking and collection of single photons emitted from the infected animal. Although different luciferases can be produced in fungi the most suitable reporter derives from synthetic codon-adapted versions of the firefly luciferase. This luciferase requires the substrate D-luciferin that is highly water soluble, non-toxic to animals and is rapidly distributed to all body sites and, thus, not only allows to track localised but also disseminated infections. In this presentation the bioluminescence imaging technique will be introduced and examples for successful monitoring of disease progression and dissemination from various fungal species will be presented. Additionally, the value in monitoring of antifungal therapy efficacy and some unexpected results concerning cryptic or non-targeted host sites will be shown.

Candida albicans Stp2p, a transcription factor regulating amino acid permeases, is required for virulence and alkalinization of the phagosome

S. Vylkova* and M.C. Lorenz

University of Texas, Houston, USA. Contact: Slavena.Vylkova@uth.tmc.edu

Candida albicans, the most important fungal pathogen, has the ability to switch from yeast to hyphal form in response to various environmental factors, including neutral pH. Hyphal morphogenesis also facilitates *C. albicans* escape from macrophage phagocytosis, but the inducing signal(s) that trigger germ tube formation and the fate of the cell within the macrophage endocytic pathway are not well understood. We have recently demonstrated that cells grown in vitro in glucose-poor and amino acid rich acidic environment, conditions that also provoke the metabolic changes observed after phagocytosis, actively co-opt amino acid catabolism to extrude acid import is crucial for this process, since cells lacking STP2, a transcription factor that regulates the expression of multiple amino acid permeases, were completely deficient in alkalinization in vitro. Phagocytosed *stp2*Δ mutant cells showed significant reduction in hyphal formation and escaped from the macrophages less readily compared to the wild-type, suggesting that amino acid uptake is necessary for morphogenesis during phagocytosis. As a result, *stp2*Δ mutant cells were killed at a higher rate by RAW264.7 macrophages than the wild-type cells, as determined by end point dilution assay. Similarly, in a macrophage cytotoxicity assay the *stp2*Δ mutant caused less damage to RAW264.7 cells compared to the wild-type. Our model is that amino acid catabolism resulting from Stp2-regulated import leads to ammonia export and alkalinization of the phagolysosome. To test this, we assayed colocalization of *C. albicans* with the acidophilic dye Lysotracker Red; our data indicates that only a small number (~20%) of the wild-type cells are found in acidic phagosomes after one hour of interaction with RAW264.7 or bone marrow-derived mouse macrophages. In contrast, the percentage of *stp2*Δ cells in acidic compartments was significantly higher, and was not significantly different than UV-killed cells, suggesting that *C. albicans* is able to neutralize the environmental pH within the phagosomes. Finally, *stp2*Δ cells were able to form hyphae and escape from phagocytosis in macrophages pre-treated with the vATPase inhibitor Bafilomycin A1, indicating that neutralization of the phagosomes is crucial for this process. Altogether our results suggest that during phagocytosis *C. albicans* utilizes amino acids to promote neutralization of the phagolysosome, and that the neutral pH is critical factor that promotes morphogenesis and escape from the macrophages.

F-box protein 15 (Fbx15) links virulence of *Aspergillus fumigatus* to protein degradation and stress response

Bastian Jöhnk[1]*, Özgür Bayram[1], Oliver Valerius[1], Thorsten Heinekamp[2], Ilse D. Jacobsen[2], Axel A. Brakhage[3] and Gerhard H. Braus[1]

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Rapid adaptation to a versatile host represents a challenge for the opportunistic human pathogen *Aspergillus fumigatus* for successful infection. F-box proteins are the adaptor subunits of E3 SCF (Skp1 cullin-1 F-box protein) ubiquitin ligases. They recognize target proteins, which are marked by the SCF complex for degradation in the 26 S proteasome. Here we have identified Fbx15 as an F-box protein, which links *A. fumigatus* virulence to protein degradation. *A. fumigatus* deletion strains which have lost fbx15 are unable to infect immunocompromised mice in a murine model of invasive aspergillosis. Fbx15 is required for growth during stress including increased temperature, oxidative stress and amino acid starvation. Fbx15 is also required for controlling the synthesis of the antiphagocytic gliotoxin. Fbx15 interacts in the nucleus with the linker protein Skp1/SkpA suggesting that SCF-Fbx15 primarily targets nuclear proteins. Four nuclear subunits of the COP9 signalosome (CSN) are putative Fbx15 interaction partners. Defects in CSN result in increased oxidative stress, impaired development and a missregulated secondary metabolism in the mold *Aspergillus nidulans*. Furthermore the Cullin-deneddylation activity of the CSN is required to prevent the accumulation of specific SCF-complexes (Nahlik et al., 2010; Von Zeska Kress et al., 2012). We propose an interdependent stabilization of Fbx15 and the COP9 signalosome, which is required to link protein degradation and stress response to virulence.

Nahlik K., Dumkow M., Bayram Ö., Helmstaedt K., Busch S., Valerius O., Gerke J., Hoppert M., Schwier E., Opitz L., Westermann M., Grond S., Feussner K., Goebel C., Kaever A., Meinicke P., Feussner I. and Braus G.H. (2010) The COP9 signalosome mediates transcriptional and metabolic response to hormones, oxidative stress protection and cell wall rearrangement during fungal development. *Mol Microbiol* 78: 964–979.

Von Zeska Kress M.R., Harting R., Bayram Ö., Christmann M., Irmer H., Valerius O., Schinke J., Goldman G.H. and Braus G.H. (2012) The COP9 signalosome counteracts the accumulation of cullin SCF ubiquitin E3 RING ligases during fungal development. *Mol Microbiol* 83: 1162–1177.

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The classical complement pathway induces phagocytosis of *Aspergillus fumigatus*

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Aspergillus fumigatus is an opportunistic pathogen causing different kind of diseases ranging from allergic bronchopulmonary aspergillosis (ABPA) to invasive aspergillosis. Particularly immunocompromised patients like haematological and transplant patients are affected and infection in these patients often leads to death. The innate immune system is the first line of defence against invading pathogens. Polymorphonuclear cells (PMNs) are the most significant cellular defence and essential in clearing fungal infections. During invasion, PMNs are attracted to the site of infection, recognize and phagocytose the pathogen. Opsonisation of the microorganism is essential for phagocytosis and is a result of complement activation. The complement system can be initiated via three different pathways, namely the alternative (spontaneous activation), classical (antibody dependent) and lectin pathway (recognition of sugars).

The human body encounters different morphotypes of *A. fumigatus*. Dormant conidia are inhaled and enter the lung alveoli. Insufficient clearance of the fungus results in swelling and germination of conidia and subsequent formation of hyphal structures invading human tissue. Current knowledge demonstrates that all morphotypes initiate the complement system via the alternative pathway, whereas complement activation on hyphae is partly classical pathway dependent. However, dormant conidia of some strains initiate the classical pathway in absence of MBL. Though, after MBL reconstitution, these strains activate only the alternative pathway. Moreover, phagocytosis by PMNs of dormant conidia is thought to be complement dependent and fully initiated via the alternative pathway.

Here we show that complement is essential for phagocytosis by PMNs of all morphotypes. Interestingly, although antibodies are present on all morphotypes, it does not result in Fc-mediated phagocytosis of fungal particles. Using different deficient sera we clearly demonstrate that C3b deposition as well as phagocytosis is dependent on the activation of the classical pathway on swollen conidia and hyphae. Complement on dormant conidia is mainly activated via the classical pathway. However, none of the deficient sera tested in this study abolish C3b deposition and phagocytosis completely, indicating that other serum components can induce some complement activation on dormant conidia.

In conclusion, the present data show that complement is essential for phagocytosis of fungal particles. The classical pathway is the main initiator of complement activation and subsequent phagocytosis. Since antibodies activate the classical pathway, antibodies against *A. fumigatus* are crucial for opsonization of the fungus, leading to effective phagocytosis by PMNs.

A transcriptomic and proteomic approach to dissect MAP kinase signaling in the mycoparasitic fungus *Trichoderma atroviride*

Albert Nemes[2], Sabine Gruber[1], Pawel Labaj[3], Martina Marchetti-Deschmann[2], David Kreil[3], and Susanne Zeilinger[1*]

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Species of the fungal genus Trichoderma are among the most successful biofungicides in today's agriculture although our understanding of the exact molecular mechanisms of their activity still is fragmentary. The biological control of plant diseases by Trichoderma includes direct antagonism of phytopathogenic fungi by parasitism. This mycoparasitic attack comprises sensing of the prey and chemotropic growth towards it followed by activation of the production of "molecular weapons" such as cell wall-lytic enzymes, secondary metabolites, and infection structures. Investigations on the underlying intracellular signal transduction pathways of *Trichoderma atroviride* revealed the involvement of the Tmk1 MAP kinase in triggering of the mycoparasitic response. Mutants missing Tmk1 show infection structures comparable to the parental strain, they over-produce chitinases, key enzymes of mycoparasitism, and show elevated antifungal activity caused by over-production of low molecular-weight metabolites. Despite these enhancements in mycoparasitism-relevant processes, delta-*tmk1* mutants exhibit reduced mycoparasitic activity against prey fungi. These findings suggests that additional still unknown genes/proteins and processes are contributing to *T. atroviride* mycoparasitism which were aimed to be identified by using the delta-*tmk1* mutant as a tool. To this end, comparative transcriptomic and proteomic approaches were applied to identify target genes and proteins being regulated by the Tmk1 MAPK upon prey recognition and playing key roles in mycoparasitism.

Septin-mediated plant tissue invasion by the rice blast fungus *Magnaporthe oryzae*

Yasin F. Dagdas, Lauren S. Ryder, Michael J. Kershaw, Yogesh Gupta, George Littlejohn and Nicholas J. Talbot*

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Magnaporthe oryzae is the causal agent of rice blast, one of the most serious diseases affecting rice production. During plant infection, *M. oryzae* forms a specialised infection structure called an appressorium. The infection cell generates enormous turgor, which is focused as mechanical force to breach the rice cuticle and facilitate entry of the fungus into plant tissue. A hetero-oligomeric septin GTPase complex is necessary for re-organisation of a toroidal F-actin network at the base of the appressorium, which allows re-establishment of polarised fungal growth. Re-modeling of F-actin at the appressorium pore is necessary for cortical rigidification and localisation of proteins associated with membrane curvature to the point of plant infection. Septin-mediated cytoskeletal re-modeling is required for development of a penetration peg that ruptures the host cuticle and leads to invasion of epidermal cells by biotrophic invasive hyphae of *M. oryzae*. Septin-mediated plant infection is controlled by NADPH oxidase activity and a regulated burst of reactive oxygen species occurs within the appressorium. A specialised Nox2 NADPH oxidase-tetraspanin complex is necessary for septin-mediated control of actin dynamics. We will also describe the potential operation of a pressure-mediated checkpoint, mediated by a cell wall mechanosensor protein, that is necessary for initiation of septin activation and the re-orientation of the cortical F-actin cytoskeleton to facilitate plant tissue invasion.

Speculations on the evolution of host-specific pathogenicity in the *Fusarium oxysporum* species complex

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Fortunately, all plant pathogenic fungi have a limited host range. It appears that this limitation is the result of several prerequisites for disease to occur when a fungus and a plant interact: (1) toxic secondary metabolites in the plant need to be neutralized by the fungus, (2) nutrients needed for fungal growth to be (made) available, (3) immune responses by the host need to be avoided or overcome, (4) colonization by the fungus leads to development of disease symptoms. Prerequisites 1-3 also hold for endophytic and symbiotic fungi.

A powerful way to uncover molecular determinants of fungal pathogenicity towards plants is to compare genomes of closely related strains with different host specificities. We take this approach with the root-infecting, xylem colonizing fungus *Fusarium oxysporum*. This species complex harbours an enormous variety of highly host-specific pathogenic strains, together infecting more than a hundred plant species, as well as harmless strains. We have found that host-specific pathogenicity is associated with the presence of specific virulence genes, encoding small, *in planta* secreted proteins. These genes reside in accessory genomic regions, some of which comprise entire chromosomes that can be transferred horizontally between strains. In contrast, the conserved or 'core' genomes of strains of *F. oxysporum* are highly similar (98-100% sequence identity).

Through a combination of comparative genomics and targeted analysis of virulence genes we are beginning to find clues to the mechanisms underlying the evolution of host-specific pathogenicity in the *Fusarium oxysporum* species complex.

Insights into Colletotrichum hemibiotrophy from genome and transcriptome sequencing

Richard O`Connell[*1,2], Stéphane Hacquard[1], Pamela Gan[3], Emiel Ver Loren van Themaat[1], Jochen Kleemann[1], Stefan Amyotte[4], Michael Thon[5], Li-Jun Ma[6], Ken Shirasu[3], Lisa Vaillancourt[4]

[1] Max Planck Institute for Plant Breeding Research, Cologne, Germany; [2] INRA-BIOGER, Thiverval-Grignon, France; [3] Plant Science Center, RIKEN, Yokohama, Japan; [4] Department of Plant Pathology, University of Kentucky, Lexington, Kentucky, USA; [5] CIALE, Universidad de Salamanca, Villamayor, Spain; [6] University of Massachusetts Amherst, Amherst, Massachusetts, USA. Contact: richard.oconnell@versailles.inra.fr

Species of Colletotrichum cause devastating diseases on numerous crop plants worldwide. These pathogens use a hemibiotrophic infection strategy that involves the formation of specialized cell-types for initial penetration (appressoria), growth inside living plant cells (biotrophic hyphae) and tissue destruction (necrotrophic hyphae). The genomes of 4 species were sequenced to date: *C. higginsianum (Ch), C. graminicola (Cg), C. orbiculare (Co)* and *C. fructicola*, which respectively infect Arabidopsis, maize, cucurbits and strawberry. All 4 genomes encode extraordinarily large inventories of plant cell wall-degrading enzymes, with the three dicot pathogens having twice as many pectinases as the monocot pathogen *C. graminicola*, reflecting adaptation to host cell wall composition. Genes encoding secondary metabolism (SM) enzymes, especially those involved in the synthesis of polyketides, terpenes and alkaloids, are also expanded compared to other sequenced fungal pathogens, suggesting Colletotrichum species are capable of producing great chemical diversity. Similar to biotrophic pathogens, all 4 species encode large, lineage-specific repertoires of putative effector proteins, including an expanded family of chitin-binding LysM proteins that may function in evasion of PAMP-triggered immunity. The transcriptional dynamics underlying hemibiotrophy were examined using RNA-Seq (Ch, Cg) and microarrays (Co). Although appressoria formed in vitro resemble those *in planta*, comparison of their transcriptomes showed >1,500 Ch genes were induced only upon host contact, suggesting that plant signals sensed by appressoria reprogram fungal gene expression in preparation for host invasion. SM enzymes and secreted effectors were among the most highly up-regulated genes in appressoria, suggesting a role for these infection structures in host manipulation. During the biotrophic phase, transcripts encoding effectors and SM enzymes were also abundant, but there was no specific induction of nutrient uptake transporters, suggesting biotrophic hyphae function primarily as organs for delivering small molecule and protein effectors rather than nutrient acquisition. At the switch to necrotrophy, genes encoding a vast array of wall-degrading enzymes, proteases and membrane transporters were up-regulated, enabling the pathogen to mobilize nutrients from dead and dying cells to fuel its rapid growth and sporulation.

Post-transcriptional regulation of *Magnaporthe oryzae* pathogenicity genes

Julio Rodriguez-Romero, Marco Marconi, Mark Wilkinson and Ane Sesma*
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Transcriptional (*de novo* transcription) and post-transcriptional (pre-mRNA maturation, localisation and translation) mechanisms regulate the coordinated expression of related genes in the fungal cell. During the polyadenylation step which occurs in the nucleus, pre-mRNAs are cleaved at their 3` ends and subsequently modified by the addition of a polyadenosine (polyA) tail. Multiple 3′ end cleavage sites can be found in one pre-mRNA molecule. This mechanism of alternative polyadenylation (APA) generates mRNA isoforms with different exon content or 3′ UTR lengths, contributing to transcript diversity in eukaryotes.

We have used a comparative genome-wide sequencing approach to carry out a comprehensive map of polyadenylation sites in a *M. oryzae* wild type strain and the *Δrbp35* mutant. The *Δrbp35* mutant is defective in the Rbp35 subunit of the fungal Cleavage Factor I complex. We have also quantified polyA site usage under different nutritional conditions (rich and minimal media, carbon and nitrogen starvation). We have found a total of 9,589 polyA sites in the wild type strain and 10,118 in *Δrbp35*. The polyA mapping has covered ~7,600 genes in each nutrient condition. Nine percent of the reads could not be assigned to any annotated transcript. In the wild type strain, 94% of polyA sites are located in annotated 3′ UTRs, and APA has been found in 1,643 genes (21,6% of all mapped genes). A nucleotide profile surrounding the polyA sites has identified recognition motifs that differ slightly from yeast. Interestingly, the specificity for the cleavage sites has been misplaced in the *Δrbp35* mutant. The average 3′ UTR length of Rbp35-dependent genes in *Δrbp35* is significantly shorter (~250 nucleotides) compared to wild type (~300 nucleotides). Our mapping analysis suggests that the recognition of UGUA motif located 50 nucleotides upstream of the cut site by the fungal Rbp35/CfI25 complex is essential for a proper cleavage reaction. In addition, 457 genes that are alternatively polyadenylated in the wild type strain, have lost one or more polyA sites in the mutant. Significantly, the Rbp35/CfI25 complex is required to avoid an inappropriate processing of pre-mRNAs containing a specific U-rich motif found 7 nucleotides upstream of the polyA site. In summary, this genome-wide comparative analysis has helped us to identify i) the nucleotide context surrounding polyA sites in fungal pre-mRNAs; ii) the potential motif recognised by Rbp35/CFI25; iii) the involvement of Rbp35/CFI25 in APA; and iv) the dual function of the Rbp35/CFI complex in the 3′ end processing of pre-mRNAs in *M. oyzae*. The 3′ end profiling of infection-related genes found in these polyadenylation maps will be presented.

III. PLENARY SESSION: CELL BIOLOGY AND TROPIC GROWTH

Alexandra Brand (University of Aberdeen, UK)

“Understanding directional growth in fungal hyphae”

André Fleißner (University of Braunschweig, Germany)

“The art of networking: Cell fusion in *Neurospora crassa*”

Andrea Genre (University of Torino, Italy)

“Fungal recognition and accommodation in arbuscular mycorrhizal plants”

Poster session talks: Plant fungal interactions

A. Di Pietro: "On target: chemotropism of *Fusarium oxysporum* towards its host plant"

Laser microdissection and transcriptomics of infection cushions formed by *Fusarium graminearum*
Marike J. Boenisch, Stefan Scholten, Sebastian Piehler, Martin Münsterkötter, Ulrich Güldener, Wilhelm Schäfer

The secreted *Piriformospora indica* effector Dld1 is involved in penetration of plant cell wall appositions
Robin Nostadt, Magdalena Hilbert, Hannah Kleyer, Jörg Martin, Andrei Lupas, Alga Zuccaro

Anthocyanin induction via a secreted fungal effector promotes virulence through preventing lignifications
Shigeyuki Tanaka*, Thomas Brefort, Jörg Kahnt and Regine Kahmann

D-galacturonic acid utilization by *Botrytis cinerea*
Lisha Zhang*, Chenlei Hua, Joost Stassen, Sayantani Chatterjee, Maxim Cornelissen, Jan A.L. van Kan

***Colletotrichum orbiculare* CoPAG1, a component of RAM network in *Saccharomyces cerevisiae*, is involved in appressorium development triggered by plant-derived signals**
Sayo Kodama, Ayumu Sakaguchi and Yasuyuki Kubo*

Understanding directional growth in fungi

Alexandra Brand, University of Aberdeen, UK. Contact: a.brand@abdn.ac.uk

Fungal hyphae are programmed to explore their surroundings in search of nutrients and, for pathogens, success can depend on locating and identifying suitable host penetration sites. Fungi have therefore evolved mechanisms that link the sensing of environmental cues with appropriate growth responses. The intracellular components involved in polarised growth in fungi are generally well-conserved and have been studied in model organisms but how environmental signals interact with the molecular machinery of hyphal tip growth is less well-understood. *Candida albicans* is an opportunistic pathogen that exhibits pre-programmed, or tropic, growth responses to specific stimuli *in vitro*, making it a useful model for dissection of the regulatory pathways that control hyphal tip behaviour. We have used a variety of external stimuli, including electric fields, surface modification and nanofabrication techniques to examine the physical properties of apical growth, such as directional memory, asymmetric tip organisation and hyphal tip force. These methods have also been coupled with reverse genetics, fluorescence protein-tagging and live-cell imaging to identify cell-polarity components that can enhance, or even reverse, the direction of hyphal growth. The evidence to date suggests that hyphal growth direction is the net output from multiple, and sometimes opposing, input signals. Perturbation of this balance by local environmental signals may determine hyphal growth behaviour *in vivo*, where a strong association between proper hyphal tip regulation and the ability to invade and damage host tissue has already been established.

The art of networking: Cell fusion in *Neurospora crassa*

André Fleißner

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Cell fusion is an essential process for the development of most eukaryotic organisms. However, the molecular mechanisms underlying cell merger are only poorly understood. In recent years, the filamentous fungus *Neurospora crassa* has been adopted as an experimental model to study cell fusion and related cell-cell signaling processes.

Germinating vegetative spores of this fungus sense each other, grow towards each other and fuse. As a result an interconnected network of germlings is formed, which further develops into the mycelial colony. MAK-2, a MAP kinase, and SO, a protein of unknown molecular function, are both essential for germling fusion. GFP fusion constructs of both proteins are recruited in an oscillating and alternating manner to the plasma membrane of fusion tips. This observation suggests that the two cells coordinately switch between signal sending and receiving, in a kind of "cell dialog". Further analysis of this unusual mode of cell communication revealed additional factors essential for germling fusion, including systems, which generate reactive oxygen species, or cell polarity proteins. We also tested the influence of the plasma membrane composition on germling fusion by analyzing ergosterol biosynthesis mutants. Interestingly, specific structural features of the various sterols accumulating in the mutants have different effects on the fusion process. For example, an additional double bound in the ergosterol side chain results in communication defects, while changes in the ergosterol ring system inhibit plasma membrane merger. In conclusion, germling fusion depends on a spatially and temporally highly regulated interplay of signaling networks and the plasma membrane. It therefore provides a tractable experimental system for studying the subcellular dynamics of signaling cascades, directed cell growth and plasma membrane merger.

Fungal recognition and accommodation in arbuscular mycorrhizal plants

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Arbuscular mycorrhizas (AM) are symbiotic associations involving up to 90% of terrestrial plants and symbiotic fungi belonging to Glomeromycota. AM fungi enhance the absorption of plant nutrients and give them resistance against pathogens by colonizing the root through the development of inter- and intracellular hyphae and the formation of arbuscules, the highly branched structures where the nutrient exchange takes place. The presence of a symbiotic interface compartment around intracellular fungal structures is a hallmark of the biotrophic condition of AM fungi, and enables fungal development inside the plant cell space, while preserving its integrity.

This presentation focuses on the plant perception of AM fungi and their accommodation within the host cell. Our results, largely based on in vivo confocal microscopy, demonstrate that the process of interface construction takes place upon recognition of the AM fungus and adhesion of a hyphopodium to the root epidermis. Epidermal cells contacted by the fungus show repetitive oscillations (spiking) of nuclear calcium concentration. These oscillations are a central element in the signaling pathway that controls the symbiosis. Indeed, activation of this pathway leads to the assembly of the prepenetration apparatus (PPA), a columnar cytoplasmic aggregation, containing all the elements of the secretory pathway. By taking advantage of a range of fluorescent protein markers we show that the proliferation of the host plasma membrane takes place within the PPA, leading to the assembly of the perifungal membrane and symbiotic interface, in advance of hyphal tip growth.

On target: chemotropism of *Fusarium oxysporum* towards the host plant

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Chemotropism is the ability of an organism to re-orient the growth axis in response to chemical cues. This process has been studied in detail for the mating pheromone response in the budding yeast *Saccharomyces cerevisiae*. In addition, hyphal chemotropism is likely to play a critical role in many key aspects of the fungal lifestyle, including colony establishment, foraging for nutrients or location of host organisms. While directional growth towards nutrients is still under debate, it is known that soil-inhabiting pathogens and mycorrhizal symbionts use chemotropic sensing of root chemoattractants to locate host plants. However, the mechanisms underlying these chemotropic responses are currently unknown.

The soil-borne ascomycete *Fusarium oxysporum* provokes vascular wilt disease in a wide range of plant species, causing devastating losses in field and greenhouse crops. Infectious hyphae of the fungus penetrate the plant preferentially through openings at the junctions of root epidermal cells, indicating that they can sense and grow towards chemical signals secreted by the host. We genetically dissected the chemotropic response of *F. oxysporum* to nutrients and root chemoattractants. We found that directed hyphal growth towards tomato roots requires a functional orthologue of the alpha mating pheromone receptor from budding yeast, as well as key elements of the conserved cell integrity mitogen-activated protein kinase (MAPK) cascade. Meanwhile, a functionally distinct MAPK pathway is responsible for chemotropic sensing of nutrient chemoattractants such as glucose or glutamate. Our results suggest that root-colonizing fungi exploit the highly sensitive sex pheromone perception system to locate plant hosts in complex environments such as the soil.

Laser microdissection and transcriptomics of infection cushions formed by Fusarium graminearum

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The fungal plant pathogen *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein) Petch) is the causal agent of Fusarium head blight (FHB) of small grain cereals and cob rot of maize worldwide. Trichothecene toxins produced by the fungus e.g. nivalenol (NIV) and deoxynivalenol (DON) contaminate cereal products and are harmful to humans, animals, and plants. We demonstrated recently, that *F. graminearum* forms toxin producing infection structures during infection of wheat husks, so called infection cushions (Boenisch and Schäfer, 2011). The aims of the presented study were to further clarify the penetration mechanism of infection cushions by histological studies and to identify molecular characteristics of infection cushions by expression analysis. Structural characteristics of infection cushions were visualized by 3D images following laser-scanning microscopy. We observed multiple penetration events underneath infection cushions by scanning electron microscopy. Colonization of the underlying plant tissue was studied by bright field microscopy and transmission electron microscopy of LR-White serial sections. To understand the molecular basis of initial colonization of the leaf surface followed by infection cushion development, a laser capture microdissection (LCM) approach was established to isolate specifically runner hyphae and infection cushions. Several hundred runner hyphae and infection cushions grown on wheat glumes were collected using the PALM system (Zeiss) avoiding contamination with plant tissue. Total mRNA of runner hyphae and infection cushions were isolated and amplified. The cDNA library of each developmental stage was used for next generation sequencing with Illumina HiSeq 2000. Quantitative expression analysis show marked differences in gene expression patterns between runner hyphae and infection cushions. Different functional pathways specific for each infection stage were identified. Thereby new insights in the initial infection process of FHB disease are gained. To our knowledge, we provide the first transcriptome data of runner hyphae and infection cushions from a fungal plant pathogen obtained under *in planta* conditions. In summary, the power of combined microscopic and molecular approaches to analyze cell type-specific gene expression during fungal-plant-interactions is demonstrated.

The secreted *Piriformospora indica* effector Dld1 is involved in penetration of plant cell wall appositions

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The mutualistic root endophyte *P. indica* colonizes the roots of a large spectrum of mono- and dicotyledonous host plants, including the non-mycorrhizal plant *A. thaliana* and the crop plant *H. vulgare* (barley). The fungus confers a variety of beneficial effects to its host including improved resistance to biotic and abiotic stresses and increased biomass production and seed yield. *P. indica* colonization of barley can be divided into two distinct phases: Between 3 and 7 days post inoculation (dpi) the fungus penetrates the cell wall of epidermal and root cortex cells and establishes a biotrophic interaction, growing inter- and intracellulary. Past 10 dpi the fungus colonizes and digests dead root cortex cells using its saprophytic traits. Genome data revealed an expanded family of genes encoding a novel family of plant responsive secreted putative effectors, sharing a regular distribution of histidine and alanine residues and a conserved C-terminal 7 amino acid RSIDELD motif. One member of the DELD family proteins, Dld1, is highly upregulated during biotrophic interaction with barley. The protein shows high affinity to several divalent metal ions and binds to iron(III) in a pH dependent manner. A Yeast-two hybrid screen with a cDNA library from A. thaliana colonized by P. indica, indicated interactions between Dld1 and a *P. indica* membrane transporter and several plant derived metal associated proteins, including a class III peroxidase. Iron(III) specific staining revealed that plant cell wall appositions (CWAs) formed during cell wall penetration attempts by pathogenic fungi contain a large amount of iron(III) (Llu et al. 2007). We show that the amount of iron(III) in barley CWAs, which formation coincides temporally with the expression pattern of PiDLD1, is lower after penetration by *P. indica*. We therefore suggest Dld1 is produced and secreted upon penetration in the apoplast where it sequesters iron(III) from CWAs inhibiting iron catalyzed reactions to fortify the plant cells wall.

Anthocyanin induction via a secreted fungal effector promotes virulence through preventing lignifications

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The biotrophic 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 a dramatic reduction of tumor formation and loss of anthocyanin induction, which are characteristic phenotypes of maize leaves infected with *U. maydis*. Introduction of the *tin2* gene into the 19A-1 mutant partially rescued tumor formation and fully restored anthocyanin induction. Here we demonstrate that anthocyanin induction by the Tin2 effector protein encoded in the 19A-1 region is beneficial for tumor induction. Tin2 protein is secreted and expressed exclusively during biotrophic growth. Expression of Tin2 protein in the cytosol of maize plants could induce anthocyanin in leaves infected with the *tin2* deletion mutant, suggesting that Tin2 protein is translocated into plant cells after secretion. A Tin2 protein lacking the C-terminal 5 amino acids had lost its biological function. In line with this, the Tin2 mutant protein could not interact with the cytoplasmic maize protein kinase ZmTTK1 identified as Tin2 interactor by yeast two hybrid screening. Transient expression assays in *Nicotiana benthamiana* revealed that ZmTTK1 is degraded proteasome-dependently through the phosphodegron-like motif DSGxS. Interestingly, co-expression with Tin2 stabilized ZmTTK1. Transient expression of a stabilized ZmTTK1 protein could induce anthocyanin in leaves with the *tin2* deletion mutant, suggesting that ZmTTK1 might regulate the anthocyanin biosynthetic pathway in maize. Quantitative expression analysis revealed that a gene from the lignin biosynthetic pathway was specifically up-regulated in leaves infected with the *tin2* deletion mutant, while anthocyanin biosynthetic genes were induced in wild type infected leaves but not induced in leaves with *tin2* deletion mutant. Lignin staining revealed that the vascular bundles in leaves infected with the *tin2* deletion mutant showed strong lignifications and only rarely contained fungal hyphae. Leaves infected with the wild-type strain showed less lignification and the vascular bundles were heavily infected. This shows that Tin2 effector protein prevents lignification of vascular bundles and this promotes massive fungal proliferation.

D-galacturonic acid utilization by *Botrytis cinerea*

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The plant cell wall is the first barrier to pathogen invasion. The fungal plant pathogen *Botrytis cinerea* produces a spectrum of cell wall degrading enzymes for the decomposition of host cell wall polysaccharides and the consumption of the monosaccharides that are released. Especially pectin is an important cell wall component, and the decomposition of pectin is required for full virulence of *B. cinerea*. An effective concerted action of the appropriate pectin depolymerising enzymes, monosaccharide transporters and catabolic enzymes is important for complete pectin utilization by *B. cinerea*.

In this study, RNA sequencing was performed to compare genome-wide transcriptional profiles in *B. cinerea* grown in media containing glucose and pectate as sole carbon sources. Transcript levels of 32 genes that are induced by pectate were further examined in cultures containing 6 different monosaccharides by quantitative RT-PCR. We identified 8 genes that are specifically induced by D-galacturonic acid, including *Bcgar2* and *Bclga1* (encoding two key enzymes in the D-galacturonic acid catabolic pathway) and two putative monosaccharide transporter genes. In addition, conserved motifs were identified in the promoters of genes involved in pectate decomposition and D-galacturonic acid utilization. The role of these motifs in regulating D-galacturonic acid-induced expression was functionally analysed in the promoter of *Bclga1* gene. We will present the data on the functional characterization of the transporter genes and the identification of motifs required for D-galacturonic acid induction.

Colletotrichum orbiculare CoPAG1, a component of RAM network in _Saccharomyces cerevisiae_, is involved in appressorium development triggered by plant-derived signals

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Many plant pathogenic fungi initiate infection of host leaves by the germination of conidia and differentiation of appressoria at the tip of germ tubes. These morphological changes are triggered by various external signals such as physical or chemical signals from the plant surface. We previously reported that cucumber anthracnose fungus *Colletotrichum orbiculare* CoKEL2, a *Schizosaccharomyces pombe tea1* homologue is essential for proper morphogenesis of appressoria on artificial substrates but is dispensable for appressorium formation on the host plant surfaces (Sakaguchi et al., 2008). Our results suggested that there is a bypass pathway that transduces plant-derived signals for appressorium formation independent of CoKEL2. These plant-derived signaling pathways for appressorium formation have not been characterized in fungal pathogens including *C. orbiculare*. To determine the specific components of the plant-derived signaling pathway that leads to appressorium formation, we obtained six *cokel2* double mutants that formed abnormal appressoria not only on artificial substrates but also on the host plant surfaces. Expectedly, reintroduction of *CoKEL2* into those *cokel2* double mutants restored normal appressorium formation on artificial substrates. We identified candidate-mutated genes by whole genome sequencing of the six *cokel2* double mutants. By blastp search, it was shown that the predicted amino acid sequence encoded by the mutated gene of *cokel2* double mutant kanI-9 had high homology to that of PAG1 (TAO3) in *Saccharomyces cerevisiae*. PAG1 is one of the components of the RAM (regulation of Ace2p activity and cellular morphogenesis), a signaling cascade that is involved in the maintenance of polarity, cell separation and cellular integrity. To define the involvement of CoPAG1 in appressorium formation, we observed the phenotypes of *copag1Δ* mutants and *copag1Δcokel2Δ* double mutants. As expected, *copag1Δcokel2Δ* showed same phenotypes as *kanI-9*, indicating that existence of signaling pathway for appressorium morphogenesis triggered by plant-derived signals and that CoPAG1 is one of specific components of this signaling pathway. In *S. cerevisiae*, PAG1 facilitate normal activation of the Ndr (nuclear Dbf2-related) family protein kinase CBK1, the downstream module of the RAM. We hypothesized that CoPAG1 functions via a similar signaling cascade in *C. orbiculare*. To investigate this, an analysis of the constitutively active strain of CoCBK1 in *copag1Δ* background by site directed mutagenesis is in progress.

Sakaguchi, A., Miyaji, T., Tsuji G. and Kubo, Y. (2008) Kelch-repeat protein Clakel2p and calcium signaling control appressorium development in *Colletotrichum lagenarium*. *Eukaryotic Cell* 7:102-111.

Poster session talks: Cytskeleton and Polarity

M. Feldbrügge: "Cytoskeleton, intracellular transport and hyphal tip growth"

Does endocytosis affect the dynamics of exocytic markers and cell wall assembly
Ramírez-Del Villar A., Echauri-Espinosa R., Bartnicki-García S. and Mouriño-Pérez R.R.*

The Rab GTPase YPT-1 coordinates vesicle traffic at the Golgi and at the Spitzenkörper in *Neurospora crassa*
Eddy Sanchez-Leon*, Barry Bowman and Meritxell Riquelme

Interaction of calmodulin with cytoskeletal proteins during colony initiation in *Neurospora crassa*
Chia-Chen Chang*, Thierry Le Bihan, and Nick D. Read

Woronin bodies: impact on stress resistance and virulence of *Aspergillus fumigatus* and anchoring at the septal pore of filamentous Ascomycota
Julia Beck and Frank Ebel*

Visualization of membrane domains, microtubule and actin cytoskeletons in *Aspergillus nidulans* by Photoactivated Localization Microscopy (PALM)
Anna Bergs*, Yuji Ishitsuka, Yiming Li, Ulrich Nienhaus, Reinhard Fischer and Norio Takeshita

IV. PLENARY SESSION: ORGANELLES AND ORGANELLAR FUNCTIONS

Ida van der Klei (University of Groningen, The Netherlands)

“The biogenesis and function of fungal peroxisomes”

Gero Steinberg (University of Exeter, UK)

“Endosome motility in fungi: The how and why”

Benedikt Westermann (University of Bayreuth, Germany)

“Mitochondrial dynamics and inheritance in yeast”

Cytoskeleton, intracellular transport and hyphal tip growth

M. Feldbrügge

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Fungal hyphae exhibit a characteristic growth mode. The growth apex is responsible for the rapid expansion of membrane and cell wall and in subapical regions, filaments are partitioned by septa forming molecular barriers. Polar growth is mediated by intensive membrane dynamics, i. e. local exo- and endocytosis that is supported by vesicle trafficking. This might be promoted by distinct membrane microdomains at the apical pole, which are rich in specific lipids such as sphingolipids and ergosterol. Two juxtaposed macromolecular units are involved in growth cone function: the Spitzenkörper and the polarisome. The latter is a multiprotein complex consisting of landmark proteins, signalling molecules such as small GTPases and formins that nucleate actin cables for short distance transport. The Spitzenkörper is thought to function as a supply centre for endocytotic and exocytotic vesicles. For example, specialised vesicles, so-called chitosomes, transport and export chitin synthases as well as building blocks for cell wall synthesis Active transport along the actin and microtubule cytoskeleton is mediated by molecular motors for short and long-distance delivery of vesicles, respectively. This process is streamlined for efficiency and any disturbances lead to reduced growth rates or a complete blockage.

Does endocytosis affect the dynamics of exocytic markers and cell wall assembly?

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The orderly migration and discharge of exocytic vesicles carrying cell wall components are responsible for morphogenesis in filamentous fungi. We have been searching for evidence that endocytosis, an opposite process, could also play a role in morphogenesis. We observed a mutant lacking coronin (*Δcrn-1*) that is a conserved actin binding protein that promotes cellular processes that rely on rapid remodeling of the actin cytoskeleton, as endocytosis. The lack of coronin in *N. crassa* leads to intermingled periods of polarized and isotropic growth of the hyphae. We used CRIB fused to GFP as an exocytic reporter of activated Cdc-42 and Rac-1. We found that CRIB-GFP was present in wild-type hyphae as a thin hemispherical cap under the apical dome. In the *Δcrn-1* mutant, the location of CRIB-GFP shifted between the periods of polarized and isotropic growth, it migrated to the subapical region and appeared as localized patches. Significantly, cell growth occurred in the places where the CRIB-GFP reporter accumulated, thus the erratic location of the reporter in the *Δcrn-1* mutant correlated with the morphological irregularity of the hyphae. Additionally, we studied the distribution and composition of the cell wall in the *Δcrn-1* mutant and WT stain. We found a different chitin content in the *Δcrn-1* mutant, it had a higher proportion of chitin than the WT strain (14.1% and 9.1% respectively). We also compared the relative cell wall area (TEM images) and we found a different ratio wall/cytoplasm between the *Δcrn-1* mutant and the WT strain. In conclusion, we have found that the mutant affected in endocytosis has an altered pattern of exocytosis as evidenced by its distorted morphology and displaced exocytic markers. A direct cause-effect relationship between endocytosis and exocytosis remains to be established.

The Rab GTPase YPT-1 coordinates vesicle traffic at the Golgi and at the Spitzenkörper in *Neurospora crassa*

Sanchez-Leon, Eddy[1*], Bowman, Barry[2] and Riquelme, Meritxell[1]

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In eukaryotic cells secretion mechanisms require the coordinated actions of Rab GTPases, protein coats, molecular motors, v- and t-SNAREs and long coil-coiled or multisubunit tethering factors to ensure cargo directionality between membranes. Vesicle traffic involves budding, transport, tethering and fusion of vesicles to the acceptor membrane. Small Rab GTPases in their active state interact with the membrane of the vesicles and promote the association with other factors before the subsequent vesicle fusion mechanisms occurs. In contrast to other eukaryotic model systems, filamentous fungi contain the Spitzenkörper (Spk), a multi-vesicular complex found at the hyphal apex to which cargo-carrying vesicles arrive before being redirected to specific cell sites. The exact regulatory mechanisms utilized by the hyphae to ensure the directionality of the secretory vesicles that reach the Spk are still unknown. Hence, we have analyzed the *N. crassa* Rab-GTPase YPT-1 (Rab1), a key regulator of the secretory pathway involved in the regulation of ER-Golgi and late endosome-Golgi traffic steps in *Saccharomyces cerevisiae*. Laser scanning confocal microscopy of strains expressing fluorescently tagged versions of YPT-1 revealed its localization at the Spk and at Golgi equivalents. Co-expression of differently labeled YPT-1 and the post-Golgi Rab GTPases SEC-4 (Rab8) and YPT-3 (Rab11) showed that YPT-1 was confined at the microvesicular core of the Spk, while SEC-4 and YPT-3 localize in the Spk peripheral macrovesicular layer, suggesting that trafficking of macro and microvesicles of the Spk are regulated by distinct Rabs. Co-localization analysis of YPT-1 with the early Golgi markers USO-1 (p115) and VRG-4, and the late Golgi marker VPS-52 indicated the participation of this Rab at early and late Golgi vesicle trafficking steps. TIRFM (Total Internal Reflection Fluorescence Microscopy) revealed anterograde and retrograde movement of YPT-1 associated vesicles, sometimes decorating tubule-like structures at distal hyphal regions; and a significant flow of vesicles reaching apical and subapical regions. FRAP (Fluorescence Recovery After Photobleaching) analysis showed a fast recovery of GFP-YPT-1 fluorescence due to vesicles arrival at the Spk, confirming TIRFM observations. On sucrose density gradients, GFP-YPT-1-associated particles sedimented mainly in fractions with a density of 1.124-1.132 g/mL, which coincides with the density of microvesicles as previously shown. We propose that YPT-1 is an important component in the regulation of secretory vesicles traffic in filamentous fungi; its intracellular distribution and dynamics at Golgi structures and at the Spk core suggests that this Rab GTPase participates during both, early and late steps of protein secretion.

Interaction of calmodulin with cytoskeletal proteins during colony initiation in *Neurospora crassa*

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Calmodulin (CaM) is the primary intracellular calcium-receptor in eukaryote cells. It is activated by intracellular calcium signals that allow it to interact with and modulate the activities of a diverse range of target proteins. In this study, calmodulin-interacting proteins were isolated from conidial germlings of *N. crassa* by immunoprecipitation and analyzed by mass spectrometry. A total of 318 putative calcium/CaM-binding proteins were identified of which 11 proteins were implicated in cytoskeletal function. Of these 11 proteins, 10 contained putative CaM-binding motifs identified from the CaM target database (http://calcium.uhnres.utoronto.ca/ctdb). Two CaM antagonists (calmidazolium and trifluoperazine) were found to selectively inhibit a form of cell fusion involving conidial anastomosis tubes (CATs) that occurs during colony initiation. Localization of CaM-GFP during colony initiation showed that it was associated with F-actin and microtubules. The F-actin polymerization inhibitor latrunculin-A prevented the pronounced accumulation of CaM at growing tips. In addition, the movement of CaM associated with the spindle pole body was suppressed with β-tubulin depolymerising agent, benomyl. Deletion of the type II myosin motor protein gene (*Δmyo-5*) resulted in defective CaM recruitment to the growing tips of germ tubes and CATs and also reduced CAT fusion.

Woronin bodies: impact on stress resistance and virulence of *Aspergillus fumigatus* and anchoring at the septal pore of filamentous Ascomycota

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Multicellular organism benefit from a well organized flow of molecules between individual cells or compartments. For this purpose, distinct types of cell-to-cell channels have evolved in the kingdoms of animals, plants and fungi. Filamentous fungi grow as hyphae that, in the Pezizomycotina, are divided into compartments that remain connected via septal pores. Due to this coenocytic organization and the high internal osmotic pressure, wounding can cause a dramatic loss of cytoplasm. To avoid this, the Pezizomycotina have evolved specialized organelles, so-called Woronin bodies, which function as a kind of emergency plug. Two major types of architecture exist: tethering of Woronin bodies at the lateral cell wall in the Neurospora-Sordaria-clade and anchoring at the septal wall in most other Pezizomycotina. In the latter, the precise spatial positioning of these organelles is an essential element of the hyphal architecture, but surprisingly little was known about the respective molecular organization. We therefore analyzed the function and localization of Woronin bodies in *Aspergillus fumigatus*. We provide evidence that Woronin bodies are important for stress resistance and virulence of this human pathogen. Moreover, we describe the molecular machinery that links Woronin bodies to the septum. Namely we have identified the tethering protein Lah and provide evidence suggesting HexA as the Lah binding partner on the Woronin body surface. This attributes an important cellular function to a protein that was so far supposed to be merely the main structural building block of the Woronin body core. The model of the tethering mechanism that arises from our data differs substantially from that described for *Neurospora crassa*. We assume that the situation in *A. fumigatus* is representative for those fungi in which Woronin bodies are anchored at the septal pore, which applies to the vast majority of the filamentous Ascomycota.

Visualization of membrane domains, microtubule and actin cytoskeletons in *Aspergillus nidulans* by Photoactivated Localization Microscopy (PALM)

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Filamentous fungi grow by their continuous tip elongation. For the maintenance of polarized tip growth, it is necessary for the cell to establish a continuous transport of secretory vesicles towards the hyphal tip to provide proteins and lipids necessary for cell wall and cell membrane extension. The vesicle transport and exocytosis are performed microtubule and actin cytoskeleton dependent.

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 size of SRDs is around a few µm, whereas the size of membrane microdomains ranges in general between 10-200 nm.

In recent years, super-resolution microscopic techniques have been improving and breaking the diffraction limit of conventional light microscopy of 250 nm. In this method, a lateral image resolution as high as 20 nm will be a powerful tool to investigate membrane microdomains and components of the cytoskeletons. To investigate deeply the relation of membrane domains and cytoskeleton components for vesicle transport and exocytosis, the distribution of microdomains in SRDs, microtubules, and actin-cables were analyzed via a super-resolution microscope technique, which is called Photoactivated Localization Microscopy (PALM). The components of interest are visualized via tagging with the photoconvertible fluorescent protein mEosFP for PALM.

Peroxisome proliferation in fungi

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Peroxisomes are ubiquitous cell organelles that play a role in various metabolic processes. Several internal and external stimuli are known that induce peroxisome proliferation. Proliferation occurs via fission of pre-existing organelles or by de novo formation. De novo peroxisome formation has been observed in *pex3* mutant cells, which lack peroxisomal membrane structures, upon reintroduction of the PEX3 gene. It has been proposed that in *pex3* mutant cells peroxisomal membrane proteins (PMPs) accumulate at the ER. Upon induction of PEX3 expression, newly synthesized Pex3 is first sorted to the ER and subsequently incorporated in preperoxisomal vesicles together with the other PMPs. According to this model Pex3 is required for the formation of these vesicles and hence for the exit of PMPs from the ER.

Using the yeast *Hansenula polymorpha*, we re-investigated this process using advanced electron and fluorescence microscopy techniques. Our data indicate that in a *pex3* mutant, peroxisomal membrane structures are already present. These structures contain a subset of peroxisomal membrane proteins and are the template for *de novo* peroxisome formation upon reintroduction of Pex3.

These findings shed new light on the de novo peroxisome formation process and the role of the ER in peroxisome formation.

How and why early endosomes move in fungal cells

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Early endosome motility along microtubules supports fungal growth and pathogenicity. It is mediated by the counteracting motor proteins kinesin-3 and dynein, which constantly change the transport direction of the organelles. Why early endosomes move and how the opposing motor activities are regulated is currently not known. In this talk I will provide most recent data that show that master control regulators exist that regulated the attachment of kinesin-3 and dynein to the early endosomes. These regulators are evolutionary conserved from humans to protists, but absent from the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. In both yeasts, microtubules have lost their role in membrane trafficking. Thus, we have identified the molecular control complex that is of central importance for bi-directional motility of endosomes in the fungal cell. Furthermore, we have identified the biological function of bi-directional endosome motility, which will also be discussed in this talk.

Mitochondrial dynamics and inheritance in yeast

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Mitochondria are essential organelles of eukaryotic cells. They are the major sites of energy production and play important roles in programmed cell death and aging. In many cell types, mitochondria show an amazingly dynamic behaviour. They continuously move along cytoskeletal tracks, and their membranes 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 programmed cell death. Budding yeast *Saccharomyces cerevisiae* is a particularly useful model organism to study these processes. Systematic screening of comprehensive yeast mutant collections has revealed novel molecular components and cellular pathways required for mitochondrial fusion, division, motility, mitochondrial DNA inheritance, and respiratory activity. These large scale genetic analyses are combined with functional characterization of newly identified proteins by biochemical and imaging techniques. Our current work focuses on the molecular mechanisms contributing to mitochondrial transport, distribution, and inheritance in yeast. We have recently identified the class V myosin, Myo2, as a motor directing anterograde mitochondrial transport, and the cell cortex-associated protein, Num1, as an important factor for retention of mitochondria in the mother cell. The ultimate goal of our ongoing work is to obtain a comprehensive picture of the molecular processes contributing to mitochondrial inheritance in a simple eukaryotic cell.

V. PLENARY SESSION: BIOTECHNOLOGY

Claus Bollschweiler (BASF SE, Ludwigshafen, Germany)

"Hydrophobins: Innovative performance proteins for industrial Biotechnology"

Ronald de Vries (CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands)

"Fungal strategies for plant biomass degradation"

Katsuhiko Kitamoto (University of Tokyo, Japan)

"Developing *Aspergillus oryzae* as a host for heterologous protein production"

Poster session talks: Gene regulation

Y. Liu: "Less is more, codon usage regulates protein expression, structure and function"

Signalling of the calcineurin responsive transcription factor CrzA in response to calcium and alkaline-pH stresses
Patricia Hernandez-Ortiz and Eduardo A Espeso*

Phytochrome and the white-collar proteins control light-induced genes in *Aspergillus nidulans* via chromatin remodeling
Stefan Rauscher*, Julian Röhrig, Maren Hedtke, Zhenzhong Yu and Reinhard Fischer

Import of peroxisomal matrix proteins in *Ustilago maydis*
Julia Ast*, Alina C. Stiebler, Johannes Freitag, Michael Bölker

Role of the small RNAs synthesis machinery on the antagonistic capacity of *Trichoderma atroviride*
Emma Beltrán-Hernández, Jorge Molina-Torres, Alfredo Herrera-Estrella*

Molecular basis of FLO11 mediated interactions in *Saccharomyces cerevisiae*
Timo Kraushaar*, Maik Veelders, Stefan Brückner, Hans-Ulrich Mösch and Lars-Oliver Essen

Hydrophobins: Innovative *Performance Proteins* for Industrial Biotechnology

Claus Bollschweiler, BASF SE, Ludwigshafen, Germany. Contact: claus.bollschweiler@basf.com

Nature offers different examples for proteins with interesting and promising properties. Technical advances in White Biotechnology especially with respect to molecular biology biochemistry and fermentation, is now enabling chemical industry to synthesize some of these proteins on an industrial scale for the first time, thereby allowing consumers to benefit from the special properties of these natural substances in their daily life.

One example of a new product from BASF's research labs comes from the class of Hydrophobins, a group of small, cysteine rich proteins of about 100 amino acids that are expressed only by filamentous fungi in small amounts. Based on differences in hydrophobicity patterns and biophysical properties, they can be divided into two categories: class I and class II. BASF has its focus on DewA, a class I Hydrophobin from *Aspergillus nidulans*. This fungal protein spontaneously adheres as a monolayer to all surfaces with the result of significant changes in surface properties with respect to surface tension on solid surfaces as well as on emulsions or foams. Researchers of BASF have isolated the gene responsible for producing Hydrophobin and transferred it to the bacterium E. coli. Based on this a process was developed to produce Hydrophobin in industrial quantities. The actual focus is the validation of potential applications. One first major learning tells us that based on an innovative protein like Hydrophobin it is unlikely to identify drop in solutions for already existing, optimized applications. BASF and a number of committed partners are just in the process to develop tailor made market opportunities for this very promising protein.

Fungal Strategies for plant biomass degradation

Ronald de Vries

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In nature, many fungi use plant biomass as their main carbon source. However, their approach to this differs significantly and is largely dependent on their biotope and lifestyle. For instance, saprobes, plant pathogens and mycorrhizae require different types of interactions with plant biomass and therefore also a different enzymatic and regulatory machinery. Still, organisms with a similar lifestyle and biotope, such as several Aspergilli, also use diverse strategies for plant biomass utilization, as revealed by proteomic and enzymatic analysis.

In the last years, our lab has explored the diversity of fungi with respect to plant biomass degradation from a physiological, enzymatic, metabolic and regulatory angle. In this presentation, highlights from these studies will be presented, focussing on the differences between fungi that directly correlate with their biotope and/or lifestyle, as well as on the differences within one genus. We recently started exploring the differences between generalist and specialist fungi, and those results will also be presented.

Developing *Aspergillus oryzae* as a host for heterologous protein production

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We had demonstrated that decreasing protease activity in *A. oryzae* through the disruption of ten protease genes led to increased productivity of heterologous proteins. Moreover, disruption of the gene coding for the vacuolar protein sorting (Vps) receptor AoVps10 led to decreased transportation of recombinant proteins to vacuoles via the Vps pathway and increased secretion of recombinant proteins in the culture medium. This finding suggests that repression of vacuolar degradation may be effective in enhancing the productivity of heterologous proteins.

Autophagy, which is a highly conserved intracellular degradation pathway in eukaryotes, functions as a survival mechanism under nutrient starvation conditions by recycling intracellular components. In addition to nutrient recycling, autophagy plays important roles in cell development and differentiation, immune responses, and cell death. Thus, autophagy is an extremely important system in eukaryotes; however, no studies have examined the mechanisms by which autophagy affects the production of recombinant proteins.

In the present study, we examined the production of heterologous protein in several autophagy (*Aoatg*) gene disruptants of *A. oryzae*. We transformed *A. oryzae* gene disruptants of *Aoatg1*, *Aoatg13*, *Aoatg4*, *Aoatg8*, or *Aoatg15*, with a bovine chymosin (CHY) expression construct and found that the production levels of CHY increased up to three fold compared to the control strain. Notably, however, conidia formation by the *Aoatg* gene disruptants was significantly reduced. As large amounts of conidia are necessary for inoculating large-scale cultures, we also constructed *Aoatg* gene-conditional expression strains in which the promoter region of the Aoatg gene was replaced with the thiamine-controllable thiA promoter. Conidiation by the resultant transformants was clearly enhanced in the absence of thiamine, while autophagy remained repressed in the presence of thiamine. Moreover, these transformants displayed increased CHY productivity, which was comparable to that of the *Aoatg* gene disruptants. Consequently, we succeeded in the construction of *A. oryzae* strains capable of producing high levels of CHY due to defects in autophagy. Our finding suggests that the conditional regulation of autophagy is an effective method for increasing heterologous protein production in *A. oryzae*.

Less is more, codon usage as a mechanism that regulates protein expression, structure and function

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Codon usage bias has been observed in the genomes of almost all organisms and is thought to result from selection for efficient and accurate translation of highly expressed genes. Many genes exhibit little codon usage bias. The lack of codon bias for a gene is thought to be due to lack of selection for mRNA translation. Alternatively, however, non-optimal codon usage may have biological significance. The rhythmic expression and the proper function of the Neurospora FREQUENCY (FRQ) protein are essential for circadian clock function. Here, we show that, unlike most genes in Neurospora, *frq* exhibits non-optimal codon usage across its entire open reading frame. Optimization of *frq* codon usage results in the abolition of both overt and molecular circadian rhythms. Codon optimization not only increases FRQ level but surprisingly, also results in conformational changes in FRQ protein, altered FRQ phosphorylation profile and stability, and impaired functions in the circadian feedback loops. These results indicate that non-optimal codon usage of *frq* is essential for its circadian clock function. Our study establsihes an example of how non-optimal codon usage is used to regulate protein expression and to achieve optimal protein structure and function.

Signalling of the calcineurin responsive transcription factor CrzA in response to calcium and alkaline-pH stresses

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Aspergillus nidulans is able to grow over a wide range of nutrient conditions or abiotic stresses. Among the later are alkalinity and abundance of monovalent or divalent cations in the medium. During the years we have been interested in understanding how this filamentous fungus elaborates a response to these stresses. Growth under alkaline conditions requires the activities of three important transcription factors, named PacC, SltA and CrzA. SltA also is involved in cation homeostasis but CrzA activity is likely restricted to provide with an adequate transcriptional response to calcium. CrzA is by regulated calcium in its cellular location, being preferentially nuclear when this cation is present in the medium. We focused now on determining the effect of abiotic stresses on the localization of CrzA and in the identification of element belonging to its signalling pathway. CrzA remains cytoplasmic and excluded from nuclei in non-stimulated cells, resting cells. Alkalinity and magnesium, stresses requiring CrzA activity, promoted nuclear accumulation of this transcription factor as observed for calcium-induced stress. Other cation or ambient-pH stresses are not recognised by the signalling pathway which includes the non-essential calcineurin phosphatase. In the absence of calcineurin phosphatase activity CrzA cellular distribution remains unaltered after stress. CrzA is post-translationally modified by calcineurin. CnaA, the catalytic subunit of calcineurin binds to CrzA through a conserved calcineurin-docking domain, CDD. A mutant form of CrzA lacking this CDD is partially functional suggesting alternative sites for CrzA-CnaA interaction and subsequent signalling. In addition to a CDD we have identified other functional regions in CrzA, such a nuclear export sequence and two nuclear localisation signals.

At least two kinases exert their activities in an opposed way to calcineurin over CrzA. Casein kinase I, CkiA, and the homologue of glycogen synthase kinase 3beta, GskA, participate in the post-translational modification state of CrzA. CkiA does not influence nucleocytoplasmic transport of CrzA but GskA activity is needed for proper nuclear import of this transcription factor in response to medium alkalinisation.

This work has identified cis and trans regulatory elements mediating nucleocytoplasmic trafficking and the post-translational modified level of CrzA in response to a specific number of abiotic stresses.

Phytochrome and the white-collar proteins control light-induced genes in *Aspergillus nidulans* via chromatin remodeling

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In *Aspergillus nidulans* light serves as a major signal and regulates many important responses like the ratio between sexual and asexual growth (1), conidial germination (2) or secondary metabolism (1). Whereas asexual development occurs in light, sexual development is preferred in the dark (1).

A. nidulans is able to sense red light with a phytochrome (FphA) and blue light with the white collar system (LreA/B) (3). Interestingly, FphA physically interacts with LreA/B (WC-1/2) and another regulator, VeA (4). Two light-induced genes, *ccgA* and *conJ* were chosen for the analysis of the mechanism of light induction (5). FphA, LreA/B and VeA were identified at both promoters. Whereas LreA was bound in darkness and released in light, FphA and VeA were recruited to the promoter after illumination. Interestingly the binding of FphA and LreA to the promoter was lost in *ΔlreA* and *ΔfphA* strains respectively. This suggests that the red and blue light receptors interact directly and function antagonistically at the promoters of light-induced genes.

Since FphA has no known DNA binding motif we were looking for new interaction partners. In *Neuospora crassa* WC-1 interacts with NGF16, a histone remodeling acetyltransferase of the GCN5 protein family (6). The orthologue in *A. nidulans* is GcnE with 73.6% sequence similarity. LreA interacted with GcnE within nuclei. As a difference to *N. crassa* GcnE also interacted with FphA. LreA - but not FphA - also interacted with HdaA, a histone deacetylase. We hypothesize that LreA and FphA control the acetylation status of histone H3 through a light-dependent control of histone-acetyl transferase and histone deacetylase.

(1) Purschwitz et al. (2008), *Curr. Biol.* 18(4), 255–259.
(2) Röhrig et al. (2013), *Curr. Genet.,* 59, 55–62
(3) Blumenstein et al. (2005), *Curr. Biol.* 15, 1833–1838
(4) Purschwitz et al. (2009), *Mol Genet Genomics* 281(1), 35–42
(5) Ruger-Herreros et al. (2011), *Genetics*, 188(4), 809 – 822
(6) Brenna et al. (2012), *Mol Biol Cell.* 19, 3863-72

Import of peroxisomal matrix proteins in *Ustilago maydis*

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Peroxisomes are ubiquitous organelles that perform important metabolic reactions such as the β-oxidation pathway for degradation of fatty acids. Peroxisomal matrix proteins are translated in the cytoplasm on free ribosomes and are imported in a folded/co-factor bound state or even as oligomers. The majority of peroxisomal proteins is imported via the conserved cytosolic receptor protein Pex5 that recognizes a short C-terminal targeting signal (PTS1) with the consensus sequence S/A-R/K-L/M/I.

The plant pathogenic fungus *Ustilago maydis* encodes two Pex5 receptors (Pex5a, Pex5b), which show a high extent of sequence similarity. We could show that Pex5a is responsible for the import of proteins with unusual PTS1-motifs, e.g. ANL*, while Pex5b prefers target proteins with canonical PTS1-motifs. Thus, Pex5a and Pex5b appear to import different subsets of peroxisomal matrix proteins. This is corroborated by the fact that deletion of *pex5a* abolished growth on oleic acid but has nearly no effect on pathogenic development. In contrast, Pex5b was found to be important for growth on fatty acids, but also for filament formation and virulence. Interestingly, both Pex5 proteins are differentially expressed during the life cycle of *U. maydis*. This suggests that the peroxisomal proteome and metabolism is modulated during different stages of the life cycle.

Role of the small RNAs synthesis machinery on the antagonistic capacity of *Trichoderma atroviride*

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RNA silencing is a process based on the recognition sequences specific for small RNA molecules to regulate gene expression and cellular processes highly conserved in plants, animals and fungi. In the latter our knowledge about this mechanism of regulation is limited. Therefore, in order to improve our understanding of the role of small RNAs in processes such as development and conidiation, we generated mutants in all components of the small RNAs synthesis machinery in the fungus *Trichoderma atroviride*.

Trichoderma atroviride is a soil saprophytic fungus that has numerous applications in biotechnology. One of these applications makes use of its antagonistic power for the biological control of plant pathogens. Trichoderma spp. display different antagonistic strategies, it is recognized as a mycoparasite, but it is also good competitor for space, using antibiotics as a barrier against other microorganisms. The antibiotics produced by Trichoderma are volatile and non-volatile compounds. Most Trichoderma antagonism studies have focused on non-volatiles compounds. Although volatile compounds have been shown to be a source of communication in plants, in fungi little is known about their function. Therefore we wonder if a highly conserved gene regulatory machinery as that mediated by RNAi (RNA interference) modulates antagonism by Trichoderma.

For this aim we evaluated the antagonistic capacity of a battery of mutants affected in the small RNAs synthesis machinery (*ago1, ago2, ago3, rdr1, rdr2, rdr3, dcr1, dcr2, dcr1dcr2*). The antagonistic effect of the mutants and the parental strain *T. atroviride* (IMI206040) as a control was evaluated in direct and indirect confrontations against a wide range of phytopathogenic fungi: *Rhizoctonia solani*, *Phytophtora capsici*, *Alternaria solani*, *Botrytis cinerea*, *Fusarium oxysporum* and *Sclerotium rolfsii*. We have found antagonistic differences in direct confrontation, antibiosis assays and in control by volatiles, in all mutants. To clarify these differences, we decided to study the metabolic profiles of volatile and non-volatile compounds in the mutants, independently and during the *T. atroviride*-pathogen interaction by gas chromatography coupled to mass spectrometry. Briefly, the Ddcr2 and Ddcr1dcr2 mutants produce high levels of important antibiosis compounds including 6 pentyl-α-pyrone, while the Dago1, 2, and 3, as well as the Drdr1, 2, 3 mutants poorly produced these metabolites or did not produce them at all, affecting the inhibitory capacity of *T. atroviride* towards certain pathogens. Additionally, we have evaluated the ability of the mutant strains to communicate with plants in dual cultures, using marker lines carrying a reporter gene under the control of promoters (*pLox2:uidA* and *PPR1:uidA*) that are induced during the plant defense response in *Arabidopsis thaliana*. In these experiments, the Dago1, 2, and 3, as well as the Drdr1, 2, and 3 mutants failed to induce plant defense responses in *A. thaliana*, correlating, apparently, with their deficiency in the production of metabolites, indicating the importance of this regulation process in direct and indirect Trichoderma biocontrol capacity.

Role of the small RNAs synthesis machinery on the antagonistic capacity of *Trichoderma atroviride*

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Molecular basis of FLO11 mediated interactions in *Saccharomyces cerevisiae*

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The cell wall of *Saccharomyces cerevisiae* harbors several proteins that allow the fungal cell to interact with its environment. Adhesins enable cell-cell as well as cell-surface contacts and are involved in many processes like biofilm formation and cell aggregation (flocculation) [1]. All fungal adhesins share a common three-domain structure consisting of a C-terminal GPI-anchor site, a highly glycosylated middle domain and an N-terminal carbohydrate or peptide binding domain [2]. Whereas the highly related FLO1, FLO5 and FLO9 genes recognize specific mannose oligomers on the cell surface via a unique Ca^{2+}-binding motif, floc formation mediated by FLO11 is Ca^{2+}-independent and mannose insensitive [1]. The presence of FLO11 seems to be necessary for diploid pseudohyphal formation, haploid agar invasion and biofilm formation. These morphogenetic events are tightly regulated demonstrated by the large promoter of FLO11, which spans more than 3 kb and contains many upstream activation sequences (UASs) as well as elements for repression [1]. Therefore, FLO11 mediated adhesion differs from classical flocculation and might operate through cell surface hydrophobicity. This view is supported by the observation, that the disruptant mutant for FLO11 shows lower hydrophobicity of the cell surface than the wild type [3]. The N-terminal domain of FLO11 belongs to a single protein family that occurs only in fungi (PFAM: PF10182). The crystal structure of the A domain of FLO11 could be solved in our group. The core domain shows a β-sandwich with a hitherto unknown topology consisting of three antiparallel β-sheets. A short α-helix is located between β-strand 1 and 2 while another one is across from the C-terminal end. Three disulfide bonds stabilize the structure. The A domain seems to be equipped with solvent-exposed aromatic residues that are arranged in two opposite rings. These rings could interact with each other through π-stacking or hydrophobic effects. We could prove a homotypic interaction mechanism by surface plasmon resonance spectroscopy revealing dissociation constants within a low micromolar range. We are now studying whether these interactions are pH-dependent because Saccharomyces cells expressing FLO11 as sole flocculin showed decreased adhesion to plastic surfaces under basic conditions whereas adhesion could be observed under acidic conditions.

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Wednesday, 2.10.2013

Poster session talks: Secondary metabolism

A. Calvo: "Advances in the knowledge of fungal secondary metabolism"

The aflatoxin-like toxin dothistromin: virulence, regulation and evolution
R.E. Bradshaw*, M.S. Kabir, P. Chettri

Two histone deacetylases, FfHda1 and FfHda2, are important for secondary metabolism and virulence in *Fusarium fujikuroi*
L. Studt*, F.J. Schmidt, L. Jahn, C.M.K. Sieber, L.R. Connolly, E.-M. Niehaus, M. Freitag, H.-U. Humpf and B. Tudzynski

The expression of a secondary metabolite cluster is strongly repressed by the asexual development regulator FlbB in *Aspergillus nidulans*
Elixabet Oiartzabal-Arano*, Marc S. Cortese, Eduardo A. Espeso, Unai Ugalde and Oier Etxebeste

The role of histone acetyltransferases in the secondary metabolism of *Aspergillus fumigatus*
Derek Mattern*, Claudia König, Volker Schroeckh, Vito Valiante, and Axel A. Brakhage

Mono- and sesqui- terpene production in the biocidal endophytic fungus, Nodulisporium sp.
Ross C. Mann*, Scott W. Mattner, Simone J. Rochfort, Ian J. Porter and German C. Spangenberg

VI. PLENARY SESSION: CELL SIGNALLING AND MORPHOGENESIS

Alex Idnurm (University of Missouri, USA)

“Light signaling in Phycomyces”

Alfredo Herrera Estrella (Langebio, Irapuato, Mexico)

“An injury-response mechanism conserved across kingdoms determines entry of *Trichoderma atroviride* into development”

Axel Diernfellner (University of Heidelberg, Germany)

“Molecular mechanisms of the *Neurospora crassa* circadian clock”

Conserved veA-dependent *rtfA* and *mtfA* regulate secondary metabolism and morphogenesis in *Aspergillus nidulans*

Vellaisamy Ramamoorthy, Sourabha Shantappa, Sourabh Dhingra and Ana M. Calvo*

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In the model filamentous fungus *Aspergillus nidulans* the global regulatory gene *veA* is required for the biosynthesis of several secondary metabolites, including the aflatoxin-related mycotoxin sterigmatocystin (ST). In order to identify additional veA-dependent genetic elements involved in regulating ST production, we performed a mutagenesis on a deletion *veA* (*ΔveA*) strain to obtain revertant mutants (RM) that regained production of toxin. The mutated genes in selected mutants RM3 and RM7 were identified and named *rtfA* and *mtfA* respectively. Both gene products, RtfA and MtfA, accumulate in nuclei. Deletion of *rtfA* or *mtfA* in a *ΔveA* background restored mycotoxin production. Interestingly, *rtfA* and *mtfA* not only regulate the expression of the genes in the ST cluster, but also control the expression of penicillin genes and terrequinone genes. Furthermore, our studies indicated that *rtfA* and *mtfA* are necessary for normal sexual and asexual development. Deletion of *rtfA* or *mtfA* in wild-type strains (*veA+*) resulted in a reduction in conidiation and loss or reduction of sexual development. We found *rtfA* and *mtfA* putative orthologs extensively conserved in numerous fungal species.

The aflatoxin-like toxin dothistromin: virulence, regulation and evolution

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Dothistromin is a non host-specific mycotoxin found in disease lesions of pine needles infected with the hemibiotrophic Dothideomycete fungus *Dothistroma septosporum*. Dothistromin has remarkable chemical similarity to the aflatoxin precursor versicolorin B and the biosynthetic pathways of these toxins share many common gene products. Genes required for biosynthesis of dothistromin in D. septosporum are unusual amongst secondary metabolite (SM) genes in being dispersed over one chromosome instead of being clustered. In recent efforts to better understand the biology of dothistromin, genetic studies made possible by the availability of the D. septosporum genome sequence have shed light on the role of dothistromin in disease, and on the regulation and evolutionary origins of dothistromin genes. Targeted deletion of dothistromin genes yielded mutants of D. septosporum that lack the ability to make dothistromin but are able to infect pine needles and complete their life cycle. However, disease lesions produced by these mutants are smaller and contain significantly fewer spores than lesions produced by wild-type strains, suggesting a role for dothistromin in virulence. Dothistromin gene expression is regulated by a pathway regulator, AflR, orthologous to the aflatoxin pathway regulator in Aspergillus spp., showing conservation across fungal Classes. Within the Dothideomycetes, the closely related tomato pathogen Cladosporium fulvum also contains dothistromin genes in a fragmented arrangement, but does not make dothistromin due to pseudogenization of key pathway genes. The fragmented dothistromin gene cluster and its inactivation in a biotroph (C. fulvum) enables key questions about SM gene cluster evolution in plant pathogens to be addressed.

Two histone deacetylases, FfHda1 and FfHda2, are important for secondary metabolism and virulence in *Fusarium fujikuroi*

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Histone modifications are crucial for the regulation of secondary metabolism in various filamentous fungi. Here, we studied the importance of histone acetylation on secondary metabolism in the phytopathogenic fungus *Fusarium fujikuroi*, a known producer of several secondary metabolites including phytohormones, pigments and mycotoxins. Deletion of three Zn^{2+}-dependent histone deacetylase (HDAC)-encoding genes, *ffhda1*, *ffhda2* and *ffhda4*, indicated that FfHda1 and FfHda2 regulate secondary metabolism, whereas FfHda4 is involved in developmental processes but dispensable for secondary metabolite production. Single deletions of *ffhda1* and *ffhda2* resulted not only in an increase or decrease, but also in de-repression of metabolite biosynthesis under normally repressing conditions. Moreover, double deletion of both genes, *ffhda1* and *ffhda2*, shows additive but also distinct phenotypes with regard to secondary metabolite biosynthesis. In addition, both FfHda1 and FfHda2 are required for bakanae-disease on the preferred host-plant rice, as *ΔΔffhda1/ffhda2* mutants resemble the uninfected control plant.
A HDAC-activity assay using the wild type and the three single deletion mutants, *Δffhda1, Δffhda2* and Δ*ffhda4*, revealed FfHda1 as the predominant HDAC in *F. fujikuroi*. Subsequently, microarray analysis with a *Δffhda1* mutant that has lost the major HDAC was accomplished in order to study the impact of FfHda1 in the regulation of the 45 putative secondary metabolite gene clusters in *F. fujikuroi*. The obtained results indicate that deletion of *ffhda1* leads to a differential expression of some but not all secondary metabolite gene clusters, that was subsequently verified by a combination of chemical and biological approaches. Our results indicate that HDACs are not only involved in gene silencing but also required for the activation of some genes. Chromatin immunoprecipitation followed by high-troughput sequencing (ChIP-seq) with the wild-type strain and the *Δffhda1* mutant revealed significant and rather puzzling alterations in the acetylation state of secondary metabolite gene clusters in the mutant thereby providing insights into the regulatory mechanism on chromatin level. Altogether, manipulation of HDAC-encoding genes constitutes a powerful tool to control secondary metabolism in filamentous fungi.

The expression of a secondary metabolite cluster is strongly repressed by asexual development regulator FlbB in *Aspergillus nidulans*

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In the model ascomycete *Aspergillus nidulans*, asexual development is induced when vegetative cells are exposed to the air. This stimulus is transduced into signals by early regulators (UDAs), which are expressed in vegetative cells, and activate the expression of the first conidiation-specific regulator *brlA*. The bZIP-type UDA transcription factor (TF) FlbB has been reported to activate the expression of a second UDA TF-coding gene known as *flbD*, and both are jointly required for the induction of *brlA*. Beside this important function, little is known about additional roles of FlbB. To identify new genes under the influence of FlbB, we analyzed the transcriptomes of a *ΔflbB* mutant and its isogenic wild type strain under two conditions: after 19 hours of vegetative growth and 5 hours after the induction of conidiation, the time-point in which a maximum *flbB*-expression peak is detected. The results showed that the expression pattern of four secondary metabolite clusters was significantly altered. We focused on one of those clusters, the DHMBA cluster (AN7893-An7903; Gerke et al., 2012), to observe that its expression remarkably increased when FlbB activity is absent. This was confirmed through RT-PCR by measuring the expression of three genes from the cluster. This de-repression also occurs in *ΔfluG* strain. Our bioinformatic analyses showed that the DHMBA cluster is not conserved in other five Aspergillus species. However, all genes conserved the position and orientation in *Talaromyces stipitatus*, an ascomycete. Thus, the presence of the DHMBA cluster in the *A. nidulans* genome could be a consequence of a horizontal gene transfer process. In an attempt to obtain more information on its role, we generated the knockout mutants of *An7895* (oxidoreductase), *An7896* (TF) and *An7901* (TF) using WT and *ΔflbB* as recipient strains. In standard minimal medium (MMA) with cycloheximide (an inhibitor of protein synthesis), colonies of the double null strains *ΔflbB;ΔAn7895, ΔflbB;ΔAn7896* and *ΔflbB;ΔAn7901* showed a 30% greater diameter than the parental strain. In MMA with NaCl, the double null mutants produced 30 times more conidia than the *ΔflbB* strain. These results suggest that DHMBA activity might be induced in response to different stress situations. The results presented here showed that the loss of FlbB activity renders important variations in the secondary metabolism signature of *A. nidulans*, repressing specific clusters but inducing others that could be necessary to respond to an hypothetic stress situation caused by the inability to induce conidiophore development in the *flbB* null strain.

The role of histone acetyltransferases in the secondary metabolism of *Aspergillus fumigatus*

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The saprophytic filamentous fungus *Aspergillus fumigatus* is able to grow in a wide variety of habitats, with its most common being the soil. Interestingly, *A. fumigatus* is also known to be an opportunistic human pathogen of immunocompromised individuals, where it can colonize the human body. The reason for this fungus to be able to adapt to such different environments could be because of its ability to produce a wide variety of secondary metabolites that are both biologically and pharmaceutically active. In *A. fumigatus* the biosynthetic genes of secondary metabolites are found in specific biosynthetic gene clusters. Under standard laboratory conditions many of these gene clusters are not activated, making it difficult to identify which secondary metabolites are biosynthesized from a particular gene cluster. One approach in identifying silent gene clusters involves the use of epignetics and the manipulation of the chromatin landscape. By creating knockouts in some of the key enzymes involved in histone modifications, this will help in understanding the exact role of these specific enzymes and whether or not they relate to secondary metabolism and/or virulence. The main focus will be on histone acetyltransferases (HATs), which are key enzymes that acetylate histones and are generally thought to be involved in gene activation. Previous studies in *Aspergillus nidulans* have demonstrated that HATs can play a role in secondary metabolism, but nothing has been shown in the pathogenic fungus *A. fumigatus*. There are approximately 46 putative HATs in *A. fumigatus* and currently work is being done on creating a knockout library of these HATs. Thus far, five knockout HAT strains have been successfully completed and characterization phenotypically and secondary metabolite profiles have been analyzed. The phenotypes range from inhibited growth compared to the wildtype (WT) to having comparable growth. In particular, one of the deletion mutants is restored to WT growth when exposed to osmostress conditions. This could indicate its potential role in the high osmolarity glycerol (HOG) pathway. Further work will be completed in order to try and determine the function of these knockout mutants.

In addition, these mutants have been grown under different growth conditions and exposed to different environmental stresses to look for any changes in secondary metabolism. Furthermore, HATs will also be looked at to see if they are also involved in virulence. Assays testing the knockout mutants against macrophages and/or neutrophils will help in making this determination. The continuing work on the HATs in *A. fumigatus* will help in identifying their exact functions and hopefully help in understanding their roles in both secondary metabolism and virulence.

Mono- and sesqui- terpene production in the biocidal endophytic fungus, Nodulisporium sp.

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Endophytic fungi are an emerging tool in agriculture, in part, due to their ability to protect their host plant against biotic stresses. The secondary metabolism of endophytic fungi is central to their unique role within their host, particularly in defence. One group of fungal secondary metabolites that are of interest as defence compounds are the terpenes, and particularly mono- and sesqui- terpenes. Extensive research has been dedicated to defence-related terpenes in plants and the genes that regulate their production, terpene synthases. However, only a limited amount of research has been conducted in fungi (e.g. mycotoxins), and very little dedicated to monoterpene production. Terpene synthases are unique in their function, in that they can synthesise multiple terpene structures from the one enzyme, which potentially offers an endophyte a diverse arsenal against pathogens and pests. To investigate terpene production in endophytic fungi a study was established to investigate the bioactive endophytic fungus Nodulisporium sp., which was found to produce a suite of volatile mono- and sesqui- terpenes, including terpenes common to plant defence (e.g eucalyptol). *In vitro* bioassays established the spectrum of activity of isolates of Nodulisporium sp. against agricultural pathogens (fungi and bacteria), pests (insects) and weeds. Metabolomic studies profiled the production of volatile metabolites from a range of Nodulisporium sp. isolates over time and established key terpenes involved in the bioactivity. The genomes of Nodulisporium sp. isolates were sequenced to identify all terpene synthases and their surrounding gene clusters, which identified an expansive array of terpene synthases, consistent with its unique ability to produce terpene metabolites. Finally comparative genomic studies evaluated genetic diversity across the terpene synthase gene clusters to ascertain genetic mechanisms responsible for terpene production. This research provides new information about the metabolomic and genomic diversity of terpene production in endophytic fungi, whilst providing the basis for future efforts to enhance terpene production that may improve disease tolerance in their plant host.

Light signaling in Phycomyces

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Phycomyces blakesleeanus is a zygomycete fungus that emerged as a model species to understand light-sensing mechanisms in fungi. Mutant strains of Phycomyces unable to respond effectively to blue light to initiate phototropism were isolated in the late 1960s-1970s, and named the mad mutants. Mutations in the *madA* and *madB* strains are in homologs of the *wc-1* and *wc-2* genes characterized from *Neurospora crassa*. The other *mad* genes have not been identified: in part, this has been due to the technical limitation of being unable to transform Phycomyces. To circumvent this challenge, traditional map-based position cloning coupled to genome resequencing now enables the identification of these historical mutants. The *madC* gene was identified by this approach. MadC is a Ras GTPase activating protein, implicating Ras signaling in the light response in this fungus.

An injury-response mechanism conserved across kingdoms determines entry of *Trichoderma atroviride* into development

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Species of the filamentous-ascomycetes genus Trichoderma are among the most commonly isolated saprotrophic fungi. They are frequently found in soil and growing on wood, bark, other fungi and innumerable other substrates, demonstrating their high opportunistic potential and their adaptability to various ecological conditions. The main mechanism for survival and dispersal of Trichoderma is through the production of conidia (asexual spores) that has evolved with several sophisticated molecular mechanisms to respond to environmental cues that trigger conidiation. This developmental process is induced not only by nutrient depletion, but also by light and mechanical injury. In this sense, although light responses in fungi are reasonably well understood, information on their response to injury is extremely limited. The response to injury is of particular interest because we have recently discovered the existence of mechanism in *T. atroviride* conserved in plants and animals.

We have used biochemical and functional genomics approaches to study injury-induced conidiation in Trichoderma. High-throughput RNAseq allowed us to identify genes responsive to this stimulus. Interestingly, functional classification of injury responsive genes suggested the involvement of reactive oxygen species, increases in intracellular calcium and the activation of calcium signaling pathways; as well as, the participation of lipids and activation of the cell cycle. Indeed, this mechanism involves the production of reactive oxygen species (ROS) by the NADPH oxidase complex, since Nox1 and NoxR mutants are affected in conidiation in response to damage. Based on these data, we have proposed a model for the signaling cascades that participate in this process involving MAPKs. Accordingly, mutants in two of the three MAPK genes present in the *T. atroviride* genome present major defects in conidiation induced by injury.

Given the observed transcriptional response to injury and the existence of the highly conserved mechanism of regulation of gene expression based on RNAi in Trichoderma, we decided to analyze the role of the components of the RNAi machinery in this process. Analysis of gene replacement mutants in all components of the machinery revealed that Dcr2 and Rdr3 play a major role in conidiation induced by injury. Transcriptome analyses of the Ddcr2 mutant in response to injury revealed major differences in transcription of genes involved in activation of the cell cycle, as well as in mRNA levels of genes encoding ROS scavenging proteins.

Molecular mechanisms of the *Neurospora crassa* circadian clock

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FREQUENCY (FRQ) is the central oscillator of the *Neurospora crassa* circadian clock. Together with the RNA helicase FRH and casein kinase 1a (CK1a) it forms the FFC complex, which regulates the activity of the circadian transcription factor WHITE COLLAR COMPLEX (WCC).

FRQ is sequentially phosphorylated in the course of a day at up to 113 sites and eventually degraded. FRQ bound CK1a is essential for the progressive phosphorylation as it triggers a conformational change in FRQ and commits the protein for degradation via a PEST signal. The kinetics of CK1a dependent hyperphosphorylation of FRQ is crucial for timekeeping on a molecular level. Here we show that FRH plays an important role in the regulation of CK1a mediated FRQ phosphorylation.

VII. PLENARY SESSION: SECONDARY METABOLISM

Joseph Strauss (University of Vienna, Austria)

“Heterochromatin influences fungal secondary metabolism and pathogenicity”

Marc Stadler (HZI, Braunschweig)

“Correlations between biodiversity and secondary metabolism in the Xylariaceae”

Sandra Bloemendal (University of Bochum, Germany)

“Strain development in fungal biotechnology: Regulation of beta-lactam antibiotic biosynthesis”

Claudio Scazzocchio (Université Paris Sud, France and Imperial College, London, UK)
"Concluding remarks: Fungal Biology in the post-genomic era"

Heterochromatin influences fungal secondary metabolism and pathogenicity

Joseph Strauss

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Gene expression in any organism is locus-dependent mainly because the chromatin structure at this locus defines accessibility of transcription factors and adaptors. Densely packed heterochromatin is transcriptionally silent but, eventually, can be converted to transcriptionally active euchromatin by removal of heterochromatic and establishment of euchromatic marks on the N-terminal tails of the histone octamer. Fungal secondary metabolite (SM) genes are organized in continuous biosynthesis gene clusters and the hetero- to euchromatin switch is known in several fungal species to participate in coordinated regulation of these large regions. When environmental conditions or a specific developmental stage prevent SM production secondary metabolite biosynthesis genes are silenced by heterochromatic marks. Conversely, when environmental or developmental conditions favor SM production, repressive histone modifications are replaced by activating marks. During the transition phase from primary to secondary metabolism – for example when fungi run out of nutrients or when they are challenged by bacterial competitors - histone de-methylases are required to remove repressive marks and the function of histone de-acetylases is turned down. Subsequently, histone acetyltransferases are recruited, usually by pathway-specific transcription factors, and this combined action establishes an open, transcriptionally active euchromatic structure inside the SM cluster, but not outside of it. Genetic and molecular data suggest that LaeA/Lae1, a conserved general activator of fungal SM, has a chromatin-related function. In the *Aspergillus* sterigmatocystin cluster, both, de-methylases and LaeA, are required to replace the repressing methylation marks on H3 by activating marks.

Interestingly, the hetero- to euchromatic switch also seems to play a role in the regulation of fungal pathogenesis. Mycotoxins are pathogenicity factors of Fusarium graminearum, a potentially severe pathogen of wheat and maize and heterochromatin mutants of this pathogen show an altered mycotoxin profile and a hyper-virulent phenotype. RNAseq data of the fungal-host interaction reveal that, in addition to SM genes, a number of pathogenicity-related genes are also subject to regulation by facultative heterochromatin.

Correlations between biodiversity and secondary metabolism in the Xylariaceae

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Fungi continue to be a rich source of unique secondary metabolites (SM) with interesting biological activities. Based on several thousands of specimens and several hundreds of cultures, morphological studies of the sexual and asexual states of these fungi were combined with chemotaxonomic studies based on HPLC-DAD/MS profiling, as well as PCR fingerprinting and molecular phylogenetic analyses [1]. Numerous novel pigments and other natural products, many of which were shown to have biological activities were identified, and their production during the life cycle of their producer organisms was followed by HPLC profiling and biological assays. Numerous new genera and species were recognised in the course of this work, and the first comprehensive world monograph of the interesting genus Daldinia is now in press [2]. Moreover, secondary metabolite production in cultures of Xylariaceae was correlated with molecular phylogenies, demonstrating the phylogenetic significance of SM profiles. Recently, it was possible to elucidate the life cycle of certain drug producing endophytes [3] and explore highly interesting correlations between endophytes, their host plants and insect vectors [4]. The Xylariaceae seem to represent an ideal "model family" for interdisciplinary work on basic as well as applied aspects of mycology.

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Strain development in fungal biotechnology: Regulation of beta-lactam antibiotic biosynthesis

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The filamentous fungus *Penicillium chrysogenum* is the main industrial producer of the pharmaceutically relevant beta-lactam antibiotic penicillin. The three penicillin biosynthesis genes are found in a single cluster and the expression is controlled by a complex network of global regulators. It is supposed that subunits of the velvet complex, which were recently detected for *P. chrysogenum*, function as global regulators, although the exact regulatory mechanisms still have to be elucidated. Core components of the velvet complex are PcVelA and PcLaeA, which regulate secondary metabolite production, hyphal morphology, conidiation, and pellet formation [1]. As novel subunits, we recently identified PcVelB, PcVelC, and PcVosA [2]. Using yeast two-hybrid analysis and bimolecular fluorescence complementation (BiFC), we demonstrated that all velvet proteins are part of an interaction network. Functional analyses using single and double knockout strains generated by the FLP/FRT recombination system clearly indicate that velvet subunits have opposing roles in the regulation of penicillin biosynthesis and light-dependent conidiation [2, 3].

Most strikingly, a direct interaction of PcVelB with an enzyme of the penicillin biosynthesis pathway, the isopenicillin N synthase (IPNS) was identified during yeast two-hybrid analysis with PcVelB as bait. This unexpexted interaction was confirmed in vivo by using a combined tandem affinity purification/mass spectrometry approach. Fluorescence microscopy of both proteins revealed a nuclear-cytoplasmic localization for PcVelB, whereas the IPNS localizes solely in the cytoplasm. Our discovery of a direct interaction of the isopenicillin N synthase with a subunit of the velvet complex implies a novel regulatory mechanism how enzymes of penicillin biosynthesis are regulated at the molecular level.

The results provided here contribute to our fundamental understanding of the function of velvet subunits as part of a regulatory network mediating signals responsible for morphology and secondary metabolism, and will be instrumental in generating mutants with newly derived properties that are relevant for strain improvement programs.

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Abstracts: Pathogenic Fungi and Fungal Interactions

Anticancer activity of Altersolanol A from endophytic fungus *Phomopsis longicolla* from *Nyctanthes arbortristis*

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Cancer can be described as a disease involving dynamic changes in the genome. With an increasing understanding of the molecular mechanisms that are involved in the development of cancer, newer potential targets to fight cancer are being developed. Natural products are a good source of potential anticancer compounds. In this project, the anticancer potential of fungal extracts was studied. It involved the use of cytotoxicity screening to determine potential cytotoxic compounds on tumour cell lines, which were non-toxic to normal cell lines. A fungal endophyte, PM0409092 isolated from *Nyctanthes arbor-tristis* showed promising anticancer activity in a panel of 10 cell lines by using High Throughput Screening. This finding led us to do the chemical investigation of endophytic culture PM0409092 for bio-assay directed isolation, through this process we identified lead compound which was responsible for anticancer activity. PM0409092 was identified as *Phomopsis longicolla*, according to a molecular biological protocol by DNA amplification and sequencing of the ITS region. The chemical investigation of this lead molecule yielded Altersolanol A, an anthraquinone derivative as a major secondary metabolite. The structure was determined on the basis of physical and chemical properties including 2D NMR spectroscopy and mass spectroscopy. Altersolanol A exhibited the mean IC50 value of 0.005mg/ml, in 34 cell lines. While the same compound showed significantly lesser toxicity in normal cell line. These findings dictate the efficacy and selectivity of pure compound towards highly proliferating cancer cells. In order to find out different molecular events involved in cancer cell apoptosis, these compounds were profiled in High Content Screening for different molecular targets. The results from molecular profiling revealed that, Altersolanol A showed a concentration dependant decrease in the phosphorylation of Akt, 4E-BP1 and p70S6 kinase. It had also inhibited the STAT3 transcriptional activity in HeLa- STAT3-Luc cell line with an IC50 of 1.8 mM. We also noticed that the compound induces the expression of *p21*, *p53* and suppresses Bcl-2 levels. Subsequently we observed prominent induction of BAX, cytochrome C and cleaved caspase 3 levels when compared to the control cells.

There are some reports of anticancer activity of altersolanols. However, this is the first report of exhaustive studies for anticancer indication. This would help the scientific community to take this compound for further studies and development.

The impact of competing insects on the fungal 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. mycotoxin) 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.

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. This led to the identification of secondary metabolite genes up- or down-regulated under these conditions. Quantitative RT-PCR was employed to analyze secondary metabolite gene expression at different time points. Fungal single, double and triple mutations of identified up-regulated genes are currently analyzed in confrontation assays to identify potential modifications in gene expression and the survival rate of larvae concerning to chemical defense reaction of fungus-insect interaction compared to wild type. This could reveal insights about the biological function of secondary metabolite genes and clusters such as stc and mdp.

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From VIGS to HIGS: Host-Induced Gene Silencing in the Asian Soybean Rust *Phakopsora pachyrhizi*

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Host- induced gene silencing (HIGS) has become a powerful tool to manipulate the gene expression of fungal pathogens during intimate host-pathogen interaction. To generate HIGS in different fungal pathosystems several techniques have proven suitable. These methods include virus-induced gene silencing (VIGS) and in planta-induced gene silencing (PITGS).

Both techniques are based on transient expression of nucleic acid sequences complementary to specific target genes in the fungus within host tissue. VIGS uses recombinant viruses, whereas PITGS exploits *Agrobacterium tumefaciens*-mediated transformation.

To date, the feasibility of HIGS has been demonstrated for different obligate biotrophic fungal pathogens, such as *Blumeria graminis* and *Puccinia striiformis* f. sp. *tritici* but not for legumes and corresponding pathogens like the Asian Soybean Rust (ASR) *Phakopsora pachyrhizi*.

With the aim of achieving methods and protocols for the establishment of host-induced gene silencing in *P. pachyrhizi*, we are working on the identification of suitable target genes, the generation and the transfer of silencing constructs and finally the assessment of induced silencing effects at the phenotypical and the transcriptional level.

Secretion of a xylem sap induced peptidase is required for full plant virulence of Verticillium

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Diseases are caused by Verticillium in a wide range of crops representing a particular threat. This soilborne vascular pathogen leads to premature plant senescence and contaminates agricultural soil with microsclerotia, long-time surviving resting structures. Both yield reduction and microsclerocial contamination of soil turn Verticillium infections into a severe economic problem. In this study we aimed to identify Verticillium proteins that are specifically expressed in xylem sap. In extracted xylem sap of oilseed rape cultivated fungal exoproteomes were compared to growth on pectin-rich media to identify xylem sap specific proteins. Xylem sap cultivation revealed a characteristic and reproducible extracellular proteome signature divergent from growth on pectin-rich media. We identified over 400 secreted proteins of which 100 proteins were similar expressed under both growth conditions. 180 proteins were down regulated and 37 were upregulated in xylem sap. The xylem sap specific exoproteome can be divided in the categories of putative adhesins with predicted carbohydrate-binding domains and carbohydrate-active enzymes like polysaccharide lyases and glycosyl hydrolases. These enzymes are probably needed for the degradation of structurally complex molecules of the plant and might play a role during the penetration of roots and cell walls and distribution within the host. In addition various members of peptidase families were enriched, which might be important for proteolysis of host substrates or host defence proteins. Furthermore several small cysteine-rich proteins, necrosis and ethylene-inducing-like proteins (NLP) and proteins of unknown function were identified, which resemble potential effectors in pathogenicity. The most highly upregulated and unique protein for xylem sap was a secreted peptidase. To verify the xylem sap approach which should reveal pathogen-associated genes the secreted peptidase was analysed for its importance during plant infection. Plant infection assays with Verticillium dahlia in Arabidopsis thaliana and Solanum lycopersicum reveal the importance of the secreted peptidase for full virulence of Verticillium. Further we discovered significant differences in impact of the secreted peptidase on the two host plants. These differences will be analysed in more detail in follow-up experiments to uncover the exact function and substrates on molecular level of this xylem sap induced peptidase.

Biological control of the oilseed rape pathogen *Verticillium longisproum* by the rhizobacterium *Pseudomonas fluorescens*

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Verticillium longisporum is a filamentous soil-borne fungus that possesses phytopathogenic properties and is responsible for increasing economical losses especially in the cultivation of oilseed rape (*Brassica napus*). Antagonistic bacteria like *Pseudomonas fluorescens* can be used as biological control agents to reduce the infection intensity of pathogenic fungi in the absence of appropriate fungicides. We study the counteractive potential of *P. fluorescens* on the development of *V. longisporum* to understand mechanisms of interaction between these organisms on a molecular level. A cultivation system of V. longisproum together with *P. fluorescens* in *B. napus* was established. *P. fluorescens* has an impact on *V. longisporum* germination and growth with a potential for biocontrol. Typical *V. longisporum* disease symptoms as formation of necrosis and secondary branching are absent in the presence of the bacterium. Plant heights or biomasses of biocontroled infected *Brassica napus* show intermediate phenotypes when compared to uninfected or infected plants without biocontrol with a significantly increased plant vitality in the presence of the bacterium. The production of a variety of secondary metabolites of *P. fluorescens* are controlled by the GacS/GacA two-component system. Bacterial deletion mutant strains affecting the general regulator gene *gacA* are unable to inhibit germination of the fungus. Consistently, the *gacA* gene is required to protect plants against the fungus. Several secondary metabolites are presumably required for fungal biocontrol, because knockout *P. fluorescens* mutant strains deficient in the production of single secondary metabolites are still able to inhibit the germination of fungal spores.

First insights into the genome of the oilseed rape pathogen *Verticillium longisporum*

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Verticillium longisporum infects predominantly Brassicaceae such as *Brassica napus* (oilseed rape, Canola), *B. oleracea* (broccoli, cabbage, cauliflower, etc.), *Armoracia rusticana* (horseradish) and the model plant *Arabidopsis thaliana*. *B. napus* plays a major role as vegetable oil producing plant in Germany but also in Northern Europe, Canada and China. *V. longisporum* is an allodiploid hybrid with long spores and almost double the amount of nuclear DNA compared to other Verticillium species. Two genomes of a virulent and an avirulent *V. longisporum* isolate from Mecklenburg (Germany) were sequenced by a combined approach via pyro-sequencing (454-Roche) and paired-end library sequencing of 0.5, 3, 8, and 20kb (454-Roche and Illumina). Gene prediction was performed by AUGUSTUS and additional hints were integrated such as predicted proteins derived of the sequences of the putative parents, peptide information derived from proteome analysis, ESTs and RNA-Seq data. The resulting gene sets were visualized in GBrowse, imported into Pathway Tools and integrated into a VertiBase web interface for the BioFung consortium. Although both genomes show high sequence identities, we have evidences that both strains could originate from separate hybridization events. Phylogenetic analysis revealed significant differences between the parental strains of the two hybrids. Both *V. longisporum* isolates contain two alleles of the MAT-1-1-1 gene and the MAT1-1-3 gene. BLAST searches failed to detect any homologs of MAT1-2-1 sequences. In depth analysis of the mating-type genes favored a hybridization event in contrast to a possible mating. Currently, we analyze which genes have two or more alleles to identify possible gene duplication events during or after the hybridization.

Identifying rust fungal effectors by finding plant interaction partners

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In order to survive, pathogens have to somehow manipulate their hosts. Special proteins known as effectors play a major role in this process. Little is known about these kinds of proteins although perception of their importance steadily increases. Clarifying the function and role of such effectors is vital to understand plant-pathogen interactions. Via sequencing haustorial trancriptomes of *Phakopsora pachyrhizi, Uromyces appendiculatus*, and *U. fabae* a large quantity of effector candidates for all three fungi has been identified.

Our goal is to identify true effectors from these candidates and to elucidate their function in plant-pathogen interactions.

Candidate effectors were chosen from families of secreted proteins, with different characteristics like being restricted to the family Pucciniaceae, the genus Uromyces or maybe even to a specific species. Others show indications of being present in all rust fungi. One family, forming an exception to the rule, can be found in distantly related pathogens like *Phytophtora infestans*, but not in other basidiomycetes - except rust fungi, which might indicate horizontal gene transfer.

Other candidates are up regulated in specific infection structures like haustoria, indicating an importance in pathogenesis. To get a broader view at some families, we are investigating homologs from more than one species.

We work under the assumption that effector proteins have to interact with specific host target proteins in order to manipulate the host plant. Therefore, the existence of a plant interaction partner is a good criterion to identify effectors. Possible interaction partners of effector proteins might be either their targets or resistance proteins.

To identify interaction partners we decided to use a yeast-two-hybrid system. The yeast-two-hybrid kit we chose is the MatchmakerTM Gold system from Clontech. In order to ensure that the effector is not exported from the yeast cell, the candidates are cloned into the respective vectors without their signal sequence. cDNA libraries of the host plants *V. faba* and *P. vulgaris* were constructed from healthy and infected leaves so that proteins that are only expressed during infection, like pathogenesis related proteins, are also represented.

By identifying effector proteins - especially the ones responsible for enabling a biotrophic lifestyle - we should be able to draw conclusions about the function of these highly interesting effector families and move a huge step forward understanding plant-pathogen interactions.

Currently our main focus lies on the effector PpEC23, which has been identified as a cell death suppressor by Qi Mingsheng, a member of our cooperating group of Iowa State University, USA. Since cell death suppression could be a key aspect in biotrophic parasitism, PpEC23 is highly interesting and we are testing interactions between this effector’s two subunits and its homologs to better understand their function.

The regulation of the alternative oxidase in *Ustilago maydis*

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The *Ustilago maydis* has an economic impact on the agriculture sector, because it is a pathogen that infects corn. Other ustilaginales are also important because they infect several important plants like rice, sugar cane or sorghum. Due to these characteristics, *U. maydis* is considered as a model for various types of research, among which the host – parasite interactions, dimorphism, and gene regulation are important. The pathogenic activity in these fungi depends on the fusion of two compatible haploid strains, which generate a dikaryotic hypha that invades the host plant. The yeast form (haploid) is unable to cause disease and can be propagated on artificial media. *U. maydis* is used to study various biological processes present in animal cells, because there are several similarities in both cell types. It was shown that this organism is more related to humans than *S. cerevisiae*.

A phenotype shared between *U. maydis* and other organisms i.e. plants, is the presence of an alternative oxidase (AOX); the AOX is a monotopic mitochondrial protein located in the mitochondrial inner membrane that allows a cyanide resistant respiration. The AOX is important in energetic metabolism. The protein has two hydrophobic regions and two or three iron binding motives, which participate in electron transfer from ubiquinol to the eventual reduction of oxygen to water. AOX is a protein that does not generate a proton motive force and therefore does not contribute to energy conservation.

Our group found that depending on the growth phase and the culture conditions used to grow *U. maydis* (YPD; minimal medium-glucose, mm-lactate, mm-ethanol; mm-glycerol), there are changes in the activity of the AOX in this yeast; however, it is not known whether this behavior is due to alterations in the expression of the AOX or the allosteric regulation of its activity.

CorA magnesium transporters are key regulators of growth and pathogenicity in the rice blast fungus *Magnaporthe oryzae*

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Magnaporthe oryzae, the causative organism of rice blast disease, infects cereal crops and grasses at various stages of plant development. Thus, a comprehensive understanding of the fungal metabolism and its implications on pathogenesis is a must to countering this devastating crop disease. As a co-factor for functionally diverse enzymes, Mg^{2+} regulates a variety of biological processes. Magnesium transporters have been shown to be essential for viability of *S. cerevisiae* and some bacterial pathogens. We present the molecular identity, function and regulation of magnesium (CorA) transporters and their role in growth and pathogenicity of the filamentous fungal pathogen, *M. oryzae*. Complementation analysis in *S. cerevisiae* showed that magnesium transporters, MoALR2 and MoMNR2, have overlapping functions. Further, we have shown that reduction of Mg^{2+} transporters using a knock-down approach leads to decrease in intracellular magnesium levels, indicating that these transporters play a crucial role in Mg^{2+} homeostasis of *M. oryzae*. Functionally, the mutants display defects in surface hydrophobicity, cell wall stress tolerance, sporulation, appressorium (infective structure) formation and infection, making MoALR2 and MoMNR2 indispensible for growth and pathogenesis of the rice blast fungus *M. oryzae*. MoMPS1 (MAPK) and MoMPK1 (MAPK) driven signaling pathways have been shown to be essential for cell wall integrity, conidiogenesis, appressorium formation and invasive growth. Expression analysis by qPCR of MoPMK1 (MAPK) and MoMPS1 (MAPK) signalling pathways, core to the above mentioned phenotypes, showed deviation from the wild type. We also detected corresponding differences in downstream MoMPS1 driven Cell Wall Integrity (CWI) signalling and the MoPMK1-regulated hydrophobin, MoMPG1, expression.

Transcriptome of *Trichoderma virens* in interaction with maize or tomato roots: evidence for plant host specificity

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Species belonging to the fungal genus Trichoderma directly antagonize soil-borne fungal pathogens. They also colonize plant roots, promoting systemic resistance. The Trichoderma-root interaction can be hosted by a wide range of plant species, including both monocots and dicots. To test the hypothesis that gene expression by the fungal partner in this beneficial interaction is modulated by the plant host, we cocultured *Trichoderma virens* with maize or tomato in a hydroponic system allowing interaction with the roots. The transcriptomes for *T. virens* alone and with tomato or maize roots were compared by hybridization on Agilent custom oligonucleotide microarrays of 11645 unique probes designed from the predicted protein-coding gene models. At the chosen cutoff for statistical significance, the transcript levels of many genes were modulated by interaction with the roots. Glycoside hydrolases and transporters are highly represented in the sets of transcripts induced by coculture with both maize and tomato. Although some transcripts are shared between the maize and tomato sets, the majority of regulated genes are specific to either the maize or tomato host. The tomato and maize – induced transcriptomes thus differ from each other and from the saprophytic growth transcriptome. Reporter genes chosen from the microarray data will be useful to follow, in time and space, how the fungal cell detects plant signals, and will provide an entry point to the signaling pathways. Secreted proteins released in response to plant signals might have a role in inducing systemic resistance in the plant.

Functional analysis of the Mps1 MAP kinase pathway in the rice blast fungus *Magnaporthe oryzae*

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Signaling pathways are essential in coordinating fungal cellular processes required for stress resistance, development and pathogenicity. The Mps1 MAP kinase pathway of the plant pathogen *Magnaporthe oryzae* is involved in cell wall integrity, sporulation and pathogenicity. *Δmps1* mutants displayed abnormal mycelial growth (reduced aerial hyphae and melanisation), did not sporulate and were non-pathogenic on plants as reported (1). Sensitivity of M. oryzae to cell wall inhibitors (CWI) and cell wall degrading enzymes (CWDE) was found to be dependent on pH. Indeed, *M. oryzae* cell walls display a resistance to enzymatic degradation at pH 5, while they are sensitive at pH 6. *Δmps1* loses this pH 5 induced cell wall resistance, while it is as sensitive to CWDE as the wild type is at pH 6. *M. oryzae* is highly resistant to calcofluor (cell wall disorganizing agent) at pH 5 (10x) compared to pH 6, but *Δmps1* loses this pH 5 induced calcofluor resistance, while it is as sensitive as the wild type is at pH 6. *M. oryzae* is more sensitive (20x) to Nikkomycin Z (chitine synthase inhibitor) at pH 5 than pH 6, while sensitivity to Aculeacin (glucan synthase inhibitor) is independent of the pH. However, *Δmps1* is as sensitive as the wild type to these inhibitors at both pH. We conclude that the pH 5 induced resistance of fungal cell walls to CWDE and calcofluor requires the Mps1 pathway. This also suggests that the Mps1 pathway is strongly activated at pH 5 compared to pH 6. To test this hypothesis, we are assaying the phosphorylation status of Mps1 at different pH as well as under several stress conditions and developmental stages to determine when this pathway is activated. Additionally we constructed an activated allele of Mkk1, the MAPKK upstream of Mps1, placed under the control of either its own promoter (2) or the repressible pNIA1 promoter. These transformants will be used to assess the effect of controlled activation of the Mps1 pathway on M. oryzae cellular functions. The different conditions of Mps1 pathway activation will be used for a comparative transcriptomic analysis of wild type and *Δmps1* mutants.

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Silencing of trehalose-6-phosphate synthase 1 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 achieved 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 significantly in their growth behaviour and metabolite contents. As trehalose is furthermore well known as stress protectant, temperature stress behaviour was also studied in the transformed strains. These results together with metabolite content will be presented.

Spreading of the Esca-fungus *Phaeomoniella chlamydospora* in vineyards

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Phaeomoniella chlamydospora (Pch) is a fungus of the grapevine trunk disease, Esca. Spreading from the Mediterranean areas Esca has become one of the most dangerous diseases in vineyards worldwide resulting in high yield losses every year. Pch and *Phaeoacremonium aleophilum* may infect the vines at an early stage causing a young form of Esca, the "Petri disease". In an older stage plants are infected by Fomitipora mediterranea, which leads to the "Esca proper" symptoms. Any wound in the wooden part of the plants may be infected by the pathogens.

As there are no effective fungicides available for the above fungi the exact way and seasonal regime of infection and spreading have to be understood. The focus of interest in this research is on Pch as this fungus causes the early form of Esca and appears most frequently in german vineyards. To investigate the ways of infection spore traps are set out in the vineyards to find different strains of Pch and the spreading of spores over the year. At the same time bark and soil samples are analyzed for spores.

After the isolation from their source, the fungi are analyzed via PCR using Intersimple sequence repeat (ISSR)-primers. These kinds of primers provide a characteristic pattern of DNA with respect to the genetic variety of the fungi. The patterns are searched for polymorphisms to distinguish the single strains of Pch. Preliminary PCR-analyses showed promising results for finding characteristic polymorphisms in strains from different geographical origins in Germany and elsewhere in Europe.

In a further step the isolated strains from the vineyard will be compared to strains already found in wood samples to give information whether the infections are spread via spores over the vineyards or if the infections have spread during the cultivation steps in the vineyard e.g. infecting different plants during winter pruning. In descriptive experiments ISSR-primer analyses have become the appropriate method for analyzing the reisolated fungi as the polymorphic patterns are sensitive enough to show the occurrence of different strains in neighboring vines.

For this reason also parts of single vines are analyzed as the plants are usually grafted. Therefore scion and rootstock can inhabit different strains. The analysis of strains in a single plant shall give an overview about the spreading behavior of Pch as the grafted area may act as a natural border for the growth of fungi.

Bionectria hyperparasite as control agent of choke disease of grasses caused by Epichloë sp. Fungi

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Choke disease, caused by fungi of genus Epichloë (Clavicipitaceae, Ascomycota), is a plant disease that has long been known to spread in populations of grasses, including cultivated species and species that are used in grassland- and pasture ecosystem-based agriculture. Fungal stromata enclose grass shoots, preventing their further development so the flowering and seed production of infected plants are restricted. Additionally, the mycotoxins produced by fungus cause numerous adverse symptoms in farm animals that feed on the infected grasses.

Some plant pathogenic fungi are successfully eradicated by other fungi that parasitise on them. A classic example is the application of Trichoderma fungi as a vaccine to eradicate pathogenic fungi such as Rhizoctonia spp., Pythium spp., *Botrytis cinerea* and Fusarium spp., which cause both soil-borne and leaf- or flower-borne diseases in agricultural plants

We report the occurrence of a fungal hyperparasites of *Epichloë typhina* stromata developing in populations of the grass *Puccinellia distans*. Green colonies of the hyperparasite were identified as *Bionectria epichloë* based on microscopic characters. DNA sequences support the positioning of the fungus within the Clonostachys/Bionectria group. Microscopic observations demonstrated a lack of asci with ascospores in the *Epichloë perithecia* and damage to the stroma mycelium in sites colonised by *B. epichloë*. This suggests the possibility of using Bionectria mycoparasite to control choke disease caused by *E. typhina* in grass populations.

Are metabolite fingerprints of grapevine leaves related to *Plasmopara viticola* resistance traits?

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The obligate biotrophic oomycete *Plasmopara viticola* infects grapevines. The European grapevine species *Vitis vinifera* is highly susceptible to this pathogen. In contrast native American species, e.g. *Vitis labrusca* or *V. riparia*, are resistant to *P. viticola*, which was native to Northern America and was unintendedly imported to Europe in the 19th century. *P. viticola* infects leaves, flowers, and small berries through the stomata. It afflicts grapevine over the whole growing phase, but infection at the time of flowering is most damaging for European winegrowers, since then it has the highest influence on yields. Once infected, the grapes can not be harvested because of low quality.
Though the infection mechanism is still not fully understood, one undisputed hypothesis is that secondary metabolites act as defence components, resistance activators and signalling compounds. To answer the question, if metabolites of grapevine leaves relate to *P. viticola* susceptibility, ten grapevine genotypes with different resistance levels to *P. viticola* were selected. Leaves were analysed by GC-MS and LC-MS regarding their patterns of volatile and non-volatile organic compounds, respectively. Non-targeted chemometrical data processing was used to screen as many metabolites as possible. The tested genotypes were the *V. vinifera* cultivars Müller-Thurgau, Riesling, Phoenix, Regent, as well as interspecific crossings cv Delaware, Othello, Concord, Isabella and two accessions of the species *V. labrusca* and *V. riparia*. They are noted in ascending order of resistance. The metabolite fingerprints were taken from grapevine leaves at the plant developmental stage of flowering (BBCH 6). Leaves were sampled in two subsequent years from the vineyard of JKI Siebeldingen and a nearby vineyard for Müller-Thurgau leaves. Metabolite fingerprints of both volatile organic compounds (VOCs) and non-volatile organic compounds (non-VOCs) were correlated to the resistant traits of grapevines. Most published analyses of grapevine metabolites only deal with either VOCs or non-VOCs, neglecting that VOCs and non-VOCs may act synergistically.
Principal component analyses (PCA) of the fingerprints aranges all *V. vinifera* cultivars close to each other. The interspecific genotypes and the *V. labrusca* samples are positioned in close proximity, and the *V. riparia* probes segregate from the above. These observations apply to the fingerprints of VOCs and of non-VOCs. PCA generates groups, which divide the grapevine genotypes after their biological filiation. In case of the non-VOC fingerprints the clustering is more prominent. The *V. vinifera* species are clearly separated from the *V. labrusca* and the crossings as well as from the *V. riparia* accessions. As stated above, the *V. vinifera* cultivars are more susceptible to *P. viticola* than the other genotypes. Therefore, the title question has to be answered with "Yes, metabolite fingerprints of the leaves are related to *P. viticola* resistance traits."

Uromycladium tepperianum, the gall rust fungus from *Falcataria moluccana* in Malaysia and Indonesia

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Batai (*Falcataria moluccana*) is a valuable tree species for forest plantations in Malaysia and Indonesia. Since 1993, a gall rust disease has caused severe damage to all growth stages, from seedlings in the nursery to mature trees in the field. The objectives of this study were to identify the fungus causing gall rust disease on *F. moluccana* in Malaysia and Indonesia, and to ascertain the mode of infection and changes in the anatomy of infected cells. The results of this study confirmed that the disease in Brumas Estate, Tawau, Malaysia and in East and Central Java, Indonesia, is caused by *Uromycladium tepperianum*. The fungus produces three longitudinally ridged teliospores on each head, with spores measuring 13-20 µm wide and 17-28 µm long. The fungus completes its entire life cycle on *F. moluccana*. This study confirmed that the teliospores themselves cannot infect the host; under favourable conditions, about 10 hours after inoculation they germinate to produce basidiospores which form penetration pegs about 6 hours later and it is the peg that penetrates the host cells directly through the epidermis. Pycnia, recognized as small brown pustules which break through the epidermis are formed seven days after inoculation.

Host-dependent colonization strategies of the root endophyte *Piriformospora indica*

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Their host range defines plant-associated fungi as either specialists, which are adapted to one or few distinct hosts, or generalists who are able to thrive in highly variable host environments. Specialists and their hosts are in an evolutionary arms race that leads to the development of weapons perfectly tailored to the respective host. Conversely, broad-host range species must evolve adaptations to cope with a plethora of different host-associated signals and host-specific defense mechanisms. The evolutionary force, in this case, drives the expansion and diversification of the fungal arsenal and the host-adapted gene expression to better suite different plants. The mechanisms underpinning broad compatibility in root symbiosis are largely unexplored. The generalist root endophyte *P. indica* that stimulates growth, alleviates salt stress and induces systemic resistance to pathogens in different hosts can establish a long lasting interaction with the roots of barley and Arabidopsis, two morphologically and biochemically very distinct plants. We show here that in these two hosts, root colonization proceeds very differently. While in Arabidopsis the fungus establishes and maintains biotrophic nutrition within living epidermal cells, in barley the symbiont undergoes a nutritional switch to saprotrophy that is associated with the production of secondary thinner hyphae (SH) in dead cortex cells. Consistent with a diversified trophic behavior, genome-wide expression profiling revealed a strong induction of genes encoding cell wall degrading enzymes and nutrient transporters in barley but not in Arabidopsis at a late colonization stage. In particular small secreted proteins (SSPs < 300 amino acids) known as effectors have been shown to facilitate infection by manipulating host defense and reprogramming plant metabolism during symbiosis. Many of these proteins are cysteine rich or possess distinctive features such as a regular pattern of histidine and alanine residues found in the 29 members of the *P. indica*-specific DELD family. Our study reveals that broad compatibility in root endophytes requires strong phenotypic plasticity and the expression of alternative lifestyle strategies in a host-dependent way.

Functional analysis of the Mps1 MAP kinase pathway in the rice blast fungus *Magnaporthe oryzae*

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Signaling pathways are essential in coordinating fungal cellular processes required for stress resistance, development and pathogenicity. The Mps1 MAP kinase pathway of the plant pathogen *Magnaporthe oryzae* is involved in cell wall integrity, sporulation and pathogenicity. *Δmps1* mutants displayed abnormal mycelial growth (reduced aerial hyphae and melanisation), did not sporulate and were non-pathogenic on plants as reported (1). Sensitivity of M. oryzae to cell wall inhibitors (CWI) and cell wall degrading enzymes (CWDE) was found to be dependent on pH. Indeed, *M. oryzae* cell walls display a resistance to enzymatic degradation at pH 5, while they are sensitive at pH 6. *Δmps1* loses this pH 5 induced cell wall resistance, while it is as sensitive to CWDE as the wild type is at pH 6. *M. oryzae* is highly resistant to calcofluor (cell wall disorganizing agent) at pH 5 (10x) compared to pH 6, but *Δmps1* loses this pH 5 induced calcofluor resistance, while it is as sensitive as the wild type is at pH 6. *M. oryzae* is more sensitive (20x) to Nikkomycin Z (chitine synthase inhibitor) at pH 5 than pH 6, while sensitivity to Aculeacin (glucan synthase inhibitor) is independent of the pH. However, *Δmps1* is as sensitive as the wild type to these inhibitors at both pH. We conclude that the pH 5 induced resistance of fungal cell walls to CWDE and calcofluor requires the Mps1 pathway. This also suggests that the Mps1 pathway is strongly activated at pH 5 compared to pH 6. To test this hypothesis, we are assaying the phosphorylation status of Mps1 at different pH as well as under several stress conditions and developmental stages to determine when this pathway is activated. Additionally we constructed an activated allele of Mkk1, the MAPKK upstream of Mps1, placed under the control of either its own promoter (2) or the repressible pNIA1 promoter. These transformants will be used to assess the effect of controlled activation of the Mps1 pathway on M. oryzae cellular functions. The different conditions of Mps1 pathway activation will be used for a comparative transcriptomic analysis of wild type and *Δmps1* mutants.

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Light matters: The transcription factor BcLTF1 regulates virulence and light responses in the necrotrophic plant pathogen *Botrytis cinerea*

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Botrytis cinerea/ Botryotinia fuckeliana is the causal agent of gray mold diseases in a range of dicotyledonous plant species. The fungus can reproduce asexually by forming macroconidia for dispersal and sclerotia for survival; the latter also participate in sexual reproduction by bearing the apothecia after fertilization by microconidia has taken place. Light induces the differentiation of conidia and apothecia, while sclerotia are exclusively formed in the absence of light. The relevance of light for virulence of the fungus is not obvious, as infections are observed under natural illumination as well as in constant darkness. By a random mutagenesis approach, we identified a novel virulence-related gene encoding a GATA transcription factor (BcLTF1 for light-responsive TF1) with characterized homologues in *Aspergillus nidulans* (NsdD) and *Neurospora crassa* (SUB-1). By deletion and overexpression of *bcltf1*, we confirmed the predicted role of the transcription factor in virulence, and discovered furthermore its extraordinary functions in regulation of light-dependent differentiation, the equilibrium between production and scavenging of reactive oxygen species (ROS), and secondary metabolism. Microarray analyses revealed 293 light-responsive genes, and that the expression levels of the majority of these genes (66%) are modulated by BcLTF1. In addition, the deletion of *bcltf1* affects the expression of 1,539 genes irrespective of the light conditions, including the overexpression of known and so far uncharacterized secondary metabolism-related genes. Increased expression of genes encoding alternative respiration enzymes, such as the alternative oxidase (AOX), suggests a mitochondrial dysfunction in the absence of *bcltf1*. The hypersensitivity of Δ*bctlf1* mutants to exogenously applied oxidative stress - even in the absence of light - and the restoration of virulence and growth rates in continuous light by antioxidants, indicate that BcLTF1 is required to cope with oxidative stress that is caused either by exposure to light or is arising during host infection.

Mycelial growth of insect-pathogenic fungi under visible light induces higher conidial stress tolerance

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Visible light exposure during growth influences primary and secondary metabolism, growth and sporulation, sexual and asexual development, and pigment formation in many fungal species. However, little is known about the phenotypic effects of light during mycelial growth on the tolerance of the developing fungal conidia to different stress conditions. Conidia of the entomopathogenic fungi *Beauveria bassiana* (ARSEF 252), *Metarhizium brunneum* (ARSEF 1187), *M. robertsii* (ARSEF 2575), *Tolypocladium cylindrosporum* (ARSEF 3392), *Isaria fumosorosea* (ARSEF 3889), *T. inflatum* (ARSEF 4877), *M. anisopliae* s.l. (ARSEF 5749), Lecanicillium aphanocladii (ARSEF 6433), *Simplicillium lanosoniveum* (ARSEF 6651), and *Aschersonia aleyrodis* (ARSEF 10276) were produced on PDA medium under continuous light, on PDA medium in the dark, and on minimal medium in the dark (MM). The conidial tolerance of these species was evaluated in relation to heat and to menadione, a potent inducer of reactive oxygen species. The conidial production under the three treatments was also evaluated. Visible light during mycelial growth increased heat tolerance only in conidia of ARSEF 5749. The tolerances to menadione were higher for the isolates ARSEF 1187, ARSEF 2575, and ARSEF 5749 when conidia were produced under light. The nutritive stress caused by MM induced increased heat tolerance to conidia of ARSEF 1187, ARSEF 2575, ARSEF 4877, and ARSEF 5749. Conidia produced under nutritive stress had higher tolerance to menadione for the isolates ARSEF 252, ARSEF 1187, ARSEF 2575, ARSEF 3392, and ARSEF 5749. Increased conidial production was induced by visible light only for the Tolypocladium species ARSEF 3392 and ARSEF 4877.

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Discovery of a novel growth inhibitor of the fish pathogen *Saprolegnia parasitica*

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Saprolegnia parasitica is the most important oomycete fish pathogen causing losses of over 10 million dollars annually in the aquaculture industry. At present, there are no means of controlling this pathogen as malachite green, which was so far used for disease control, has been banned worldwide because of its mutagenic and carcinogenic effects. The infective stage of the parasite is the zoospore/cyst stage of development. The arrest or inhibition of cyst development represents a good strategy to control Saprolegnia infections.

A library of 360 bioactive molecules that inhibit pollen tube germination in plants was identified recently. Pollen tube and cyst germination in oomycetes share similar characteristics as both occur by tip growth. We have therefore screened the above-mentioned library to identify new drugs that inhibit the germination of Saprolegnia cysts.

Results from our screen identified one drug ("B4") as the most potent inhibitor of cyst germination. Concentrations of B4 as low as 0.25 µM were inhibitory when the cysts were exposed to the drug for at least 2 h. However, a relatively higher concentration of 10 µM was needed to inhibit the further germination of already germinated cysts. B4 also inhibited mycelial growth of several members of the Saprolegniales order, but with varying effects on different species. B4 (100 µM) caused a 75% growth inhibition of *S. parasitica* mycelium while *Saprolegnia ferax* and *Saprolegnia diclina* were inhibited by about 60 % and 65 %, respectively, in the same conditions. This suggests that *S. parasitica* is more sensitive to B4 than the other species. Microscopic observations of the hyphae revealed that B4 affects vesicle trafficking and provokes an aggregation of vesicles as well as an increased vacuolation. In vitro assays suggest that B4 does not interfere with the actin or microtubule cytoskeleton. In addition, the drug has no effect on the activity of cell wall biosynthetic enzymes (e.g. glucan synthases). Additional work is ongoing to gain further insight into the molecular mode of action of the B4 drug.

AfuFlo8 is required for development and adhesion of human pathogen *Aspergillus fumigatus*

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Adhesion, well characterized in *Saccharomyces cerevisiae*, plays a role in cellular development and pathogenicity. In *S. cerevisiae*, invasive growth, flocculation in haploid and diploid pseudohyphal growth were regulated by adhesins (Flo1 and Flo11), and expression of adhesins was controlled by the Flo8 transcription factor. One protein which has conserved LUFS domain at N-terminal was found in *Aspergillus fumigatus* (AfuFlo8). AfuFlo8 binds to the promoter of *Flo11* and complements adhesion in flo8-defecient mutant in *S. cerevisiae*. In *A. fumigatus*, defect in AfuFlo8 results no adhesion for abiotic surface and loss of asexual development. The *brlA*, major transcription factor for conidiation, had no expression in AfuFlo8-knockdown strain. Our data suggest AfuFlo8 regulates central regulatory genes for conidiophores formation and adhesive protein expressions for abiotic adhesion.

Determination of microfungal biodiversity of potential infectous risk in a neonatal intensive care unit in Izmir City in Turkey

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The aim of this study was to determine the indoor microfungal biodiversity, of potential intectous risk of Neonatal intensive Care unit of Ege University Medical School in Izmir in Turkey. We have investigated fungal air quality variation of neonatal intensive care Unit in medical school of Ege University where babies are hospitalizing, they born very early or not improved adequately. We also considered to seasonal variation and measured intensity level of airbone microfungi that effective for hospital infection.

Samples were taken from 29 selected sampling points, 15 of them are inside of baby incubator in hospital unit, every month between May 2011 and April 2012, by using microbial air sampler and Potato Dekstroz Agar (PDA) as isolation medium. An air sample of 100L was aspirated from each points of inside of hospital unit and 50 L from single point of outside of hospital unit in two parallels medium. We determined the distribution of microfungi and identified them as genus level.

As a result of twelve months of this study; we calculated 3186 microfungi colonies. We have recorded highest total account of microfungi colony in October sampling (7,3x103 cfu/m3), lowest account of microfungi colony in February (0,3x103 cfu/m3). According to identification results on genus level, Aspergillus (% 7,51), Penicillium (%10,50), Alternaria (%7,65), Cladosporium (%32,03), yeasts (%20,13), Miselia sterilia (%12,79), and other genera (%9,38) were determined as mostly genera.

The Gpr1 7-transmembrane receptor from the mycoparasite *Trichoderma atroviride*, its targets and interactors

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The application of mycoparasitic fungi being able to antagonize fungal phytopathogens is a promising alternative to hazardous chemical fungicides in plant disease control. The market for fungal biological control agents is dominated by *Trichoderma spp.*; however, our understanding of the cellular and molecular mechanisms of the mycoparasitic behavior of Trichoderma still is fragmentary. Mycoparasitism is preceded by chemotropic growth towards the prey fungus and pre-contact induction of "molecular weapons" such as cell wall-lytic enzymes and secondary metabolites in Trichoderma, consequently the receptors and signaling pathways involved in sensing and responding to the prey are of special interest.
The *Trichoderma atroviride* Gpr1 7-TM receptor has been shown to be essential for regulating mycoparasitism-relevant processes and it is implicated in the recognition of prey-derived signals. Here we provide results from a comparative transcriptomic study of *T. atroviride* wild-type as well as *gpr1* mutants in order to identify target genes being regulated by the Gpr1 receptor upon prey recognition. Furthermore, the identification of direct interactors of Gpr1 by the split-ubiquitin yeast two-hybrid system and the preliminary functional characterization of one of the identified interacting proteins will be reported.

Contact-dependent behaviour of *Candida albicans* hyphae

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Candida albicans is a pleiomorphic fungus that lives as a commensal yeast in the human body but can become pathogenic in susceptible patient groups. Virulence is strongly linked with the production of penetrative hyphae that can adhere to and invade a wide range of substrates, including blood vessels, organ tissue, keratinised finger-nails and even soft medical plastics. Using live-cell imaging and nanofabricated surfaces, we are characterising the spatio-temporal dynamics of contact-induced hyphal tip behaviour.

Localization of fluorescent protein markers for the Spitzenkörper and the Polarisome (Mlc1-YFP and Spa2-YFP, respectively) illustrated that *C. albicans* hyphal tips grow in an asymmetric, 'nose-down' manner on a surface. Additionally, hyphal tips can detect surface stiffness and show a distinct preference for nose-down growth on the softer of two substrates.

Live-cell imaging of hyphal growth revealed that *C. albicans* performs directional memory, during navigation of a mazed topography. This memory is lost when apical compartments undergo septum formation, prior to encountering open space in the maze. Further, septum formation also abolishes the nose-down tip morphology, which may be a pre-cursor to directional memory.

Finally, we developed an assay to measure the pressure generated by the hyphal tip against a substrate, where a distinction was present between glucose-grown and lactate-grown hyphae. Our results suggest that *C. albicans* hyphae can exert sufficient force to penetrate human epithelial tissue without the need for secreted enzyme activity. This is consistent with the observed hyphal penetration of medical-grade silicone, which has a similar Young's modulus to human cartilage.

The allergenic potential of fungal communities on organic and integrated farm management and a handy tool for its evaluation in future studies

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Fungi are an important cause for allergies, following pollen as 2nd most significant aero-allergens outdoors. Besides conurbations, our main outdoor environment consists of agricultural landscapes. Their composition regarding the growth of potential allergen producers like saprotrophic fungi can therefore have great impact on our daily lives. This study helps to enlighten the influence of the long-term agricultural regime on the existence and growth of known allergenic fungi in soil and decomposing plant matter and delivers a tool for the standardized assessment of the outdoor fungal allergenic potential in future experiments.

Decaying plant litter (*Triticum aestivum* L.), former rhizosphere and bulk soil where sampled at several time points following harvest. Source of the samples were organically or integrated farmed plots at the Scheyern Research Farm near Munich, which are in use under defined crop rotation and farm management for more than 15 years. A distinct washing procedure was used for the isolation of more than 400 fungi actively growing in and on the decomposing matter.

In a subsequent approach frequency and amount of the most relevant allergenic fungal species were measured via a quantitative PCR procedure especially adapted for the comparative use with soil, rhizosphere, plant litter and mixtures thereof.

Culture based and molecular estimations of the allergenic fungi present in organically and integrated farmed plots suggest a significant influence of the agricultural regime on the community composition - an important issue with organic farming being on the rise.

Apart from that, our adapted qPCR system allowed for an uncomplicated assessment of the fungal allergenic potential in small scales of space and time as it will be necessary in future studies to reveal the actual importance of fungal outdoor allergy.

Characterisation of the *Aspergillus fumigatus* mating-type idiomorphs

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Sexual reproduction of the human pathogen *Aspergillus fumigatus* (teleomorph: *Neosartorya fumigata*) was assumed to be absent or cryptic until fertile crosses among geographically restricted environmental isolates were described in 2008. The existence of cryptic sexuality in this species had been proposed before, based on genomic and genetic analyses revealing presence of mating type idiomorphs (MAT1-1 and MAT1-2) and several putative genes orthologous to recognized determinants of pheromone signalling, mating, karyogamy, meiosis, or fruiting body formation in the fertile species *Aspergillus nidulans*. Furthermore, the products of *A. fumigatus MAT1-2* and *MAT1-2* genes were shown to be functional in *A. nidulans*. We provide evidence for mating, fruiting body development, and ascosporogenesis accompanied by genetic recombination between unrelated clinical isolates of *A. fumigatus*, which reveals the generality and reproducibility of this long-time undisclosed phase in the lifecycle of this heterothallic fungus. Furthermore, we demonstrate that successful mating requires the presence of both mating-type idiomorphs MAT1-1 and MAT1 2, as does expression of genes encoding factors presumably involved in this process. Given the narrow conditions that favour sexual development in *A. fumigatus* accompanied by the strict need for the opposite mating type, we became interested in the creation of a presumed homothallic strain expressing both mating-type idiomorphs and therefore being capable of progression through the initial stages of sexual development and fruiting body formation. Also, a congenic pair of strains differing only in their mating-type identity was created with the aim to systematically scrutinize any phenotypical differences based on the MAT1 genotype.

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 and its arsenal of virulence factors, *A. fumigatus* is also able to infect and colonise the human body. The asexual conidia are able to reach the lower lung alveoli and withstand the immunological clearance to some extent. 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 number 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 associated with 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.

By generation of *A. fumigatus* GPCR knock-out strains, we could show that deletion of some GPCRs caused a significant decrease in spore formation and growth rate, whereas germination was only slightly affected. Furthermore, an increased sensitivity towards chemicals provoking stress against reactive oxygen intermediates was observed.

A major question concerning receptors of all organisms is the identification of agonists and antagonists modulating their activity. Using the Biolog System, we were able to identify potential metabolites that led to a significantly changed growth of GPCR knock-out strains compared to the wild type, leading to the assumption that the recognition and/or corresponding signal transduction processes might be altered in these mutant strains. Interestingly, addition of these metabolites in excess can complement the growth defect of the respective mutants, giving a strong hint that the corresponding GPCRs are important for sensing these molecules.

Finally, to link GPCR sensing with intracellular signal transduction pathways via cognate G protein complexes, using the split-ubiquitin yeast 2 hybrid system, a protein-protein interaction study was performed to elucidate the formation of receptor-signal transducing complexes. The ongoing analysis allows define the function of different GPCRs in this important opportunistic filamentous fungus.

Monitoring arbuscular mycorrhizal fungi distribution patterns in India with computational databases and platforms

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Understanding natural distribution of Glomeromycota or AMF is hampered by their microscopic nature and hidden habitat in soil, tedious and lengthy procedures for pot culturing and identification. Search for alternative methods have lead to standardisation of molecular biology and information technology procedures and there has been revolutionary changes in understanding of the distribution AM fungi using computational databases and platforms. In the present study distribution pattern of AM fungi is studied across several states of India.

A collection of random 64 citations referring to occurrences of AM fungal species in different states of India from year 2005 onwards was made to judge the present biodiversity status of these fungi across these regions in India. A database with complete information about the biodiversity status of AMF species in different states of India was build using MS access 2007 and visual basic was used to retrieve information about location and abundance of different species from the above citations. The principal findings in this context are as follows:

1. There exists about 171 species of AMF fungi across 18 states of India namely: Andhra Pradesh, Assam, Bihar, Goa, Haryana, Jammu and Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Meghalaya, Orissa, Punjab, Rajasthan, Tamil Nadu, Uttarkhand, Uttar Pradesh and West Bengal.
2. These species belong to 18 genera with Glomus being represented by highest number of species. The list of genera arranged in decreasing order according to number of representative species (is given in brackets here) is: Glomus (65), Acaulospora (30), Scutellospora (17), Gigaspora (11), Sclerocystis (10), Funneliformis (7), Rhizophagus (5), Racocetra (5), Ambispora (4), Claroideoglomus (3), Entrophospora(3), Pacispora (3), Paraglomus (2), Dentiscutata (1), Piriformospora (1) , Archaeospora (1), Redeckera (1), and Diversipora (1).
3. The 10 most widely distributed species arranged based on their representation in more number of states in India are Funneliformis mosseae, *Acaulospora spinosa, Rhizophagus fasciculatus*, Acaulospora laevis, *Acaulospora scorbiculata*, *Glomus aggregatum*, *Rhizophagus intararadices*, *Claroideoglomus claroideum* and *Claroideoglomus etunicatum*. On the other hand 62 species are more localised in their distribution and are present only in one state.
4. Further this survey shows the states falling in the Western Ghats region i.e. Karnataka, Goa and Maharashtra contain more than 68.9 % of total AM fungal species available in India.

Undoubtedly India is vastly rich in biodiversity of AM fungi. The present database provides a reference dataset for enlisting of Glomeromycota and stores available information about their geographical distribution, occurrence in different habitats and climatic regions, in different states of India. Emerging patterns – including the uneven distribution of Glomeromycota among different states and climatic zones in India, or the tendency of certain of AM fungal species known to associate with a wide taxonomic range of host plants in many states or just limit itself to one region – can serve as a basis to generate testable hypotheses for future study.

Trichoderma coconut- aroma plays a role in plant resistance mechanisms

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Trichoderma species are used as biocontrol agents since they produce a wide array of secondary metabolites. The aim of this study was to identify volatile compounds emitted from Trichoderma that could induce resistance in plants and/or modulate plant growth. A total of 10 strains of Trichoderma were screened for bioactive volatiles. *Trichoderma koningii* (T.5) was isolated from the rhizosphere of *Zea mays* and produced many volatiles. These T.5 volatiles inhibited mycelial growth and spore germination of *Alternaria brassicicola* (A.b) and *Botrytis cinerea* (B.c). After 6-days of co-cultivation 40 % and 36% inhibition was observed, respectively.

Co-cultivation of T.5 with 3 days old seedlings of *Arabidopsis thaliana* Col-0 resulted in reduction of root length and fresh weight compared to control plants. Furthermore, these plants accumulated high levels of anthocyanin and the stress compound H_2O_2. In contrast, T.5 volatiles induced promotion of fresh weight (45%) and extensive root branching when applied to 10 days old *A. thaliana*.

The major volatile of Trichoderma T.5 was identified to be 6-pentyl-α-pyrone (6-PP), a lactone with coconut-like aroma. Up to 450 ng/µl of 6-PP accumulated in the head space of Petri dish after 8 days of cultivation. This high emission level of 6-PP prompted us to study whether this lactone effects *A. thaliana's* growth: *A. thaliana* plants were cultivated on soil in Weck containers for 28 days before 6-PP was added to the soil. 24 hours after this treatment the plants were challenged with *A. brassicicola* and *B. cinerea* and 5 days post infection the lesion sizes were measured. The lesion sizes of the treated plants were reduced more than 50% indicating that 6-PP treatment increased plant resistance.

This study provides new insights into the poorly studied plant- Trichoderma volatile interactions and will help to better understand their communication interplay in the soil. Furthermore, these results may establish a future development in biological control such as `Trichoderma volatile therapy`.

Developing a new model grass-fungal pathosystem

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A successful colonization of plants by pathogens requires active effector-mediated suppression of plant defense responses. We are interested in studying effectors as a manipulative toolbox for plants. The *Ustilago maydis* - *Zea mays* pathosystem is a well-established model system to investigate basic principles of biotrophic plant-pathogen interactions. However, due to the long generation time, space requirements, and difficulties in transformation of maize studying the plant side is demanding. Recently, it has been shown that the yet uncharacterized smut fungus *Ustilago bromivora* infects Brachypodium, a model grass (1). Short generation time, small size, sequenced genome, and accessible reverse genetics make this monocot highly suitable for the analysis of biotrophic interactions, which focus on the pathogen as well as the plant side. Here we report about our progress to develop tools in *U. bromivora* as well as in Brachypodium to enable functional effector studies in this new pathosystem.

(1) Barbieri, M., Marcel T. C., Niks E. R. (2011) Host Status of False Brome Grass to the Leaf Rust Fungus *Puccinia brachypodii* and the Stripe Rust Fungus *P. Striiformis*. *Plant Disease*, 95, 1339-1345.

Establishment of an assay for host entry of *Ustilago maydis* effectors

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A successful colonization of plants by pathogens requires active effector-mediated suppression of defense responses and host tissue reprogramming. During the early stages of maize infection, the biotrophic fungus *Ustilago maydis* penetrates the plant tissue and becomes encased by the invaginating host plasma membrane. The close apposition of the fungal cell wall and the plant plasma membrane creates a biotrophic interface, a compartment where all the subsequent exchanges between pathogen and host will occur. In the *U. maydis* / maize system all effectors are first secreted to this interface. Those with an activity in the host cytosol then need to cross the plant plasma-membrane in order to reach their intracellular target. Little is known about the molecular mechanisms of effector uptake and the only *U. maydis* effector that is translocated to the host cytoplasm is the active chorismate mutase, Cmu1 (Djamei et al., 2011). Here we report the establishment of new assays to assess in a reproducible and specific manner effector translocation to the host cell. This will provide the foundation for determining which of the 300 *U. maydis* effectors are translocated and allow to investigate their intracellular activity. In addition, we discuss how these new assays may help to determine the molecular basis of effector uptake.

Djamei A, Schipper K, Rabe F, et al. (2011) Metabolic priming by a secreted fungal effector. *Nature* 478: 395-398.

Host-related nitrogen availability affects the switch from biotrophic to cell death associated phase in *Piriformospora indica*

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The mechanisms underpinning broad compatibility in root symbiosis are largely unexplored. The generalist root endophyte *Piriformospora indica* establishes long-lasting interactions with morphologically and biochemically different hosts stimulating their growth, alleviating salt stress and inducing local and systemic resistance to pathogens. Comparative analysis revealed the presence of two ammonium transporters (AMT) in the *P. indica* genome and the absence of nitrate transporters. One of the AMT transporters PiAmt1 proved to be plant responsive by microarray and qPCR analyses. Its ammonium import function was verified by yeast complementation. Silencing of the PiAMT1, whose transcripts are accumulating during nitrogen starvation, resulted in enhanced colonization of barley whereas it had no effect on the colonization of Arabidopsis. Decreased expressions of plant defense gene and fungal saprotrophic gene, increased levels of free amino acids and reduced enzymatic activity for the cell-death marker VPE (vacuolar processing enzyme) in colonized barley roots coincided with an extended biotrophic lifestyle of *P. indica* upon silencing of PiAMT1. This suggests that PiAmt1 functions as a nitrogen sensor mediating the signal that triggers the *in planta*-activation of the saprotrophic program.

Fungal endophytes in elderberry (*Sambucus nigra* L.) in dependence of seasonal effects

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Austria is one of the main producers of elderberry (*Sambucus nigra* L.) worldwide. With more than 1400 ha, elder covers 12.9 % of the Austrian fruit cultivation area in 2012. But since about ten years, the fungal diseases corymb wilt and anthracnose spread in commercial elderberry orchards and cause severe harvest losses even up to 100 %. In our study we investigated occurrence and development of endophytic fungi within five Styrian orchards in dependence on seasonal conditions and fungicide treatment. In total, we isolated 1275 endophytic fungi from 650 plant samples on eight sampling dates along one vegetation period. In each orchard half of the samples were drawn from fungicide treated shrubs, the other half from untreated ones. The fungal isolation was carried out by surface sterilization of the plant samples and placing of small plant pieces onto potato dextrose agar containing ampicillin to avoid bacterial growth. Outgrowing fungi were placed onto new PDA plates to obtain pure cultures. The data analysis showed that the colonization frequency for fungicide treated plant samples was 46 % whereas for untreated ones it was 76 %. Similarly, nearly three quarters (72 %) of all isolated fungi originated from untreated plants. Sequencing of the ribosomal ITS region and identification of the fungi via BLAST NCBI database comparison revealed 37 different genera, with Alternaria, Fusarium, Colletotrichum, Phomopsis, Cladosporium, Phoma, as well as members of the Xylariaceae family being the most frequently isolated. On average 7.2 ± 2.7 genera have been isolated per elder tree, the maximum was twelve. Our special emphasis was placed on the genera Phoma, Fusarium and Colletotrichum, comprising the hitherto known pathogens. These three genera together cover 36 % of the 1050 identified isolates, showing the severeness of disease pressure within the orchards. We found genus-specific development patterns over the season, where Fusarium isolations increase from beginning of summer until harvest, Phoma isolations stay in the same range over the vegetation period, and Colletotrichum only becomes important in the last two weeks before harvest with a steep increase of isolations. Further, the distribution pattern upon different plant parts is genus-specific. Whereas Fusarium, Phomopsis and Phoma were most frequently isolated from berries, Colletotrichum and Cladosporium were dominant in umbel stalks. Members of the Xylariaceae occurred mostly in leaves and Alternaria was numerous in all three plant parts.

By analyzing fungal endophyte diversity in cultivated elderberry trees via ITS sequence data, we were able to detect genus-specific differences in occurrence and development of the fungi influenced by seasonal and fungicide treatment effects. These results build the basis for further studies in order to establish effective disease management strategies against anthracnose and corymb wilt.

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Formation of plant tumors in the *Ustilago maydis* – maize interaction requires organ-specific activity of effector proteins

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Ustilago maydis is a biotrophic smut fungus, which infects all aerial organs of its host plant maize. Disease progression goes along with comprehensive reprogramming of the plant tissue, which ultimately results in formation of tumors. This tumor induction is likely being triggered by small secreted proteins by the fungus, so called effectors. 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 tissues. To further investigate the role of individual organ specific effectors in modulating biotrophy, we performed a candidate gene approach based on transcriptional regulation and sequence divergence of effector genes. This approach identified a whole set of novel *U. maydis* effectors that contribute to fungal virulence in an organ-specific manner. One such effector is See1 (Seedling efficient effector 1), whose expression is strongly induced in seedling leaves but only weakly expressed in tassels and ears. *U. maydis* deletion mutants for *see1* show a strong reduction of tumor formation in maize seedlings but not in floral tissues. Laser scanning confocal microscopy shows that the mutant hyphae successfully enter the leaf tissue but might be blocked during pre proliferation stages in the mesophyll tissue of the leaf. Moreover, by labeling replicating DNA by 5-ethynyl-2-deoxyuridine (EdU) we observed that maize seedling colonized by Δ*see1* do not show mitotic activity during infection, while cell division in leaves is specifically induced in wildtype infected host cells. In contrast, the Δ*see1* mutant induces normal tumor formation in tassels and also shows the stable cell division rate in colonized anthers. To understand its organ-specific function for *U. maydis* virulence, See1 interaction partners have been identified using a yeast two-hybrid screen. Recent progress on the functional characterization of See1 will be presented.

Manipulation of host metal ion homeostasis as efficient colonization strategy of *Piriformospora indica*

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In order to enter the plant cells and to be able to establish an interaction with the host, the microorganisms have to deal with sophisticated plant defense responses. It is already known, that microbial pathogens hijack the cellular program of their hosts by producing small, secreted proteins called effectors. Recently, the influence of fungal effector proteins on the establishment of mutualistic interactions was described. The genome analyses of the root endophyte *Piriformospora indica* revealed the presence of a novel putative effector gene family encoding proteins with a highly conserved pattern of seven amino acids "RSIDELD" at their C-terminus. Analyses of Dld1, a member of this protein family, demonstrated its strong affinity to Fe3+ and Zn2+. These metal ions play a very important role as cofactors for many different plant enzymes, which are activated in response to microbial attacks. Additionally, in colonized barley roots a co-expression of PiDLD1 with a gene encoding a transmembrane protein was observed. Blast analysis of this protein revealed a strong similarity to fungal metal ion transporters. We speculate that these two proteins could be involved in host metal acquisition by sequestering metal ions through Dld1 and subsequent metal ion relocation to the fungal cell by the transmembrane metal ion transporter. Yeast-two-hybrid analysis could confirm the interaction between the Dld1 and the putative transporter. Further analyses using *P. indica* RNAi transformants as well as a yeast mutant *Δfet3fet4* defective in both high- and low-affinity iron transport are supposed to explain the role of these two proteins in manipulation of host metal ion homeostasis during colonization of barley roots.

Arbuscular mycorrhizal fungi: A biocontrol agent against common bean Fusarium Root Rot Disease

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Effectiveness of arbuscular mycorrhizal fungi in the protection of common bean plant (*Phaseolus vulgaris* L.) against Fusarium root rot disease was investigated in the present study under natural conditions in pot experiment. A mixture of arbuscular mycorrhizal fungi consists of propagated units of *Glomus mosseae*, *Glomus intraradices, Glomus clarum, Gigaspora gigantea* and *Gigaspora margarita* in suspension form (10^6 units/L in concentration) was used at dilution of 5 ml/L water. The obtained results demonstrated that, arbuscular mycorrhizal colonization significantly reduced the percentage of disease severity and incidence in infected bean plants. On the other hand, mycorrhizal colonization significantly increased the tested growth parameters and mineral nutrient concentrations. While, infection with Fusarium root rot disease negatively affected on the mycorrhizal colonization level in bean roots. Finally, mycorrhizal colonization led to a significant increase in the phenolic content and the activities of the investigated defense related enzymes (Phenylalanine ammonia-lyase, Polyphenol oxidase and Peroxidase enzyme). From the obtained results, it can be concluded that the application of arbuscular mycorrhizal fungi as a biocontrol agent played an important role in plant resistance and exhibit greater potential to protect bean plants against the infection with *F. solani*.

The role of cytokinins in the *Claviceps purpurea* plant interaction

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Cytokinins (CKs) are phytohormones influencing various physiological and developmental processes. They are most famous for their stimulatory effect on cell division, apical dominance and the transmission of nutritional signals. However, it is becoming more and more evident that CKs are not unique to plants but can be found in a wide range of organisms. Interestingly, plant-interacting bacteria are able to use CK biosynthesis to manipulate host-plant development in a pathogen advantageous way. In many gall-forming bacteria, for example, CK biosynthesis is used to alter the host morphology and therefore depicts a virulence factor. So far, little is known about the role of CKs during development and pathogenesis of fungi. Though, it is particularly noticeable that they have only been detected in micorrhizal, biotrophic and hemibiotrophic fungi. Up to now, no biosynthetic pathway or proteins involved in CK synthesis have been identified.

Thus, we are currently investigating the CK production in *Claviceps purpurea* and the role of CKs in the interaction with its host plants. *C. purpurea* is a biotrophic fungus that solely infects the ovaries of flowering grasses. It replaces the ovarian tissue and finally leads to the formation of a sclerotium instead of a caryopsis. In general, chemical analyses prove that *C. purpurea* produces CKs in axenic cultures and most likely also *in planta*. Genome wide analyses reveal three putative CK biosynthesis genes. An in vitro assay of the heterologously expressed proteins shows that they have the expected catalytical activity. To analyze the biological function of CK biosynthesis deletion mutants will be analyzed *in planta*.

Occurrence of microorganisms inside dying rape plants identified with the assistance of DNA-ITS sequencing

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During the period of early spring, dying *B. napus* plants can be observed on fields of rapeseed oil plants. This can be caused either by autumn damages by insects, damages caused by game animals or spore invasions of sac fungi from the genus of Leptosphaeria spp. In conditions of the climate in Poland, infestation with the above-mentioned pathogens is recorded every year and this fact is frequently connected with yield losses of rape seeds. During a period of four years (2009-2012), experiments were carried out to study the occurrence of microorganisms, which settled inside infected fragments of *B. napus* plants, i.e. root necks and dying apexes. Following external sterilisation, the dying parts were appropriately prepared and then applied onto PDA substrates. After the period of 5 days, growing hyphae of mycelium were transferred into Eppendorf tubes and frozen. Next, the isolated species were identified with the assistance of sequencing of DNA, ITS-1 or ITS-2 fragments. The species composition of the examined microorganisms differed in individual years. In 2009, the following species were identified: *Phoma eupyrena, Alternaria sp., Fusarium sp., Verticillium, Mucor hiemalis, Leptosphaeria maculans*, Trichosporon. In 2010, in the same location: *Alternaria alternata, Lewia infactoria, Epicoccum nigrum, Botryotinia fuckeliana, Leptosphaeria maculans, Leptosphaeria biglobosa, Fusarium culmorum, Fusarium redolens, Fusarium oxysporum, Fusarium tricinctum, Apiospora montogenei, Giberella avenacea species were recorded. In the following year (2011), Botryotinia Botryotinia fuckeliana, Leptosphaeria maculans, Leptosphaeria biglobosa, Fusarium culmorum, Fusarium redolens, Fusarium oxysporum, Fusarium tricinctum, Fusarium equiseti, Mucor hiemalis, Bionectria ochroleuca, Mortierella elongata, Pythium salpingophorum, Sclerotinia sclerotiorum* species were identified, while last year (2012), *Leptosphaeria maculans, Leptosphaeria biglobosa, Fusarium sp., Alternaria sp., Cladosporium sp., Pleosporales sp., Trichoderma koningiopsis, Rhizopus sp., Sclerotinia sclerotiorum* species were found in the same location. In order to detect the above-mentioned species, the total of 445 DNA ITS sequencing analyses were performed. During the 4-year period of analyses (2009-2012), the proportion of the most dangerous rapeseed oil pathogens, i.e. fungi from the Leptosphaeria sp. genus was found to be at approximately 10%. *Sclerotinia sclerotiorum* pathogen, also dangerous for *B. napus* plants, occurred during early spring only occasionally.

Signalling in *Magnaporthe oryzae*: Involvement of histidine kinases in differentiation, stress response and pathogenicity

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HIKs in fungi coordinate distinct parts in differentiation processes, hyphal- and spore development, pathogenicity, secondary metabolism and they are mediators of environmental stress responses, for example in adaption to high osmolarity or salt stress.

The aim of this study is a functional analysis of all putative HIKs in the phytopathogenic fungus *Magnaporthe oryzae*, which is known to be the causal agent of rice blast disease. We identified ten HIK encoding genes within the *M. oryzae* genome, generated gene inactivation mutants and characterized the resulting mutant strains. MoSln1p and MoHik1p were previously reported to be signalling proteins involved in the high osmolarity glycerol (HOG) pathway. However, studies concerning the further 8 HIKs have not been reported to date. MoSln1p and MoHik1p are part of a signalling cascade involved in salt stress adaption. MoHik1p has been furthermore involved in the mode of action of the fungicides fludioxonil and iprodione. Physiological characterization of generated mutants revealed the role of HIKs in sporulation, appressorium formation, pathogenicity and adaption to different stress conditions. The *ΔMoSLN1* mutant is stronger affected by salt stress (NaCl) than *ΔMoHIK1* whereas *ΔMoHIK1* is stronger affected by osmotic or sugar stress (sorbitol) in contrast to *ΔMoSLN1*. The double mutant *ΔMoHIK1*+*ΔMoSLN1* is unable to grow on both stress-inducing solutes indicating the redundant function of both HIKs in reaction of salt and sugar stress via the HOG pathway. Analysis of phosphorylated MoHog1p indicates that additional proteins have to be involved in the HOG signalling pathway. Compared to yeast, the HOG signalling cascade in phytopathogenic fungi is a multi component system with more components involved.
The HIKs MoSln1p, MoHik5p and MoHik8p were found to be essential for full virulence of the fungus. It was found that the mutant strain *ΔMoHIK5* and *ΔMoHIK8* both show abnormal spore morphology. Furthermore *ΔMoHIK5* is unable to form appressoria. The ability to form functional infection structures is strongly reduced in the mutant *ΔMoSLN1* and *ΔMoHIK8*. As a consequence the HIKs MoHik5p and MoHik8p appear to be essential for pathogenicity since the mutants produce a decreased number of spores and fail to infect and colonise rice plants.

Vag2 is a virulence effector of the maize head smut pathogen *Sporisorium relianum*

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The closely related biotrophic smut fungi *Sporisorium reilianum* and *Ustilago maydis* infect maize but lead to different symptoms. *S. reilianum* infects maize at seedling stage, and spreads through the plant initially without visible symptoms. Symptoms appear when the plant starts flowering and the inflorescences are substituted by spores and/or show phyllody. *U. maydis* leads to symptoms within one week after plant penetration, forming tumors near the penetration site, in which fungal spores develop. To identify genes involved in symptom formation, the genomes of *S. reilianum* and *U. maydis* were compared, which revealed the presence of genomic regions containing genes mainly encoding weakly conserved secreted proteins. 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 responsible for the early leaf wilting and virulence phenotypes. Deletion of one of these effectors (*vag2*, virulence associated gene 2) led to the highest reduction in virulence and early leaf wilting. qRT-PCR analysis revealed that *vag2* is highly upregulated during biotrophic growth of *S. reilianum*. Using yeast two-hybrid analysis, potential interaction partners were identified that include plant proteins predicted to be involved in hormone production and plant disease resistance. This suggests that the largest divergence region contains effector genes that contribute to symptom formation possibly via modulation of plant hormone levels, or suppression of plant defense.

Botrytis cinerea **BcPMR1 gene is involved in germination, cell wall integrity and is essential for full virulence**

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Botrytis cinerea is a necrotrophic fungal plant pathogen that is the causal agent of grey mould in vineyards and can cause infections in a few hundred plant species. Fungal cells are surrounded by cell walls that are multilayered composite structures. In general, the cell walls of higher fungi are composed of linear polymers of chitin, b-glucans and glycoproteins. Glycoproteins contain both N- and O-linked oligosaccharides. The N-linked glycans are attached to asparagines residues of proteins and O-linked glycans are attached to serine or threonine residues. PMR1 gene encodes for a Golgi P-type ATPase ion pump responsible for transporting calcium and manganese ions. PMR1-disruption phenotype in different microorganism includes reduced O- and N-linked glycans, defects cell shape, hypersensitive to antifungal and virulence. We studied the effect of lack PMR1 gene in *B. cinerea* and their role in cell wall integrity and virulence. For them, we made the Bcpmr1 knockout using the *B. cinerea* strain B05.10 and disrupted using a hygromycin B-resistance cassette. A Southern-blot and PCR analyses were done to check that the disruption cassette was at the correct locus. The initial results show that the Bcpmr1 mutant increases the sensitivity to cell-wall perturbing agents such as Calcofluor White (CFW), Congo Red (CR) and Caffeine. Bcpmr1 mutant also displays reduced virulence in tomato leaves and apple fruits compared to the parental strain. Moreover, the mutant displayed slow rate growth and significant reduction of conidia production. These results demonstrate that Bcpmr1 mutant is involved in cell wall integrity, sporulation and is essential for full virulence.

Molecular taxonomy, population dynamics and host specificity of *Botrytis cimerea* and related species

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The genus Botrytis comprises almost thirty described species. Most Botrytis spp. are host specific, infecting only one or few host species or genera, most of which are monot plants. In contrast, the major grey mould fungus *Botrytis cinerea*, is reported to be the only wide host range species within the genus, comprising more than 200 dicot host plants. *B. cinerea* is closely related to two host-specific dicot-infecting species, *B. fabae* and *B. calthae*.

During a survey of fungicide resistance of Botrytis populations in German strawberry fields, we have discovered a novel genotype, called Botrytis group S, which was found by multiple gene sequencing to be closely related but distinct to both *B. cinerea* and *B. fabae*. Botrytis group S was found in similar frequencies as B. cinerea or dominating in German strawberry fields. In contrast, Botrytis group S was almost absent from grapevine berries, indicating a host preference different from *B. cinerea*. Furthermore, a novel, stronger efflux-mediated multidrug resistance phenotype, called MDR1h, caused by a small deletion in the drug efflux regulator Mrr1, was discovered only in Botrytis group S but not in *B. cinerea*. This is of practical relevance because group S MDR1h strains often contained multiple target resistance mutations, and therefore seriously compromise fungicide control efficiency. PCR indel markers were developed for rapid identification of the main grey mould species infecting dicots (*B. cinerea, B. fabae, B. pseudocinerea, B. calthae*), and three subgroups of the newly discovered Botrytis group S. Different patterns of occurrence of each Botrytis species or clade were found, depending on host plant, fungicide treatments, time of season, and geographic region. Artificial infection tests confirmed different host preferences of the dicot-infecting Botrytis species and genotypes. Genome sequences of several dicot-infecting Botrytis spp. and the newly discovered Botrytis groups were obtained to clarify their genetic and taxonomic relationships, and to reveal the molecular basis of their markedly different host specificity. In parallel, a detailed comparative phenotypic analysis of several isolates from each of these species/ groups and crossing experiments are underway and will be presented.

Reference: Leroch et al. (2013) Appl Environ Microbiol. 77:2887-97.

The protein PMT4 is involved in morphogenesis and virulence in the plant pathogen *Botrytis cinerea*

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Botrytis cinerea is a phytopathogenic fungus that infects different plants. The primary contact of the fungus with its host takes place at the cell surface. The fungal cell wall is composed of β-glucans, α-glucans, chitin and glycoproteins. The genome of *B. cinerea* contains over 100 putative glycoproteins that can be anchored to the cell wall through a glycosylphosphatidylinositol (GPI) moiety or can be linked directly to the β-glucans. Glycoproteins are N- and O-glycosylated polypeptides; glycosylation occurs by a process that involves various enzymes, such as those encoded by the MNT and PMT gene families. Members of the protein manosil transferasas (PMT) family catalyze the transference of one mannose residue to the serine and threonine amino acids. The PMT family is classified into PMT1, PMT2 and PMT4 subfamilies, which differ in protein substrate specificity and number of genes per subfamily. In filamentous fungi and yeast PMT genes are essential for growth, crucial for cell wall integrity, morphology and virulence. In *B. cinerea* only three PMT genes: BofuT4_P160540.1/CCD54765 (*BcPMT1*), BofuT4_P003410.1/CCD47182 (*BcPMT2*), and BofuT4_P109250.1/CCD48541 (*BcPMT4*), one in each of the subgroups, have been reported. The aim in this study was determining the role of the *BcPMT4* gene in B.cinerea. For them the *PMT4* gene was disrupted by homologous recombination in the B05.10 strain using a hygromycin resistance cassette. Phenotypes analyses have shown that *Bcpmt4* mutant increases the sensitivity to cell-wall perturbing agents such as Calcofluor white, Congo Red and Caffeine. On the other hand, the *Bcpmt4* mutant also displays reduced virulence in tomato leaves and apple fruits. In the complementation assay using the *BcPMT4* gene under glucose promoter and incorporated in the *Scpmt4* mutant in *S. cerevisiae* demonstrated that the *B. cinerea PMT4* gene is able to rescue the phenotype. These results support the hypothesis that *BcPMT4* gene is involved in morphogenesis and virulence.

Host preferences, population genetics and molecular taxonomy of novel Botrytis genotypes closely related to *Botrytis cinerea*

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The grey mould fungus *Botrytis cinerea* is the major representative of the genus Botrytis, which comprises almost thirty described species. Grey mould isolates from commercially grown strawberry fields revealed the existence of novel Botrytis genotypes, closely related to but distinct from *B. cinerea*. Originally referred to as Botrytis group S, they seem to belong to at least three different subgroups, tentatively called group Sa, Sb and Sc, based on multiple gene sequencing. PCR RFLP and indel markers were developed for rapid identification of the closely related Botrytis spp. infecting dicots (*B. cinerea, B. fabae, B. pseudocinerea, B. calthae*), and the three newly discovered groups. Botrytis group Sa was found to occur worldwide, in particular on strawberries. In Germany, Botrytis group Sa isolates were often dominant in strawberry fields, probably due to their ability to accumulate multiple fungicide resistance mutations. One of these mutations, leading to a stronger efflux-mediated multidrug resistance phenotype, called MDR1h, was only observed in Botrytis group Sa strains, and was correlated with a small deletion in the drug efflux regulator Mrr1. Preliminary population studies indicated that all MDR1h isolates in Germany are derived from a single founder cell, and that they can rapidly invade fungicide treated fields. Depending on the host plant, time of season, and geographic origin, different patterns of occurrence of each of the dicot-infecting Botrytis species or genotypes were found. Botrytis group Sa, Sb and Sc strains were found to occur on several host plants, but revealed different host preferences. Interestingly, the host plants of the host-specific species *B. fabae* (*Vicia faba*) and *B. calthae* (*Caltha palustris*), were sometimes extensively colonized by the generalist species *B. cinerea*, *B. pseudocinerea* and groups Sa-Sc. In conclusion, grey mould populations are mixtures of genetically diverse genotypes with different host preference, which undergo dynamic changes in the field.

Reference: Leroch et al. (2013) Appl Environ Microbiol. 77:2887-97.

Functional characterization of a putative mutualistic effector from *Laccaria bicolor*

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Ectomycorrhizal symbiosis are mutualistic interactions between soil-born fungi and roots of trees. Sequencing of mycorrhizal fungi (Martin et al, 2008) sheds the light on hundreds of Mycorrhizal induced Small Secreted Proteins (MiSSPs). Among them, MiSSP8 is a 8-kDa protein highly expressed during symbiosis. RNAi knockdown of MiSSP8-encoding gene impairs *Laccaria bicolor's* mycorrhization ability. Localization of synthetic MiSSP8 fused to fluorescein shows an apoplastic localization, highlighting the hypothesis that MiSSP8 could remain in the apoplastic space to bind fungal carbohydrates in order to avoid recognition of the fungal colonization by Pattern Recognition Receptor (PRR) or to build the biotrophic interface.

Role of MAP kinase pathways in the pathogenicity of the wheat pathogen *Mycosphaerella graminicola*

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Mitogen-activated protein kinases (MAPKs) are essential components of fungal signaling pathways involved in different developmental processes and are required for host plant infection. *Mycosphaerella graminicola*, the causal agent of *Septoria tritici* leaf blotch (STB) of wheat, has three MAPK pathways that are all required for infection (*MgFUS3* , *MgHOG1*, *MgSLT2*; Cousin et al., 2006; Mehrabi et al., 2006a, Mehrabi et al., 2006b). We showed that *Mgfus3* null mutants are non-pathogenic on intact wheat leaves (paint brush inoculation), but highly-reduced in pathogenicity when infiltrated into leaf tissues by syringe injection (reduced necrosis, low number of pycnidia). This suggests that MgFUS3 is involved in fungal penetration, host colonization and pycnidia formation. *Mghog1* null mutants have pathogenicity defects similar to *Mgfus3* null mutants. This result highlights that the role of HOG1 in pathogenicity on plants differs among fungi (Segmüller et al., 2007). *Mgslt2* null mutants are fully non-pathogenic on inoculated wheat leaves either by paint brush inoculation or injection. This phenotype is unusual among *slt2* null mutants from other fungi. Therefore, *Mycosphaerella graminicola* MAPK pathways may have evolved to control regulatory networks differing from other fungal plant pathogens. To identify which genes are under the control of the MgSLT2 signaling pathway, we are developing different transcriptomics analyses. Expression profiling relies on the comparison of transcriptomes of *Mgslt2* null mutants and wild type strains grown under conditions corresponding to either an active or an inactive SLT2 pathway. Additional transcriptomics analyses will be performed using an allele encoding a conditionally active MAPKK expressed under the control of an inducible/repressible promoter. Genes whose expression requires an active SLT2 MAPK will be further studied for their role in development and infection using reverse genetics.

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Genome analysis of secondary metabolism in *Ustilago maydis*

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Much of natural product chemistry concerns a group of compounds known as secondary metabolites. These low-molecular-weight metabolites often have potent physiological activities. Recent advances in molecular biology, bioinformatics and comparative genomics have revealed that the genes encoding specific fungal secondary metabolites are clustered and often located near to the telomeres. It is also known that the analysis of the increasing number of whole genome sequences indicate that fungi encode the genetic information for the biosynthesis of plethora of compounds that are not observed when cultured under standard laboratory conditions.

In our case we are interested in the phytopathogenic fungus *Ustilago maydis*, the causative agent of maize smut. As a pathogen, it is able to withstand the defense reactions of its host plant corn and to subvert the plant metabolism for its own purposes. During these interactions, small molecules produced by the fungus serve important functions in the communication with its host and other organisms. We strongly believe that there might be other secondary metabolites produced by *U. maydis* waiting to be discovered, specially for those involved in plant-pathogen interaction.

We expect that many gene clusters are expressed only under highly specific and even transient conditions. Therefore, they will start silent if tested under most environmental conditions and thus cannot be detected by genome expression profiling. Reactivation of such silent clusters, however, can be easily reached if they are regulated by pathway specific transcription factors. In this case, forced expression of this transcription should result in activation of silent cluster. Therefore it is planned to identify potential transcription factor genes located in regions enriched for genes with potential biosynthetic functions. We will systematically analyse all genes annotated as transcription factors (about 350) for their localization on the chromosome and for neighbouring genes.

Potential candidates for pathway specific regulators will then be expressed under control of a set of inducible promoters. Quantitative RT-PCR will be used to determine the expression profiles of neighbouring genes depending on the presence of the respective transcription factors. For those transcription factors that induce expression of their immediate adjacent genes, the region of co-regulation will be determined by further expression studies. One has to be aware, that this method might also detect clusters responsible for specific degradation of specific nutrients or other chemical substances.

Clusters likely to contain potential biosynthesis genes will be characterized in more detail. First, extracts will tested under inducing conditions for the appearance of novel metabolites. If such substances can be detected, single genes of the cluster will be mutated to determine, which genes are critical for the production of these metabolites.

Two formae speciales of *Sporisorium reilianum* induce distinct responses in maize and sorghum

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Smuts are phytopatogenic fungi with a very narrow host range. Head smut of maize and sorghum is caused by *Sporisorium reilianum*. This fungus exists in two host-adapted formae speciales (SRS and SRZ) that either cause disease on sorghum (SRS) or on maize (SRZ). To investigate the differences leading to host specificity in S. reilianum, we microscopically followed each fungal variety during colonization of sorghum and maize. Both varieties were able to penetrate and multiply in sorghum and maize leaves. In sorghum, SRS preferentially colonized bundle sheath cells, entered the vascular bundles and reached the apical meristem, while SRZ did not show a preference for vascular bundles and was never found in apical meristems. Fungal DNA of SRS was prominent in inoculated leaves, nodes and apical inflorescences, but relative DNA quantity of SRZ decreased from the inoculated leaves to the other parts. To find out the reasons that make SRZ unsuccessful in sorghum and SRS on maize, we investigated plant defenses responses in both hosts, which were observed by microscopy of stained samples and quantitative real time PCR of marker genes. Penetrating and proliferating hyphae of SRZ induced a local response of increased H_2O_2 at 1 day after inoculation (dai), and at 2 dai the plant deposited callose in the cell walls of infected cells. At 3 dai, red dots were observed in leaves, demonstrating the presence of antimicrobial phytoalexins. In maize, we observed a very weak production of H_2O_2 and callose, which did not show differences between the two fungal varieties. These data suggest that host colonization of sorghum by *S. reilianum* is the result of a multilayered adaptation to either suppress or evade the different plant defense responses, while host adaptation in maize seems to have different reasons. The investigation of additional plant defenses (programmed cell death, lignin deposition) and the analysis of host- and tissue-specific gene expression profiles will lead to a molecular understanding of the factors that determine host selection in *S. reilianum*.

Immuno-localization and biochemical characterization of potential effectors from the Asian Soybean Rust fungus *Phakopsora pachyrhizi*

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Effector proteins play a crucial role in host parasite interaction. Especially in Rust fungi with their obligate biotrophic growth, effectors mediate the long lasting interaction with a living host plant by suppressing plant defense mechanisms. The hallmark of obligate biotrophy is the formation of specialized hyphae inside the plant cell, called haustoria. These structures represent the most intimate contact zone between pathogen and host and play a vital role in the uptake of nutrients and the release of effector proteins. These proteins are secreted into the extrahaustorial matrix or even into the plant cell, where they are expected to interact with plant proteins. Knowledge about these interaction networks might provide valuable clues for plant protection in the future.

The project presented, is focused on the identification and further analysis of novel effectors from *Phakopsora pachyrhizi*, the causal agent of Asian Soybean Rust (ASR). For this purpose we have generated specific antibodies targeted against putative effector proteins and use them for suitable downstream applications including immuno coprecipitation, pull down assays and immuno localization. Our results will help to gain a more profound understanding of the complex signalling pathways established between host and pathogen.

Population history and pathways of spread of the plant pathogen *Phytophthora plurivora*

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Many dramatic recent declines and dieback phenomena in forest ecosystems worldwide have been associated with fungus-like Phytophthora species. However, to date little is known about the population history of plant pathogens in this oomycete genus. *Phytophthora plurivora* affects a broad range of host plants in forests, nurseries, and parks. Resident populations of this species are reported in Europe and the US but the centre of origin and pathways of spread of the pathogen remain unclear. Given that this pathogen is self-fertilizing when reproducing sexually (forming oospores), but also reproduces asexually (forming sporangia), and has a broad host range, we would expect *P. plurivora* to be clonal in a given locality, but populations to be differentiated regionally and globally. In the present study, we analysed a large number of *P. plurivora* samples derived from European and US populations to determine the population genetics and demographic history of this important plant pathogen using sequenced nuclear and mitochondrial genes, as well as microsatellite loci. Our analyses show that *P. plurivora* populations are moderately diverse and differentiated, but show migration among and within continents resulting in admixture. *P. plurivora* has most likely been introduced into the US from Europe, which may be the centre of origin of the pathogen. A genetic signal of a recent expansion is present in the global *P. plurivora* population. International nursery trade may have allowed the pathogen to colonise new environments and/or hosts, resulting in a population growth.

Antimicrobial photo treatment as an alternative to control plant-pathogenic fungi

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Increasing tolerance to currently-used fungicides both in clinical and agricultural areas stimulates the development of new strategies to control pathogenic fungi. The non-conventional light-based approach antimicrobial photo treatment (APT) is a promising alternative to conventional fungicides that can be used to control localized mycoses in animal hosts or to kill fungi in the environment. In the present study, we investigated the in vitro antimicrobial photo treatment (APT) of conidia of the plant-pathogenic fungi *Colletotrichum acutatum* and of the model ascomycete *Aspergillus nidulans* with the commercial furocoumarin 8-MOP and with one furocoumarin (isopimpinellin) and with a mixture of two coumarins (7-methoxy coumarin and citropten) extracted from the Tahiti acid lime (*Citrus latifolia*). Subcellular localization of the PS was also determined in *C. acutatum* conidia. Additionally, the effects of APT on the leaves of the plant host *Citrus sinensis* were determined. APT with 8-MOP (50 µM) resulted in a reduction of approximately 4 logs in the survival of the conidia of both species. APT with the mixture of the two coumarins (12.5 mg l-1) resulted in a reduction of approximately 4 logs for A. nidulans and 3 logs for *C. acutatum* and APT with the isopimpinellin (50 µM) resulted in a reduction of 4 logs for *A. nidulans* and less than 2 logs for *C. acutatum*. Washing the conidia to remove unbound PS before light exposure reduced the photodynamic inactivation of *C. acutatum* both with 8-MOP and the mixture of the two coumarins. The reduction was smaller for A. nidulans. 8-MOP spread throughout the cytoplasm and accumulated in structures such as lipid bodies of *C. acutatum* conidia. No damage to orange trees leaves was observed after APT with any of the PS. This result opens the interesting perspective of using antifungal photo treatment to control plant-pathogenic fungi.

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Ectomycorrhizal functional plasticity and environmental stress

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Assessing ectomycorrhizal functions in ecosystems is still an experimental challenge. In a number of studies, potential enzyme activities of ectomycorrhizae were used to assess effects of environmental stress conditions (ozone, heavy metals, drought) on important functional traits.

Enzyme activities of ectomycorrhizae were rather resilient under diverse stress conditions. However, enzyme activities related to nitrogen cycling and to oxidative stress reactions were identified as potentially critical functions in ectomycorrhizal communities. To get deeper insights into mechanisms of stress regulation, drought related reactions were studied using a proteomic approach. For this study, pure culture mycelia of *Cenococcum geophilum* were subjected to drought treatments. Drought stress increased the level of oxidised proteins. Moreover, specifically upregulated proteins could be identified for specific protection mechanisms related to drought tolerance or repair mechanisms.

Spatial relations in grass infection caused by the endophytic fungus *Epichloë typhina* and geostatistical prediction of its occurrence

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Over the last several years, the number of reported grass species infected with the fungi of the genus Epichloë (Clavicipitaceae, Ascomycota) has significantly increased in Europe. In the sexual stage fungi form stromata that enclose plant shoots and prevent their further development – it is a symptom of so called choke disease. In the asexual stage, the fungus is a grass endophyte that grows intercellularly and symptomlessly within all the plant tissues, also generative. Grasses inhabited by Epichloë fungi are toxic, both to the farm animals and other herbivores living in grassland ecosystems, due to presence of various fungi-derived alkaloids.

So far only a few single environmental factors, such as carbon dioxide, salinity, availability of nutrients, substratum water content, habitat fragmentation etc. conductive to fungal infection has been diagnosed

In our research we analysed the influence of not just one but the combination of soil factors in the field conditions. The study included two non-agricultural populations of weeping alkaligrass (*Puccinellia distans*) in Central Poland. The soil data on salinity (soil conductivity of soil suspension), pH and substratum water content were collated together with information on the state of infection in the examined grass individuals. The RTK GPS and low-height remote sensing techniques were used, not only for the location of single objects under study or preparation of point maps showing the actual differentiation of plants in space in relation to the presence of absence of infection, but also for predictive maps, illustrating the possible status of infection in the whole studied area. Also, geostatistic analyses were used, which made it possible to transform the obtained point data into a spatial and continuous image, which corresponds to the actual ecological relationships, including the interaction between soil factors and fungal infection.

We have found that the distribution of infection shows a similar spatial autocorrelation to soil parameters, especially to the salinity. It probably means that there must be some connection between them – we can assume that the similarity of the spatial variability of these two phenomena depends on the same factor or factors. Basing on the measurement results and using kriging method, maps showing soil factor values for the entire area were created. With use of the information about the location of the infected and healthy individuals it was possible to prepare maps of the estimated probability of infection across the whole research area. This kind of images may be very useful for agricultural purposes.

Lessons from a T-DNA mutagenesis screen: The cell wall integrity MAPK cascade is crucial for the mutually beneficial symbiotic interaction between *Epichloe festucae* and *Lolium perenne*

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The mutualistic symbiosis of the fungal endophyte *Epichloe festucae* and its host *Lolium perenne* is an interesting model to study signals and mechanisms involved in symbiosis maintenance. Growth of *E. festucae* in its grass host is highly synchronized and involves a switch from fungal tip growth in the plant meristem to intercalary extension of hyphae in the developing leaf. The fungal cell wall is tightly attached to the plant cell wall during apoplast colonization and formation of a hyphal network is facilitated by hyphal fusion. Previously we identified several fugal genes for the symbiotic interaction including the NADPH oxidase NoxA and the transcription factor ProA. The aim of this study was to identify additional genes required for maintenance of the mutualistic symbiotic interaction by screening T-DNA mutants.

We report here on one of these mutants, TM1066 showed a dramatic *in planta* phenotype, rendering the usual symptomless association to a pathogenic one with the fungus eventually killing the plant. The host plant was stunted, and was accompanied by an increase in tiller number and fungal biomass. Light and electron microscopic analysis of TM1066 revealed proliferative colonization of the plant tissue by the mutant, including the vascular bundle system, which is never observed for the wild type.

Analysis of the single copy T-DNA integration revealed that two adjacent genes were partially deleted, namely the 3' region of a gene with homology to a *truD/pus7* pseudouridine synthase and the 3' region of a gene with homology to the cell wall integrity MAPKK *mkk2* of *S. cerevisiae*. Reintroduction of the *E. festucae* MAPKK homolog (*mkkB*) into TM1066, but not of the TruD/Pus7 homolog restored the plant phenotype, demonstrating a crucial role for the CWI MAPK pathway in symbiosis maintenance. This result was confirmed by targeted gene replacement of the E. festucae CWI MAPKK (*mkkB*) as well as the downstream acting MAPK (*mpkA*). We found that the CWI MAPK pathway mutants do not only show an elevated sensitivity to cell wall stress agents and growth at high temperature, but TM1066, *ΔmkkB* and *ΔmpkA* are also defective in hyphal fusion, a phenomenon frequently found in wild type. Interestingly, *E. festucae noxA*, *noxB*, *noxA/noxB*, *noxR* and *racA* mutants are fusion negative as well (Kayano et al., 2013).

Recently, it has become apparent, that the CWI pathway in filamentous fungi plays not only a role in adaptation to cell wall stress and in morphogenesis (infection structures, sexual reproduction structures, conidiogenesis) but also has a role in protection against ROS, iron starvation response, cell to cell communication, and hyphal fusion. Interestingly *E. festucae* mutants involved in these processes (ROS: NoxA, Tanaka et al., 2006; NoxR, Takemoto et al., 2008; Iron starvation: SidN, Johnson et al., 2013, hyphal fusion: SOFT, Craven et al., 2012) show a plant phenotype similar to the CWI MkkB and MpkA KO phenotype.

We will discuss the implications of the CWI pathway in *E. festucae* for fungal plant interaction, vegetative growth, cell wall stress response, and hyphal fusion in planta as well as under axenic conditions.

Adhesins from *Pichia pastoris* - A structural basis for a symbiotic lifestyle?

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Fungi and yeasts are able to colonize most known ecological niches. This enables them to adopt a broad variety of different lifestyles, from biofilms to floc formation and even as pathogens. Different lifestyles are achieved by cell-cell communication as well as by adhesion based on the unique fungal cell wall. In yeast, adhesion is accomplished by a set of GPI-anchored cell-wall proteins, where many of them belong to the fungal adhesin superfamily with three distinct regions. By the A-domain carbohydrate residues in the cell walls of other cells or surfaces are recognized in a lectin-like manner via a Ca^{2+}-ion and a unique motif of two consecutive aspartate residues linked by a cis-peptide bond. Selectivity and specificity is mediated by a set of variable loops around the Ca^{2+} binding site. Therefore it is thought that each adhesin is capable to confer a different function to the yeast cell. The highly repetitive B-region is believed to present the A-domain by its stalk-like shape on the outside of the fungal cell wall. The C-domain serves as fixation point in the cell envelope via a GPI-anchor and subsequent transglycosylation. On the basis of two previously known adhesion proteins, Epa1 from *Candida glabrata* [1], serving in adhesion to human epithelial cells, and Flo5, which mediates floc-formation in *Saccharomyces cerevisiae* [2], we now present fungal adhesion proteins from Pichia pastoris. We report structural, biochemical and cell biological results that indicate adhesion to chitin core-residues by one of these adhesins. This may be the first molecular clue for a social relationship between Pichia yeasts and insects, which are known for several strains from the Pichia genus but have not been biochemically studied. A second protein of the same fold indicates involvement in *P. pastoris* self-aggregation. This gives us, in comparison with Flo5 and Epa1, a broad view on the impact of adhesion in fungal lifestyles.

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Stable transformation of the obligate biotrophic rust fungus *Uromyces fabae*

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The manipulation of genomes provides a powerful tool to get deeper insights into the lifestyle of organisms and the underlying principles of their interplay with the environment by generating loss of function mutants, fusion-proteins, or overexpression lines. However, for one of the most devastating groups of plant pathogens, the obligate biotrophic rust fungi, a universally applicable system for stable transformation is still missing. Here, we report of a transformation system established for our model pathogen *Uromyces fabae*, the causative agent of broad bean rust. Urediospores produced for mass distribution in the summer were chosen as target cells. For gene delivery, biolistics and *Agrobacterium tumefaciens* mediated transformation (ATMT) have been established. Using a biolistic approach, transient transformation events with fluorescent color marker (dsRed coupled with a nuclear localization signal) under control of *U. fabae* plasma membrane -ATPase 1 regulatory elements occur at a frequency of about 10-5. Furthermore, an in planta selection approach to achieve stably transformed lines has been established. As selective agent, we are using the fungicide carboxin in combination with a point mutation within the Succinate-Dehydrogenase 1 gene (*SucDH1r*). PCR analysis shows the presence of the transgene for up to ten rounds of selection. Initial results using SNP (single nucleotide polymorphism)-PCR indicate the integration of transgene at the wt-*SucDH1* locus without PMA1 regulatory elements via homologous recombination. However, propagation of transformants under selection pressure still remains a challenge, yielding only limited amounts of spores. So far, this limitation and the fact that transgene and wildtype allele only vary in a single point mutation has made final proof of transformation by genomic Southern blot analysis impossible. Further work is needed to circumvent these limitations. In a first step we optimized the resistance cassette by introducing an additional EcoRI restriction site to intron 4 of the *SucDH1r* gene to achieve a clear discrimination between wildtype and transgene during Southern hybridization. Screening procedures were also improved by omitting selection pressure after several rounds of selection to ensure high amounts of spores for gDNA isolation. One of the major tasks now is the optimization of our transformation toolbox with special focus on the requirements of a platform for functional analysis. We will further adjust marker cassettes by adding flanking sequences of the 18S and 28S rDNA region to attain integration of the transgenes at one of multiple rDNA loci and add additional selection markers to our toolbox. As soon as transformation of *U. fabae* is functional on a routine basis, we could draw a big step closer to understand these special plant pathogens.

Differential expression of five endopolygalacturonase genes and functional demonstration of MfPG1 as a negative virulence determinant in the brown rot pathogen *Monilinia fructicola*

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The production of cell wall-degrading enzymes (CWDEs) by the fungal pathogen *Monilinia fructicola* (G. Wint.) Honey has been thought to be required for the occurrence of brown rot blossom blight and fruit rot on Prunus spp. To better understand their roles in lesion development and fungal virulence, five polygalacturonase (endo-PG) genes, designated *MfPG1*, *MfPG2*, *MfPG3*, *MfPG5* and *MfPG6* were cloned and characterized in *M. fructicola*. Quantitative reverse-transcriptase PCR (q-rt-PCR) and RNA blot analysis revealed that the five MfPG genes are differentially expressed in culture under various pH, carbon and nitrogen sources as well as during pathogenesis. *MfPG1* encodes the major endo-PG and is expressed to significantly higher levels compared to the other four MfPGs in culture and in planta. The function of MfPG1 in M. fructicola pathogenicity was determined by examining the phenotypes and expression patterns of MfPG1 overexpression strains and strains carrying β-glucuronidase (*GUS*) reporter gene fused with *MfPG1*. In situ expression of the *MfPG1* gene fused with *GUS* revealed that *MfPG1* was expressed in conidia and hyphae inoculated on flower petals. Q-rt-PCR analysis confirmed that the expression of *MfPG1* during pathogenesis. *MfPG1* overexpression strains produced smaller brown lesion on the petals of peach and rose as well as on peach fruit than those produced by wild-type. The results suggest that *MfPG1* functions as a negative virulence factor in the Prunus - *M. fructicola* interactions.

Mycoviruses in the rust fungi *Uromyces appendiculatus* and *Phakopsora pachyrhizi*

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Four putative viral sequences were found during 454-pyrosequencing of the haustorial transcriptome of *Uromyces appendiculatus* and ten in *Phakopsora pachyrhizi*. First experiments show that these could be genomes of double-stranded RNA (dsRNA) mycoviruses. We were able to purify dsRNA using a protocol based upon the fact that dsRNA specifically binds to cellulose at an ethanol concentration of 16.5 per cent.

Rust fungi such as *U. appendiculatus*, the causal agent of common bean rust, and *P. pachyrhizi*, which causes rust on soybean plants, can provoke infections in many important crops and lead to severe yield losses of up to 80 per cent. Since rust fungi are obligate biotrophic parasites, which means that they require living host tissue for their propagation, they are difficult to work with on a molecular level. Only a small number of obligate biotrophic organisms have been genetically transformed so far.

Mycoviruses, or fungal viruses, are widespread in all major fungal groups. Most mycoviruses are symptomless (cryptic) but some seem to have beneficial or negative effects on their host. For instance *Cryphonectria hypovirus* 1 (CHV1) is already being used for the control of the chestnut blight pathogen *Cryphonectria parasitica*. Maybe it is possible to develop one of our viruses into a biocontrol agent as well. Viruses and mycoviruses as well can be used as tools in molecular biology for both silencing and overexpressing genes in their hosts. Until now such tools are rare for rust fungi therefore our goal is the establishment of one each of the mycoviruses in *U. appendiculatus* and *P. pachyrhizi* as molecular tools.

Before that we want to characterize these viruses. The first step will be verification of the viral sequences using standard PCR and the genome ends using RLM-RACE-PCR. By trying to isolate virus particles we can ascertain the organization of the viruses and the number of different mycoviruses in the fungi.

We also want to clarify the distribution of the mycoviruses. For this we plan to screen as many isolates of *P. pachyrhizi* and *U. appendiculatus* as possible from all over the world for the presence of mycoviruses. In this context we also hope to find virus-free isolates, which should work better in infection experiments and for physiological comparing of infected and non-infected strains.

Then we will begin with the construction of infectious clones. They will be tested in artificial inoculation experiments using biolistics and *Agrobacterium tumefaciens* mediated transformation. Later we will adapt the viruses for silencing or overexpressing interesting genes like effectors or candidate effectors or putative pathogenesis proteins. We would also like to couple candidate effectors to fluorescent proteins for subcellular localization experiments.

HIGS of *Uromyces appendiculatus* on common bean?

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Phaseolus spp. are the most important legumes for human consumption, while the common bean (*Phaseolus vulgaris* L.) including dry beans and green (snap) beans can grow in almost all parts of the populated world. The most important disease of common bean is caused by *Uromyces appendiculatus*.

The rust fungus completes its entire life cycle on *P. vulgaris*. Thus it is called an autoecious and macrocyclic rust fungus. When urediniospores land on the surface of leaves, *U. appendiculatus* undergoes a high degree of morphological and physiological differentiation from urediospores to germ tube, infection hypha, and haustorium. After few days, fresh uredinia burst from both the adaxial and abaxial leaf surfaces.

It is believed that the calcium-signalling pathway is involved in the initial infection and biotrophic growth stages of rust fungi. Several calcium related genes were found in EST collections of different growth stages *of U. appendiculatus*.

Virus-induced gene silencing (VIGS) was developed in plants for rapid functional analysis of plant genes using viruses to deliver silencing constructs. Host-induced gene silencing (HIGS) is a newly developed RNAi technology to indirectly silence parasite genes by expressing an RNAi construct in vivo in the host. HIGS was also successfully used in obligate biotrophic fungi, for example a BSMV-based vector for silencing fungal genes in *Blumeria graminis* and *Puccinia striiformis f. sp. tritici*.

With the goal of clarifying the importance of calcium signalling in rust pathogenesis, we chose genes with sequence homologies indicating a function in calcium signalling from EST collections of *U. appendiculatus*. We measured transcript levels of these genes in different infection stages using quantitative RT-PCR. Using bean pod mottle virus (BPMV) as VIGS vector, we try to establish HIGS on *P. vulgaris* to silence these genes. We expect that silencing calcium related genes could severely debilitate the pathogen. This adds high potential in biotechnological control *of U. appendiculatus* to our project.

Unraveling the role of two fungal effector proteins in the establishment of arbuscular mycorrhiza symbiosis

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The Arbuscular Mycorrhiza (AM) symbiosis is the most widespread mutualistic symbiosis in the majority of all land plants. It is formed by fungi of the phylum Glomeromycota and the plant root system. The most important benefit of this fungal-plant interaction is the bidirectional nutrient exchange. Despite its early origin of about 450 million years ago, only little is known about how the host plant is able to distinguish between a mutualistic symbiotic and a harmful pathogenic fungus. One explanation could be the secretion of so called effector proteins. Effectors are secreted molecules targeted towards the host plant, allowing the fungus to bypass or suppress plant defense responses and to reprogram the host cell. This leads to a facilitated infection process or to the establishment of long-term biotrophic relationships.

Using a YSST-Screen (Yeast Signal Secretion Trap) a novel family of putative effector proteins from the AM-fungus *Rhizophagus irregularis* (formerly *Glomus intraradices*) was identified. SP7 (Secreted Protein7) was the first characterized effector protein from this family with a function in establishing the biotrophic status of the host plant by counteracting the activation of the defense-related transcription factor ERF19 (ethylene-response factor19) (Kloppholz et al. 2011).

A second putative effector protein from this group, called SP31, showing a significant similarity in its domain structure to SP7, is being characterized. SP31 mRNA accumulation was only induced when the fungus was in contact with the plant, but no longer expressed at later stages of mycorrhization, suggesting a specific role for SP31 at earlier stages of the symbiosis. Detailed localization assays are in progress, whereby first results hint at a localization of SP31 within the plant nucleus. All these facts point to a common role for SP7 and SP31. Recently performed microarray analysis of *Medicago truncatula* hairy roots expressing SP7 and SP31, respectively, result in a whole set of common regulated plant genes as well as some genes that seem to be exclusively regulated by SP7 or SP31. Thus a shared regulatory mechanism of both effectors is proposed, whereas each of them seem to execute a specific function. Further analysis of the obtained transcriptomic data is in progress.

Transcriptome of *Trichoderma virens* in interaction with maize or tomato roots: evidence for plant host specificity

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Species belonging to the fungal genus Trichoderma directly antagonize soil-borne fungal pathogens. They also colonize plant roots, promoting systemic resistance. The Trichoderma-root interaction can be hosted by a wide range of plant species, including both monocots and dicots. To test the hypothesis that gene expression by the fungal partner in this beneficial interaction is modulated by the plant host, we cocultured *Trichoderma virens* with maize or tomato in a hydroponic system allowing interaction with the roots. The transcriptomes for *T. virens* alone and with tomato or maize roots were compared by hybridization on Agilent custom oligonucleotide microarrays of 11645 unique probes designed from the predicted protein-coding gene models. At the chosen cutoff for statistical significance, the transcript levels of many genes were modulated by interaction with the roots. Glycoside hydrolases and transporters are highly represented in the sets of transcripts induced by coculture with both maize and tomato. Although some transcripts are shared between the maize and tomato sets, the majority of regulated genes are specific to either the maize or tomato host. The tomato and maize – induced transcriptomes thus differ from each other and from the saprophytic growth transcriptome. Reporter genes chosen from the microarray data will be useful to follow, in time and space, how the fungal cell detects plant signals, and will provide an entry point to the signaling pathways. Secreted proteins released in response to plant signals might have a role in inducing systemic resistance in the plant.

Control of fungal development by targeted activation of the unfolded protein response

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The unfolded protein response (UPR) is a conserved eukaryotic signalling pathway counteracting endoplasmic reticulum (ER) stress during situations of increased demands on the secretory pathway. We identified and characterized the homologs of the central UPR regulators, Hac1 and Ire1 in the plant pathogenic fungus *Ustilago maydis*. The UPR is closely interlinked with the regulatory circuits that control sexual and pathogenic development. Exact timing of UPR-activation is required for faithful execution of various developmental transitions. A C-terminal extension of the *U. maydis* Hac1 homolog, Cib1, mediates direct interaction with Clp1, an essential regulator of fungal development. This interaction leads to stabilization of Clp1, increased ER stress resistance and prevents deleterious hyperactivation of the UPR during in planta development of *U. maydis*. Our data suggest that UPR activation serves as a checkpoint to time developmental progression and the increased activity of the secretory pathway to facilitate effective colonization of the host plant.

Ectomycorrhizal functional plasticity and environmental stress

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Assessing ectomycorrhizal functions in ecosystems is still an experimental challenge. In a number of studies, potential enzyme activities of ectomycorrhizae were used to assess effects of environmental stress conditions (ozone, heavy metals, drought) on important functional traits.

Enzyme activities of ectomycorrhizae were rather resilient under diverse stress conditions. However, enzyme activities related to nitrogen cycling and to oxidative stress reactions were identified as potentially critical functions in ectomycorrhizal communities. To get deeper insights into mechanisms of stress regulation, drought related reactions were studied using a proteomic approach. For this study, pure culture mycelia of *Cenococcum geophilum* were subjected to drought treatments. Drought stress increased the level of oxidised proteins. Moreover, specifically upregulated proteins could be identified for specific protection mechanisms related to drought tolerance or repair mechanisms.

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* [1] and *hdp2*, respectively. We show that *Δhpd2* strains are completely blocked in appressoria formation, whereas *Δbiz1* 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. Systematic deletion analysis led to the identification of *pst1* and *pst2*, both encoding *U. maydis* specific effectors predicted to be secreted. *Δpst1* and *pst2* cells are still able to penetrate the plant surface, but subsequently fail to invade and colonize the plant, resembling the *biz1* deletion phenotype. Based on a bioinformatical approach we identified further *U. maydis* genes encoding pst-like proteins (*plp1*, *plp2*, *plp3*). Deletion of *plp2* causes loss of pathogenicity similarly to the deletion of *pst1/2*. The deletion strain stops after penetration and fails to successfully colonize the plant. *plp1* and *plp3* deletion strains are able to colonize the plant tissue. However, whereas infections with *Δplp3* cells result in less and smaller tumor formation than wild type infections, *Δplp1* cells show no tumor formation. Currently we are characterizing further details of the *Δplp1*, *Δplp2* and *Δplp3* mutant phenotypes.

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Abstracts: Cell Biology

The SPF27-Homologue Num1 Connects Splicing and Cytoplasmic Trafficking Processes in *Ustilago maydis*

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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 and distribution 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 (NTC), which is an integral component of active spliceosomes and required for intron removal during pre-mRNA splicing. In addition to regulating spliceosome formation and splicing fidelity, the complex is involved in the cellular response to DNA damage, cell cycle checkpoint control or formation and nuclear export of mRNP-particles.

Hyphae of *num1* deletion strains exhibit pleiotropic polarity defects and in line with the described NTC functions, the *num1* mutation affects cell cycle regulation and survival upon UV irradiation. In addition, the *num1*-deletion influences splicing, as RNA-Seq analysis revealed reduced splicing efficiencies on a global scale. Using the Yeast Two-Hybrid system and Co-immunoprecipitation analyses, we identified Cdc5 and Prp19, two further conserved components of the Prp19/CDC5 complex, as Num1 interactors. Remarkably, all four introns of the gene encoding the master regulator of filamentous hyphal growth, Rbf1, display increased rates of intron retention, in turn leading to reduced Rbf1-protein levels as well as a deregulation of rbf1-dependently expressed genes. Aberrant splicing of transcription factors or key proteins of the affected processes might be sufficient to explain the observed phenotypes. Surprisingly, however, we also identified several proteins with putative functions during vesicle-mediated transport processes in a Yeast Two-Hybrid screen; in particular the kinesin 1 motor protein Kin1 was shown to physically interact with Num1. Overlapping phenotypes with respect to filamentous and polar growth, vacuolar morphology, Dynein localization as well as endosomal motility corroborate the genetic interaction of Num1 and Kin1.

Our data implicate a previously unidentified connection between a component of the splicing machinery and cytoplasmic trafficking processes. As the *num1*-mutation also affects cytoplasmic mRNA-transport, the protein may constitute a novel functional interconnection between these two disparate mechanisms of splicing and trafficking. According to our model the Num1 protein functions in the coordination of pre-mRNA splicing with nuclear-pore complex dependent export of mRNP-particles and microtubule-based mRNA-transport.

A homologue of the human SPF27 protein stabilizes the NineTeen splicing-Complex in *Ustilago maydis*

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The posttranscriptional modification of mRNA via splicing is an important process in eukaryotic cells. It is mainly catalyzed by the spliceosome, which consists of numerous components. Besides the well-known small nuclear ribonuclear particles (snRNPs), the Nineteen-Complex (NTC) is a major part of the spliceosome and is indispensable for correct snRNP recruitment and thus contributes to splicing efficiency and fidelity.

The human NTC is a protein complex mainly consisting of the four core components SPF27, CDC5, PRP19 and PRL1, which can be found in most eukaryotes. Each of the four proteins plays additional roles in other important cellular processes, like cell cycle control, DNA damage repair as well as mRNP formation and export. Therefore it is not remarkable that in most organisms the deletion of either of the NTC core component is lethal.

Ustilago maydis is a biotrophic Basidomycete, which is known for causing smut disease in maize. The *U. maydis* Num1-protein turned out to be the homologue of the human SPF27 protein. Interestingly, we were able to generate a *num1* deletion strain, indicating that the protein is not essential in *U. maydis*. Using the yeast-two-hybrid (Y2H) system and co-immunoprecipitation (Co-IP) analysis, Prp19 and Cdc5, two *U. maydis* homologues of human NTC core components were shown to be Num1 interaction partners. In addition, the Y2H screen led to the identification of several cytoplasmic interaction partners of Num1, which implicate additional and yet unknown functions for an NTC-component.

The aim of our study is to determine whether the composition of the NTC is different in *U. maydis*, and why the Num1 protein is not essential in *U. maydis*. For this purpose, Prp19-variants fused to an HA- or TapTag were generated, which were then used for Co-IP in wildtype and num1-deletion strains. Our first results after Prp19 Co-IP via HA-tag and subsequent mass spectrometry show that there are less NTC components precipitated in the absence of Num1. This suggests that num1-deletion leads to a decrease of NTC stability and/or that Num1 is missing as a link to other NTC components. The finding that peptides for the core NTC components, with the exception of Prp19, are at least three times less abundant in the *num1*-deletion strain also hints to a reduced stability.

In parallel, Co-IP with Num1-HA-tag led to a list of 89 potential direct or indirect interaction partners which were not present in the WT control. In addition to all known components of the NTC, we also found proteins predicted to be involved in mRNA transport, nuclear export and cytoplasmic transport. To support and further investigate these findings, Co-IPs with the cytoplasmic fraction of cells will be performed.

On the role of the kinesin-3 motor UncA in *Aspergillus nidulans* and *Neuropora crassa* in microtubule specificity, early endosomal transport and polarized growth

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Biological motors are molecular nanomachines, which convert chemical energy into mechanical forces and use microtubules (MTs) as track to transport their cargoes. The combination of mechanoenzymes with structural components, such as the cytoskeleton, enables eukaryotic cells to overcome entropy, generate molecular gradients, and establish polarity. Hyphae of filamentous fungi are among the most polarized cells, and polarity defects are most obvious.

The *Aspergillus nidulans* kinesin-3 UncA uses preferably modified MTs as tracks for vesicle transportation. Posttranslational MT modifications are numerous; however, the biochemical and cell biological roles of those modifications remain mostly an enigma. 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) (1). Our data show that specificity determination depends on the tail rather than the motor domain, in contrast to what has been demonstrated for kinesin 1 in neuronal cells.

To further substantiate our findings in *A. nidulans* we studied an orthologue, NKIN2, in *Neurospora crassa*. We found that NKIN2 localizes as fast-moving spots in the cytoplasm of mature hyphae. To test whether the spots represented early endosomes, the Rab5 GTPase YPT52 was used as an endosomal marker. NKIN2 colocalized with YPT52. Deletion of nkin2 caused strongly reduced endosomal movement. Combined, these results confirm the involvement of NKIN2 in early endosome transport. Introduction of a rigor mutation into NKIN2 labeled with green fluorescent protein (GFP) resulted in decoration of microtubules. Interestingly, NKIN2rigor was associated with a subpopulation of microtubules, as had been shown earlier for *A. nidulans* UncA (2).

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Order needs energy: The compartmentation of fungal plasma membranes

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The discovery of MCC (Membrane Compartment of Can1) and MCP (Membrane Compartment of Pma1), stable microdomains of plasma membranes of *S. cerevisiae* cells, will briefly be summarized. The observation that MCC correspond to membrane invaginations and accumulate ergosterol will be documented. A number of MCC proteins are protected from endocytosis and turnover as long as they do not leave the compartment. This new regulatory phenomenon is named "Control by Change of Location", CCL. Examples of CCL manifestations will be discussed.

When the plasma membrane potential $\Delta\Psi$ is decreased, certain protein members of MCC as well as ergosterol leave the compartment within seconds and distribute homogenously in the membrane (1). The process is reversible. Concomitantly general membrane properties like detergent susceptibility, ergosterol accessibility and passive permeability change dramatically. Thus the plasma membranes of de-energized cells are much less susceptible to a number of detergents and they also bind significantly less ^{3}H-Triton X100 (2). It is suggested that the plasma membrane changes its overall order and packing due to a change of the membrane potential and/or due to an interference of energy dependent associations of cytoskeletal elements with components of the plasma membrane.

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Structural-functional interconnection between components of polar growth apparatus in basidiomycete *Schizophyllum commune* on different stages of life

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The Polar growth apparatus (PGA) is an essential part of growing fungal cells that provides their elongation in a chosen direction. PGA also promotes branching of hyphae that multiplies the number of simualtaneously growing cells and leads to the formation of a mycelium. PGA is a complex intracellular structure occurring at the extreme apex of the terminal cell of actively growing hyphae. It requires a specific kind of plasmatic membrane at the site of growth enriched with sterols and sphingolipids (sterol rich domains, SRDs), cytoskeletal components such as microtubules (MTs) and actin filaments (AFs) with their motor proteins. PGA also includes several specific organelles like the polarisome and Spitzenkörper (Spk) and lots of different regulatory factors.

Schizophyllum commune is a common wood-destructive basidiomycete fungus, which can form cyphelloid fruiting bodies and demonstrates classical “hymenomycetal” life cycle. At all stages morphogenetic processes are always connected with constant growth of different hyphae. We performed a comparison between PGAs in growing hyphae from different stages of *S. commune* life cycle: germinating basidiospores, monokaryotic hyphae, dikaryotic vegetative hyphae and dikaryotic hyphae from developing FBs. Following PGA’s components were selected for examination: AFs, class V myosin motors, MTs and SRDs. Visualization of these components was implemented by means of fluorescence microscopy with rhodamine-phalloidin staining for AFs, filipin staining for SRDs and with antibodies against actin, myosin V and α-tubulin.

Functional interconnections between the components of PGA were examined by means of specific inhibitors: cytochalasin A for AFs` disassembling (CyA; 50 and 80 mkg/ml), 2,3-butadione monoxime (BDM; 50 and 100 mM) and ML7 (20 mkg/ml) for myosin V deactivation, nocodazole (2 mkg/ml) for MTs` depolimerization and myriocin (80 mkg/ml) for inhibition of sphingolipid biosynthesis and disturbance of SRDs` structure. Exposure times were separately fitted to each inhibitor. All experiments were conducted at 25°C in the dark with exceptions for developing primordia for which incubation with day light and 18°C was applied.

Resting basidiospores have shown uniform distribution of actin and myosin V *per se* under their envelopes. Initial step of the germ hypha formation was characterized by an appearance of SRDs in the plasma membrane and a terminal actin spot directly below it at the growth point. During the germination process MTs in the germ tubes were never observed prior to first septation. Growing monokaryotic and dikaryotic hyphae demonstrated submembrane localization of actin and myosin V at the extreme apex. Membrane of the apical dome was always imbued by filipin suggesting its composition of SRDs.

AFs` destruction led to disappearence of SRDs from apex membrane and vice versa inhibition of sphingolipids biosynthesis resulted in dying-out of AFs. Hyphae in both cases shared the same morphology with rounded tips and demonstrated switching from polarized to isotropic growth. After treatment with BDM the growth rate was reduced in all cases and the formation of thin filiform hyphae was observed sustaining polarized type of this growth. Moreover, these hyphae demonstrated an extremely fine cell wall compared to the wild type.

Comparison of the localization and dynamics of myosins class I, II and V of *Neurospora crassa*

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Myosins are motor proteins associated with actin filaments. Depending on the class, myosins can have transport or scaffolding functions. There are several kinds of myosins in eukaryotic cells but in *N. crassa* there are just three, MYO-1 (Class I), MYO-2 (Class II) and MYO-5 (Class V). We compared the localization and dynamics of the three *N. crassa* myosins tagging them with fluorescent proteins and observed them using confocal microscopy and TIRFM. Additionally we deleted the *myo-1*, *myo-2* and *myo-5* genes to observe the cell phenotype. MYO-2-GFP localizes at developing septa six minutes before any sign of plasma membrane invagination, decorating actin filaments, it is part of the septal actomyosin tangle and also of the contractile actomyosin ring. MYO-2-GFP is not present in the apex, and its mutation seems to be essential. MYO-2 appears to be a protein organizing the actin cytoskeleton in places of septation. MYO-5-GFP is present in the apex occupying part of the Spitzenkörper and the region of the apical dome, there is just a faint fluorescence in subapical and basal parts of the hyphae. MYO-5 is not essential and it is probably associated to the transport of specific secretory vesicles. MYO-1 seems to be essential and it is seems to be associated to the formation and transport of endocytic vesicle.

Development of molecular genetic tools for *Acremonium chrysogenum* to construct a formin deletion strain

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Acremonium chrysogenum is the natural producer of the β-lactam antibiotic cephalosporin C. Due to its antibacterial activity against gram-positive and gram-negative bacteria, this β-lactam antibiotic is of great biotechnological and medical relevance. To optimize cephalosporin C production efficiently, continuous and directed improvement of industrial strains is required. A typical morphological feature of *A. chrysogenum* is the fragmentation of vegetative mycelium into arthrospores. These are uni- or binuclear cells, which develop during a prolonged cultivation under limited nutrient supply. Due to the known correlation of cephalosporin C production and arthrospore formation, we are interested to identify specific regulatory factors affecting both, cephalosporin C biosynthesis and morphological development. However, the genetic manipulation of *A. chrysogenum* is still rather difficult because it lacks a known sexual cycle and produces only few conidiospores. Here, we demonstrate the development of several molecular tools for the genetic engineering of *A. chrysogenum*. Besides the generation of an *Acku70* deletion strain for homologous recombination, the functionality of a xylose-inducible promoter from *Sordaria macrospora* (*Smxyl*) was demonstrated using *egfp* as reporter gene. In further studies, the *Smxyl* promoter was used to establish a one-step FLP/FRT recombination system in *A. chrysogenum*. The combined use of all molecular tools resulted in the construction of a strain, lacking the formin gene. Formin proteins act as nucleators within the assembly of actin filaments and have a controlling function in the dynamic remodeling of the actin cytoskeleton. They are further involved in septation processes of fungal hyphae and might consequently act as putative regulators of arthrospore formation.

Functional analysis of selective autophagy-related genes, *Aoatg11* and *Aoatg26* in the filamentous fungus *Aspergillus oryzae*

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Macroautophagy (hereafter autophagy) is a highly conserved degradation pathway that mediates the turnover of bulk cytoplasmic protein and organelles for quality and quantity control of cytoplasmic components. Generally, autophagy was considered a non-selective pathway induced as a survival strategy in response to cellular stresses such as nutrient starvation. However, selective autophagy, the process, which the substrates are selectively recognized and transported to vacuoles, was also found. Selective autophagy includes cytoplasm-to-vacuole targeting (Cvt) pathway, pexophagy, and mitophagy. The Cvt pathway is a transport route that aminopeptidase (Ape1) is selectively transported to vacuoles and converted to active form. Pexophagy and mitophagy are processes that peroxisomes and mitochondria are selectively delivered to vacuoles, respectively. Although these types of autophagy have been mainly studied in the yeasts *Saccharomyces cerevisiae* and *Pichia pastoris*, it is not clear whether these pathways exist in filamentous fungi. The filamentous fungus *Aspergillus oryzae* is an important microorganism in Japanese fermentative industries, as it plays a role in the production of sake, miso and soy sauce. In addition, *A. oryzae* has been described as an excellent host for the production of homologous and heterologous enzymes. We have studied non-selective autophagy in *A. oryzae*; however selective autophagy in this organism is poorly understood.

Selective autophagy-specific genes were identified among autophagy-related genes in yeast. *S. cerevisiae* Atg11 is known as a common adaptor protein in the Cvt pathway, pexophagy, and mitophagy. Atg11 functions in recognizing and transporting the substrates to pre-autophagosomal structure (PAS), the specific site where variety of autophagy-related proteins are assembled. In this study, to investigate selective autophagy in *A. oryzae*, we analyzed the function of a homolog of Atg11 (AoAtg11). In *Aoatg11* disruptants, the delivery of mitochondria in long term cultivation and peroxisomes induced by oleate to vacuole were decreased. This indicates that mitophagy and pexophagy exist in *A. oryzae*, and AoAtg11 is involved in these processes. Interestingly, AoAtg11 was not required for transporting of AoApe1 to vacuoles, while AoAtg1, which is a core protein for induction of autophagy, was essential for this transporting under the normal growth condition. Further, to focus on pexophagy in A. oryzae, we analyzed a homolog of Atg26 (AoAtg26). Atg26 is essential for pexophagy of large-sized peroxisome in *P. pastoris*, but not in *S. cerevisiae*. In *Aoatg26* disruptants, accumulation of incomplete autophagosomes or intermediate structures was observed in cytoplasm, indicating that AoAtg26 is required for formation of large-sized autophagsomes. Moreover, Aoatg26 disruptants showed a decreased pexophagy. These results suggest that the function of AoAtg26 was not limited to pexophagy, and AoAtg26 may determine the size of autophagosomes in *A. oryzae*.

Involvement of the karyopherin exportin-5 in *Magnaporthe oryzae* pathogenenicity

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The rice blast fungus *Magnaporthe oryzae* is an important plant pathogenic ascomycetous fungi. *M. oryzae*, together with *M. poae* and *Gaeumannomyces graminis*, are economically significant fungal pathogens that belong to the same family, the Magnaportheae. Both *M. poae* and *G. graminis* are soil-borne fungi. By contrast, *M. oryzae* has been studied for causing aerial infections. However, few years ago it was demonstrated that under laboratory conditions *M. oryzae* also produce lesions on roots. The knowledge about requirements of soil-borne fungus like *G. graminis* or *M. poae* to infect roots is limited due to their genetic intractability. Consequently, *M. oryzae* is a good tool to study fungal pathogenic mechanisms developed during leaf and root colonisation. *M. oryzae* Exp5 is the ortholog of the karyopherin exportin-5 from humans and Msn5p from *Sacharomyces cereviseae*. Karyopherins are involved in the translocation of proteins and/or RNAs, between the nucleus and the cytoplasm. The *Δexp5* mutant shows less virulence in root infection assays, suggesting that RNAs or proteins translocated by Exp5 might play an important role during fungal root infection.

We are using different approaches to identify cargo proteins and/or RNAs transported by Exp5. We have tagged the *M. oryzae* Exp5 protein with HA-FLAG to generate amino and carboxi protein fusions. These constructs have been reintroduced into the *Δexp5* mutant. Western blots using anti-HA antibodies have allowed us to select two transformants expressing the functional protein. Both Exp5-HA-FLAG amino and carboxi constructs can restore *Δexp5* defects, indicating that the tag does not alter Exp5 function. Currently co-immunoprecipitation experiments followed by mass spectrometry are underway using these two transformants.

The human and yeast EXP5 orthologs can recognize RNA binding proteins and several transcription factors. To confirm any link between Exp5 and RNA metabolism in *M. oryzae*, we have developed a cellular fractionation protocol which allows the isolation of small RNAs from total, nuclear and cytoplasmic fractions using wild-type (WT) and *Δexp5* strains. Northern assays have been carried out with the probes U6 (small nuclear RNA) and t-RNA-met. These probes are specific RNA markers for the nuclear and cytoplasmic fractions, respectively. Additional probes, including mil-RNA1, mil-RNA2 and mil-RNA3 identified in *Neurospora crassa*, have been used to compare the RNA profile patterns between WT and *Δexp5* strains.

Karyopherins are one of the major nuclear transport receptors in eukaryotes. *S. cerevisiae* has 14 and the corresponding karyopherin orthologues are present in *M. oryzae*. To study the expression levels of these karyopherins and their relationship with Exp5, WT and *Δexp5* strains have been grown in different media: We have obtained total RNA from those samples and used them for carrying out a qPCR assay. Results from these experiments aiming to characterize Exp5 function in *M. oryzae* will be presented and discussed.

Proper actin ring formation and septum constriction requires coordination of SIN and MOR pathways through the germinal centre kinase MST1

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Nuclear Dbf2p-related (NDR) kinases control polar morphogenesis and cell division. In fungi, they function as effector kinases of the morphogenesis (MOR) and septation initiation (SIN) networks and are activated by the pathway-specific germinal centre (GC) kinases POD6 and SID1, respectively. We identified a third GC kinase, MST1, in *Neurospora crassa* that connects both kinase cascades. Genetic and biochemical interactions with SIN components and life imaging identified MST1 as SIN-associated kinase acting in parallel with the GC kinase SID1. SID1 and MST1 were both regulated by the upstream SIN kinase CDC7, yet in an opposite manner, indicating that MST1 is required for fine-tuning the SIN. Lifeact- and formin-GFP reporter constructs revealed the formation of aberrant cortical actomyosin rings in *Δmst-1*, which resulted in miss-positioned septa and irregular spirals. These defects phenocopy those of mor mutants, and we determined that MST1 also interacted with the central MOR kinases POD6 and COT1. MST1 functions as promiscuous enzyme by activating the SIN and MOR effector kinases DBF2 and COT1. In summary, our data identify an antagonistic relationship between the SIN and MOR during septum formation that is, at least in part, coordinated through the GC kinase MST1.

The translocating proteins PMA-1 and PMB accumulate at the plasma membrane independently of the Spitzenkörper in *Neurospora crassa*

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Two proteins, the H^+-translocating ATPase PMA-1 and the arginine transporter PMB were tagged with fluorescent markers to characterize their delivery routes to the plasma membrane in *Neurospora crassa*. PMA-1 is delivered via the classical secretory pathway (ER to Golgi) to the cell surface, where it pumps H^+ out of the cell, contributing to generate a large electrochemical gradient that supplies energy to H^+-coupled nutrient uptake systems, such as PMB. In conidia, both PMA-1 and PMB were evenly distributed at the plasma membrane. During germination and germ tube elongation, PMA-1 and PMB were found all around the conidial plasma membrane and extended to the germ tube plasma membrane, but fluorescence was less intense or almost absent at the apex. In mature hyphae, PMA-1 and PMB localized at the plasma membrane in distal regions and in completely developed septa, but not at the apex. FRAP analysis of PMA-1-GFP at the plasma membrane was carried out to examine vesicular traffic. The source of PMA-1-GFP newly incorporated to the plasma membrane was shown to be primarily the cytoplasm, whereas lateral diffusion from adjacent plasma membrane regions did not contribute significantly in the recovery of fluorescence in bleached areas. Brefeldin A (BFA), an inhibitor used to block the classical ER-to-Golgi secretory pathway, caused the disappearance of PMA-1-GFP found at tubular endomembranes in hyphal subapical regions, suggesting a role of these tubular endomembranes in protein recycling. Upon prolonged exposure to BFA, PMA-1-GFP started to accumulate in putative Brefeldin bodies. FRAP analysis in globular vacuoles in hyphae treated with BFA indicated that the endosomal recycling route was blocked by BFA. Collectively, these results suggested the existence of an additional secretory pathway, by which proteins get incorporated directly at the plasma membrane in hyphal distal regions independently of the Spitzenkörper. Although a similar distribution pattern was observed for PMA-1 and PMB in *N. crassa*, the corresponding homologs in *Saccharomyces cerevisiae* have been shown to occupy different subregions within the plasma membrane. We are further investigating whether this compartmentation of the plasma membrane occurs in *N. crassa*.

Coordination of polarized secretion by the exocyst complex is critical for filamentous growth and cytokinesis in *Ustilago maydis*

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To establish and sustain their polarity, cells have to transport proteins and membrane lipids to defined locations at the growing tip. This is achieved by directional transport of vesicles that fuse with the plasma membrane. Vesicle fusion and active exocytosis requires the presence of an octameric protein complex, the exocyst. In *S. cerevisiae*, two proteins of the exocyst complex, Sec3 and Exo70, were shown to serve as landmark proteins for exocytosis. The other components of the exocyst tether secretory vesicles carrying the Rab GTPase Sec4 to the membrane. Fusion of secretory vesicles occurs via interaction of the exocyst with SNARE proteins. To elucidate the function and regulation of the exocyst complex and its associated proteins in *Ustilago maydis*, we have characterized the Rab GTPase Sec4 and the exocyst proteins Sec3, Exo70 and Sec15 by genetic, cell biological and biochemical approaches. We found that of the two landmark proteins, only one is important for polar growth in *U. maydis*. Interestingly, this gene is not essential, suggesting that in *U. maydis* exocytosis sites can be also marked by alternative mechanisms.

Another essential player for polar growth in *U. maydis* is the exocyst subunit Sec15, which mediates the interaction of the exocyst with incoming secretory vesicles. Conditional mutants of *sec15* are defective in hyphal tip growth and are affected in long-distance transport of secretory vesicles. In contrast to *S. cerevisiae* where Sec4 vesicles are transported along the actin cytoskeleton, long distance transport of vesicles depends in *U. maydis* on the microtubule cytoskeleton. Furthermore, we studied mutants of different motor proteins to get insights into the molecular mechanisms of secretory vesicle trafficking.

Subcellular localization and kinase activity of GK4, a *Phytophthora infestans* GPCR-PIPK involved in actin cytoskeleton organisation

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For dispersal and host infection plant pathogens largely depend on asexual spores. Pathogenesis and sporulation are complex processes that are governed by various cellular signaling networks including G-protein and phospholipid signaling. Oomycetes possess a family of novel proteins called GPCR-PIPKs (GKs) that are composed of a seven trans-membrane spanning (7-TM) domain fused to a phosphatidylinositol phosphate kinase (PIPK) domain. Based on this domain structure GKs are anticipated to link G-protein and phospholipid signalling pathways. Our studies in the potato late blight pathogen *Phytophthora infestans* revealed involvement of one of twelve GKs (i.e. PiGK4) in spore development, hyphal elongation and infection. Moreover, ectopic expression in *P. infestans* of subdomains of PiGK1 and PiGK4 fused to a fluorescent protein showed that the GPCR domain targets the GKs to membranes surrounding different cellular compartments. To further elucidate the function of the PIPK domain we tested kinase activity of PiGK4 both in vivo and in vitro and analysed the relationship between PiGK4, phosphoinositide signaling and the organisation of the actin cytoskeleton using complementation in yeast combined with various live-cell markers.

A PAM2 protein is important for endosomal mRNA transport

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In Eukaryotes, mRNA transport and subsequent localized translation is an important mechanism to regulate gene expression on a spatio-temporal level. This process is conferred by RNA-binding proteins that recognize and bind cargo mRNAs, which leads to the formation of large ribonucleoprotein (mRNP) complexes. The mRNPs are actively transported by molecular motors along the cytoskeleton. Several recent studies provide evidence, that mRNA transport is closely connected to membrane trafficking, but the underlying mechanisms are still unknown. In the filamentous fungus *Ustilago maydis* the RNA-binding protein Rrm4 mediates mRNP transport on Rab5a-positive endosomes, which are transported by the Kinesin-3 Kin3 and split dynein Dyn1/2 along the microtubule cytoskeleton. Rrm4 contains three RRM domains for RNA binding and a C-terminal MLLE domain, which is also found in the poly(A) binding protein Pab1. The MLLE domain confers protein –protein interaction by binding to the conserved PAM2 motif, found in several mRNA-associated proteins. Furthermore, the MLLE domain of Rrm4 is crucial to recruit mRNPs to shuttling endosomes. Based on these information we investigated a potential interaction partner of Rrm4 designated Upa1 (Ustilago PAM2 protein 1). We provide evidence for the hypothesis that Upa1 is a novel component of endosomal mRNA transport.

Live-cell imaging and analysis of modes-of-action of a new generation of small, synthetic antifungal peptides

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The significance of fungal infections has been grossly underestimated, few drugs are available to treat life-threatening fungal infections, and resistance against these drugs is rising. Natural and synthetic antifungal peptides (AFPs) are being actively explored as novel pharmaceuticals. We are investigating the modes-of-action of various small rationally designed AFPs and synthetic AFPs derived from plant defensins. For this purpose we are using the fungal model *Neurospora crassa* and the human pathogen *Aspergillus fumigatus* combined with live-cell imaging of fluorescently labelled AFPs and other live-cell probes, inhibitor treatments and mutant analyses. PAF26 is a de novo-designed hexapeptide possessing two well-defined motifs: N-terminal cationic and C-terminal hydrophobic regions. We have characterized how each motif is responsible for PAF26's dynamic antifungal mechanism of action involving the electrostatic interaction with cells, cellular internalization, and cell killing. PAF26 increases cytosolic free Ca^{2+} and several Ca^{2+} signalling/homeostatic mutants are resistant to the AFP. Using our understanding of the mode of action of PAF26, we are synthesizing new peptides and peptoids with improved antifungal activity. Overall our results provide new mechanistic insights into the mode-of-action of AFPs that should help us design new synthetic AFP-based drugs with improved activity and stability.

Transcriptome-wide insights into Rrm4-mediated mRNA-transport in the pathogen *Ustilago maydis*

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Posttranscriptional control, conveyed by RNA-binding proteins, is an important regulatory aspect during the development of eukaryotic organisms. A prerequisite for the successful infection of host plants by the pathogenic fungus *Ustilago maydis* is the morphological switch from yeast-like to filamentous growth. Increasing evidence indicates that RNA-binding proteins (RBPs) are involved in this developmental program. Rrm4 is an ELAV-like RBP, sharing the canonical domain architecture of three N-terminal RRM domains. The protein is the key factor for microtubule-dependent mRNA transport in *U. maydis* and deletion of *rrm4* leads to impaired filamentous growth. Previously, a small set of bound transcripts was identified encoding proteins important for growth, e.g. polarity factors. This approach however was not saturated and up to now a clear binding motif remains elusive. In order to understand underlying principles of Rrm4-mediated mRNA transport in more detail and at the transcriptome-wide level, we applied the in vivo UV-crosslinking technique iCLIP. iCLIP experiments showed that potential binding sites were predominantly enriched in the 3´UTR of target transcripts. Furthermore, a novel binding motif was identified, which was shown to be required for the in vivo interaction with Rrm4 in a heterologous system. Overall, Rrm4 binds a large number target transcripts encoding for a multitude of functionally diverse proteins. Importantly, a large fraction of the identified target transcript was not found in previous approaches. Interestingly, a number of novel potential target mRNAs encode for proteins involved in membrane-trafficking. Rrm4-mediated mRNA-transport appears thus to play a global role during filamentous growth of *U. maydis*.

The 1/3 inositol polyphosphate kinase Asp1 regulates polarized growth in the fission yeast *Schizosaccharomyces pombe*

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Polarization of a cell can be defined as the generation and upkeep of a specific cellular organization. In fungi the read-out of cell polarization is polarized growth, which occurs by allowing growth only at specific regions of the cell. The rod-shaped fission yeast *Schizosaccharomyces pombe* maintains its single celled yeast form by restricting growth to the cell tips. Growth site selection in this organism is cell cycle controlled as during G2 phase a sharp transition from monopolar to bipolar growth (called NETO) occurs. Expansion of the yeast cell depends on a localized crosstalk between the actin and microtubule cytoskeletons as well as the cell membrane. Two external signals, namely nutrient limitation and mating pheromones lead to a switch of the *S. pombe* intrinsic vegetative growth mode. Both signals lead to a dramatic redirection of growth. However the input signaling and morphological changes induced by these extrinsic signals are vastly different.

We find that the conserved *S. pombe* Asp1 protein regulates these types of polarized growth as it is required for NETO and the ability to adjust the growth mode in response to extrinsic signals. Asp1 is a member of the highly conserved Vip1-like 1/3 inositol polyphosphate kinase family. Members of this family have a dual domain structure consisting of an N-terminal kinase domain that generates specific inositol pyrophosphates and a C-terminal domain with homology to acid phosphatases. The latter domain has no enzymatic activity, instead we have shown via *in vivo* and *in vitro* assays that it negatively regulates the function of the kinase domain. Dissection of the Asp1 protein revealed that the generation of inositol pyrophosphates is a prerequisite for the regulation of polarized growth. NETO and the dimorphic switch in response to nutrient limitation do not occur in the absence of Asp1 kinase activity, while the adaptive growth in response to pheromone is delayed. How inositol pyrophosphates specifically regulate polarized growth is presently under investigation. However the modulation of microtubule dynamics and thus an altered deposition of so-called landmark proteins, which define growth zones are changed in cells that express non-physiological levels of inositol pyrophosphates.

The role of different microtubule populations in *Aspergillus nidulans*

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The microtubule (MT) cytoskeleton is not as rigid and uniform as the name implies, but is characterized by its dynamic instability. In addition, MTs can be made up of different tubulin isoforms and can be post-translationally modified (PTM). MT modifications such as posttranslational acetylation and polyglutamylation are evolutionarily old "inventions" and occur in primitive eukaryotes such as *Giardia lamblia*, whereas detyrosination appeared later during evolution. In this work we found that the kinesin-3 of *Aspergillus nidulans* and of *Neurospora crassa* bind preferentially to a subpopulation of MTs, which are more stable during mitosis (1, 2).

Detyrosination of alpha-tubulin is a reversible reaction in which the C-terminal tyrosine is removed by a carboxypeptidase and re-added by a tubulin-tyrosin-ligase (TTL). In rat neurons kinesin-1 can distinguish between tyrosinated and detyrosinated MTs with a specific TERF sequence in the ß5L8-loop of the motor domain (3). With introduction of this sequence in *A. nidulans* kinesin-1, we could show that kinesin-1-ß5L8 prefers the same subpopulation like kinesin-3. Those results suggest that different MT populations exist in *A. nidulans*.

To confirm the presence and impact of detyrosinated tubulin in *A. nidulans*, we searched for microtubule modifying enzymes. We identified a putative tubulin-tyrosin-ligase (TtlA) and constructed a triple mutant strain in which we deleted *ttlA*, *tubB* and the terminal tyrosine of TubA (TubAΔY). Thus this strain exclusively should produce detyrosinated alpha-tubulin. The strain shows a reduced growth rate and produces less spores. Specific antibodies against detyrosinated TubA decorated all microtubules in this strain, whereas in wild type only very faint signals were obtained. We also constructed a strain in which detyrosinated TubB was created. This strain did not display any obvious phenotype, similarly to the strain in which only TubA was detyrosinated. However, the combination of detyrosinated TubA and detyrosinated TubB, resulted in a phenotype similar to the one of the triple mutant *(ΔtubB, ΔttlA, tubAΔY*). These results show that tyrosinated tubulin has specific functions, which cannot be fulfilled by detyrosinated tubulin. Deletion of *ttlA* in the TubAΔY background produced a similar phenotype, suggesting TubB as substrate for the tubulin tyrosine ligase (TTL). The biochemical analysis of tubulin and modified tubulin by Mass-spec analysis is under way.

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Analysis of septal microtubule organizing centres in *Aspergillus nidulans*

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In *Aspergillus nidulans* spindle pole bodies (SPBs) and septum-associated microtubule-organizing centres (sMTOCs) polymerize cytoplasmic microtubules. Previously, we identified a novel MTOC-associated protein, ApsB (*Schizosaccharomyces pombe mto1*), whose absence affected microtubule formation from sMTOCs more than from SPBs, suggesting that the two protein complexes are organized differently (Suelmann et al., 1998; Veith et al., 2005). Surprisingly, we discovered that ApsB localizes to a subclass of peroxisomes apparently without a peroxisomal targeting motif. However, we found that ApsB interacts with the Woronin body protein HexA, which has a PTS1 motif at the C-terminus (Zekert et al., 2010). Our hypothesis is that ApsB is imported to peroxisomes by a piggyback import mechanism along with HexA, but HexA is not the only transporter for ApsB. To further investigate the role of peroxisomes in microtubule orgnization, we created a deletion mutant of *pexC*. PexC is an essential protein for peroxisomal biogenesis (Heiland & Erdmann, 2005). The *pexC* mutant partly phenocopied the *apsB* mutant phenotype, which further suggests that peroxisomes play a role in microtubule organization via ApsB.

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Functional and structural investigation of ScSUN4 a fungal inner-cell-wall protein required for cell wall biogenesis and cell septation

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SUN4 is the fourth member of the SUN gene family from *Saccharomyces cerevisiae*. The founding members of this family SIM1, UTH1 and NAC3 share high sequence homology in their 258 amino acid spanning C terminal domain. These members are involved in different cellular processes like ageing, DNA replication or mitochondrial biogenesis. The recently identified members AfSUN1p from *Aspergillus fumigatus* and CaSUN41p from *Candida albicans* belong to a novel glycoside hydrolase family (GH132) of β-1,3-glucan modifying enzymes with transglycosylation activity. Although SUN4 hydrolyses β-1,3-oligomers, from dimers up to insoluble β-1,3-glucan are by SUN4 hydrolysed, SUN4 proteins show much lower activity in comparison to other beta-glucosidases (e.g. Laminarinase) much lower hydrolysis activity. To understand how the hydrolysis and transglycosylation activity proceed mechanistically and to explain the overall low activity, structural insights are necessary. A high-resolution protein crystal structure (1.10 Å) of ScSUN4p from *S. cerevisiae* could be solved by single-wavelength anomalous diffraction (SAD) experiments. ScSUN4p revealed a two-domain architecture consisting of a thaumatin-like fold and a second domain of yet novel structural organisation. Highly conserved amino acid residues form a groove with a central cavity where β 1,3-glucan polysaccharide chains can be bound and hydrolysed. Because of the ubiquitous occurrence of SUN proteins they are an interesting pharmaceutical target for fungal infections.

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In vitro analysis of the Chitin synthase III complex in *Saccharomyces cerevisiae*

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The cell wall of fungi is essential for survival in hostile environments. Though the fungal cell wall varies in composition and structure, one common component is chitin. In its pure form, chitin is a β-(1-4)-linked linear polymer of N-acetyl-glucosamines, while chitin from natural sources usually contains also a varying percentage of deacetylated glucosamine moieties. The general pathway of chitin synthesis is highly conserved. The last polymerization step is catalysed by a membrane-integral glycosyltransferase known as chitin synthase (Chs).

The number of chitin synthases in fungi varies from one to seven. In *Saccharomyces cerevisiae*, there are three chitin synthases, of which one is part of a complex that produces more than 90 % of the cell wall chitin in vivo. This complex is named CSIII and consists of the catalytic subunit Chs3 and the regulatory subunit Chs4.

Previous studies demonstrated that genetic deletion of Chs4 impairs chitin formation in yeast, while Chs3 is still transported correctly to the plasma membrane. A similar localization was observed for Chs4 in chs3 strains suggesting different trafficking routes to the membrane. Furthermore, yeast-two-hybrid studies indicate that Chs4 interacts with the N-terminal domain of Chs3.

To obtain a better understanding of Chs4 and its role in chitin synthesis, we focus on protein-protein-interactions between Chs4 and two different N-terminal domains of Chs3, which had been suggested to be involved in binding of Chs4. For this purpose, his-tagged versions of Chs4 and the two Chs3 domains were expressed with the maltose binding protein as recombinant fusion proteins in *E. coli* and purified via Ni-NTA and amylose columns. Binding studies and far-western blot analysis could not confirm a physical interaction between the Chs3 domains and Chs4 so far.

Targeting of Chs3 to the bud neck is controlled by phosphorylation. To map possible phosphorylation sites, we treated the purified Chs3 domains with protein kinase A and detected a protein shift in Phos-tag gels for both domains indicating phosphorylation in vitro. The final identification of the phosphorylation sites can now be analyzed by mass spectrometry.

Microtubule organizing centers in *Neurospora crassa*

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Microtubule organizing centers (MTOCs) are multipeptide complexes found on the minus ends of microtubules (MTs). Gamma-tubulin is the major MT nucleator found in eukaryotic cells. In order to find other proteins involved in the MTOCs of *N. crassa*, we tagged gamma-tubulin, GRB (a pericentrin homolog to Pcp-1 of *S. pombe*) and APS-1 (*A. nidulans* ApsB homolog) with GFP and/or mCherry Gamma-tubulin was present in the Spindle pole bodies (SPB) of nuclei as a single bright spot in interphase nuclei and as two opposite spots in mitotic nuclei, there was no gamma-tubulin in any other location. The pericentrin GRB is responsible of the recruitment of the gamma-tubulin to the inner plate of the SPB, and it is important to ensure the correct mitotic spindle assembly. GRB was observed in the SPBs of the nuclei. It completely co-localizes with gamma-tubulin and both have the same dynamics. GRB co-localizes with centromeric histone 3 CenH3 in interphase nuclei and it is embedded in the nuclear envelope. It seems that the positioning of the gamma-tubulin complex in the inner side of the SPB is GRB dependent but it was not possible to be corroborated because the deletion of *grb* gene is lethal. APS-1 is present in the SPB co-localizing with gamma-tubulin and also is present as gamma-tubulin free accumulations in the cytoplasm but not in septa. Gamma-tubulin in *N. crassa*, as in other organisms, is essential but not APS-1. To conclude, Gamma-tubulin and GRB are present exclusively in the SPBs and both are essential. APS-1 is a possible Gamma-tubulin free MTOC, but this needs to be confirmed.

The cell end marker TeaA and the microtubule polymerase AlpA contribute to microtubule guidance at the hyphal tip cortex of *Aspergillus nidulans*

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In the absence of landmark proteins, hyphae of *Aspergillus nidulans* lose their direction of growth and show a zigzag growth pattern. Here, we show that the cell end marker protein TeaA is important for localizing the growth machinery at hyphal tips. The central position of TeaA at the tip correlated with the convergence of the microtubule (MT) ends to a single point. Conversely, in the absence of TeaA, the MTs often failed to converge to a single point in the cortex. Further analysis suggested a functional connection between TeaA and AlpA (MT polymerase XMAP215 orthologue) for proper regulation of MT growth at hyphal tips. AlpA localized at MT plus ends, and bimolecular fluorescence complementation assays suggested that it interacts with TeaA after MT plus ends reached the tip cortex. In vitro MT polymerization assays showed that AlpA promoted MT growth up to seven-fold and that the C-terminal region of TeaA increased catastrophe frequency. Thus, the AlpA activity controlled through TeaA may be a novel principle for MT growth regulation after reaching the cortex. In addition, we present evidence that the curvature of hyphal tips also could be involved in the control of MT growth at hyphal tips.

Takeshita, N., Mania, D., Herrero de Vega, S., Ishitsuka, Y., Nienhaus, G.U., Podolski, M., Howard, J. & Fischer, R. (2013) The cell end marker TeaA and the microtubule polymerase AlpA contribute to microtubule guidance at the hyphal tip cortex of *Aspergillus nidulans* for polarity maintenance. J. Cell Sci., in press.

A functional orthologue of the human tumor suppressor APC protein plays a role in polarity determination in the filamentous fungus *Aspergillus nidulans*

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Polarized growth is an essential mechanism conserved in all kingdoms and very obvious in filamentous fungi like *A. nidulans*. The cell needs a well-functioning polarization machinery to initiate and sustain a highly polarized structure such as a hypha. The microtubule and the actin cytoskeleton along with microtubule associated proteins (MAPs) such as microtubule plus-end tracking proteins (+TIP's) play key roles in establishing and maintaining an internal polarity axis. In order to ensure a continuous supply of cell wall or plasma membrane components, vesicles and other cargos are transported anterograde to the hyphal tip along the microtubule and the actin cytoskeleton. In addition, microtubules define the site of actin polymerization through the delivery of cell end marker proteins (1).

Here we describe KarA from *A. nidulans*, which is the first functional orthologue of the human tumor suppressor adenomatous-polyposis-coli (APC) protein in filamentous fungi. APC is an essential regulator of radial glial polarity and construction of the cerebral cortex in mice (2). Furthermore it regulates axon arborization and cytoskeleton organization. KarA is related to Kar9p from *Saccharomyces cerevisiae*. It interacts with the membrane associated ApsA protein and is involved in spindle positioning during mitosis. This function is conserved in comparison to *S. cerevisiae*. *A. nidulans* KarA forms a homodimer and is able to bind filamentous α-tubulin. Moreover, KarA is also associated with septal and nuclear microtubule organizing centers (MTOCs) and is transported in an EbA-dependent manner to assembling and retracting microtubule plus-ends. This characteristic classifies KarA as a +TIP. In contrast to Kar9p, KarA has another unexpected function. It is required for microtubule convergence and correct localization of the cell end markers TeaR and TeaA at the hyphal tip. KarA also interacts with the class V myosin MyoV (3). Taken together we propose an active actin-myoV-karA-dependent guidance mechanism of microtubules in the hyphal tip, which we specified as KarA-pathway. Hence, actin and microtubule organization depend on each other.

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Mechanistic investigation of uniparental mitochondrial DNA inheritance in *Ustilago maydis*

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The *U. maydis a2* mating type locus genes *lga2* and *rga2* are critical for uniparental mitochondrial DNA (mtDNA) inheritance during the sexual cycle. Specifically, Lga2 triggers elimination of a1-associated mtDNA, while Rga2 protects a2-associated mtDNA from Lga2. In addition, the mitochondrial p32 family protein Mrb1 plays an essential role in controlling the impact of Lga2 such that only one parental mtDNA population is lost. To gain mechanistic insight into selective mtDNA elimination the mitochondrial proteom was analysed in response to conditional Lga2 overexpression. In particular, this revealed increased levels of Mrb1 as well as of eight mitochondrial ribosomal proteins. Interaction between Mrb1 and a subset of these proteins was verified based on the yeast-two-hybrid system. Together, this suggests that Mrb1 enhances mitochondrial protein expression to compensate for adverse Lga2 effects during selective mtDNA elimination. Furthermore, a yeast-two-hybrid screen revealed the *U. maydis* homolog of yeast Mdm12, which is crucial for vegetative mtDNA inheritance, as potential physical interaction partner of Rga2. The identification of higher order complexes involving either Lga2 or Rga2 is currently approached by tandem affinity purification. In addition, a role of mitophagy in uniparental mtDNA inheritance was addressed.

Characterization of the unconventionally secreted endochitinase Cts1 from *Ustilago maydis*

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The corn smut fungus *U. maydis* contains a versatile set of secreted proteins that play important roles in nutrition, cell morphology and plant infection. The majority of these proteins is secreted by the conventional pathway encompassing transit through the ER and Golgi. Recently, the unconventional secretion of a bacterial-type endochitinase, Cts1, has been demonstrated. Enzymatic activity towards a chitotrioside substrate can be detected on the cell surface and in culture supernatants although the protein lacks a canonical N-terminal secretion signal. Until now, the mechanism of Cts1 export is unknown. However, it has been shown, that the key RNA-binding protein for long-distance mRNA transport along microtubules, Rrm4, is essential for efficient secretion of Cts1. This suggests a novel connection between mRNA transport and secretion of distinct fungal proteins.

To further characterize Cts1, the full-length protein was produced heterologously in *E. coli*. Comparable to the situation in the natural host, the purified protein showed hydrolytic activity towards the chitotrioside substrate, demonstrating its enzymatic function. The recombinant protein was furthermore able to bind chitin, although no typical chitin-binding domain could be identified by bioinformatics analyses. Interestingly, albeit its chitin-binding properties, the enzyme was not able to degrade crystalline chitin isolated from crab shells and furthermore, did not display antagonistic activities to other fungi in confrontation assays. Further advances in studying the function of this fungal endochitinase will be presented.

Identification of proteins involved in the cellular localization of the *Aspergillus nidulans* asexual developmental regulator FlbB

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Asexual development (conidiation) in the model ascomycete *Aspergillus nidulans* is induced when submerged hyphae make contact with the air. This event triggers the function of Upstream Developmental Activators (UDAs), a set of proteins, which regulate the early stages of formation of asexual structures or conidiophores. UDAs also activate a conidiation-specific set of regulators, which complete the developmental process, leading to the biogenesis of conidia. FlbB is a bZIP-type transcription factor (TF), which is required for induction of asexual development. This TF displays a complex subcellular localization pattern in vegetative cells, being located at the hyphal apex and the most apical nucleus. The actin cytoskeleton plays an essential role in the establishment of the apical localization. Latrunculin B allows the transport of a constitutively expressed GFP::FlbB chimera to subapical regions but inhibits its accumulation at the hyphal apex.

FlbE is a protein situated at the hyphal apex in an actin-dependent manner. FlbB and FlbE are mutually required for the apical localization and form a complex. The conservation of a functional bZIP domain is sufficient for FlbB has binding to FlbE, but not for apical localization.

Interestingly, *flbE* shares its promoter with a putative small (sigma) subunit of the clathrin-associated heterotetrameric adaptor complex AP-2 (An0722). This protein has been attributed a role in protein sorting at the plasma membrane during Clathrin Mediated Endocytic vesicle formation. We observed that in the *ΔAn0722* background apical localization of FlbB was lost. The null *An0722* mutant showed severe defects in polarity establishment and vegetative growth. Furthermore, it had remarkable abnormalities in conidiophore production: conidiophore vesicles did not present swelling, they produced a limited number of metulae which budded abnormally into phialides. Despite these features, *ΔAn0722* generated some viable conidia.

We also observed that specific myosin motor proteins (MyoB and MyoE) were important for determining the correct localization of FlbB. *myoE* deletion provoked a variation in the apical distribution of FlbB, probably as a consequence of the effects of MyoE absence on the organization of the Spitzenkörper. However, both *myoB* and *myoE* deletions strongly inhibited the accumulation of FlbB at the apical nucleus.

Taken together, the results of this work show that FlbB nuclear and apical localizations are severely dependent on the actin cytoskeleton, and open an new avenue for more detailed molecular studies on the molecular mechanisms that control FlbB localization and function.

Abstracts: Gene Regulation

A cyclopropane fatty acid synthase gene is essential in fruiting body development of *Coprinosis cinerea*

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Coprinopsis cinerea is an inkcap that is cultured in some Asian and African countries on agricultural wastes for human consumption. In science, the fungus serves as an ideal model fungus to study fruiting body development in basidiomycetes since *C. cinerea* completes its whole life cycle on artificial media in the laboratory within two weeks. Fruiting body initiation is controlled by environmental conditions such as light, temperature, nutrients, and humidity as well as genetically by the A and B mating type genes as the master regulators of sexual development. Fruiting bodies normally form therefore only on the dikaryon [1]. However, specific mutations in the two mating type loci (*Amut* and *Bmut*) allow mushroom development also on mutant homokaryons without a need of previous mating [2]. In consequence, *Amut Bmut* homokaryons are used for the generation and screening of dominant as well as recessive mutations in the fruiting process. In our laboratory we possess a large collection of mutants from homokaryon *AmutBmut* (ca 1200 different clones) that have either been generated by UV- or by REMI mutagenesis [3; unpublished]. UV-mutant 6-031 was the first strain analyzed from our collection. The mutant is defective in formation of hyphal aggregates (secondary hyphal knots) as an early step in fruiting body development. Complementation studies of the recessive mutant gene with a wildtype library detected a gene *cfs1* whose protein product has strong sequence similarity with bacterial cyclopropane fatty acid synthases [4].

In *Escherichia coli*, cyclopropane fatty acid synthase (Cfa) is synthesized when a bacterial culture enters the stationary phase. The enzyme alters the acyl chains of membrane phospholipids in response to changing environmental conditions. Cfa transfers a methylene group from an S-adenosyl-L-methionine to the double bond of an unsaturated fatty acid chain. As a result, a cyclopropane ring is formed on the alkyl chain. Production of cyclopropane fatty acids helps to make membranes better resistant to acids [5]. In this study, we make use of a cfa mutant of *E. coli* in an acid-resistance test system. The defect in acid-resistance in the *E. coli* mutant can be complemented by transformation of the wild-type gene [5]. In this work, we cloned an isolated cDNA from *cfs1* of *C. cinerea* behind a bacterial promoter and transformed the construct into the *E. coli cfa* mutant. As the E. coli cfa wild-type gene, introduction of the cfs1 gene from *C. cinerea* confers acid resistance to the bacterial cells.

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Growth response of *Penicillium rubens* after drought

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Fungal growth in the indoor environment affects human health. To prevent indoor fungal growth it is important to understand how indoor fungi are able to grow under the difficult conditions of the indoor environment, such as fluctuating humidity and porous substrates. To understand the response on drought of the indoor fungus *Penicillium rubens*, its growth was tested both on malt extract agar (MEA), and on a commonly used porous building material: gypsum ($CaSO_4 \cdot ½H_2O$). For experiments with MEA, conidia of *P. rubens* were inoculated on a polyvinylidene fluoride membrane (pvdf), and placed on MEA with high water activity (aw =0.99) to grow. At different life phases the pvdf membrane, with growing colony, was transferred to MEA with low water activity (aw=0.4). After this dry period the pvdf membrane with colony, was transferred back to MEA (aw=0.99), and the lag time for regrowth and growth rate were measured.

In a subsequent experiment a suspension of *P. rubens* was spray-inoculated on gypsum samples. Again the effect of different dry periods, at different stages in the life cycle of *P. rubens*, was tested. Digital images were analyzed using Matlab code to quantify and measure sporulation on gypsum, as an indirect measure of growth.

For the experiments on MEA we observed that exposure to a dry period before germination tubes had formed, did not have an effect on the germination time. When a dry period was introduced during germination, we did observe a delay in germination. A dry period after germination tubes had been formed, caused a lag time for re-growth. A short dry period; 30 min, 1h or 4h, showed a lag time for re-growth more or less equal to the time a remaining conidium would take to germinate and grow from the sporulating zone to the edge of the colony. However, when the dry period was extended to 48 h, the lag time increased more than can be explained only with conidial germination and growth to the edge of the colony.

For the experiments on gypsum we measured that the most vulnerable moment to drought is approximately after 48h of incubation at high relative humidity (RH=97%), that is ±30h after onset of germination. We propose that on gypsum a large spread in germination time between conidia, serves as a survival mechanism to the erratic conditions of the indoor environment such as fluctuations in humidity.

In addition on gypsum, we measured that exposure to a short dry period; 1h up to 12h, had no or little effect on the moment of sporulation. However, increasing the dry period to 24h or 48h we did observe an increase in sporulation time. After a dry period of 48h, the required time until sporulation was equal to the time remaining conidia would need to start germination and form conidiophores. Indicating that during a long dry period the existent mycelium is not capable of survival.

From these studies we conclude that for growth of *P. rubens* on MEA, and on gypsum, mycelium is most vulnerable to drought. Mycelium is only capable of recovery after shorter dry periods (<24h). The weakest moment for a growing colony is when almost all conidia have germinated, but no sporulation has yet occurred. Furthermore we conclude that after longer periods of drought, conidia have an essential role in re-growth of the colony.

A submerged culture using whole barley is associated with the up-regulation of amylases and post-translational pathway closer to a solid culture of *Aspergillus kawachii*

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We previously described a novel submerged batch culture system that produced high levels of amylase by *Aspergillus kawachii* using whole barley (WB), the surface of which is covered by its husk. High levels of glucoamylase and acid-stable α-amylase were produced in this culture, and expression levels of amylases, as well as glucose-repressive genes including high-affinity glucose transporter and peroxidase/catalase were also high. On the other hand, the morphology of mycelia was altered, with swollen, bulbous, multi-septum hyphae and conidiophores that normally form in a solid culture being partially generated. Furthermore, cell cycle and post-translational modification-related gene expression levels were altered, and were similar to those in the solid culture. These findings suggest that high amylase productivity in the submerged culture using WB is accompanied by both the up-regulation of amylase genes and activation of post-translational modifications due to fungal morphological changes being brought closer to those in the solid culture.

Functional analysis of genes in the mating type locus of *Botrytis cinerea*

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Botrytis cinerea is a heterothallic ascomycete with two mating types, MAT1-1 and MAT1-2, each containing two genes. Besides the archetypal genes encoding the MAT1-1-1 (alpha-domain) protein and the MAT1-2-1 (HMG-box) protein, each idiomorph contains one additional gene, designated MAT1-1-5 and MAT1-2-4, respectively. Homologs of these genes are only found in closely related taxa, and their function is as yet unknown.

Knockout mutants were generated in all four genes in the *B. cinerea* MAT locus, either in the MAT1-1 strain SAS56 or in the MAT1-2 strain SAS405. Mutants were crossed with a strain of the opposite mating type, either the wild type or a knockout mutant, in all possible combinations.

Knockout mutants in the MAT1-1-1 gene and the MAT1-2-1 gene fail to show any sign of primordial outgrowth and are entirely sterile. This confirms the essential role of the alpha-domain protein and the HMG-box protein in the mating process. By contrast, mutants in the MAT1-1-5 gene and the MAT1-2-4 gene do produce stipes, but these fail to develop further into an apothecial disk. The MAT1-1-5 and MAT1-2-4 mutants show identical phenotypes, suggesting that these two genes jointly control the transition from stipe to disk development.

RNAseq data were obtained from a cross between two wild type strains and from a cross involving a MAT1-1-5 knockout mutant, from tissue at the stage of transition from stipe to disk. Differential gene expression analysis was performed to identify genes that are possibly involved in development of the apothecial disk.

In conclusion, our study revealed that the MAT1-1-1 and MAT1-2-1 are required for sexual reproduction of the heterothallic ascomycete *B. cinerea*. We were able to demonstrate that MAT1-1-5 and MAT1-2-4 play a key role in the apothecium disk development.

Interaction between AraR, XlnR and GalR in *Aspergillus nidulans*

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The xylanolytic regulator XlnR and the arabinanolytic regulator AraR control pentose catabolism in *A. niger* and *A. nidulans*. The role of AraR differs between these species as *A. niger ΔxlnRΔaraR* is not able to grow on L-arabinose, whereas *A. nidulans ΔxlnRΔaraR* still grows on this substrate. The D-galactose oxido-reductive pathway in *A. nidulans* makes use of pentose catabolic pathway enzymes and one gene encoding such an enzyme has been shown to be under control of the galactose-responsive regulator GalR. GalR is unique to *A. nidulans*, which might explain the observed difference in L-arabinose catabolism between *A. niger* and *A. nidulans*. In this study the interactions of XlnR, AraR and GalR were investigated in more detail by studying the phenotype of double and triple disruptant strains of these regulators in *A. nidulans. A. nidulans ΔxlnRΔaraRΔgalR* was able to grow on L-arabinose and expression of *ladA*, larA and *xdhA* was identical to that in *ΔxlnRΔaraR*. Thus, the presence of GalR does not explain the difference in pentose utilization between *A. niger* and *A. nidulans*. However, araR does seem to have a role in D-galactose catabolism as growth of the *ΔaraR* strain was strongly reduced on D-galactose and galactitol.

XlnR and AraR strongly effect physiology of *Aspergillus niger* solid cultures with wheat bran as carbon source

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Aspergillus niger is a highly efficient degrader of plant biomass and one of the main regulatory factors in this proces is the (hemi-)cellulolytic regulator XlnR. In response to the presence of D-xylose, this regulator activates genes involved in cellulose and hemicellulose degradation, but also some genes of the pentose catabolic pathway. We recently described a homolog of XlnR, AraR, that has a similar role in that it activates genes involved in release and conversion of L-arabinose. These two regulators interact with each other and together are essential for an efficient utilization of plant biomass by *A. niger*.

Most studies into regulation of plant biomass degradation in *A. niger* are performed using submerged cultures. In this study we dissected the influence of XlnR and AraR during growth on wheat bran on solid media to be closer to the natural situation of the fungus. Mutants were generated in which either *xlnR* or *araR* or both were disrupted and the resulting strains were analyzed for enzyme activity, gene expression and protein production in 5 zones of the colony, from center to periphery.

Clear differences were observed between the mutants and the wild type, in particular with respect to genes involved in plant biomass degradation. Results from this study will be presented.

Study of the MAPK signaling cascade in response to mechanical injury in *Trichoderma atroviride*

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Trichoderma atroviride is a filamentous fungus that lives in soil and can be associated with plant roots. *T. atroviride* develops asexual structures (conidiophores) in response to different stresses such as light, nutrient limitation and mechanical damage. The latter is of particular interest because the existence of a conserved mechanism in *T. atroviride* similar to that found in plants and animals has been discovered. This mechanism involves the production of reactive oxygen species (ROS) by the NADPH oxidase complex, since Nox1 and NoxR mutants are affected in conidiation in response to damage. However, the mechanism by which the signal is transduced has not been elucidated. It has been reported that the activation of MAPK (Mitogen Activated Kinases) cascades regulates various types of stresses including oxidative stress, development and spore functions in filamentous fungi. Interestingly, the activation of MAPKs can be regulated through reactive cysteines to form intra or intermolecular disulphides bonds in response to oxidative stress.

Therefore, we aim to understand the role of the MAPKs Tmk1 and Tmk3 and their mechanism of activation that leads to the development of conidiophores in the response to injury. The *Δtmk1* and *Δtmk3* mutants show an important decrement in yield of conidia due to a lower production and defects in the formation of conidiophores. Both MAPKs were phosphorylated after mechanical damage. Activation of Tmk1 was observed during up to 30 minutes after injury in the absence of NADPH oxidase activity. Whereas, Tmk3 presented maximal phosphorylation at 1 to 5 minutes after injury in a Nox dependent fashion. Therefore both MAPK pathways are activated by different signaling molecules, Tmk1 could regulate early and late asexual development gene, while Tmk3 is activated by ROS and it could regulate stress realted genes and early asexual development gene. We propose a mechanism based on redox regulation through cysteines present in Tmk3 that would allow sensing of ROS, to then trigger a specific transcriptional response. Preliminary results suggest that Tmk3 is regulated through oxidation of its cysteines and can form a protein complex by disulphides bonds in the first minutes after injury. In future work we will identify the cysteines involved in the oxidative modification.

The circadian clock in *Aspergillus nidulans* – identifying the core members in circadian regulation

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Circadian clock systems appear from bacteria to human, and provide the organism an adaption to environmental changes throughout the day. In most cases the system consists of an autoregulatory negative feedback loop, although the players are different. There are three basic properties, which confirm a clock as a clock: There has to be a free cycling of about 24h in for example protein level or visible biological activity. This cycling has to be entrainable for example via light or temperature. The constant cycling period has to be stable at different environmental circumstances, for instance at different constant temperatures – this is called temperature compensation [1].

A well-studied system for the circadian clock is the filamentous fungus *Neurospora crassa*. Here the positive clock element is represented by the White Collar Complex (WCC) consisting of WC-1 and WC-2. The negative element is the FFH Complex consisting of a frequency (FRQ) homodimer interacting with the RNA helicase FRH [2].

In *A. nidulans* circadian expression was reported for the *gpdA* gene, suggesting the presence of a clock [3]. However, one central element from the *N. crassa* clock, FRQ, cannot be identified in the genome of *A. nidulans*. Therefore, we analysed the circadian clock using two genes, which are regulated in a circadian manner in *N. crassa*, *ccg-1* (*ccgA*) and *con-10* (*conJ*).

Using luciferase as reporter, we show that *A. nidulans ccgA* and *conJ* both are expressed in a circadian manner. The clock can be entrained by light and by temperature shifts. The free-running period was about 24 h. Both, white collar and phytochrome appear to interact with the clock, although neither of them seems to be a central component. Experiments to test cryptochrome and VeA as parts of the clock are under way.

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The putative transcription factor Blu7 controls sensitivity to light in *Trichoderma atroviride*

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Asexual reproduction of the ascomicete fungus *T. atroviride* in response to light involves changes in the morphology of the colony forming structures called conidiophores. The processes of photoconidiation requires de novo synthesis of RNA and proteins. Light is perceived by the protein BLR1 (Blue Light Regulator 1), and most likely in a complex with BLR2, regulates light responsive genes that determine entry into asexual reproduction. Microarray analysis and high-throughput RNA sequencing by 454, allowed us to identify genes regulated by light, including a subset encoding transcription factors, as well as to determine those that are BLR1 dependent. Five of the genes coding for transcription factors have been poorly studied or not studied at all. One of them, Blu7, a C2H2 zinc finger type encodes a 213 amino acids protein, and appears to be restricted to filamentous fungi. Based on this information, we obtained gene replacement mutants in order to evaluate its role in photoconidiation.

The *Δblu7* mutants showed a reduction in photoconidiation after a pulse of light and under constant light. Likewise, growth of the mutant decreased in the presence of light and was carbon source-dependent. In the presence of glucose and glycerol the *Δblu7* mutant showed a more evident decline in growth and photoconidiation. Transcriptional analysis of the response to constant light in the presence of glucose, revealed 134 induced genes in *Δblu7* and 125 in the wild type, while 84 genes were repressed in *Δblu7* and 106 in the wild type, respectively. The majority of the genes with functional annotation by BLAST2GO, were present in both strains, but in *Δblu7* mutants the GO cellular process category of biological processes were less represented than in wild type strain. Interestingly, from the genes induced only in WT, we found mannitol-1-phosphate dehydrogenase, the first enzyme in the biosynthesis of mannitol an enzyme implicated in stress responses in fungi. Conversely, *Δblu7* induced genes were related to detoxification and transport and metabolism of carbohydrates. Whereas the transcriptional response of the Dblu7 mutant to a pulse of blue light showed more genes responsive to the stimulus than the WT (897 and 322 respectively), suggesting a repressive role in gene regulation. The role of Blu7 in the response to light as a regulator of photoconidiation and growth in *T. atroviride* is further supported by the fact that strains overexpressing this gene have an exacerbated phenotype in constant light and after a light pulse.

Bioinformatics-informed functional domain analysis of the bZIP transcription factor FlbB: a key activator of *Aspergillus nidulans* asexual development

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Sensing of environmental cues and their transduction into signals is a key process for the activation of development in fungi. In the model ascomycete *Aspergillus nidulans*, the transition from submerged to aerial growth, under light, provoke the induction of asexual development. Under these conditions, a set of proteins expressed in vegetative hyphae that include several transcriptional factors (TF), hence called *Upstream Developmental Activators* (UDAs), trigger conidiophore development. UDAs finally hand over control of development to the first asexual development-specfic TF *brlA*.

FlbB is a bZIP-type UDA TF which apparently plays multiple roles. At actively growing vegetative hyphae, it accumulates at the hypha tip as well as the most apical nucleus. Constitutive expression of FlbB tagged with the photo-switchable fluorescent protein DENDRA2 showed that apical localization requires an active polar-growth machinery and is not cell-cycle dependent. A bioinformatic analysis identified domains within FlbB. Key residues were then selectively mutated to alanines in each domain, to selectively disrupt function. These point mutations indicated that the bZIP-dimerization domain, located at residues 97-133, is required for the interaction with the UDA factor FlbE and localization at the hypha tip. In addition, two conserved FlbB regions, B (254-281) and C (296-306), as well as Cys382 are also required for stable retention at the tip.

In order to enter the nucleus, FlbB requires conservation of residues 57-70, where we predict a nuclear localisation domain. Once in the nucleus, FlbB is necessary to activate the expression of *flbD*, which codes for a cMyb-type UDA TF. Both TFs interact, and are essential for the induction of *brlA* expression through direct binding to its promoter. Changes in the DNA binding domain (DBD) of the bZIP (N86A; R87A) did not alter localisation, but the fluffy phenotype of the DBD mutants suggested that this region is exclusively required for the transcriptional activation of asexual development.

Taken together, this directed mutational analysis provides an overview of the main functional domains of FlbB as a developmental regulator, as a prelude to more detailed studies on the specific functions of the protein.

Dual targeting via ribosomal stop codon read-through in *Ustilago maydis*

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In eukaryotic cells single genes often encode distinct proteins that are located in different subcellular locations. This phenomenon is termed dual targeting. We could show that in the basidiomycetous fungus *Ustilago maydis* some glycolytic enzymes are dual localized in the cytoplasm and peroxisomes, e.g. the enzymes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoglycerate kinase (PGK). For Pgk1 dual targeting is reached by ribosomal stop codon read-through during translation creating an elongated protein. This short elongation contains a C-terminal peroxisomal targeting signal (PTS1), which causes import into the peroxisomes. The same mechanism using a cryptic targeting signal via stop codon read-through was also observed in other fungi like the ascomycetes *Neurospora crassa* and *Aspergillus nidulans*.

We established a test system to characterize sequences relating to their effect on stop codon read-through. This system allows us to identify new candidate proteins for dual targeting via ribosomal read-through. Furthermore we could identify a short sequence that induces efficient stop codon read-through in *U. maydis*. Genomic data analyses indicate that this sequence is conserved not only among fungi but also in mammals.

Structural and functional analysis of *Saccharomyces cerevisiae* cell surface adhesions

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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 neighbouring 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 our 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, confers only weak 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.

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

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The sugar alcohol mannitol is a major compatible solute in Neosartorya fischeri and accumulates in conidia and ascospores. Biosynthesis of mannitol in this fungus mainly depends on the presence of the enzyme mannitol 1-phosphate dehydrogenase (MPD). In this study a functional analysis of mpdA was performed. Expression of mpdA was observed in aerial hyphae and conidiophores, but was highest in ascomata and ascospores. Disruption of mpdA reduced mannitol as much as 85% of the wild type and increased. Trehalose levels were increased to over 400%. Decreased mannitol accumulation had no effect on mycelial growth during temperature and oxidative stress. Some increase of stress sensitivity of conidia against heat and oxidative stress was observed. The most distinct phenotype of mpdA deletion was the complete absence of fully formed ascospores. Ascomata, asci and ascospore initials were formed as judged by light- and electron microscopy. After 6 days of development many ascospores showed distorted cell contents and a malformed cell wall. The ascus cell wall however remained intact in mutant strains, while in the wild type the ascus cell wall disappeared and numerous ascospores accumulated within the ascomata. Addition of the Mpd inhibitor nitrophenide to the wild type strain resulted in disturbed ascospore formation. Taken together, these results show a novel function for mannitol in fungal growth and sexual development.

Hyphal heterogeneity in Aspergillus is the result of dynamic closure of septa by Woronin bodies and promotes growth during stress conditions

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Hyphae of higher fungi are compartmentalized by septa. These septa contain a central pore, which allows for inter-compartmental and inter-hyphal cytoplasmic streaming. The cytoplasm within the mycelium is therefore considered to be a continuous system. In this study, however, we demonstrate by laser dissection that part of the septa of young exploring hyphae of aspergilli such as those of *Aspergillus oryzae* and *Aspergillus niger* are closed. For instance, about 50% of the septa of *A. niger* were closed during standard growth conditions. Septal plugging increased in *A. niger* during exposure to 4 °C and hypertonic conditions. During these conditions, only 10%, and 17.5% of the septa were open, respectively. Changing the environmental conditions did not affect the incidence of septal plugging in the other aspergilli. Closure of septa in aspergilli correlated with the presence of a peroxisome-derived organelle, known as Woronin body, near the septal pore. The location of Woronin bodies in the hyphae was dynamic and, as a result, plugging of the septal pore was reversible. Septal plugging in *A. oryzae* was abolished in a *ΔAohex1* strain that cannot form Woronin bodies. Notably, hyphal heterogeneity was also affected in the *ΔAohex1* strain. Wild-type strains of *A. oryzae* showed heterogeneous expression of the glucoamylase gene *glaA* and the α-glucoronidase gene *aguA* between neighboring hyphae at the outer part of the colony. Two populations of hyphae could be distinguished; one that highly and one that lowly expressed *glaA* or *aguA*. In contrast, *glaA* and *aguA* expression showed a normal distribution in the case of the *ΔAohex1* strain. Taken together, Woronin bodies cause hyphal heterogeneity in a fungal mycelium by impeding cytoplasmic continuity. We present evidence that reduced heterogeneity between hyphae impacts growth of Aspergillus during stress conditions.

fluG **and** ***flbA*** **homologs in basidiomycetes and establishing their functional roles in** ***Coprinus cinerea***

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In the ascomycete *Aspergillus nidulans*, the *fluG* and *flbA* genes work concomitantly to activate expression of the *brlA* gene, which is required for conidiation and production of sterigmatocystin [1]. Deletion of either of these two genes results in a fluffy colony. FluG works upstream to activate the developmental specific regulatory cascades including activation of the regulator of G protein signaling FlbA [2]. FluG contains an N-terminal amidohydrolase and a C-terminal glutamine synthase I (GSI)-like domain. For function as a regulator, the GSI-like domain is essential [3] but the domain does not confer glutamine synthase activity [4]. FlbA homologs have been studied in many more fungi and shown to participate in regulation of processes such as vegetative growth, asexual sporulation, mating, mycotoxin and pigment production and pathogenicity. FlbA contains two DEP (Dishevelled, Egl-10, and Pleckstrin) domains and an RGS (regulator of G protein signaling domain superfamily) domain [2,5].
FluG homologs have not been yet investigated in basidomycetes and homologs of FlbA only in two species. We aim to examine the roles of *fluG* and *flbA* homologs in development and secondary metabolism of basidiomycetes, using the mushroom Coprinopsis cinerea as a model. First, we screened for *fluG*-like genes in fungal genomes. Most of the filamentous ascomycetes do have complete fluG genes. In exceptional cases, the two protein domains are split into independent proteins and some species have lost the information for the N-terminal domain. Most yeasts have no information for either of the two domains. Only two of the tested basidiomycetes have full-length *fluG* homologs. The majority of the Agaricomycotina have the information split into separate genes. Several but not all of the more basic Agaricomycotina, the Ustilagomycotina and the Pucciniomycotina have no fluG homologous sequences. For *flbA*, we are currently on the way to elucidate the distribution of homologs of this gene within fungal genomes. In *C. cinerea*, there are two *fluG*-homologous genes (*FLU1-II* genes) coding only for the C-terminal GSI-like domain, which for conidiation in *A. nidulans* was reported to be sufficient [3]. *C. cinerea* also has a *flbA* homolog referred to as *crg1*. Genes *crg1* and *crg2* in the basidiomycetous yeast *Cryptococcus neoformans* and gene *thn1* in the filamentous *Schizophyllum commune* are shown to be functionally linked to G protein signaling in the pheromone- and the cAMP-response pathways [6-9]. Homologous gene targeting and overexpression of the *fluG*- and *flbA*-homologous genes will be used to characterize their functions in asexual sporulation, development of fruiting bodies and metabolism of *C. cinerea*.

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Characteristic mycelial traits in cultrues of coprinoid mushrooms

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Taxonomically, the coprinoid mushrooms (inky caps) distribute over two different families and genera (Agaricaceae: Coprinus; Psathyrellaceae: Coprinellus, Coprinopsis, Parasola). Inky caps are fast-growing mushrooms with fragile gills on which dark-brown to black spores are formed. In many species, the basidiospores are released in liquid droplets via autolysis of the cap [1]. Different coprinoid mushrooms are commonly difficult to distinguish. Due to the fast decomposition of the mushrooms, it is problematical to bring them in intact form to the lab for thorough morphological classification. Mycelial cultures might however be won from spore germination or from cap or stipe tissue [2]. Cultures can then serve to define morphological characteristics of the mycelia and potential asexual stages of reproduction as further features for morphological identification [3]. Cultures of Coprinellus species are most easy to describe since they often form eye-catching yellow to red-brown mycelial strands. Such coloured mycelium sterilium has also been observed in nature and described under the anamorph name Ozonium [4]. Whereas the name is a nomen dubious, an ozonium-stage appears to be an excellent taxonomic criterium to define *Coprinellus species* [1,3]. Ozonium formation has been observed in species *curtus, disseminatus, domesticus, ellisii, micaceus, patouillardii, radians, truncorum, xanthrotrix* and others. Ozoniums can be produced by monokaryotic and dikaryotic mycelia. A typical ozonium is characterized by parallel growing undifferentiated hyphae with stained cell walls and multiple anastomoses between. Single hypha can enter or leave a specific ozonium bundle and can connect different bundles with each other. Formation of new parallel growing hyphal branches starts laterally with firmly curved hyphal tips that fastly attach to the parental hypha. Typical are also coloured hyphal loops found amongst the ozonium strands that form by single hypha growing in circles. Some Coprinellus species (e.g. *domesticus, radians, xanthotrix*) in addition produce abundant asexual spores (oidia, arthroconidia) on specialized oidiophores. An anamorphic name Hormographiella has been introduced for mycelia with such structures [5]. However, oidia production is also very common amongst species of the genus Coprinopsis [6,7]. Specific parameters of spores and oidiophores can be defined for different species [5-7]. Other reproductive structures found in cultures of coprini are thallic arthrospores, blastoconidia-like mitospores, terminally and/or intercalary formed chlamydospores and mycelial cystidia. Simple hyphal coilings were observed in cultures from clades Coprinus and Coprinopsis. The extent of the taxonomic relevance of all these structures remains to be established.

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Impact of the quality control mechanism autophagy on aging of *Podospora anserina*

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Maintenance of mitochondrial function is of crucial relevance in the life cycle of organisms allowing them to cope with endogenous and exogenous stressors. Several different pathways like ROS scavenging, mitochondrial protein quality control via proteases and mitophagy are active in keeping mitochondria functional over time. In recent years, we have studied various mutants impaired at different stages of these pathways in the filamentous fungus *Podospora anserina*. The characterization of these mutants revealed unexpected effects on aging and lifespan. For example, although lack of mitochondrial SOD, PaSOD3, in *P. anserina* leads to increased paraquat sensitivity, lifespan is not affected in the mutant. From these data we hypothesize that lack of components of the ROS scavenging pathway may be compensated by the induction of other pathways like autophagy, which may act as a 'back-up' protection pathway. To test this idea in *P. anserina*, we developed or adopted appropriate tools and methods: (i) a PaATG8 reporter strain allowing the microscopic tracking of autophagy, (ii) the measurement of the degradation of GFP fusion proteins via autophagy and (iii) a strain lacking a central component of the autophagy machinery, PaATG1. With these tools we were able to demonstrate that during aging of *P. anserina*, the number of autophagosomes strongly increases as well as the autophagy-dependent degradation of a GFP fusion protein. Significantly, the PaAtg1 deletion strain is characterized by a significant reduction in lifespan, stressing the role of autophagy as pro-survival mechanism during aging. This protective role of autophagy is also relevant in the PaSod3 deletion mutant and demonstrates that autophagy is able to compensate for impairments of the ROS scavenging pathway.

An age-dependent transcriptome analysis of *Podospora anserina* identifies autophagy as a potential mechanism to compensate age-related proteasomal impairments

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Most biological systems are characterized by time-dependent functional impairments known as aging, which finally lead to death. The underlying mechanisms are depending on environmental conditions, stochastic processes and the genetic constitution of the individual. In order to systematically study age-dependent changes in gene expression, we performed a genome-wide longitudinal transcriptome analysis (SuperSAGE) of the fungal aging model *Podospora anserina*.

In this study, transcript abundance was quantitated for seven days in the lifetime of *P. anserina* – from young to old - which led to more than 10,000 transcript profiles. Using different bioinformatics approaches, we analyzed these expression profiles and identified some major pathways, which are affected during aging. We found that genes significantly down-regulated during aging are associated with "ribosomes" and the "proteasome", while genes involved in "autophagy" are significantly up-regulated during aging. We suggest that autophagy, as part of the cellular quality control system, is induced during aging due to impairments of the proteasome system. Furthermore, we found the transcript levels of genes involved in the cellular energy metabolism, mitochondria, and especially the respiratory chain to fluctuate during aging, but exhibiting strong differences between young and old individuals. Finally, the comparison of a former published transcriptome analysis of the copper depleted mutant grisea with our transcriptome data confirmed a relationship of the copper metabolism with cellular aging and provides a list of new putative target genes for the transcription factor GRISEA.

Autophagy and fruiting-body development in *Sordaria macrospora*

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Autophagy is a degradation process involved in various developmental aspects of eukaryotes. However, its involvement in developmental processes of multicellular filamentous ascomycetes is largely unknown. Here, we analyzed the impact of the autophagic proteins SmATG8 and SmATG4 on the sexual and vegetative development of the filamentous ascomycete *Sordaria macrospora*. A yeast complementation assay demonstrated that the *S. macrospora Smatg8* and *Smatg4* genes can functionally replace the yeast homologs. By generating homokaryotic deletion mutants, we showed that the *S. macrospora* SmATG8 and SmATG4 orthologs were associated with autophagy-dependent processes. *Smatg8* and *Smatg4* deletions abolished fruiting-body formation and impaired vegetative growth and ascospore germination, but not hyphal fusion. We demonstrated that SmATG4 was capable of processing the SmATG8 precursor. SmATG8 was localized to autophagosomes and SmATG4 was distributed throughout the cytoplasm of *S. macrospora*. Furthermore, we could show that *Smatg8* and *Smatg4* are not only required for nonselective macroautophagy, but for selective macropexophagy as well. Our results suggest that in *S. macrospora* autophagy seems to be an essential and constitutively active process to sustain high energy levels for filamentous growth and multicellular development even under nonstarvation conditions.

Voigt O, Pöggeler S (2013) Autophagy genes *Smatg8* and *Smatg4* are required for fruiting-body development, vegetative growth and ascospore germination in the filamentous ascomycete *Sordaria macrospora*. Autophagy 9:33-49

The role of superoxide dismutases in lifespan control of *Podospora anserina*

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Within biological systems reactive oxygen species (ROS) like superoxide and hydrogen peroxide are formed as by-products of the respiratory chain, during photosynthesis or are actively generated by enzymes like NADPH oxidases. These ROS can serve as signaling molecules but, at higher levels, can also damage vital cellular compounds like DNA, lipids and proteins and consequently lead to a decline of cellular functions and aging.

In biological systems ROS levels are controlled by enzymatic or non-enzymatic ROS scavenging systems. The first step in the enzymatic detoxification is the dismutation of superoxide to hydrogen peroxide and oxygen. This reaction utilizes superoxide dismutases (SODs), which are the only enzymes capable of degrading superoxide.

The fungal aging model *Podospora anserina* encodes four putative superoxide dismutases. PaSOD1 represents the Cu/Zn isoform located in the cytoplasm and partially in the mitochondrial inter-membrane space, PaSOD2 and PaSOD3 are proteins with a manganese-binding domain. Only PaSOD3 contains a clear mitochondrial targeting sequence (MTS) and localizes in the mitochondrial matrix while PaSOD2 localizes to the perinuclear ER (Zintel et al. 2010). PaSOD4 has not been investigated so far.

Here we report investigations analyzing the role of mitochondrial SODs in the ascomycete *P. anserina*. These results contain first studies on the role and localization of PaSOD4 and the influence of manganese sulfate on lifespan control and the ROS detoxification network in PaSod3 mutant strains.

Zintel et al. (2010) *Exp Gerontol* 45: 525-532

Transcriptional regulation during paraquat stress and ageing in the model organism *Podospora anserine*

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Ageing is associated with an accumulation of reactive oxygen species (ROS) which are able to oxidise different components of the cell (DNA, RNA, proteins, lipids) resulting in molecular damage, functional impairments and finally death of the system. One of the major ROS, the superoxide anion, is generated as by-product of mitochondrial respiration. It can undergo further reactions that lead to the formation of other ROS like hydrogen peroxide or hydroxyl radicals.

Podospora anserina is an established ageing model and like all organisms, has a variety of strategies to cope with the adverse effects of ROS accumulation. Besides its damaging features, ROS and especially hydrogen peroxide, have a vital function as signalling molecule. However, the effects of ROS on transcriptional regulation are not well understood yet.

In order to analyse the effects of ROS, we performed a whole transcriptome analysis with RNA isolated from juvenile, middle aged and senescent *P. anserina* in the presence and absence of the superoxide producing agent paraquat. In this study we found that paraquat treatment of juvenile *P. anserina* cultures led to up regulation of 681 and down regulation of 1172 of the 10640 *P. anserina* genes by a factor of three or larger. During ageing 509 transcripts increased in abundance while 767 transcripts decreased. 89 genes are up regulated during both, ageing and oxidative stress, suggesting important functions at these conditions. We also observed a strong impact of paraquat stress on expression of genes located at mitochondria, on some genes for ROS scavengers and on several other pathways.

Conserved signaling complexes regulate fungal development: Structure-function relationship of PRO40 (SOFT) and PRO45 (HAM4)

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Fungal development, i.e. vegetative growth, hyphal fusion, pathogenic and symbiotic interactions, and development of sexual or asexual fruit bodies, is controlled by a plethora of conserved and fungal-specific proteins. In the model ascomycete *Sordaria macrospora*, sexual fruit body (perithecia) development starts with the formation of ascogonia, which are enveloped by sterile hyphae, generating spherical protoperithecia. Further cell differentiation inside the protoperithecia leads to mature perithecia ejecting ascospores.

Two conserved signaling pathways have been found to control perithecia formation in *S. macrospora*. First, the STRIPAK (striatin-interacting phosphatase and kinase) complex was shown to regulate the transition of ascogonia to protoperithecia as well as the transition of protoperithecia to perithecia. Second, the cell wall integrity (CWI) MAP kinase pathway was found to be involved in protoperithecia formation as well as protoperithecia to perithecia transition. Although the composition of both complexes is largely known, the exact working mechanism of all involved proteins is not completely understood.

Here, we focus on the molecular function of developmental protein PRO40 and STRIPAK element PRO45. PRO40, a WW domain containing protein homologous to *Neurospora crassa* SOFT, interacts with components of both the STRIAPK complex and the CWI pathway. PRO45, homolog of *N. crassa* HAM-4 and human SLMAP (sarcolemmal membrane-associated protein), interacts with striatin PRO11 and phocein SmMOB3 and contains coiled-coil domains, a transmembrane domain, and an FHA domain. Using derivatives of the two proteins in protein-protein interaction studies, in vivo functional analysis, and state-of-the-art fluorescence microscopy, we provide mechanistic insights into PRO40 and PRO45 function. Due to the evolutionary conservation of the protein complexes under investigation, our data have implications to insect and mammalian systems.

The highly conserved STRIPAK complex is involved in signalling of sexual development

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The major serine/threonine protein phosphatase 2A family (PP2A) is highly conserved throughout eukaryotes and dephosphorylates a multitude of cellular proteins involved in e.g. cell cycle control and gene regulation. PP2A balances various signal transduction pathways by negatively regulating kinases or other proteins. PP2A typically assembles as a heterotrimer including a structural A-, a regulatory B- and a catalytic C- subunit.

Here, we present an approach using protein-protein interaction studies, among others, to survey a putative protein supracomplex controlling the development of reproductive structures in *Sordaria macrospora*. Previously, this fungus served as a model organism for sexual development and therefore the formation of fruiting bodies [1]. To determine their role for sexual differentiation we deleted the genes encoding the catalytic subunits of PP2A, PP2Ac1 and PP2Ac2. The ∆PP2Ac1 strain shows a sterile phenotype, whereas preliminary data suggest that deletion of *pp2Ac2* is lethal. Data from tandem affinity purifications followed by mass spectrometry using MudPIT (multidimensional protein identification technology) with PP2Ac1 as bait revealed a strong interaction of PP2Ac1 with PRO22. Moreover, PP2Ac1 may connect different signalling pathways leading to a controlled reaction on a multitude of signals. Via yeast two-hybrid analysis we discovered that both catalytic subunits of PP2A (PP2Ac1 and PP2Ac2) interact with subunits from the striatin interacting phosphatase and kinase (STRIPAK) complex and PRO40-MAPK interaction network. Further putative interactions of PP2AA (regulatory subunit A of PP2A) and components of the PRO40-MAPK complex were confirmed. The STRIPAK complex is highly conserved within eukaryotes and first fungal STRIPAK complex was characterized in *S. macrospora* [2]. This complex consists of at least six subunits, among them PP2AA and PRO22. The PRO40-MAPK interaction network comprises PRO40 and the three kinases of the cell wall integrity pathway. PRO40-MAPK and STRIPAK complex play a crucial role in signalling during the transition from vegetative growth to sexual development.

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The transcriptional landscape of *Δnox1* protoperithecia: insights into cellular processes involved in fruiting body development of *Sordaria macrospora*

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NAD(P)H oxidase 1 (NOX1) was identified as an important regulator of developmental processes in ascomycetes. The regulating signaling molecules produced by NOX1 are reactive oxygen species (ROS), which are able to change reversibly the structure and function of effectors in distinct amounts. Although the function of NOX1 was investigated in many ascomycetes, so far only cytoskeleton remodeling was identified as a NOX1 regulated cellular process in *M. oryzae*.

To gain a deeper insight in the processes regulated by NOX1, we performed RNAseq analysis. Using the laser micro dissection technology, RNA was collected from wild type and Δnox1 protoperithecia of *Sordaria macrospora*. To identify NOX1 regulated genes we compared sequenced RNA reads, which map to the *S. macrospora* genome in Δ*nox1* and the wild type strain. About 700 genes up and down regulated were identified, including those encoding mating type factors, pheromone precursors and receptors and other proteins involved in sexual development. This result is in concord with the sterile phenotype of *nox1* deletion mutant. Further, we identified several homologues *N. crassa* genes involved in sexual development. Finally, we identified among others genes involved in cellular processes like metabolism, ubiquitin dependent cell cycle regulation, and cdc42 dependent actin polymerization. Our findings will provide insights into NOX1 regulated cellular processes that may underpin developmental processes in filamentous fungi.

DNA-methylation and HP1 do not affect effector gene expression in *Fusarium oxysporum*

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In the tomato pathogen *Fusarium oxysporum* f. sp. *lycopersici*, most known effector genes reside on a pathogenicity chromosome that can be exchanged between strains through horizontal transfer. Expression of these effector genes is induced upon infection, but the mechanism by which this is regulated is unknown. We noticed that targeted deletion of the effector genes on the pathogenicity chromosome has a particular low rate of succes, when compared to genes on the core genome. Possibly, the pathogenicity chromosome has a more compact (less accessible) chromatin structure. It has been shown that release of chromatin condensation can be a way to regulate gene expression, for example of secondary metabolite gene clusters in Fusarium [Reyes-Dominguez et al, FGB 2012]. To test whether DNA methylation in *F. oxysporum* can influence effector expression, knock-outs of HP1 (heterochromatin protein) and DIM2 (DNA methylase) were tested for expression of the effector gene SIX1. No differences compared to wild-type were observed. Previously it was shown that expression of SIX1 requires Sge1, a conserved transcription factor encoded in the core genome. Loss of DNA methylation did, however, also not bypass the requirement of Sge1 for SIX1 expression (in *Δhp1Δsge1* and *Δdim2Δsge1* double mutants). Both DIM2 and HP1 are not required for pathogenicity of *F. oxysporum* f.sp. *lycopersici*, and DNA methylation in this strain in general seems to be very low. To obtain more insight in the regulation of effector gene expression we are currently focussing on the potential targets of the transcription factors encoded on the pathogenicity chromosome itself.

Light-dependent fruiting body formation of *Pyronema confluens*: Blue light dependency of apothecia and carotenoid production

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In recent years many genomes of many filamentous ascomycetes have been sequenced in order to study evolution of species and to investigate specialized morphology and physiology. However, while for each group of ascomycetes at least ten or more genomes are available, the basal group of Pezizomycetes only features one sequenced genome, from the black truffle. Therefore, we sequenced the genome and transcriptiome of the Pezizomycete *Pyronema confluens*. During the late 19th and early 20th century *P. confluens* was studied as model organism for fruiting body development; now the 50 Mb sequenced genome is available, coding for 13369 predicted protein-coding genes. In contrast to most fungi, sexual development of *P. confluens* is strictly light-dependent, with a minimum of 12 h light exposure to produce orange pigmented apothecia. Growth tests under different wavelengths revealed that blue light (450–550 nm) is the effective part of the visible spectrum. Within the *P. confluens* genome, homologs of known light receptors as well as light regulated genes, e.g. *wc1*, *wc2*, *phy1*, *phy2*, *orp*, *frq* and more, could be annotated. However, homologs of the blue light receptor vivid (*vvd*) and the rhodopsin-type receptor *nop-1* are missing in the genome of *P. confluens*. qRTPCR analysis of light-dependent transcription under different wavelengths confirmed the upregulation of annotated photoreceptor genes and light-dependent genes, e.g. *al* genes. Furthermore we analysed transcription rates depending on short- and long-term illumination under white, blue and green light. Our data confirm a major influence of blue light towards most photoreceptor gene transcription and homologs of the early and late light response genes, e.g. *sub-1/pro44*. An influence of green light could also be detected although *P. confluens* lacks *nop-1*, which is discussed to act as a putative green light receptor in *Neurospora crassa*. Short- and long-term data indicated that the *orp* gene transcription is regulated via blue and green light, with a stronger effect of green light. Furthermore our data of these light induction experiments suggest that early and late light response mechanisms in *P. confluens* might be comparable to mechanisms in the distantly related species *N. crassa*. In order to determine if *P. confluens* could be used as a model organism for fruiting body development and circadian rhythmicity, future experiments will investigate if circadian rhythmicity can be detected in this fungus as well.

The *Aspergillus nidulans* SrkA protein kinase requires the MAPK SakA to be translocated to the nucleus in response to stress and regulates sexual development

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To transduce environmental stress signals fungi use a phosphorelay system coupled to specialized MAP kinases. The *Aspergillus nidulans* MAPK SakA is activated in response to stress signals and during asexual development, and is also required to repress sexual development. The phosphorylation state of SakA regulates the transition between dormancy (spore) and growth (germination), and this is conserved in other fungi. Here we examined the role of the SrkA protein kinase in these processes. A fission yeast SrkA homolog is required for osmotic stress resistance and regulates G2/M cell cycle arrest. We found that ΔsrkA mutants are not sensitive to osmotic or oxidative stress. However, a functional SrkA::GFP fusion is translocated to the nucleus under stress conditions, in a SakA-dependent fashion. In addition, ΔsrkA strains show derepressed sexual development. Our results suggest that SrkA is part of the SakA stress pathway and also mediates SakA repressing functions during sexual development.

Establishment of an efficient Golden Gate cloning system for the generation of gene-replacement mutants in fungal model organisms

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During the past decades *Ustilago maydis* evolved as a well-studied eukaryotic model organism for basic research. Genetically, the fungus is easy to handle and genetic manipulations like gene replacement, deletion or insertion by homologous recombination are well established. Nevertheless, the generation of the linear DNA constructs that correspond to the different applications still constitutes a limiting step during daily laboratory work, which is often the reason for the delay of follow-up experiments. The aim of this project was the engineering of this time consuming bottleneck by adopting an existing vector library containing various resistance cassette modules such as promoter cassettes, gene-insertion cassettes, gene-deletion cassettes, gene-replacement cassettes or reporter-gene cassettes, for the use with the so called Golden Gate cloning strategy. For the Golden Gate cloning strategy the type IIs restriction endonuclease BsaI was selected. This enzyme hydrolyzes the DNA besides its own recognition site and hereby gives the user the opportunity to design a total number of 256 specific and unique DNA overhangs. These specificities enable the use of only one enzyme for combining multiple fragments which results in highly specific overhangs and thereby can be used for one pot cloning procedures with high cloning efficiencies. As a proof-of-principle we generated a subset of vectors containing resistance cassettes and modules for gene replacements in *U. maydis* and subsequently demonstrated their functionality *in vivo*. We will present recent data proving that the novel method is suitable for minimizing the time for the generation of linear transformation constructs to an average of only four days with a high efficiency and a strongly diminished workload. Furthermore, the amount of consumed material and therefore the expenses could be significantly reduced. Importantly, to proof the applicability of this novel strategy in other fungal model organisms, we conducted gene deletions in the filamentous ascomycetes *Neurospora crassa* and *Aspergillus nidulans*. Thus, we present a highly efficient and economic cloning strategy for the quick generation of gene-replacement mutants speeding up reverse genetics approaches in fungal model organisms.

Identification and characterization of the novel poly(ADP-ribose) glycohydrolase of the filamentous fungus *Aspergillus nidulans*

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Poly(ADP-ribosyl)ation is a post-translational modification of protein in which the ADP-ribose moiety of NAD+ is transferred to amino acids in proteins, altering their structure and function. This protein modification is catalyzed mainly by a nuclear poly(ADP-ribose) polymerase (PARP) in response to several stress, most notably DNA damage. In mammalian cells, poly(ADP-ribosyl)ation is involved in many cellular processes, including DNA repair, chromatin remodeling, transcription, telomere function, mitotic spindle formation, and apoptosis (1). PARP encoded by *prpA* in genome of the *A. nidulans* is a highly conserved enzyme in all eukaryotes except for yeast. PrpA is an essential protein involved in the DNA damage response, programmed cell death and for asexual development. Although it is known that poly (ADP-ribose) is degraded by poly(ADP-ribose) glycohydrolase (PARG), the physiological role of PARG is not fully understood. In higher eukaryote, PARG is encoded by only one gene in genome, and knockdown of the parg gene results in the lethal sensitivity against DNA-alkylating agents or gamma-irradiation (2). It is also reported that knockdown of parg caused the derangement of biological clock in *Arabidopsis thaliana* (3). PARP orthologs are found in mammals, plants, metazoans, protists and filamentous fungi, but not in yeast, while PARG homologs are identified in all eukaryotes, besides fungi.

Here, we searched for a parg orthlog in the genome sequence of a model fungus *A. nidulans*. First, we picked up seven genes as candidates of a fungal parg gene. Proteins coded by the genes were successfully expressed as soluble proteins using *Escherichia coli* expression system. Among them, only one protein was found to hydrolyze poly(ADP-ribose). The PARG activity was enhanced by the addition of 10 mM MgCl. These findings are consistent with the previous reports for PARGs of the other organisms, and thus we designated the novel enzyme as fungal PARG (fPARG). It was reported that the expression of *parp* and *parg* genes was activated by DNA-alkylating agent MMS in mammals. We examined the effect of MMS on the transcription of *fparg*. As expected, the reagent also up-regulated the transcription of fparg, suggesting that it might plays a role in DNA damage response. In conclusion, this is the first report for identification of PARG in filamentous fungi.

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A transposable element reveals new insights in *Schizophyllum commune* thin mutation

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The white rot fungus *Schizophyllum commune* has been used as a model organism for studying the function of mating type genes and sexual development. In this context, spontaneous occurring mutants were observed showing an alteration in mate recognition. For *S. commune* a transposable element, designated as *scooter*, is known which interrupts the Bβ2 pheromone receptor gene *bbr2*. Another copy of this transposon is responsible for the mutation of *thn1* encoding a putative member of the regulator of G-protein signaling (RGS) family. Proteins with a RGS domain family are multi-functional and promote the GTP hydrolysis by direct interaction with the alpha subunit of G proteins. Hereby they accelerate the signal termination and act as a key regulator element in signal transduction. The disruption of *thn1* leads to the formation of wavy or corkscrew-like hyphae and in many cases to a reduced aerial growth, but less is known about gene expression and protein synthesis. Subsequent experiments could give new insights in the function of Thn1 and the background of *thin* mutation. Since *thin* strains possess a specific alcoholic smell, the composition of these substances should be analyzed to identify involved mechanisms.

Genetic and molecular aspects of heterokaryosis and vegetative compatibility in Verticillium

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The asexual phytopathogenic ascomycete *Verticillium dahliae* is the causal agent of Verticillium wilt disease of more than 400 agronomically important plant species worldwide. The parasexual cycle in Verticillium, starting with hyphal fusion between compatible strains and subsequent heterokaryon formation, has been experimentally established and appears to be a major genetic process in this asexual fungus, providing the means for the occurrence of mitotic genetic recombination. However, the genetically different hyphae often fail to fuse and/or establish heterokaryotic growth, a phenomenon described as heterokaryon or vegetative incompatibility. Depending on the frequency and vigour of complementation between appropriately marked mutants, *V. dahliae* strains have been classified in a number of Vegetative Compatibility Groups (VCGs), which are predicted to differ in physiological and pathogenicity-related traits. Research in our lab has been lately re-focused on the genetic and molecular study of heterokaryosis and heterokaryon incompatibility in Verticillium species, and a number of recent findings are presented here. (a) Nitrate-non-utilizing (nit) mutants, which are extensively used for grouping of isolates in VCGs, were produced from a large population of *V. dahliae* strains. Their stability and complementation were tested and it was shown that mutants with high reversion rates are consistently produced from several isolates of the fungus, underlining that compatibility grouping assignments based solely on their complementation testing is unreliable. (b) The use of auxotrophic and drug-resistant mutants for VCG classification, in comparison with nit mutants, confirmed that depending on the type of mutants used for testing, different compatibility grouping behaviour was exhibited from the same strains. (c) A comparison of complementation grouping with phylogenetic profiling of *V. dahliae* populations based on rDNA IGS sequence variation and group-I intron content in the nuclear SSU gene, is presented and discussed. (d) We report the isolation and cloning of five *V. dahliae* homologues of putative heterokaryon incompatibility (het) genes and the construction of knock-out (KO) mutants for these genes. The KO strains were tested phenotypically for alterations in their ability to form heterokaryons and their compatibility grouping behaviour. We expect the combination of classical genetic analysis of heterokaryosis and vegetative compatibility of *V. dahliae* with the functional study of genes putatively involved in the control of the phenomenon to shed light on the fundamental genetics of an important plant pathogenic fungus.

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CarS, a central protein in the regulation of carotenogenesis in Fusarium

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The ascomycete *Fusarium fujikuroi* is a model organism for the investigation of secondary metabolism. This fungus stands out for its capacity to produce gibberellins, growth-promoting plant hormones widely used in intensive farming. *F. fujikuroi* has proved to be a useful model for other secondary metabolite pathways, such as those for bikaverin, fusarins or carotenoids biosynthesis. Carotenoids are terpenoid pigments produced by all photosynthetic species and by some bacteria and fungi. Our group has focused his attention on the synthesis of neurosporaxanthin, an acidic apocarotenoid originally identified in Neurospora, and of retinal, the predicted light-absorbing prosthetic group of rhodopsins. The structural genes needed for the synthesis of neurosporaxanthin and retinal by Fusarium, *carRA*, *carB, carT, carD* and *carX*, have been identified, providing a valuable tool for the analysis of their regulation at transcriptional level. The major regulatory factors controlling the expression of the car genes are light and nitrogen, and the proteins responsible of this regulation are under investigation. In addition, the pathway is derepressed in carotenoid-overproducing mutants, deeply pigmented strains generically called *carS*, which accumulate large amounts of neurosporaxanthin under any culture conditions. Similar mutants have been identified in *Fusarium oxysporum*, which exhibit the same regulation pattern. The gene mutated in these strains, *carS*, has recently been identified in *F. fujikuroi* and *F. oxysporum*. The protein CarS contains a RING finger and a LON domain, similar to those found in the ubiquitin ligase from Mucor circillenoides, CrgA.

In order to characterize the Fusarium CarS, this protein was fused to glutathion-S-transferase (GST), expressed in Escherichia coli and the resulting fusion protein GST-CarS purified through affinity chromatography. We also obtained an antibody against a CarS epitope whose validity was checked using the purified fusion protein. As a negative control, cell-free extracts from a *carS* mutant, SG1, were used. SG1 allele, due to a premature stop mutation, produced a short CarS that did not contain the epitope recognized by the antibody. The results confirmed that the antibody was specific for CarS and useful to investigate its presence by western analyses. We hypothesized a negative correlation between CarS protein levels and carotenoid biosynthetic activity based on the phenotype carS. However, higher amounts of the protein were detected in the *F. fujikuroi* wild type (WT) grown in the light than in the dark. Similar results were also obtained for *F. oxysporum* WT. Mutants from both *F. fujikuroi* and *F. oxysporum* seemed to accumulate more CarS than the WT. Subcellular fractionation experiments suggest that CarS is a cytoplasmic protein. Accordingly, preliminary electroforetic mobility shift assay (EMSA) showed that GST-CarS did not bind to a bidirectional promoter of the structural genes *carRA* and *carX*. The available data point CarS out as probably involved in the interaction with one or more regulatory proteins, responsible of the transcriptional activation of the carotenoid genes.

Mating system studies in Flaviporus (Polyporales) species

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The mating type genes of basidiomycetes are responsible for determining sexual compatibility between different individuals. Polypore species that require different mating types for cell fusion and reproduction are called heterothallic and can be of two types: bipolar or tetrapolar. The bipolar species present multiallelic genes in a single locus (A) and two mating types can be segregated from a single basidiome. Tetrapolar species, on the other hand, present multiallelic genes in two loci (A and B) and four mating types can be segregated from one basidiome. In tetrapolar species, A contains genes for homeodomain transcription factors, while B contains pheromone precursors and receptors. In bipolar species, the mating type locus (B) of the pheromone receptor and pheromone precursor genes was lost.

The biology of the polypore genus Flaviporus is poorly studied and information on mating system is known only for *Flaviporus venustus*, which presents bipolarity. In order to expand the knowledge on biological characteristics of the genus, mating type studies were conducted on *Flaviporus subhydrophilus* and *Flaviporus liebmanii*. Sporeprints were obtained from fresh basidiomes collected in southern Brazil and inoculated on PDA and Water Agar. Due to the difficulty of spore germination in *F. liebmannii* in previous attempts, the plates were dusted with activated charcoal after the inoculation of basidiospores in order to remove inhibitory substances in agar. *F. subhydrophilus* spores germinated 5-6 days after inoculation, while *F. liebmannii* germination started only a month after inoculation. Pairings of eight monosporic cultures of *F. subhydrophilus* showed that it presents a tetrapolar mating system. In addition, preliminary data on pairings of ten monosporic cultures of *F. liebmannii* indicate that it is also tetrapolar. The difficulty of spore germination in Flaviporus species can be a hindrance in biology studies of the genus. This is the first record of spore cultures in *F. liebmannii* and *F. subhydrophilus*. So far, our attempts to obtain spore cultures of *F. brownii*, type species of the genus, have not succeeded. In polypores, the occurrence of more than one mating type in a single genus is known but uncommon. Further studies including polarity and molecular data are necessary to clarify this question and help understand the biology of the group and its relation with other genera.

The response to light and injury and their regulation by RNAi machinery in the filamentous fungus *Trichoderma atroviride*

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All living organisms must sense and respond appropriately to different environmental stimuli in order to survive. *Trichoderma atroviride* is a filamentous fungus with wide adaptability to different environmental conditions and is considered a good morphogenetic model because it respond to light and injury producing asexual reproductive structures (conidia). The mechanisms used by this fungus to respond to these stimuli have been studied independently and models of perception and signal transduction for each stimulus have been proposed. In our research group, we found that the *Δdcr2* mutant, involved in small RNA biogenesis, is affected in conidiation in response to light and injury, indicating that conidiation is regulated by small RNAs. In this work we discovered that when the *Δdcr2* mutant receives simultaneously light and injury, it is able to conidiate. Based on this observation, we propose that there is a central core of genes needed to coordinate the response to both stimuli and in the *Δdcr2* the signaling pathways act synergistically to achieve the correct expression of these genes in order to conidiate.

To test the above hypothesis we analyzed the transcriptome in response to light and injury of the wild type (WT), and identified 38 genes that have the same expression profile in response to the two stimuli, 19 of them are induced and 19 are repressed, called the central core genes, most of the up-regulated genes are involved in RNA processing, ribosome biogenesis, chromatin remodeling and other cellular processes indicating that this core regulates gene expression to respond to the stimulus. While genes that are repressed are mainly involved in lipid, carbohydrate and protein metabolic processes suggesting that a metabolic arrest is necessary to respond to both stresses. The expression analysis of this core of genes in the Δ*dcr2* revealed some of them are deregulated, but other genes respond similarly to WT in either light or injury in Δ*dcr2*, so this group of genes could be regulated by the RNAi machinery.

Apocarotenoid signals regulate sex in mucoralean fungi

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A haploid asexual phase predominates life cycle in Mucorales, but adverse extrinsic factors along with darkness promote "sexual communication". The sequential cleavage of β-carotene and exchange of trisporoid intermediates among complementary mating partners produce sex hormones known as trisporic acids. Little is known about the physiological functions of the enzymes and products involved in the sexual phase. We hypothesise that each of the intermediate C18 trisporoid have a specific regulatory function upon the genes linked to sex pheromone production varying with their species, vegetative and sexual phases. Real-time quantitative PCR kinetics conducted in *Blakeslea trispora* (Choanephoraceae), displayed a very high transcriptional upregulation for carotenoid cleavage dioxygenase *tsp3*, only during sexual phase. For *Mucor circinelloides f.lusitanicus* (Mucoraceae), two putative carotenoid cleavage oxygenases (CarS and AcaA) were identified based on phylogeny and amino acid sequence similarity among 4 other mucoralean members. Unlike Bt, during sex Mcl had no significant temporal transcriptional up regulation for carotenoid cleavage oxygenases, while *carS* and *acaA* had a high m-RNA level in (+) and (-) partners. A repressor of carotenogenesis, CrgA has no regulatory function on carotenoid cleavage as transcript levels of *carS* or *acaA* were on par among wild type Mcl (-) and its Δ*crgA* mutant. Cloning and heterologous co-expression of TSP3 with β-carotene overproducing plasmid in *E. coli* for in vivo enzyme assay and LC-MS analysis led to the identification of β-apo-12′-carotenal, as first apocarotenoid in *B. trispora*. β-apo-12′-carotenal induced *acaA* gene expression in Mcl(-). Trisporin C stimulated gene transcription in Mcl(-) and had no impact upon Bt. Supplementation of the first C18 trisporoid, β-apo-13-carotenone (D'orenone), entailed a positive feedback regulation on *carRP/carRA* and *acaA/tsp3* gene expression in Mcl (-) and Bt (+). Our data supports the hypothesis that mode of action of trisporoids varies among genotypes and growth phases which in turn lead to the concept of "specific chemical dialect" rather than a universal language. *M. mucedo* (Mucoraceae) was an ideal choice for the in situ localization of sex hormones as they produce trisporic acids copiously and have a very distinct morphological differentiation from zygophore to zygosporangium. A Raman mapping of β-carotene and pure trisporic acid extracted from fungal tissue enabled us to get the specific vibrational raman shift that distinguish 2 compounds. Coherent anti-Stokes Raman Spectroscopy (CARS) is a three dimensional non-invasive, novel approach and we successfully detected both sex hormone and its highly abundant precursor β-carotene in the gametangial phase. Moreover GC-MS analyses of solid agar extracts clarified that detectable amounts of trisporic acid production is limited to mated cultures in *Mucor mucedo*.

In vitro binding of the HapX-HapB/C/E complex to the promoter regions of iron-related genes

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The CCAAT sequence is one of the most common cis elements in eukaryotes. The CCAAT-box binding factor, which is a highly conserved heterotrimeric complex among eukaryotes, consists of the HapB, HapC and HapE subunits in Aspergillus species. We have isolated HapX as a novel protein that interacts with the HapB/C/E complex (1). Afterward, it was shown that various iron dependent pathways are repressed under iron-depleted conditions by interaction of HapX with the HapB/C/E complex (2). Previously we have carried out the in vitro binding analysis of the HapX-HapB/C/E complex to the promoter of the cytochrome c gene cycA of *Aspergillus nidulans*, which is one of HapX-dependent genes. We found that the HapB/C/E complex, a CCAAT-box and an adjacent sequence were necessary for HapX-binding to the *cycA* promoter. From the results we concluded that both CCAAT-box and the adjacent sequence (putative HapX binding sequence) of the cycA promoter are required for the recognition by the HapX-HapB/C/E complex, suggesting that both the DNA-binding ability of HapX and specific binding to the HapX-dependent genes result in the different regulation of HapX-dependent genes from the other HapB/C/E-dependent genes (3). In this study, we extended our findings to other iron-related genes, *acoA* (aconitase gene), *catB* (catalase gene), *lysF* (homoaconitase gene), and *sreA* (iron repressor gene). DNase I foot printing analyses and EMSAs (electrophoretic mobility shift assays) with various DNA probes were carried out. We found that the HapB/C/E-dependent binding of HapX to all the iron-related gene promoters we examined. From the results, the specific recognition mechanisms of HapX-HapB/C/E complex against iron-related genes and the consensus binding sequence for the complex will be discussed.

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Does thermal stress induce priming in fungi?

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Fungal survival and growth requires a specific and stable internal condition that can withstand fluctuations of external conditions such as temperature, osmolarity, pH, availability of nutrients and presence of toxic compounds. In order to cope with variable and even drastic environmental changes, most fungi have developed autonomous mechanisms. One of these mechanisms induces tolerance to a severe stressor after sensing fluctuations or mild changes of the same or different stressor(s) in their environment. This response, known as "acquired stress resistance" in yeast, involves a rapid adjustment of gene expression to the new condition and seems to be stressor-specific instead of being a commonly activated environmental stress response. As it can prepare fungi for future environmental changes it potentially provides a major fitness benefit.

This study addresses the question if saprophytic fungi and arbuscular mycorrhizal fungi (AMF) exhibit a priming response to temperature-related stress. In order to explore the experimental conditions needed to induce a priming response in fungi, we exposed a strain of *Saccharomyces cerevisiae* to both heat and cold stress conditions and tested for priming effects. Changes in the number of viable cells during the recovery period were then determined by plating the cells on solid medium and by alive/dead staining measurements in a flow cytometer. Additionally the responses of yeast cells to thermo-stress were measured by analyzing their growth and metabolic profile. A priming response was evident when a cell suspension (105-106 cells*ml) was exposed for 2 h to 35°C as priming stimulus and for 5 h to 40°C as triggering stimulus after a 24 h-memory phase at optimal temperature (28°C). Yeast growth was also affected after heat treatments. Cells that suffered both stimuli showed a shorter lag phase and faster growth in comparison to the cells that were exposed only to the second and stronger stressor. In a separate series of experiments, a significantly higher viability for cold conditions (0oC or -5°C) was observed if the cells were stressed in a 10X diluted YPD medium.

According to the results of these first experiments, specific parameters were selected to analyze and compare the priming effect in other yeast strains as well as in filamentous fungi with different growth strategies. On the basis of these preliminary results we will extend our study to other saprophytic and symbiotic fungi to find out whether or not functional traits related to fitness are affected after priming and triggering stimuli. Further analyses will be performed for testing physiological/ biochemical responses that are related to fungal fitness and to find out how fungal species along the r/K-strategy continuum and in different phylogenetic groups differ in priming effectiveness.

Benefits of sex and ascospore production in *Aspergillus nidulans*

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

SltB a new element in the Slt-cation/alkalinity stress response pathway

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Many microorganisms, including fungi, have developed genetic strategies to survive environmental stresses, such as variations in pH, temperature, nutrient availability, reactive oxygen or diverse saline concentrations. In the model filamentous fungus *Aspergillus nidulans*, tolerance to an alkaline ambient pH requires the activities of three principal transcription factors: PacC, CrzA and SltA. We have described the role of SltA, a C2H2 zinc-finger transcription factor, in tolerance to alkalinity and to high concentrations of certain mono and divalent cations. Although PacC and CrzA homologues are widely distributed among fungal kingdom, SltA homologues are found only in filamentous fungi.

To understand how SltA is activated and how it mediates its regulatory action, we have isolated mutations affecting this cation/pH response pathway. A source of new slt- mutations was the isolation of extragenic suppressor mutations of the inviable phenotype caused by certain null vps alleles. Several of these mutations mapped in *sltA* and others allowed the identification of a novel member of this pathway. The new locus has been denoted as *sltB*. This gene encodes for a protein of 1272 amino acids, also specific to filamentous fungi. A strain carrying a null allele of *sltB* was generated and characterized, and displayed an identical phenotype to that shown by a null *sltA* mutant. In these null allele genetic backgrounds we have also determined the transcriptional relationships between SltA and SltB.

Finally, in silico analysis of SltB protein showed two putative functional domains: a pseudo-kinase (Ps-kinase) domain between residues 1 and ~590, and a C-terminal trypsin-like protease domain. Additional searches with the Ps-kinase domain of SltB showed the presence in *A. nidulans* genome of another gene predictably coding for a Ps-kinase protein, also specific of filamentous fungi, whose potential role in the Slt-cation/pH8 signalling pathway has been studied. This is a first study of the role of Ps-kinases in signalling in *A. nidulans*.

Abstracts: Biotechnology and Secondary Metabolism

Endophytic fungi: A treasure trove of bioactive compounds

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There are numerous under explored microbes, which reside inside the plant as endophytes. Their potential has hardly been harnessed in pharmaceutical, agrochemical and industrial application since they have been seldom isolated and studied for their bioactive potential. Muscodor is sterile endophytic fungus that produces a mixture of volatile organic compounds (VOCs) that possess broad spectrum antimicrobial activity. The present study report the existence of four novel Muscodor species in *Cinnamomum malabaricum*, *Cinnamomum camphora* and *Aegle marmelos* growing in Western Ghats and North-east Himalayan regions of Indian subcontinent. Microscopic and Scanning electron microscopic study reveals difference in morphology from the earlier reported Muscodor species. These isolates exhibit novel volatile antimicrobial compounds like 4-octadecylmorpholine, Kopsinyl alcohol etc. Further molecular studies revealed 92–96% identity to that of Muscodor species and found to be novel species on basis of molecular data, SEM studies and gas chemistry. #16 AMLWLS, a Muscodor isolate from *Aegle marmelos* exhibited maximum antimicrobial activity by 100% inhibition of 26 pathogens where as #6610 CMSTITBRT completely inhibits the growth of 9 out of a total of 35 tested pathogens comprising plant and human pathogenic fungi, yeast and bacteria. Apart from these the three Muscodor isolates #1 CCSTITD, #2 CCSTITD and #16 AMLWLS possess potential Xanthine oxidase inhibitory (XOI) activity in the range of 75% to 89% which can be used in anti-hyperuricemic therapy as the effective preventive measure of the gout. #6610 CMSTITBRT also exhibits a potential L- Asparaginase activity an enzyme used for treatment of acute lymphoblastic leukemia. Other important endophytes producing different activities of clinical and industrial importance shall also be presented and discussed highlighting endophytes to be a treasure chest of bioactivities and bioactive compounds for medicinal and agrochemical applications.

Detection of aflatoxin from some Aspergillus sp. isolated from wheat seeds

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From (2) samples of wheat, 10 genera of fungi were isolated and identified (Alternaria sp., Aspergillus sp., Aureobasidium sp., Cladosporium sp., Dreschslera sp., Penicillium sp., Rhizoctonia sp., Stemphylium sp., Mucor sp. And Rhizopus sp.), where the highest frequency of seed-borne fungi was observed on wheat cultivar site Mol14. Their mean and standard deviation was (5.5 ±1.69), while the lowest fungal frequency isolated were Dreschslera sp. and Rhizopus sp.. Their mean and standard deviation (0.1 ±0.64) were recorded.

The results indicate that the plate 5 colony 4 (P5CO4) and plate 7 colony 4 (P7CO4) of *Aspergillus sp.* Isolated the detection of aflatoxin which grow on coconut milk agar using UV light by presence of influorescence as compared to the control. The aflatoxin-producing isolates appeared as gray or black colonies in the UV photographs, whereas nonproducing isolates appeared as white colonies, while plate 5 colony 4(P5CO4) show positive results, which means the presence of aflatoxin as compared to the controls which show the negative results. The dish was inverted and 1 or 2 drops of concentrated ammonium hydroxide solution are placed on the inside of the lid. The undersides of aflatoxin-producing colonies quickly turn plum-red after the bottom of the Petri dish has been inverted over the lid containing the ammonium hydroxide. Essentially no color change occurs on the undersides of colonies that are not producing aflatoxins. The main objective of this study is the isolation, identification and rapid detection of aflatoxin wheat seed-borne fungi.

Genetically shaping morphology of shear-sensitive marine-derived fungus *Aspergillus glaucus* for improving antitumor polyketide production

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The conventional strategies against shear-sensitive problems in fungal fermentation usually focus on adapting agitation, impeller type, bioreactor configuration and type. This work proposed a new strategy that genetically shaping shear resistant fungal morphology to establish an efficient fermentation process. A marine-derived shear-sensitive fungus *Aspergillus glaucus* HB1-19 producing an anti-tumor compound aspergiolide A was used as a model. Hyphal morphology shaping by modifying polarized growth genes was applied to reduce its shear-sensitivity and enhance aspergiolide A production. Degenerate PCR and genome walking were used to obtain polarized growth genes *AgKipA* and *AgTeaR*, followed by construction of gene-deficient mutants by homologous integration of double crossover. Deletion of *AgKipA* and *AgTeaR* caused meandering hyphae, and the hyphal curve of *ΔAgTeaR* mutant seemed larger than that of *ΔAgKipA* mutant. The germination of a second germ tube from conidium of the mutants also became more random while the growth rate and development showed no difference. Morphology of *ΔAgKipA* and *ΔAgTeaR* mutants turned to be compact pellet and loose clump in liquid culture, respectively. The curved hyphae of both mutants showed no remarkably resistant to glass bead grinding comparing with the wild type strain. However, they generated greatly different broth rheology which further caused growth and metabolism variations in bioreactor fermentations. By forming pellets, the *ΔAgKipA* mutant created a tank environment with low-viscosity, low shear stress and high dissolved oxygen tension, which then led to productions of aspergiolide A (121.7±2.3 mg/L) and catenarin (2.3±0.1 g/L) that were 82.2% and 475% higher than the wild type, respectively.

The biosynthesis of ochratoxin A by Penicillium as one mechanism for adaptation to NaCl rich foods

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The FAO estimates that up to 25% of the world's crop harvest is contaminated with filamentous fungi or their mycotoxins. For this reason it is important to understand the regulation of mycotoxin biosynthesis in order to develop prevention strategies to reduce the mycotoxin contamination of food and feed. In this study, the regulation of the biosynthesis of ochratoxin A (OTA) and citrinin (CIT), two hepato- and nephrotoxic mycotoxins produced by *P. verrucosum*, were investigated. In *P. verrucosum* the production of OTA and CIT is mutually regulated. On NaCl-rich media the biosynthesis of CIT is reduced, whereas that of OTA is increased. It could be shown that the production and excretion of the chloride-containing OTA molecule ensures cellular chloride-homeostasis under hypersalinic growth conditions. Changes in the concentration of NaCl in the environment are transmitted to the transcriptional level by the HOG-MAPK signal cascade, which results in an adaptation of gene expression. It could be demonstrated that the HOG-MAPK-pathway is substantial for the regulation of the ochratoxin biosynthesis. Western Blot experiments showed a correlation between the phosphorylation status of the HOG1-homologue of *P. verrucosum* (ACCN: KC618446) and induction of OTA biosynthesis. Inactivation of *hog1* in *P. verrucosum* by gene knock out abolishes OTA biosynthesis under high NaCl conditions. These results suggest that in *P. verrucosum*, the biosynthesis of OTA apparently act as an adaptation mechanism to hypersaline stress conditions.

Genomics-driven discovery of the pneumocandin biosynthetic gene cluster *Glarea lozoyensis* (Leotiomycetes, Helotiaceae)

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The antifungal therapy caspofungin is a semi-synthetic derivative of pneumocandin B0, a lipohexapeptide, and was the first member of the echinocandin class approved for human therapy. Pneumocandins are produced by the fungus *Glarea lozoyensis* (ATCC 20868) from Madrid, Spain. The nonribosomal peptide synthetase (NRPS)-polyketide synthases (PKS) gene cluster responsible for pneumocandin biosynthesis from *G. lozoyensis* has not been elucidated to date. We report the elucidation of the pneumocandin biosynthetic gene cluster by whole genome sequencing of the G. lozoyensis.

Sequencing of the *G. lozoyensis* with an 80× genome coverage revealed a high resolution 39.6-megabase genome with 0.5% repeat content. The *G. lozoyensis* genome encodes a rich repertoire of natural product-encoding genes including 24 PKSs, six NRPSs, five PKS-NRPS hybrids, two dimethylallyl tryptophan synthases, and 14 terpene synthases. Until now, only the gene clusters for melanin and 6-methylsalicylic acid have been linked to their end products. The fungus also produced isolecanoric and pseudogyrophoric acids, orsellinic acid dimers and trimers, likely synthesized by GLPKS23, an orsellinic acid synthase orthologue.

The pneumocandin biosynthetic gene cluster comprises a tandemly opposed NRPS (GLNRPS4) and PKS (GLPKS4), two cytochrome P450 monooxygenases, seven other modifying enzymes, and genes for L-homotyrosine biosynthesis, a component of the peptide core. Thus, the pneumocandin biosynthetic gene cluster appears significantly more autonomous and organized than that of the recently characterized echinocandin B gene cluster. Disruption mutants of GLNRPS4 and GLPKS4 no longer produced pneumocandins (A0 and B0), and the ∆glnrps4 and ∆glpks4 mutants lost antifungal activity against the human pathogenic fungus *Candida albicans*.

Characterization of the gene cluster provides a blueprint for engineering new pneumocandin derivatives with improved pharmacological properties. Whole genome estimation of the secondary metabolite-encoding genes from *G. lozoyensis* provides yet another example of the potential for drug discovery from fungal natural products.

Proteome Analysis of *Penicillium verrucosum* grown in light of short wavelength reveals an induction of stress-related proteins associated by a modified mycotoxin biosynthesis

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Mycotoxins are toxic secondary metabolites from filamentous fungi whose ingestion could lead to several health defects in human and animal. On the other hand fungi are able to produce many useful substances like hydrophobins, antibiotics, vaccines and anti-cancer substances. Because of this ambivalence, a comprehensive knowledge about the molecular mechanisms affecting biosynthesis of fungal metabolites is desirable. In this study the differential expressed protein population of *Penicillium verrucosum* incubated either in the dark or under light of 450 nm has been analyzed. Light of short wavelength leads to oxidative stress in the fungal cell, under this condition the mycotoxin biosynthesis revealed a mutual shift from ochratoxin A to citrinin. Using a proteomic approach combining an optimized protein extraction method with SDS-Page and HPLC-ESI-TOF-MS/MS mass spectrometry, several significantly diverging proteins could be detected and further identified by peptide fragment spectrum analysis using the MASCOT/SWISSPROT database alignment. Most of the identified proteins are related to metabolic processes, adaptation and response to stress. Proteome analyses are necessary to understand in general the regulation of secondary metabolite biosynthesis on translational level. This will enable possibilities to identify e.g. stress resistance proteins and triggering factors which lead to modified mycotoxin biosynthesis. This is the first proteome analysis of *P. verrucosum*.

Affecting of ergot alkaloid production in *Claviceps purpurea* by genetic manipulation

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Ergot alkaloids produced by the parasitic fungus *Claviceps purpurea* are pharmaceutically important substances widely used for production of anti-migraine drugs, uterotonics, anti-Parkinson agents, etc. Improving of ergot alkaloid production of this fungus could significantly reduce the costs of ergot alkaloid based drugs. To improve the alkaloid production we selected and overexpressed three genes from ergot alkaloid gene cluster that could lead to increased production, however this was not confirmed yet. Using the Next Generation Sequencing techniques we are analyzing the transcriptomes of four commercial strains, which differ in the spectrum of the produced alkaloids. We suppose that comparison of differential expression of genes directly or indirectly involved in ergot alkaloid biosynthetic pathway between these strains might help us to find new candidate genes, which could be manipulated to increase the production. Since the fungus strains, which we are using for genetic manipulations can produce alkaloids only when infecting a host, we are studying the process of infection as well. Interestingly, we found that two selected strains differ not only in the spectrum of produced alkaloids, but also in the progress of infection.

Next-generation genome and transcriptome based methods for the exploration of secondary metabolites from marine fungi for the treatment of cancer

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Fungi of marine origin are a potent group of secondary metabolite producers. However, they are not well-characterized and underutilised in terms of biotechnological applications. We aim for sustainable exploration of marine fungal isolates and their encoding natural products as drugs against cancer under the EU-funded project marine fungi (www.marinefungi.eu). Besides isolation of new fungal strains from unique marine habitats, the molecular development of effective producer strains is in the focus. Genomes of selected candidate strains originating from our unique strain collection of marine fungi are currently characterized with respect to secondary metabolite production.

Next-generation sequencing (NGS) techniques have changed the facets of genomics and its application. We have established the genomic sequences from three marine isolates, *Scopulariopsis brevicaulis*, Pestalotiopsis sp. and Calcarisporium sp. by the use of different next-generation sequencing methods (Roche 454, Illumina and ion-torrent).

We report on different properties of genome assemblies and annotations for these fungi. Several gene families and superfamilies have been analyzed to explore genetic peculiarities of these species along with repeats and transposable element contents. The assembled genome of *Scopulariopsis brevicaulis* is ~32 Mb in size with N50 equals to 88 kb and 935 contigs containing 16298 genes with average intron length equals to 129.4. During the annotation process, we were able to annotate 9340 genes (57.31 %) while 6958 genes (43.69 %) remained non-annotated in *Scopulariopsis brevicaulis* genome. 17 genes encoding for non-ribosomal peptide synthetases (NRPSs), 18 polyketide synthases (PKSs) and one gene encoding a hybrid NRPS-PKS were found. Similarly, the genome size for Pestalotiopsis sp. is ~46 Mb with N50 equals to 71.9 kb and 4186 contigs containing 23492 genes, which is surprisingly high for an ascomycete. The average intron length and the average intron per gene are 126.8 and 2.2, respectively. During annotation process, we annotated 60% genes of Pestalotiopsis genome with 44 NRPSs, 62 PKSs and 7 hybrid NRPS-PKS genes. The assembled genome size of Calcariosporium sp. is about 35 Mb genome with N50 equals to 91.9 kb and 2464 contigs containing 15459 genes. The percentage GC% for this genome is 50.7%. The average intron length and the average intron per gene are 121 and 2.1, respectively. During annotation process, we annotated 72% genes, while 28% genes remained non-annotated for Calcariosporium genome with 52 NRPSs, 66 PKSs and 7 hybrid NRPS-PKS genes.

Predicted genes are presently in process of validation using illumina based RNA-seq. We are also comparing wild type phenotypes with higher-yielding mutants of these fungi with special interest on specific natural compounds.

Itaconic acid biosynthesis of the phytopathogenic fungus *Ustilago maydis*

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Itaconic acid is a dicarbonic acid that is widely used in industrial productions. It is produced biotechnologically by fermentation of the filamentous fungus *Aspergillus terreus*. The biosynthesis is well characterized and depends on the key-enzyme cis-Aconitate-decarboxylase (CAD). Production of Itaconic acid has also been described for some other basidiomycetous fungi e.g. *Pseudozyma antarctica* and *Ustilago maydis*. Interestingly, the genome of *U. maydis* contains no ortholog of the *A. terreus* CAD-gene. Therefore we searched for an alternative pathway for Itaconic acid biosynthesis and were able to identify a gene cluster, which is most likely responsible for Itaconic acid production in *U. maydis*.

Identification and characterisation of new biosurfactants from basidiomycetes

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Many fungi are able to synthesize complex secondary metabolites. These are not essential for viability but procure the fungi advantages over other microorganisms. One important group of secondary metabolites are glycolipids. These amphipathic, surface-active compounds act as biosurfactants. In general they increase the availability of hydrophobic nutrients and enhance attachment to nonpolar surfaces. In some cases they also display antimicrobial activity and play an important role in biofilm formation.

The basidomycetous fungus *Ustilago maydis* is known to produce large amounts of two structurally different extracellular glycolipids under nitrogen starvation. Ustilagic acid (UA), which is a cellobiose lipid with antimicrobial activity and the extracellular oil mannosylerythritol lipid (MEL). The genes, which are essential for the synthesis of both biosurfactants were identified, and showed clustered formation in the genome.

In our project we want to identify new biosurfactants first from basidiomycetes and later from other fungi, which are collected by our research partners from the Integrative Fungal Research Group (Frankfurt/Main). We will sequence the genomes and identify the gene clusters of the biosurfactants. Then the new biosurfactants will be characterised concerning their biotechnological applications.

A comparative systems analysis of polysaccharide utilization in *Neurospora crassa* reveals molecular mechanisms of carbon adaptation as well as new factors with impact in biotechnological applications

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The need for a stable and sustainable source of energy is one of the grand challenges of our generation. Using lignocellulosic plant biomass as a renewable feedstock can contribute substantially on our way to meet this goal. While this approach drastically increases the carbon efficiency, lignocellulose evolved naturally to be highly resistant to breakdown processes, rendering the depolymerization step one key bottleneck in the industrial process. Due to their importance in global carbon recycling in particular fungi are among the most promising tools to overcome the resistance of the cell wall. While enzyme cocktails derived from them are already being used by the industry for plant cell wall deconstruction, they are typically not flexible enough to be used with similar efficiency on different feedstocks. A more profound knowledge of the fungal interaction with different plant substrates will therefore be highly informative for efforts to achieve a cost-effective biofuel production process.

In the presented work, we initiated a systematic approach and analyzed the fungal perception of the three major plant-derived polysaccharides (cellulose, xylan and pectin) by using the model filamentous fungus *Neurospora crassa*. The comparison of the individual responses allowed us to deduce fundamental knowledge on fungal adaptation strategies to the carbon composition in its environment. These included specific adaptations, such as a re-organization of the endoplasmatic reticulum upon sensing of cellulose, but also commonalities, such as a set of enzymes that is cross-induced on all carbon-sources and could thus be part of a special polysaccharide scouting system. Our study moreover revealed new factors involved in specific sugar utilization pathways with an impact in biotechnological sugar fermentation processes. Here, we report on the identification of novel MFS-type transporters for the uptake of pectic monosaccharides, many of which cannot be consumed by currently used yeast strains. For example, we describe the first eukaryotic high-affinity transporter for the main pectic backbone sugar galacturonic acid, GAT-1. To test its applicability, GAT-1 is utilized in combination with a fungal galacturonic acid metabolism pathway, to engineer a yeast strain able to grow on this monosaccharide. Further improvements will allow for an efficient utilization of pectin-rich agricultural waste streams to be used for example for biofuel production.

Degradation of biodegradable plastics by plant pathogenic fungi

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Recently, synthetic plastics have been widely used in many industries as basic materials. However, they have also caused severe environmental problems because most of these plastics persist in environment for many years. To date, many aliphatic polyesters which can be degraded in compost and moist soils, such as poly-(butylene succinate) (PBS) and poly-(butylene succinate-co-butylene adipate) (PBSA) have been developed as biodegradable plastics (BPs) with excellent formability like polyethylene. Biodegradable mulch film is already used in agriculture to reduce labor input for disposing regular plastics materials after use. On the other hand, degradation speed of BPs in agricultural fields is sometimes very slow, as it is largely affected by the environmental conditions. Techniques for promoting the degradation of BPs are needed.

The aerial parts of plants are covered by a continuous extracellular membrane of hydrophobic polymerized lipids called the cuticle. Both BPs and the cuticular layer of phylloplane are reported to be made of polyesters of fatty acids (Heredia 2003). This fact has led us to speculate that phylloplane microorganisms may effectively degrade BPs. Following this idea, we recently discovered that many phylloplane yeasts on rice and vegetables have strong abilities to degrade PBS and PBSA mulch film (Kitamoto et al. 2011). Since phylloplane is a typical habitat for various fungi, we have speculated that effective BP-degraders may also be present among phylloplane fungi. Since fungi with the ability to BPs were thought to inhabit plant surfaces, they were isolated from the leaf surface of gramineous crops (grown in the fields of Tsukuba, Japan) and cultured on a selective medium supplemented with PBSA emulsion as the only source of carbon (Koitabashi et al. 2012). Seventy strains of emulsified PBSA-degrading fungi were selected, including plant pathogens such as Alternaria, Fusarium, and Phoma. Out of 68 fungal phytopathogens belonging to the abovementioned genera stocked in the National Institute of Agrobiological Sciences Genebank (Japan), 18 strains were found to have emulsified-PBSA ability. While the degrading ability varied among different *Phoma destructiva* strains, all *Fusarium solani* strains isolated from sweet pea, passion fruit and devil's tongue (*Amorphophallus konjack*) were capable of degrading emulsified-PBSA. Among the fungi that exhibited the emulsion-degrading ability, eight strains, including *Alternaria porri*. *F. solani f. sp. pisi* and *Phoma destructiva*, were capable of degrading agricultural PBSA and PBS-based mulchfilms, which are commercially available in Japan.

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Genomic and metabolomic analysis of polyketide production in the endophytic fungus, *Ascocoryne sarcoides*

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Polyketides are an important group of fungal secondary metabolites that exhibit a wide range of structural diversity and function, with many reported to be involved in protection (e.g. pigments) and defence (e.g. antibiotics). The structural diversity of polyketides is largely attributed to the polyketide synthases that catalyse their synthesis, due to their exceptional assemblage and combinatorial power. Research was conducted to evaluate polyketide production in the endophytic fungal species, *Ascocoryne sarcoides*. *A. sarcoides* was found to produce an array of non-volatile linear polyketides, which were structurally characterised via HREIMS and NMR, indicating the metabolites were novel. The unique structure of the polyketides consisted of a central polyol (likely mannitol), flanked by two tetraketides. The polyketides exhibited varying levels of reduction along the central carbon chain, which is indicative of biosynthesis via a highly reducing iterative polyketide synthase. These polyketides exhibited bioactivity against the Gram positive bacteria *Bacillus subtilis*. The genome of the isolate of *A. sarcoides* was sequenced, assembled and annotated, which enabled genome mining for polyketide synthases and other secondary metabolite genes. A total of three polyketide synthases were identified, which contained domains associated with highly reducing iterative polyketide synthases. Genetic diversity studies were conducted to ascertain relatedness to functional polyketide synthases of fungi (e.g. lovastatin, fumonisin, T-toxin). The gene clusters surrounding the polyketide synthases were also profiled to ascertain genetic mechanisms involved in polyketide production (i.e. transporters, regulators, ligases, etc). This study has used advances in metabolomics and genomics to conduct an in depth analysis of polyketides from *A. sarcoides*, and the genes that regulate their production. Future studies will look to functionally characterise these genes and determine their potential application in the agricultural sector.

Malate production by *Aspergillus orzyae*

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L-malic acid is a C4 dicarboxylic organic acid and considered as a promising chemical building block. It can be applied as food preservative and acidulant, in rust removal because of its chelator properties and as polymerization starter unit due to its bifunctionality. Up to now it is produced chemically from crude oil via maleic anhydride. The mould *Aspergillus oryzae* produces malic acid in large quantities from glucose and other carbon sources. The microbial production of organic acids from renewable sources has the potential to be a sustainable alternative to petroleum and to reduce greenhouse gases as CO_2 fixation is involved in microbial biosynthesis.

The aim of this study was to optimize production conditions in shaking flask experiments and to establish a bioreactor fermentation to produce malic acid in large quantities.

A. oryzae was cultivated in preculture medium in shaking flasks for 17 hours. Due to nitrogen limitation and an excess of glucose in the production medium the fungus started to produce malic acid. In shaking flask experiments the optimal production temperature was determined. After optimization a bioreactor process was established in 1.5 L scale. Organic acid concentration was measured by HPLC.

The optimal production temperature was determined to be 35°C. Up to 52 g/L malic acid were produced which corresponds to a molar yield of 1.13.

Production of Bioethanol from Rice Hulls by *Aspergillus niger* MA1 and *Saccharomyces cerevisiae*

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First step in our strategy in utilization of rice hulls (RH) for bioethanol production is isolation of fungal isolate produce considerable amount and suitable mixture of cellulases which can be used efficiently in hydrolysis of RH cellulose to glucose. For this purpose, more than 1103 mesophilic fungal isolates were isolated from different samples of RH. The most active isolate is *Aspergillus niger* MA1 which produce 11.60, 3.68 and 61.25 U/gm RH for CMCase, FPase and β-glucosidase respectively, by solid state fermentation after only 2 days of incubation.

CMCase, FPase and β-glucosidase of *A. niger* MA1 showed a relatively high activity in broad temperature and pH range, with maximum activity at 50°C and pH 5. Statistical optimization of solid medium revealed increases in activity of CMCase by 3.5 fold increase from 6.0 to 21.2 U/gm, FPase are increase from 0.5 U/gm to 4.6 U/gm (9 fold) and β-glucosidase activity are resulted 3.9 fold increase from 18.2 to 71.2 U/gm.

For extraction of cellulases after fermentation, addition of 0.25% brej 35 to tap water or saline is very effective. Enzymatic saccharification of crud and pretreated RH using *A. niger* cellulases revealed that amount of reducing sugars produced from pretreated RH with steam explosion only are higher than that of combined pretreatment steam explosion with 7.5% H_2O_2 (16.36 and 14.3 g/l respectively). Amount of ethanol produced by fermentation of hydrolyzate by *Saccharomyces cerevisiae* after 24 h are 9.42 and 8.39 g/l for steam exploited RH and steam explosion with 7.5 % H_2O_2 respectively.

Tackling the protease problem to optimize unconventional protein secretion in *Ustilago maydis*

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Establishing and applying novel protein expression systems is essential to produce the full repertoire of economically relevant proteins. To fill existing gaps, *Ustilago maydis* was established and applied as a novel protein expression platform. Recent findings demonstrated that protein expression in this eukaryotic microorganism is extensively regulated at the posttranscriptional level. In particular, the key RNA-binding protein for long-distance mRNA transport, Rrm4, is essential for efficient unconventional secretion of the endochitinase Cts1. We are using the Rrm4-dependent Cts1 secretion apparatus to export various heterologous proteins such as enzymes like β-glucuronidase or biopharmaceuticals like single-chain antibodies (scFv) and higher antibody formats.

It was observed that during secretion of these heterologous proteins, degradation by the host proteases led to lower molecular weight products and hence reduced stability of secreted heterologous proteins. To solve this protease problem, the gene for a central activator protein (Cac) for proteases was deleted in expression strains and reduced protein degradation was observed. However, the deletion of *cac* also led to a peculiar phenotype and lowered growth rates, which may lead to problems during bioreactor studies. Hence in the next step key proteases were identified based on homology to other known key proteases of filamentous fungi. The respective genes were deleted and resulting strains then tested for increased protein secretion.

As the elimination of single proteases was likely not sufficient, the most promising approach to tackle this protease problem is to generate a multiple-protease deficient expression strain including the previously identified candidates. To this end, a FLP-FRT marker recycling system was used to generate such a multiple-protease deficient expression strain. Using this system, the same selection marker is used again and again in repeated rounds of protease gene deletions in a single strain. This modified strain was then checked for reduced heterologous protein degradation and hence enhanced full-length protein secretion of heterologous proteins. Thus, protease deletion strategy constitutes one of the key optimizing steps for generating an improved protein expression system in *U. maydis*.

Spiking enzyme cocktails with additional enzymes for better saccharification

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Saccharification of plant biomass is an essential step in the conversion of plant matter to high-value products, such as 2nd generation biofuels. Several commercial enzyme cocktails are available that are able to hydrolyse part of the plant biomass, but their efficiency is insufficient to obtain an economically sustainable process. To improve the efficiency, we not only need to identify the missing enzymes, but also determine the optimal ration in which these enzymes are present in the mixture.

In this project we have initiated studies to answer some of these questions. We used a collection of *Pichia pastoris* strains (Bauer et al., 2006) producing various plant biomass-degrading enzymes from Aspergillus to purify specific enzymes. These enzymes were then used in combinations with each other as well as commercial enzyme cocktails for saccharification of different plant biomass substrates to identify which activities are lacking or insufficiently available in the commercial mixtures. Highlights of this study will be presented.

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A model chimeric transcription factor: construction and characterization of an AmyR::XlnR hybrid transcription factor

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Aspergillus oryzae is extensively used for the production of traditional Japanese fermented foods and industrial enzymes, because it has ability to produces a large amount of enzymes such as amylases and xylanases. Expression of the genes encoding amylases and xylanases is controlled by the specific $Zn(II)_2Cys_6$-type transcription factors, AmyR and XlnR, respectively. Although approximately 200 genes with the $Zn(II)_2Cys_6$ motif are found from the *Aspergillus oryzae* genome sequence, most of them are uncharacterized. To find new transcription factor and its regulation is useful for the application of the fungus. However, it is difficult to know the inductive (or repressive) conditions of such an `orphan transcription factor`. Furthermore, while N-terminal regions containing the DNA binding motif are highly conserved among the $Zn(II)_2Cys_6$ family, amino acid sequence of C-terminal regions are not conserved each other, which is not informative to speculate their functions. To gain insight into such orphan transcription factors, a chimeric transcription factor with a DNA binding domain of interest and the C-terminal moiety of well-characterized transcription factor, in combination with the microarray analysis or whole transcriptome shotgun sequencing, would be a powerful tool, since the inductive conditions are already known.

In this study we constructed an artificial AmyR::XlnR chimeric transcription factor as a model transcription factor by replacing the XlnR DNA binding domain with that of AmyR. The gene encoding the chimeric protein of an N-terminal region containing the DNA-binding domain of AmyR (1-68aa) and the C-terminal moiety of XlnR (159-973aa) with FLAG-His-tag at the N-terminus was expressed in the *amyR* deficient mutant strain. Expression of the AmyR::XlnR transcription factor was confirmed by the western blot analysis with anti-FLAG antibody. As expected, the strain expressing AmyR::XlnR produced significant amount of amylase in the presence of xylan or xylose, which is an inducer of xylanese. On the other hand, starch did not induce amylases in the strain as judged by amylase activity. These results suggest that the replacement of the DNA binding domains was successfully carried out and that the strategy of using a chimeric transcription factor is effective to analyze uncharacterized transcription factors.

Biomolecular mechanisms involved in the degradation of black slate

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Schizophyllum commune is a filamentous basidiomycete used for physiological and genetic studies for several decades. Because of its ability to excrete exoenzymes, the fungus can decompose lignin and other organic compounds from wood. Some of these enzymes have a wide substrate specificity and so they are capable to degrade other substrates like rocks. Due to this, *S. commune* can affect and decompose organic matter from rocks like low grade metamorphic black slates. It was shown that excreted laccases are involved in the degradation process and seem to have an effect on the carbon mobilization from black slates. To detect other enzymes taking part in this biomolecular mechanism, the secreted proteome of *S. commune* grown with black slate was analyzed by mass spectrometry. The results showed differences in protein composition and their expression between monokaryotic and dikaryotic strains. A quantitative analysis showed upregulations e.g. for oxidoreductases as well as downregulations e.g. for glycoside hydrolases of different proteins compared to samples without black slates. The proteins involved in the degradation process seem to be a complex network in which the detailed role of some proteins for *S. commune* is still not clear. Thus, overexpressions of some genes are planned to gain an explicit insight into their function.

A point mutation in Xyr1 causes a glucose-blind, inducer independent enzyme production phenotype in *Trichoderma reesei*

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The saprophytic ascomycete *Triochoderma reesei* is industrially exploited as a high yielding producer of cellulases which are used in different industrial applications. The availability of cheap cellulases is considered to be a crucial prerequisite for the production of competitive 2nd generation biofuels. A great proportion of the production costs arise from the inducers necessary for cellulase expression. Most industrially used *T. reesei* strains are derived from the strain Rut-C30, in which cellulase and xylanase expression is released from carbon catabolite repression mediated by the transcription factor Cre1. Nevertheless, inducing substances are still necessary for a satisfactory amount of enzyme formation.

Here, we report on a strain secreting high levels of xylanases and cellulases in a glucose-blind manner. Xylanases are produced completely inducer independent while cellulases expression can still be slightly induced by sophorose. We identified a single point mutation in the gene encoding the Xylanase regulator 1 (Xyr1) to be responsible for this phenotype. Inserting this mutation in other Trichoderma production strains caused an identical phenotype. This point mutation is localized in a so-called fungal transcription factor regulatory middle homology region (FTFRMH region). Xyr1 additionally bears a Gal4-like DNA-binding domain. A lot of fungal transcription factors share a similar architecture.

Consequently we conclude that engineering a key transcription factor of a target regulon is a promising strategy in order to increase enzyme yields independent of the substrate or inducer used. Furthermore this regulatory domain, in which the described mutation is located, is certainly an interesting research target for all organisms that also depend on certain inducing conditions so far. These findings have already been published in "Biotechnology for Biofuels".

Unconventional splicing of *hacA* intron is essential for growth of *Aspergillus oryzae* under induction condition of amylolytic gene expression

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The endoplasmic-reticulum (ER) endonuclease IRE1 is a sensor protein of ER stress. During ER stress condition, activated yeast IRE1 cleaves the intron of HAC1 mRNA, encoding a transcription factor regulating unfolded protein response. This unconventional splicing results in frame-shift of HAC1, and functional HAC1 protein is translated from intronless HAC1 mRNA (inducible HAC1; HAC1i). In filamentous fungi, it has been suggested that *ireA* (orthologous gene of IRE1) is an essential gene for growth of *Aspergillus niger*. In contrast, however, recent report showed that knockout strain of *ireA* in *Aspergillus fumigatus* was viable. Although we tried to generate the *ireA* knockout strain of *Aspergillus oryzae*, heterokaryotic knockout strain could not be obtained. Therefore, we generated the conditional expression strain of *A. oryzae ireA*, and function of IreA under induction condition of amylolytic gene expression was examined.

To generate the *ireA* conditional expression (*ireAc*) strain, promoter region of *ireA* was replaced by promoter region of *nmtA*, harboring a riboswitch within its 5´-untranslated region. The resultant *ireAc* strain showed growth defect on glucose medium under repression condition. Interestingly, *ireAc* strain was inviable on maltose and starch media, suggesting that IreA was essential for growth on these media. In the presence of maltose, *A. oryzae* produces copious amounts of amylolytic enzymes, and induction of amylolytic enzyme encoding genes are regulated by transcriptional activator AmyR. To investigate the effect of amylolytic gene expression on growth of *ireAc* strain, we deleted *amyR* gene of *ireAc* strain. The *amyR* deleted *ireAc* strain was viable on maltose medium, suggesting that IreA is essential for growth under induction condition of amylolytic gene expression.

To examine the involvement of unconventional splicing of *hacA* intron in growth of *ireAc* strain, synthetic *hacAi* DNA fragment was introduced into *ireAc* strain. The resultant transformant is viable on maltose and starch media. In addition, we successfully obtained the homocaryotic *ireA* knockout strain by using synthetic *hacAi* expression strain as a host strain, and this *ireA* knockout strain was also viable on maltose and starch media. These results suggest that unconventional splicing of *hacA* intron by IreA is essential for growth of *A. oryzae* under induction condition of amylolytic gene expression.

Chromatin rearrangement analysis of Xyr1 regulon genes in *Trichoderma reesei*

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Trichoderma reesei (Hypocrea jecorina) is a filamentous ascomycete of various industrial applications because of its high secretory capacity of hydrolytic enzymes i.e. cellulases and xylanases. Besides already well-studied transcriptional regulation of Xyr1 regulon genes, including as well a carbon catabolite repression mediated by Cre1 and associated transcription factors (Ace1, Ace2, Hap2/3/5, putative repressor Xpp1), hardly anything is known about chromatin-dependent regulation due to rearrangement dynamics. The aim of this work was to employ the Chromatin Accessibility Real Time-PCR (CHART-PCR) to monitor and quantify chromatin rearrangements of genes under control of Xyr1 in *T. reesei*. The core promoter regions of *cbh1, cbh2, xyn1, xyn2* and *xyr1* in the wild-type QM6a strain and the hypercellulolytic mutant RUT-C30 were subjects of investigations under inducing (D-xylose and α-sophorose) and repressing conditions (D-glucose). Our results demonstrated that the method established is sensitive enough to discern chromatin remodeling in different growth conditions employing the same strain or while comparing different strains at the same growth condition. In QM6a it was observed a specific chromatin remodeling upon sophorose treatment for *cbh2*. On the other hand, in RUT-C30 this same effect could be observed for all promoters analyzed and with a higher accessibility in comparison to QM6a. Another important finding is that for the *xyr1* promoter RUT-C30 presents a more accessible chromatin structure in all conditions tested. Moreover, chromatin loosening in the analyzed promoters does not always correlate with higher expression profiles, reinforcing the dependence of an inducer. Transcription analysis of genes related to chromatin remodeling proteins such HDA1 and SIR2, two putative histone deacetylases and DIM2, a putative DNA-methyltransferase was also carried out. In QMa6 hda1 and sir2 were down-regulated in glucose and up-regulated in xylose and sophorose while presented a constant down-regulated expression profile in RUT-C30. A similar pattern was observed for dim2, with a pronounced induction in sophorose in QM6a while remaining constant in RUT-C30. Taken together these data suggest that RUT-C30 presents a less tight control of gene regulation due chromatin remodeling. The possible role of DNA-methylation in Xyr1 regulon genes is currently been investigated by bisulfite DNA modification followed by High-Resolution Melt (HRM) analysis.

Fungi challenge food security and plant ecosystem health

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Fungal diseases have been increasing in severity and scale since the mid 20th Century and now pose a serious challenge to global food security and ecosystem health (Gurr et al., 2011, Fungal Biology Reviews 25 181-188). Indeed, we have demonstrated recently that the threat to plants of fungal infection has now reached a level that outstrips that posed by bacterial and viral diseases combined (Fisher et al., 2012 Nature 484 185-194).

This presentation will highlight some of the more notable persistent fungal and oomycete plant diseases of our times. It will draw attention to i) the emergence of new pathotypes affecting crop yields and to fungi and oomycetes decimating our natural and managed landscapes ii) some recent work looking at the movement of pests and pathogens polewards in a warming world (Bebber, Ramatowski and Gurr., Nature Climate Change (in press)) iii) our model, which predicts "missing" pathogens in a given country and iv) at the determinants of the global distributions of crop pests and pathogens (Bebber et al, under review). The presentation will conclude with a consideration of the "mechanisms" driving emergence of fungi in natural and crop ecosystems and of future threats and challenges.

The evolution of aldo-keto reductases in ancient fungi

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Ancient fungi comprise paraphyletic groups, which are traditionally classified to the Zygomycetes and Chytridiomycetes encompassing ancient terrestrial and aquatic fungi, respectively. Aldo-keto reductases embrace a superfamily of enzymes that catalyze redox transformations involved in biosynthesis, intermediary metabolism, and detoxification. They exhibit a wide variety of substrates including glucose, steroids, glycosylation end-products, lipid peroxidation products, and environmental pollutants. The evolution of this interesting enzyme group is studied in order to explore its diversity and potential to catalyze redox transformations involved in biosynthesis, intermediary metabolism, and detoxification spanning a link between primary and secondary metabolism.

Functionalization of surfaces by fusion proteins containing hydrophobins from *Aspergillus nidulans*

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Fungal hydrophobins are small amphiphilic proteins that can self-assemble into extremely stable monolayers at hydrophilic/hydrophobic surfaces. Their ability to change the nature of interfaces makes them a perfect tool for coating and functionalizing surfaces. One of their possible applications is fusion of hydrophobins with synthetic antimicrobial peptides (AMPs) to prevent biofilm formation on implants and medical devices. Antimicrobial peptides are 9 to 50 amino acids long and are widely spread in nature as part of the innate immune system. They are effective against gram-negative and gram-positive bacteria, fungi, parasites and enveloped viruses. Certain AMPs can be tethered to a surface and remain functional. We fused hydrophobin DewA from *Aspergillus nidulans* to the AMPs Tet009 (RRWKIVVIRWRR) and Tet026 (WIVVIWRRKRRR) and used the proteins for surface coating and functionalization. The immobilized Tet009-DewA fusion protein caused death of *Pseudomonas aeruginosa* and *Staphylococcus aureus* and Tet026-DewA has prevented even initial bacterial attachment, illustrating the capability of AMP-hydrophobin constructs to prevent biofilm formation.

Using conserved regulatory patterns for prediction of secondary metabolite gene clusters in fungi

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Fungi are known to produce a wide range of structurally diverse and pharmaceutically important secondary metabolites (SMs), such as antibiotics, toxins, etc. The enzymes responsible for the biosynhesis of the SMs, polyketide synthases (PKS), non-ribosomal peptide synthetases (NRPS) or PKS-NRPS hybrids, are multimodular megasynthases that are relatively easy to detect in the genome due to their large size and specific multidomain structure. The genes involved in the biosynthesis, transport, and regulation of these metabolites are often organized in clusters, i.e., they are co-regulated and co-localized. The latter means that the genes are contiguous, however, insertions of non-cluster genes are not excluded. The sets of genes involved in clusters largely varies between the clusters and there are no marker genes besides the PKSs or NRPSs. This makes *in silico* prediction of clusters a challenging problem. On the other hand, such predictions become more and more important for the experimental design and elucidation of potential product`s structure and function.

Most of the methods developed so far for the detection of SM clusters are similarity based. Given the low number of characterized clusters and the diversity in their contents, such methods are error-prone and tend to overestimate the clusters` lengths. However, such important feature as co-regulation, which is the basic idea of the cluster definition, has never been considered as a basis for cluster prediction.

We suggest a novel method to predict SM gene clusters based on consideration of regulatory patterns in cluster promoters. The basic idea is that the cluster-specific TFBSs should be enriched in the cluster in comparison to other parts of the genome, i.e., the cluster-specific motifs occur with higher density within the cluster region. Yet, they may occur outside the cluster. We characterize promoters by cluster-specific motif occurrences and consider the density of the motifs as the main feature of the cluster region. We call our approach the motif density method (MDM).

In the first step, the algorithm scans an interim set of promoters around the SM anchor gene (PKS or NRPS) for over-represented motifs (several interim sets are considered). Next, each significant motif is submitted to a search in all promoter sequences genome-wide. In the following, we switch to consideration of promoters as units characterized by occurrences of a particular motif. Thus, instead of the real genomic sequence we consider a string of numbers, which represent the motifs' occurrences in a unit. This number string is than scanned by a sliding window, counting the number of motifs` occurrences per frame. Consideration of different frame lengths allows us to determine the real cluster length.

MDM was validated on the examples of known SM clusters from different fungal species. We demonstrate that it successfully re-identifies the clusters and tends to outperform the most prominent similarity-based tool SMURF. To this end, MDM can separate closely localized clusters, the task that is failed by similarity based methods. We also show that the method works on the completely unknown clusters. MDM not only allows to reliably predict the SM clusters but also provides information about the potential regulators of the cluster (by description of their TFBSs).

Construction of synthetic pathways in *Penicillium chrysogenum*

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The recent development of recombinant DNA techniques in fungi allow to manipulate genes or groups of individual genes that affect metabolic pathways. These techniques can be used to improve industrial production strains or to design strains capable of producing completely new compounds.

The aim of this work is to get more insights in the concept of synthetic pathway design and construction for the well-known b-lactam antibiotic producing fungi *Penicillium chrysogenum*.

For synthetic pathway design several aspects have to be taken into account like for example the biosynthesis route and the corresponding enzymes, their expression level, regulation and activity, the order of genes, targeting spots, unwanted side activities etc.

As a first step towards synthetic pathway design in *P. chrysogenum*, we are investigating the effect of different chromosomal integration sides by reintegration of the penicillin biosynthesis pathway in a host strain with genomic deletion of the original penicillin cluster.

Therefore we reassembled the penicillin biosynthesis pathway genes (*pcbAB*, *pcbC*, *pcbDE*, 18 kb) together with a fungal selection marker and different flanking regions for targeted integration in the genome.

Comparing the penicillin production of the transformants obtained will allow conclusions on the performance of the newly assembled pathway and the influence of the integration sides investigated.

Endophytic fungi from some medicinal plants in Northern Thailand: Biodiversity, secondary metabolites and application

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Endophytic fungi have been recognized to be valuable sources of secondary metabolites. The bioactive compounds from endophytes may original produce by plants or endophytic microbes activate plant to produce novel bioactive compound usually derive from novel endophytic microorganisms. In this study, diversity of endophytic fungi from some medicinal plant and their potential to produce antimicrobial compounds were investigated. A total of 3,261 culturable endophytic fungi were isolated from 2,625 samples taken from healthy leaves (vein and intervein) and stems of *Cinnamomum bejolghota* and *Lagerstroemia loudoni* collected from mature plants in northern Thailand. *Colletotrichum gloeosporioides*, Phomopsis spp. and Xylariaceous were the most frequency isolated endophytic fungi. The endophytes were classified into 12 ascomycetes, 1 basidiomycete and 31 mitosporic fungi (12 coelomycetes and 19 hyphomycetes) using traditional morphological methods. Volatile and non-volatile metabolites were evaluated from 202 endophytic fungi by dual culture volatile assay and agar well diffusion assay, respectively. The result showed that non-volatile metabolites from *Mycoleptodiscus terrestris* CMU-Cib179 culture showed the highest inhibition activity against tested organisms. Interestingly, we found three volatile-producing endophytic fungi belong to *Muscodor cinnamomi* CMU-Cib461 and *Nodulisporium* sp. CMU-Cib1018 and CMU-UPE34. The volatile compounds were identified by gas chromatograph and mass spectrometer. *Muscodor cinnamomi* and *Nodulisporium* sp. CMU-UPE34 and CMU-Cib1018 produced 2-methylpropanoic acid, eucalyptol and 1,3,2, dioxannorolan,4,4-dimethyl-5-oxo-2-ethyl as the major, respectively. Fumigation to control microorganisms was confirmed *in vitro* and *in vivo*. The results showed that volatile producing fungi have high potential to control wide range of plant pathogens and has no harmful residue.

Substrate specific enzyme production by *Podospora anserina*

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Due to the highly diverse nature and composition of plant biomass, fungi need to produce a broad range of enzymes to efficiently degrade this substrate. Many saprobic fungi largely depend on this substrate as a carbon source in natural biotopes and have therefore developed extensive enzyme sets. While some fungi occur in many different biotopes (e.g. Aspergillus), other fungi are more restricted in their biotope choice. One such fungus is *Podospora anserina* that is only found as a late colonizer of herbivore dung.

The main carbon source in this biotope is lignocellulose. A plant-based complex consisting of cellulose, hemicellulose and lignin. Earlier studies have demonstrated that the *P. anserina* genome is enriched in genes encoding cellulases and hemicellulases. In contrast, genes encoding enzymes related to other polysaccharides that are not common in dung (e.g. pectin, inulin) are reduced in the *P. anserina* genome.

In this study we have grown *P. anserina* on three plant biomass substrates (wheat straw, cotton seed hulls and soy bean hulls) and compared the secretome on different time points. In addition, we have tested the enzyme mixtures produced on these substrates for their ability to hydrolyse a variety of feed stocks.

Direct ethanol production from glucose, xylose and sugarcane bagasse by the corn endophytic fungi *Fusarium verticillioides* and *Acremonium zeae*

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The fungus *Fusarium verticillioides* produced ethanol from glucose, xylose and a mixture of these two sugars in limited oxygen conditions with yields of 0.47, 0.46 and 0.50 g/g of ethanol per sugar utilized. The fungus *Acremonium zeae* produced ethanol from glucose, xylose and mixture of these two sugars with yields of 0.37, 0.39 and 0.48 g/g of ethanol per sugar utilized. Both fungi were able to co-ferment glucose and xylose. *Fusarium verticillioides* and *A. zeae* produced high endoglucanase and xylanase activities using sugarcane bagasse as substrate. Ethanol production from 40 g/L of pre-treated sugarcane bagasse was 4.6 and 3.9 g/L for *Fusarium verticillioides* and *A. zeae*, respectively. Both fungi studied were capable of co-fermenting glucose and xylose at high yields. Moreover, they were able to produce ethanol directly from lignocellulosic biomass, indicating to be suitable microorganisms for consolidated bioprocessing.

Study the growth of *Aureobasidium pullulans* on the oil-impregnated wood related to the degree of polymerization of the oil

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Biofilms of *A. pullulans* can be formed on the oil-impregnated wood. Such an oil-biofilm system has the potential of protecting the wood against water penetration, UV and bio degradation, and creating a homogeneously black surface with self healing properties. Consequently, it is a good candidate to be used as an alternative for traditional wood coatings. To this end, low cost, fast, large-scale production, and stable functional performance in time are required. For all these issues fundamental understanding of the growth mechanism related to its influential factors is needed. A qualitative study of the growth of *A. pullulans* on different woods impregnated with different vegetable oils suggests that, regardless of the type of wood, growth on oils with lower tendency to polymerization is more abundant. Motivated by this observation, we present a theoretical study on the effect of degree of polymerization of the oil on fungal growth. A mathematical model for growth coupled to the nutrient uptake mechanisms (including enzyme secretion, enzyme-substrate reaction, and enzyme/nutrient transport) is developed. The results are in agreement with our qualitative experiment and show that as the degree of polymerization of the oil increases, the growth decreases due to decrease in mobility of both enzyme and nutrient particles.

Chrysoporthe cubensis: a new source of cellulases and hemicellulases to application in biomass saccharification processes

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The plant pathogenic fungus *Chrysoporthe cubensis* was cultivated under solid state employing different substrates and the highest endoglucanase (33.84 U/g), FPase (2.52 U/g), β-glucosidase (21.55 U/g) and xylanase (362.38 U/g) activities were obtained using wheat bran as carbon source. Cellulases and xylanase produced by *C. cubensis* showed maximal hydrolysis rate at pH 4.0 and in a temperature range of 50-60°C. All enzymatic activities were highly stable at 40 and 50°C through 48h of pre-incubation. Saccharification of alkaline pretreated sugarcane bagasse by crude enzyme extract from *C. cubensis* resulted in release of 320.8 mg/g and 288.7 mg/g of glucose and xylose, respectively. On another hand, a similar assay employing commercial cellulase preparation resulted in release of 250.6 mg/g and 62.1 mg/g of glucose and xylose, respectively. Cellulolytic extract from *C. cubensis* showed a great potential to be used in biomass saccharification processes.

Involvement of *bem46* on auxin biosynthesis in *Neurospora crassa*

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The bud emergence (BEM) 46 proteins are evolutionarily conserved members of the α/β-hydrolase super family. The exact function of the protein remains unknown. Our interests are focused on the Bem46 protein in *Neurospora crassa*. Phenotypical investigations of RNAi, over-expressing and knock-out lines show, that the most apparent phenotype is an impaired hyphal germination from ascospores of RNAi and over-expressing transformants. These results indicate a role of BEM46 in maintaining cell type-specific polarity in *N. crassa*. The BEM46 protein is localized in the perinuclear endoplasmatic reticulum and shows co-localization (Mercker et al., 2009) with the eisosomal protein LSP1. 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), an enzyme involved in the tryptophan biosynthesis. The interaction was confirmed *in vivo* by employing bimolecular fluorescence complementation assays. Tryptophan is a precursor molecule for auxin (Allegri et al., 2003). Auxin production in various fungi (Gruen, 1959), in particular for *N. crassa* (Tomita et al., 1987), has already been described, but the biosynthetic pathway in *N. crassa* is still unknown. We discuss the effect of *bem46* on polar growth being caused by its involvement on the tryptophan dependent auxin biosynthesis in *N. crassa*. Hence, we analysed the transcription of identified auxin biosynthetic genes in different strains by qRT-PCR. In addition transcription of the putatively eisosomal localized tryptophan transporter *mtr* was investigated. We also provide data regarding the potential co-localization of MTR with the eisosomal protein LSP1.

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Velvet components in *Penicillium chrysogenum*: Regulation of secondary metabolism by direct binding to enzymes of penicillin biosynthesis in the cytoplasm?

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The filamentous fungus *Penicillium chrysogenum* is the main industrial producer of the pharmaceutically relevant beta-lactam antibiotic penicillin. The three penicillin biosynthesis genes are found in a single cluster and the expression is controlled by a complex network of global regulators. It is supposed that subunits of the velvet complex, which were recently detected for *P. chrysogenum*, function as global regulators, although the exact regulatory mechanisms still have to be elucidated. Core components of the velvet complex are PcVelA and PcLaeA, which regulate secondary metabolite production, hyphal morphology, conidiation, and pellet formation [1]. As novel subunits, we recently identified PcVelB, PcVelC, and PcVosA [2]. Using yeast two-hybrid analysis and bimolecular fluorescence complementation (BiFC), we demonstrated that all velvet proteins are part of an interaction network. Functional analyses using single and double knockout strains generated by the FLP/FRT recombination system clearly indicate that velvet subunits have opposing roles in the regulation of penicillin biosynthesis and light-dependent conidiation [2, 3].

Most strikingly, a direct interaction of PcVelB with an enzyme of the penicillin biosynthesis pathway, the isopenicillin N synthase (IPNS) was identified during yeast two-hybrid analysis with PcVelB as bait. This unexpexted interaction was confirmed in vivo by using a combined tandem affinity purification/mass spectrometry approach. Fluorescence microscopy of both proteins revealed a nuclear-cytoplasmic localization for PcVelB, whereas the IPNS localizes solely in the cytoplasm. Our discovery of a direct interaction of the isopenicillin N synthase with a subunit of the velvet complex implies a novel regulatory mechanism how enzymes of penicillin biosynthesis are regulated at the molecular level.

The results provided here contribute to our fundamental understanding of the function of velvet subunits as part of a regulatory network mediating signals responsible for morphology and secondary metabolism, and will be instrumental in generating mutants with newly derived properties that are relevant for strain improvement programs.

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Changes of freshness in *Pleurotus eryngii* according to package weight and oxygen permeability of package film

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Recently, *Pleurotus eryngii*, King oyster mushroom is the main export item in South Korea mushroom industry. For appropriate transportation to Europe and USA markets, it is necessary to elucidate suitable conditions of storage and treatment of post-harvest during long time shipping.

Whole mushrooms were packed with polypropylene anti-fog film (30 μm) without trimming. The range of package weight and oxygen permeability of film were 200 g~400 g and 2000 ~ 5000 cc/m 2,24h,atm, respectively. The weight loss ratio was increased with increasing storage time. There were no big differences in weight loss ratio according to package weight and oxygen permeability of film at low temperature storage (4℃). The ratio of pileus elongation was showed big increase after 25 days in all treatment. In case of package weight is 400 g and oxygen permeability of film is 3000cc/m2,24 h,atm, the pileus elongation ratio was showed lower than other packaging films for storage period. As a results of changes of freshness, the optimum package weight and oxygen permeability of film of *P. eryngii* were 400 g and below 2000 cc/m 2,24 h,atm, respectively.

Carbon metabolism of *Aspergillus niger* during growth on plant biomass

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Plant biomass is a major substrate for many fungi. It consists mainly of polysaccharides and lignin, which themselves again consist of a variety of monomeric components. Monosaccharidea are the main components of the biomass and an important carbon source for many fungi. These monosaccharides are converted through a variety of carbon catabolic pathways.
While glucose is often considered the best carbon source for many fungi, data in our lab demonstrated that growth of *Aspergillus niger* on plant biomass is much better than on glucose. To investigate the importance of individual pathways for growth on plant biomass, we generated mutants that were blocked in several of the pathways (e.g. glycolysis, pentose catabolic pathway). Growth of these mutants on plant biomass was performed and compared to the wild type to determine the relative importance of each pathway for efficient growth. This demonstrated that during growth on plant biomass *A. niger uses* a variety of pathways in parallel, suggesting a simultaneous rather than sequential utilization of different monosaccharides. In addition we observed that these pathways are closely linked in that blocking one pathway also affected the use of other pathways. Initial results of this study will be presented.

Linseed oil as selective substrate for the formation of a fungal biofilm on wood

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Protective systems of wood or other materials are mainly based on the strategy to block UV light and prevent the growth of microorganisms. Recently a new protection system has been patented (US20040581157, 20041201, TNO): a biofilm for wood protection. This dark biofilm occurs naturally on pine wood impregnated with raw linseed oil after outdoor exposure for at least half a year. Test results are indicating that Aureobasidium is the dominant fungus.

The linseed oil or its components are probably a selective factor in terms of the presence of carbon sources. Hypothetically these carbon sources are easily consumed by Aureobasidium. The chemical composition of linseed oil varies per batch and in time. Since the chemical composition of the linseed oil present in outdoor wooden specimens is not known yet, the starting material (non-polymerized raw linseed oil) has been selected to test if Aureobasidium can grow on linseed oil as sole carbon source.

In order to quantify the growth of Aureobasidium on raw linseed oil, the number of yeast-like cells in a liquid minimal medium enriched with raw linseed oil has been determined in time. A minimal medium without raw linseed oil was used as a reference. The cell concentration of Aureobasidium in the medium enriched with raw linseed oil showed an increase. This indicates the use of linseed oil as a carbon source for Aureobasidium growth on linseed oil treated wood. The selective properties of linseed oil to restrict the growth of other organisms on this medium will be examined. In addition the influence of the transformation of raw linseed oil to polymerized linseed oil on biofilm growth is part of future research.

Cohaerins G - K, novel Azaphilones from *Annulohypoxylon cohaerens*

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Azaphilones are a family of fungal polyketides that contain a highly oxidized pyrone-quinone bicyclic core with a chiral quaternary centre. Up to now, over 350 members of this class have been isolated [1]. As many azaphilones contained in stromata of Xylariaceae are characteristic at species or genus level, they serve well as chemotaxonomic marker molecules [2].

Annulohypoxylon cohaerens is known to produce cohaerins A – F [3]. By HPLC, we isolated four additional azaphilones from the methanolic stromatal extract of *A. cohaerens*, which were named cohaerins G – K (1 – 4). Their planar structures were determined by NMR spectroscopy and by mass spectrometry. While their core structure is identical with cohaerin C and F, respectively, subgroups 2-hydroxy-6-methylphenyl and (1R,2R,4S)-4-hydroxy-2-methyl-6-oxocyclohexyl account for the structural diversity as substituents at C 3 of the azaphilone core. The stereochemistry of the new cohaerins G – K was fully characterized by a combination of CD spectroscopy, NOESY correlations, molecular modeling and derivatisation with Mosher's acid (MPTA). Furthermore, the metabolites showed cytotoxic effects besides a weak antimicrobial activity.

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Detection of aflatoxin from some Aspergillus sp. isolated from wheat seeds

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From (2) samples of wheat, 10 genera of fungi were isolated and identified (Alternaria sp., Aspergillus sp., Aureobasidium sp., Cladosporium sp., Dreschslera sp., Penicillium sp., Rhizoctonia sp., Stemphylium sp., Mucor sp. And Rhizopus sp.), where the highest frequency of seed-borne fungi was observed on wheat cultivar site Mol14. Their mean and standard deviation was (5.5 ±1.69), while the lowest fungal frequency isolated were Dreschslera sp. and Rhizopus sp.. Their mean and standard deviation (0.1 ±0.64) were recorded.

The results indicate that the plate 5 colony 4 (P5CO4) and plate 7 colony 4 (P7CO4) of *Aspergillus sp.* Isolated the detection of aflatoxin which grow on coconut milk agar using UV light by presence of influorescence as compared to the control. The aflatoxin-producing isolates appeared as gray or black colonies in the UV photographs, whereas nonproducing isolates appeared as white colonies, while plate 5 colony 4(P5CO4) show positive results, which means the presence of aflatoxin as compared to the controls which show the negative results. The dish was inverted and 1 or 2 drops of concentrated ammonium hydroxide solution are placed on the inside of the lid. The undersides of aflatoxin-producing colonies quickly turn plum-red after the bottom of the Petri dish has been inverted over the lid containing the ammonium hydroxide. Essentially no color change occurs on the undersides of colonies that are not producing aflatoxins. The main objective of this study is the isolation, identification and rapid detection of aflatoxin wheat seed-borne fungi.

Moisture dependencies of *P. rubens* on a porous substrate

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Fungal growth indoors can lead to both disfigurement of the dwelling and medical problems such as asthma. It is generally accepted that the primary cause for mould growth is the presence of moisture. Strategies to prevent fungal growth are therefore often based on controlling indoor humidity. Still, mould is frequently encountered in ventilated buildings that are considered to be relatively dry. Preliminary experiments have shown that fungi can survive on porous materials due to short intervals of favorable circumstances; even when - on average - conditions for growth are not met. This suggests that the interactions between porous materials and the fluctuating indoor humidity play an important role in a colony's survival. We study this interplay between indoor climate, substrate water household and fungal growth.

A property of water that is crucial for fungal growth is water activity (aw), which determines a fungus's ability to take up water. The effect of aw on fungal growth has been determined in the past by extensive growth experiments on agar, and many previous studies of growth on building materials take this parameter into account. Up till now, however, little attention has been paid to the water content (θ) of a substrate, which represents the amount of water that is physically present in a system. In most porous materials, even when aw is relatively high, only little water is present. We suspect therefore that, on porous substrates, growth is limited by water content (whereas on agar, θ is always close to 100% and will therefore be of little concern).

We performed growth experiments with *P. rubens* inoculated on gypsum while separately controlling θ and aw. Video microscopy was used to monitor the germination and subsequent growth of hyphae. The early development of the fungus was then quantified by determining parameters such as germination time and growth speed from the movies. The first experiments show that the germination rate, growth speed and growth density of *P. rubens* on gypsum increase with θ while aw is constant, and increase with aw while θ is constant. We conclude from this that θ and aw have separate effects on growth on porous substrates.

We think the influence of θ on fungal growth is related to the transport properties of the porous substrate. A decrease in θ will result in a sparser water network throughout the substrate, limiting both the flow of water and the diffusion of nutrients. These changes would both negatively impact the fungal growth. Follow up research will focus on modeling and explaining these effects.

Analysis of energy efficiency in *Pleurotus ostreatus* bottle cultivation according to light type

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Recently, Light Emitting Diode is begun to study agricultural adaptation as a useful light source for saving-energy. Oyster mushroom, *Pleurotus ostreatus*, is main item in South Korea for a long time. In previous studies, we selected suitable mixed color of LED during development of fruit body in *P. ostreatus*. In this study, we aim to analyze electricity usage for control of growing environments according to light type. There were no big differences temperature and relative humidity among light type. However, temperature of pileus surface below incandescent light bulb showed 1.0~1.5℃ higher than that of LED and fluorescent lamp. The CO_2 content below LED lamp until 26hr is lower than any other lamps. As a result of electricity usage analysis, total electricity usage of LED is the lowest than that of other lamp because electricity amount of lamp is the lowest.

Understanding auxin biosynthesis pathway and transport in filamentous fungi through structure-function correlation and its evolutionary consequences

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The phytohormoneindole-3-acetic acid (IAA) maybe synthesized from the amino acid tryptophan (Trp) via different pathways. The YUCCA (YUC) pathway, the indole-3-pyruvic acid (IPA) pathway, the indole-3-acetamide (IAM) pathway and the indole-3-acetaldoxime (IAOx) pathway are four proposed pathways for biosynthesis of IAA from Trp in green plants (Zhao 2010, Sugawara et al. 2009). While auxin production in filamentous fungi has been described earlier (Nakamura et al. 1978),the exact pathway for auxin production is poorly understood so far. Tryptophan aminotransferase (TAM), indole-3-pyruvic acid decarboxylase or pyruvate decarboxylase (IPD) and indole-3- acetaldehyde dehydrogenase (IAD) are three important intermediate proteins involved in auxin biosynthesis pathway in green plants (Zhao 2010, Sugawara et al. 2009). Similarly auxin transport has not yet been elucidated in filamentous fungi. Employing a computational approach we examine the involvement and putative role of above mentioned enzymes in auxin biosynthesis pathway and transport in filamentous fungi. In addition we analyze structure-function conservation throughout the evolution. For this study we have used the filamentous fungus *Neurospora crassa* as the focal species.

Our primary results suggest that *iad1, ipd* and *tam1* genes are present in a wide range of fungi across the fungus kingdom. We found that the gene NCU00589.7 present in *N. crassa* has a homolog in *Arabidopsis thaliana* with a similar annotation as `auxin efflux carrier family protein`, and used a computational characterization approach for the *N. crassa* gene products. In particular, we have investigated the structure-function conservation and correlation of the encoded proteins of the candidate genes using homology modeling. The structure-function relations of some of these are known in green plants and/or other eukaryotic system and thus have been used in our study. Predicted models for gene products from *N. crassa* exhibit similar structural conformation with known structures. Interestingly we also have found that putative ligand-binding sites of predicted models are very much similar to the known structures. In the context of ligand binding properties we have also studied for overall topology of the predicted structures and checked for the plausible binding sites through specific motifs if present any.

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Compartmentation of glycolipid production in *Ustilago maydis*

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Under nitrogen starvation the smut fungus *Ustilago maydis* produces a bunch of secondary metabolites. Among these are the glycolipids Ustilagic acid (UA) and Mannosylerythritol lipid (MEL), which consist of a sugar moiety esterified with fatty acid side chains of variable length (from C2 – C18). The biosynthesis of UA is encoded by the UA gene cluster (11 genes). MEL production depends on the MEL gene cluster composed of the genes *mat1, mmf1, mac1, emt1* and *mac2*. Deletion of *mac1, mac2* or *emt1* in *U. maydis* resulted in the complete loss of MELs.

Medium-length fatty acids (C4-C14) are derived from longer fatty acids (C16-C18) by partial peroxisomal β-oxidation. After bioinformatic analysis we have identified bona fide peroxisomal targeting sequences 1 (PTS1) at the C-termini of the two mannosylerythritol lipid acyltransferases Mac1 and Mac2 but not in any other protein involved in the biosynthesis of the MELs or the UAs.

Here we show that Mac1 and Mac2 localize in peroxisomes, and that this localization depends on the PTS1 motifs.

The analysis of glycolipid production by thin layer chromatography and mass spectrometry from wild type strain MB215 revealed a mixture of MELs with different length of the fatty acid side chains ranging from C12, C14 and C16. Strains expressing both cytoplasmic variants Mac1Δpts and Mac2Δpts showed a reduction of diversity of MELs. Furthermore, in this mutant the UA production is affected indicating the need for compartmentation of MEL and UA production in *U. maydis*.

Assessment of sterilization function of an electric precipitator against air-borne fungal spores

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Fungal spores in air are airborne particles of biological origin and omnipresent in human surroundings. Exposure to them in indoor environments could cause a wide range of adverse health effects. One of active tools for removing and/or reducing fungal spores is the use of an electric precipitator that is installed in an air conditioning system to purify the air by collecting and removing contaminant such as dust contained in the air. The aim of this study was to evaluate the function of an electric precipitator in fungal spore precipitation and sterilization. To add sterilization function against the fungal spores collected by the electric precipitator, we designed an electric precipitator composed of two connected units that were resist to high voltage. First unit was for charging air borne-particles such as microorganisms and dust and collecting the charged particles. Second unit was for collecting the charged particles which was not captured by the first unit. Using a power supplier, high voltages were applied to these units to generate an electric field, which could give fungal spores damage. For the evaluation test, spores of *Aspergillus versicolor*, *Penicillium chrysogenum*, and *Cladosporium cladosporoides* formed on each PDA plate were aerosolized by a gas generated unit and collected using an electric precipitator designed in this study. The collected fungal spores were treated for 10 min to 30 min with 5 to 7 Kv, harvested, and spread on DG18 agar medium to assess their viability. The percentage of survived fungal spores of *A. versicolor, P. chrysogenum*, *C. cladosporoides* was 1%, 10% and 9%, respectively. The fungal viability was significantly affected by the range of the voltage. A scanning electron microscope images demonstrated that high voltage treatment distorted fungal spores. Our results show that the newly designed electric precipitator should be a useful tool to control airborne fungal spores in indoor environment.

Selection of improved Ustilago biocatalysts

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Fungi of the genera Ustilago are well known for their plant pathogenicity, and *U. maydis* is a paradigm for plant-pathogen interaction 1). However, Ustilago and related genera can also produce a wide variety of biotechnologically interesting chemicals. Among the broad biodiversity are producers of organic acids (itaconate, malate, succinate), polyols (erythritol, mannitol) and glycolipids (mannosyl-erythritol lipids, ustilagic acid) 2, 3). Several *U. maydis* strains are known for their production of itaconic acid, and *U. maydis* strain MB215 is extensively studied in our lab 4). Although this strain has distinct advantages over the established itaconic acid producer *Aspergillus terreus*, such as a unicellular morphology during haploid growth, the yield, titer and rate of itaconic acid production need to be increased in order to achieve an economically viable process.

In order to identify biocatalysts for specific value-added chemicals, approximately 90 species and strains of Ustilago, Sporisorium, and Macalpinomyces were screened for production of organic acids and polyols from glucose. Surprisingly, almost all tested strains produced significant concentrations of organic acids and/or polyols, although only a few major products were found. Many of the strains transiently produced malic acid. In addition, production of erythritol and itaconic acid was also observed.

Among the biodiversity library were 55 wildtype strains of *U. maydis*, which virtually all produced itaconic acid. However, the range of produced concentrations varied considerably. Although the established strain MB215 was in the higher range of itaconic acid production, certain strains proved to produce itaconic acid with a considerably higher yield and rate. The best strains will be selected for further optimization by process development and metabolic engineering.

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Novel method for bulk isolation of basidiospores from wild mushrooms with low risk of contaminations

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The typical life cycle of basidiomycetes starts with sexual basidiospores that germinate into monokaryotic mycelia. If competent in mating type, such mycelia can fuse to form a dikaryon on which fruiting bodies may develop for basidiospore production [1]. For use in research and in applications (such as in mushroom cultivation and enzyme production), isolation of spores is important to subsequently obtain mono- and dikaryotic mycelia from these. Commonly, spores from mushrooms collected in the wild are isolated in form of spore-prints. For this, mushroom caps are placed onto a sterile paper or tin foil onto which spores fall down by gravity. When spores are then plated onto fungal growth medium, contaminations by bacteria, yeasts and molds often hinder the growth of the fungus wanted. Bacteria might be suppressed by addition of suitable antibiotic but contaminating fungi usually remain a problem [2,3]. Avoiding contaminations in isolation and cultivation of basidiospores is thus not an easy task.

The sexual spores of mushrooms are ballistospores and are forcibly discharged from their sterigmata on the basidia. The required physical forces are generated through the fusion of the osmotic active Buller´s droplet formed at the hilar appendix of the mature spore on the sterigmata with another drop that develops on the spore in an adaxial dent. The spores propel into the air space between two opposing hymenia, thereby using up all the energy provided. Then, they fall down by gravity [4,5]. However, basidiospores have electrostatic charges [6]. Here, we present experiments that show that mushrooms in up-side-down orientation release their spores, which can fly up into the air by an electrostatic attraction. Spores are attracted to the lids of plastic Petri dishes but not by glass lids. Moreover, the spores strongly attach to the plastic lids. These features can be made use of in collecting spores under reduced risks of contaminations from mushrooms collected from the wild. Plating the spores collected from the lids onto fungal media revealed that bacterial and fungal contaminants are often not present or in only very low numbers, unlike if spores are harvested from caps positioned onto surfaces in their natural direction. We successfully tested the up-side-down plastic lid method on multiple species from the gill-forming Agaricales as well as from the pore-forming Boletales. Mushrooms for spore isolation might be just matured or also older and even partially decayed or loaded with grazing insect larvae and still, (nearly) pure spore suspensions are obtained.

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Abstracts: Biodiversity

Biodiversity of fungi from some forest plant parts of Andhra Pradesh, India

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India with its varied topography, climate, forest type, soil type, altitudes and specialized ecological niches possesses a much diversified mycoflora. India has been the cradle for fungi and ⅓ of global fungal diversity exists in India. The forests of Bhadrachalam, Kothagudem and Paloncha of Khammam District, A.P., India which were not explored earlier were surveyed for micro- and macro-fungi colonizing litter, fruit, bark, humid soils, wood and dung. One hundred thirty one fungal species were found associated with diversified substrates and of which 96 were micro-fungi and 35 macro-fungi. Thirty four fungi were cultured, while the remaining could not be cultured. Substrate relationship in terms of fungal association with plant parts was also evaluated. Amongst various substrates or habitats surveyed, litter was found colonized by 65 fungal species belonging to Anamorphic group (Hyphomycetes), followed by humid soil and fallen leaves which were colonized by 18 and 17 fungal species, respectively. Humid soils supported mainly higher fungi while fallen leaves were found colonized by Anamorphic fungi. Dead and dried wood and fruits were found associated with 14 and 10 fungal species respectively. Dead wood supported only macro-fungi while fallen fruits were colonized mainly by micro-fungi. Bark (3), water (2) and Dung (2) were colonized by least number of fungal species. Anamorphic fungi dominated the mycofloristics (56%) followed by Basidiomycotina (26%), Ascomycotina (13%) and Zygomycotina (5%) members. It is interesting to note that the survey has resulted in the discovery of two new genera, four new species besides ten fungi being new additions to the fungi of India.

Macrofungal distribution of Pos Forest (Aladağ) from Turkey

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This study has been carried out from 2004 to 2012 in order to determine macrofungi distribution of Aladağ. As a result of the field and laboratory studies, 204 taxa belonging to 2 divisio and 56 families have been identified. Of them, 8 Families and 14 species belong to Ascomycota and 48 families and 190 species belong to Basidiomycota. The largest families are Tricholomataceae 26, Agaricaceae 16, Strophariaceae 17, Mycenaceae 14, Inocybecae 9, Schizophoraceae 8, Geastraceae, Marasmiaceae and Russulaceae 6 for each. The other families have less than 6 species. The study area supports an abundantly rich mycobiota that grows in various habitats, such as black pine forest, fir forest, cedar forest, oak forest, and Juniperus forest. Habitat distribution according to species numbers is as follows: *Abies cilicica* stands 170, *J. excelsa* stands 40, *P. nigra* stands 37, *Cedrus libani* stands 23, Quercus ps. stands 19, *J. foetidissima* stands 9, *Pinus nigra*-Quercus sp. stands 8, Cedrus-Abies stands 2, *J. excelsa*-*P. nigra* stands 2, *J. oxycedrus* stands 2, Abies-Quercus sp. stands 1, Populus sp. stands 1.
Diatrypella quercina (Pers.) Cooke, *Cortinarius latus* (Pers.) Fr., *Antrodia sinuosa* (Fr.) P. Karst., *Ramaria fennica* (P. Karst.) *Ricken var. cedretorum* (Maire) Schild, *Gymnopus putillus* (Fr.) Antonín, Halling & Noordel., *Tomentellopsis pusilla* Hjortstam and *Tricholoma saponaceum* (Fr.) *P. Kumm. var. lavedanum* Rolland were identified for the first time from Turkey.

Impact of habitat fragmentation on the genetic population structure of *Armillaria cepistipes*

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Armillaria cepistipes is a common wood-rotting basidiomycete fungus found in most forests in Central Europe. In Switzerland, the habitat of *A. cepistipes* is fragmented because of the presence of major geographical barriers, in particular the Alps, and past deforestation. We analysed the impact of habitat fragmentation on the current spatial genetic structure of the Swiss *A. cepistipes* population. A total of 167 isolates were sampled across an area of 41.000 km^2 and genotyped at seven microsatellite and four single nucleotide polymorphism (SNP) loci. All isolates belonged to different genotypes, which, according to the Bayesian clustering, originated from a single gene pool. FST indicates that the overall *A. cepistipes* population shows little, but significant genetic differentiation. Such a situation suggests gene flow is strong, possibly due to long-distance dispersal of airborne basidiospores. This hypothesis is supported by the fact that we could not detect a pattern of isolation by distance. Gene flow might be partially restricted by the high mountain ranges of the Alps, as indicated by a signal of spatial autocorrelation detected among genotypes separated by less than about 80 km in the entire population and ad hoc subpopulations crossing the Alps. In contrast, past deforestation seems to have no significant effect on the current spatial population structure of *A. cepistipes*.

Fungal decay of naturally infected wood of *Quercus robur*

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The durability of *Quercus robur* is related to the presence of polyphenols. It should be proven whether quercetin is present in the wood of *Q. robur*. Three stem disc areas were differentiated from bark to near pith, sapwood, transition zone (sapwood to heartwood) and heartwood. To investigate the distribution of the phenolic compounds in detail, all three wood zones were examined by UV-microspectrophotometry (UMSP), high performance liquid chromatography (HPLC-DAD and HPLC-ESI-MS/MS). Ellagic acid castalagin, grandinin, and trigalloyl-glucose were detected but quercetin was not present in any zone.

Quercus robur wood from a bridge was investigated in view of fungal degradation. The outer layer of the wood was attacked by soft-rot fungi and the middle part by white-rot species. Fruit-bodies of the white-rot basidiomycetes *Hymenochaete rubiginosa* and *Stereum hirsutum* were identified. Presence of *Fuscoporia ferrea* and *Mycena galericulata* was demonstrated by rDNA-ITS sequencing. Light microscopy showed hyphae of blue-stain fungi and white- and soft-rot decay. Field emission scanning electron microscopy (FE-SEM) exhibited white rot in vessels, fibres and ray cells. Transmission electron microscopy (TEM) revealed soft-rot with cavity formation in the S2 layer of the fibre cell walls. The topochemistry of lignin degradation within individual cell-wall layers was determined by cellular UV-microspectrophotometry at 278 nm wavelength. Increased delignification occurred in compound middle lamella regions (CML), secondary wall layers of fibres (S2), longitudinal and ray parenchyma cells as well in vessels. The highest lignin content at initial and medium decay was recorded in the CML, whereas in advanced decay secondary wall layers of the vessels are characterised by the highest content of remaining lignin. In all stages of degradation, the S2 layers of fibres revealed the lowest lignin amount. The degradation patterns was studied by analytical pyrolysis combined with gas chromatography/mass spectrometry (Py-GC/MS). Relative peak areas were calculated for pyrolysis products arising from carbohydrates and lignin. The pyrograms of control and decayed wood showed the same degradation products but with different quantities. The lignin/carbohydrate ratio increased, whereas the lignin syringyl/guaiacyl ratio decreased. This was due to the preferential degradation of the carbohydrates and syringylpropanoid structures.

Isolation and identification of filamentous fungi isolated from Pinewood Nematode Infested Korean Pine and Japanese Black Pine Wood in Korea

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Pinewood nematode (PWN, *Bursaphelenchus xylophilus*) is a serious pathogenic worm that quickly dry pine trees to death. Recently, PWN has been devastating huge amounts of conifer trees in Korea. As a first step to explore the association and ecological roles of filamentous fungi in PWN life cycle in Korea, in this study we first isolated and indentified fungi from PWN-infested Korean pine (Pinus koraiensis) and Japanese black pine (Pinus thunbergii) wood sampled six different area of Jinju, Sacheon, Pocheon, Chuncheon, Gwangju, and Hoengseong in Korea. A total of 144 fungal isolates were obtained from Japanese black pine wood and 264 fungal isolates from Korean pine wood. Their morphology and nucleotide sequences of the ITS rDNA and b-tubulin gene were examined for species identification. Ophiostoma ips, Botrytis anthophila, Penicillium sp., Hypocrea lixii, Trichoderma atroviride, O. galeiforme, Fusarium proliferatum were identified from Japanese black pine wood. *Leptographium koreanum*, *L. pini-densiflorae*, *Ophiostoma ips*, *Penicillium raistrick*, Trichoderma sp. were isolated from Korean pine wood. *O. ips* and *L. koranum* were the major species on the two different PWN-infected pine tree. Ophiostoma ips was also isolated from PWN and PNW vectoring insect, Japanese pine sawyer beetle. The cultivation of PWN on fungal mat of the identified species did not much enhance PWN reproduction. The properties of indentified fungi were described.

Complete mitohondrial genome sequence of the snow mold fungus *Sclerotinia borealis*

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We used WGS approach to determine the complete mitochondrial genome (mtDNA) of the snow mold fungus *Sclerotinia borealis*. The mtDNA is a circular molecule of 203,051 bp with a GC content of 32%. It is a largest sequenced fungal mitochondrial genome. RNA-encoding gene set include, besides 32 tRNA genes and genes for large and small ribosomal RNA (rnS, rnL), also predicted *rnpB* gene encoding the subunit of mitochondrial RNAse P, known to be responsible for tRNA processing. Protein-coding genes include those for ATP-synthase subunits, subunits of cytochrome oxidase (*cox1*, *cox2* and *cox3*), apocytochrome b (*cob*), one ribosomal protein (*rps3*), and NADH dehydrogenase subunits. Group I and group II introns, frequently interrupting yeast and filamentous fungi mitochondrial genes were found. About 90 ORFs has homology with homing endonucleases of LAGLIDAG family. All genes except DNA polymerase B gene are located on one strand and apparently transcribed in one direction. In our report we describe general organization of *S. borealis* mtDNA, gene order and content and analyse its phylogenetic relationships.

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Acknowledgement

We hope you enjoyed the meeting in Karlsruhe and felt that it was organized well. The meeting was organized without the help of a professional congress management company (except the payment through amiando) in order to keep the registration fees moderate and attract many young scientists. However, we had some help and support from different people, who we would like to thank here. Without them the meeting would not have been possible in this way.

We would like to thank Dr. Gudrun Knedlitschek and her staff (Institute of Toxicology and Genetics, BioInterfaces Program, KIT) for setting up the registration software and taking care of the registration.

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We would also like to thank our secretaries Fabienne Cochard-Rein, Tamara Bürger and Aline Deubel as well as all our students.

TUESDAY, 01.10.2013

08:30 – 10:30 **Poster session talks: Cytskeleton and Polarity**

PLENARY SESSION: ORGANELLES AND ORGANELLAR FUNCTIONS

Chairs: **Michael Bölker**
Marburg, Germany
Jürgen Wendland
Copenhagen, DK

11:00 – 11:30 **Ida van der Klei**
Groningen, The Netherlands
"The biogenesis and function of fungal peroxisomes"

11:30 – 12:00 **Gero Steinberg**
Exeter, UK
"Endosome motility in fungi: The how and why"

12:00 – 12:30 **Benedikt Westermann**
Bayreuth, Germany
"Mitochondrial dynamics and inheritance in yeast"

PLENARY SESSION: BIOTECHNOLOGY

Chairs: **Jesus Aguirre**
Mexico City, Mexico
Gerhard Braus
Göttingen, Germany

14:00 – 14:30 **Claus Bollschweiler**
BASF SE, Ludwigshafen, Germany
"Hydrophobins: Innovative performance proteins for industrial Biotechnology"

14:30 – 15:00 **Ronald de Vries**
Utrecht, The Netherlands
"Fungal strategies for plant biomass degradation"

15:00 – 15:30 **Katsuhiko Kitamoto**
Tokyo, Japan
"Developing *Aspergillus oryzae* as a host for heterologous protein production"

17:30 – 19:30 **Poster session: Gene regulation**

WEDNESDAY, 2.10.2013

08:30 – 10:30 **Poster session talks: Secondary metabolism**

PLENARY SESSION: CELL SIGNALLING AND MORPHOGENESIS

Chairs: **Michelle Momany**
Athens, USA
Luis Corrochano
Sevilla, Spain

11:00 – 11:30 **Alex Idnurm**
Kansas City, USA
"Light signalling in Phycomyces"

11:30 – 12:00 **Alfredo Herrera Estrella**
Irapuato, Mexico
"An injury-response mechanism conserved across kingdoms determines entry of *Trichoderma atroviride* into development"

12:00 – 12:30 **Axel Diernfellner**
Heidelberg, Germany
"Molecular mechanisms of the *Neurospora crassa* circadian clock"

PLENARY SESSION: SECONDARY METABOLISM

Chairs: **Bettina Tudzynski**
Münster, Germany
Ulrich Kück
Bochum, Germany

14:00 – 14:30 **Joseph Strauss**
Vienna, Austria
"Heterochromatin influences fungal secondary metabolism and pathogenicity"

14:30 – 15:00 **Marc Stadler**
Braunschweig, Germany
"Correlations between biodiversity and secondary metabolism in the Xylariaceae"

15:00 – 15:30 **Sandra Bloemendal**
Bochum, Germany
„Strain development in fungal biotechnology: Regulation of beta lactam antibiotic biosynthesis“

15:30 – 16:00 Poster awards

16:15 – 17:00 **Claudio Scazzocchio**
Paris, France
„Concluding remarks: Fungal Biology in the post-genomic era"

19:00 **Farewell Party in "Schloss Schwetzingen"**

FUNGAL BIOLOGY CONFERENCES

The International Fungal Biology Conference series started in 1965 in Bristol, UK and was held since then at different places around the world. The last meeting was organized 2009 in Ensenada, Mexico. These meetings always focus on exciting new fields at the frontiers of fungal biology. In order to promote and motivate young scientists, preference will be given to them as speakers in the poster sessions.

STEERING COMMITTEE (FUNGAL BIOLOGY CONFERENCES)

Jesús Aguirre - México
Gordon Beakes - U.K.
Louise Glass - USA
Neil Gow - U.K.
Harvey Hoch - USA
Regine Kahmann - Germany
Kurt Mendgen - Germany
Robert Roberson - USA
José Ruiz-Herrera - México

KARLSRUHE

Karlsruhe is located in the South of Germany and is amongst the warmest regions in the country. It is located in the Rhine valley just across the french border. Two mountain areas are close by, the beautiful Black Forest (Schwarzwald) and the Palatinate (Pfalz), a lovely area well known for its wines. Just take a few extra days and experience a wonderful fall season.

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