12th European Conference on Fungal Genetics

Seville (Spain) March 23-27, 2014

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Rethink Tomorrow

12th EUROPEAN CONFERENCE ON FUNGAL GENETICS

Seville, March 23-27, 2014

PROGRAMME OVERVIEW

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ECFG committes

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J. Ignacio Ibeas, University Pablo de Olavide, Seville
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Arthur Ram, University of Leiden, The Netherlands
Paul Tudzynski, Westfälische Wilhelms-Universität Münster, Germany
Gillian Turgeon, Cornell University, USA

Coordination of ECFG12 activities

Coordinator of concurrent sessions
Unai Ugalde, University of Basque Country, Spain

Coordinators of Satellite Meetings
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David Cánovas, University of Seville, Spain

Coordinators of fellowships
Javier Avalos, University of Seville, Spain
José Pérez-Martín, IBFG, CSIC, Spain

Coordinators of ECFG12 poster awards
J. Ignacio Ibeas, University Pablo de Olavide, Spain
Peter Philippsen, Biozentrum, Switzerland

Welcome

Dear Colleagues,

It is with great pleasure that we welcome you to Seville for the 12th European Conference on Fungal Genetics (ECFG12). ECFGs are held every two years in a European country in coordination with the Fungal Genetics Conferences that are also held every two years in Asilomar (USA). The central theme of ECFGs is fungal genetics, but the contents have expanded to cover other relevant areas in fungal biology such as molecular and cell biology, genomics and evolution, biotechnology, and pathogenesis. We have prepared a program for ECFG12 with three keynote lectures, fifteen plenary lectures, nine concurrent sessions, and two poster sessions for more informal discussions. These sessions are complemented with six satellite meetings. A total of 91 oral presentations by experienced and novel speakers have been scheduled for the conference, and we have tried to offer a variety of topics and experimental organisms. ECFG12 has about 700 participants, with 30% of PhD students. About 500 posters are scheduled for the two poster sessions. Most participants come from European countries, but 28% come from outside Europe. This is a truly international conference, as citizens from 40 countries will attend ECFG12.

Organizing this conference has been a major effort and would not have been possible without the help of the local organizing committee. Miguel Peñalva, the Spanish representative in the international ECFG committee, chaired the Spanish scientific committee that prepared the scientific program. The congress office of “El Corte Inglés” was in charge of the conference secretariat and handled all the major organizational details for the conference. Organizing a conference is always a team effort, and I have been very fortunate to have the help of an outstanding team that have contributed to make ECFG12 a successful and enjoyable event.

I would like to acknowledge the support that we have received from several sponsors. They are listed elsewhere in the conference book, but I would like to highlight the contributions of the Spanish Foundation for Science and Technology (FECYT) and the Federation of European Microbiological Societies (FEMS). FECYT provided funds to cover part of the cost of invited speakers and the organization of several events in Seville. These events (a round table, a public demonstration, and two lectures) have been organized to communicate the science behind ECFGs to the non-scientific public. FEMS has provided partial support for invited speakers and fellowships for young scientists. About 60 fellowships have been awarded to young attendants that have been funded by FEMS and the ECFG12 fellowship fund. In addition, several private sponsors have provided funds to support ECFG12. I would like to highlight the contributions of Novozymes and Union Biometrica for their continuing support of ECFGs.

I have always enjoyed attending ECFGs and we hope that you all enjoy ECFG12 and your stay in Seville. I would like to finish this letter with a final advice, in particular, for young attendants: talk! Talk to other students and to senior participants. Don’t be shy! This conference is a good opportunity to share our scientific thoughts, to look for help with our technical problems in the lab, and to look for scientific advice. We have plenty of time to talk during coffee breaks, lunches, and poster sessions. Don’t waste it!! We hope that you return home with new ideas, new contacts, new friends, and the burning desire to go back to the bench to try new experiments. If you do so, then ECFG12 will be a success and will compensate all the effort that we have dedicated to the organization of the conference.

Luis Corrochano
Chair of the local organizing committee of ECFG12
Seville, March 2014

Scientific programme

Sunday, March 23

12.00-18.00 Registration

18.00-20.00 **Welcome address** (Lecture Hall Velázquez)

Opening Lecture
Chair: Miguel A. Peñalva

KL1 **γ-Tubulin: A multitasking cell organizer**
Berl Oakley

20.00-22.00 Welcome reception

Monday, March 24

09.00-12.00 **The *Yanofsky* plenary session** (Lecture Hall Velázquez)
Chairs: Arthur Ram and Marc-Henri Lebrun

09.00-09.30 PS1.1 **Gene silencing, heterochromatin formation and DNA methylation in *Neurospora***
Eric Selker
09.30-10.00 PS1.2 **Early endosome motility spatially organizes polysome distribution**
Gero Steinberg
10.00-10.30 PS1.3 **HookA is a novel dynein-early endosome linker critical for endosome movement *in vivo***
Xin Xiang
10.30-11.00 Break
11.00-11.30 PS1.4 **Photobiology in model and clinical fungi**
Jennifer Loros
11.30-12.00 PS1.5 **Light and time in *Aspergillus nidulans***
Reinhard Fischer

12.30-14.00 Lunch

14.00-16.00 **Poster session 1** (Manolo "Caracol" Hall)
posters 001-037, 075-121, 169-210, 253-288, 324-366, 411-427, 444-467

16.00-19.00 **Concurrent sessions 1-3**

Unconventional gene regulation (Lecture Hall Velázquez)
Chairs: Rosa Ruiz-Vázquez and Joseph Strauss
16.00-16.20 CS1.1 **Histone de-methylases regulate primary and secondary metabolism in *Aspergillus nidulans***
Joseph Strauss
16.20-16.40 CS1.2 **Regulatory networks and regulators of chromatin structure governing global responses to changes in light and time**
Jay Dunlap
16.40-17.00 CS1.3 **Heterochromatin controls γH2A localization and genome stability in *Neurospora crassa***
Zacharias Lewis
17.00-17.20 CS1.4 **The functional characterization of the *Neurospora crassa* HAC-1 transcription factor reveals a crucial role for the unfolded protein response in plant cell wall deconstruction**
Luis Larrondo

17.20-17.40 Break

17.40-18.00 CS1.5 **Regulation of endogenous functions by small RNAs in the pathogenic fungus *Mucor circinelloides***
Rosa Ruiz-Vázquez

18.00-18.20 CS1.6 **Mechanisms of small non-coding RNA pathways in *Neurospora***
Yi Liu

18.20-18.40 CS1.7 **RNAi-dependent epimutations evoke antifungal drug resistance in the zygomycete fungal pathogen *Mucor***
Silvia Calo Varela

18.40-19.00 CS1.8 **Spliceosomal twin introns in fungal nuclear transcripts: structure and evolution**
Michel Flipphi

Fungal development (Lecture Hall Aleixandre)
Chairs: Minou Nowrousian and Eduardo Espeso

16.00-16.20 CS2.1 **Comparative genomics and transcriptomics to analyze fruiting body development**
Minou Nowrousian

16.20-16.40 CS2.2 **Tracking the evolution of perithecium morphology through transcriptomics**
Frances Trail

16.40-17.00 CS2.3 **Sexual development and female fertility in *Trichoderma reesei***
Monika Schmoll

17.00-17.20 CS2.4 **A retinoic-acid biosynthetic enzyme involved in morphology and sexual development in *Fusarium verticillioides***
Violeta Díaz Sanchez

17.20-17.40 Break

17.40-18.00 CS2.5 **Deciphering the role of the Flb-apical complex in asexual development of *Aspergillus***
Eduardo Espeso

18.00-18.20 CS2.6 **Regulation of morphogenesis during development in the filamentous fungus *Aspergillus nidulans***
Steven Harris

18.20-18.40 CS2.7 **Investigating the role of the exocyst complex in appressorium-mediated tissue invasion by rice blast fungus *Magnaporthe oryzae***
Yogesh Gupta

18.40-19.00 CS2.8 **Light-responsive transcription factors (LTFs) regulate differentiation and virulence in the gray mold fungus *Botrytis cinerea***
Julia Schumacher

Social fungal biology (Lecture Hall Turina)
Chairs: Natalia Requena and Barry Scott

16.00-16.20 CS3.1 **Defining the line between mutualism and parasitism**
Natalia Requena

16.20-16.40 CS3.2 **Dld1, a novel fungal histidine-rich effector-protein that binds to metal ions to perturb plant immunity**
Alga Zuccaro

16.40-17.00 CS3.3 **The effector protein Missp7 of the mutualistic ectomycorrhizal fungus *Laccaria bicolor* interacts with *Populus* Jaz proteins**
Claire Veneault-Fourrey

17.00-17.20 CS3.4 **Trojan horse strategy and fair trade among symbioses: how one fungal species can invade thousands of plant species**
Mathilde Malbreil

17.20-17.40 Break

17.40-18.00 CS3.5 **Cell fusion is required to maintain an *Epichloë festucae* symbiotic hyphal network in *Lolium perenne***
Barry Scott

18.00-18.20 CS3.6 **Genomic analyses of *Mortierella elongata* and associated bacterial endosymbiont (*Candidatus glomeribacter sp.*)**
Jessie Uehling

18.20-18.40 CS3.7 ***Verticillium* transcription activator of adhesion Vta2 suppresses microsclerotia formation and is required for systemic infection of plant roots**
Susanna A. Braus-Stromeyer

18.40-19.00 CS3.8 **Phylogenomics of Hypocreales and the evolution of secondary metabolism**
Joseph W Spatafora

19.00-20.00 **Special lecture** (Lecture Hall Velázquez)
Chair: Reyes González-Roncero

KL2 **Chromatin structure as a mediator of transcription- and R-loop-associated genome instability**
Andrés Aguilera

Tuesday, March 25

09.00-12.30 **The *Clutterbuck* plenary session** (Lecture Hall Velázquez)
Chairs: David Archer and Gillian Turgeon

09-00-09.30 PS2.1 **The initiation of asexual development in *Aspergillus nidulans***
Unai Ugalde

09.30-10.00 PS2.2 ***Aspergillus nidulans* septins in multicellular development**
Michelle Momany

10.00-10.30 PS2.3 **Morphological and metabolic adaptation to environmental conditions by *Penicillium marneffei* and its role in the host**
Alex Andrianopoulous

10.30-11.00 Break

11.00-11.30 PS2.4 **Evolution of sexual reproduction: a view from the fungal kingdom**
Joseph Heitman (dedicated to the memory of Prof. Lorna Casselton)

11.30-12.00 PS2.5 **Fruiting-body development in *Sordaria macrospora*-A matter of recycling**
Stefanie Pöggeler

12.30-14.00 Lunch

14.00-17.00 **Concurrent sessions 4-6**

Infecting the host (Lecture Hall Velázquez)
Chairs: Regine Kahmann and Antonio Di Pietro

14.00-14.20 CS4.1 **A secreted *Ustilago maydis* effector promotes virulence by targeting anthocyanin biosynthesis in maize**
Shigeyuki Tanaka

14.20-14.40 CS4.2 **Effector specialization in a lineage of the Irish potato famine pathogen**
Sophien Kamoun

14.40-15.00 CS4.3 **Hotspots of recombination shape the evolution of virulence in the wheat pathogen *Zymoseptoria tritici***
Daniel Croll

15.00-15.20 CS4.4 **Characterization of a circadian clock in *Botrytis cinerea* and its role in pathogenesis using *Arabidopsis thaliana* as a plant model**
Montserrat Hevia

15.20-15.40 Break

15.40-16.00 CS4.5 **Chemotropic sensing in the fungal pathogen *Fusarium oxysporum***
David Turrà

16.00-16.20 CS4.6 **Modulation of pathogenicity by pH regulation in the host**
Dov Prusky

16.20-16.40 CS4.7 **Investigating the role of tyrosine catabolism and pyomelanin production during in vivo growth in the human pathogen *Penicillium marneffei***
Kylie Boyce

16.40-17.00 CS4.8 **Pathogenicity chromosomes in host-specific toxin-producing *Alternaria* species**
Motoichiro Kodama

Sensing the environment (Lecture Hall Aleixandre)
Chairs: Alfredo Herrera-Estrella and Michael Brunner

14.00-14.20 CS5.1 **An injury response mechanism conserved across kingdoms**
Alfredo Herrera-Estrella

14.20-14.40 CS5.2 **Interplay between self and nonself recognition mechanisms regulate chemotropic interactions and cell fusion in *Neurospora crassa***
N. Louise Glass

14.40-15.00 CS5.3 **Class III peroxidases secreted by tomato roots trigger hyphal chemotropism in *Fusarium oxysporum***
Mennat El Ghalid

15.00-15.20 CS5.4 **Composition of the MAK-2 MAP kinase cascade in *Neurospora crassa***
Stephan Seiler

15.20-15.40 Break

15.40-16.00 CS5.5 **Cooperation of the GATA type transcription factors WCC and SUB1 in light-induced transcription**
Michael Brunner

16.00-16.20 CS5.6 **Light sensing in *Phycomyces blakesleeanus***
Alex Idnurm

16.20-16.40 CS5.7 **The novel sensor-globin Fungoglobin is involved in low oxygen adaptation of *Aspergillus fumigatus***
Falk Hillmann

16.40-17.00 CS5.8 **Genome-wide transcriptional response to ambient pH changes in *Fusarium graminearum*: A large metabolic reorganization controlled by Pac1**
Christian Barreau

Putting fungi to work (Lecture Hall Turina)
Chairs: José Arnau and Peter Punt

14.00-14.20 CS6.1 **Systems biology approaches for organic acid production in filamentous fungi**
Peter Punt

14.20-14.40 CS6.2 **Genetic characterization of itaconic acid biosynthesis in *Ustilago maydis***
Sandra Przybilla

14.40-15.00 CS6.3 **Cellulase and hemicellulase regulation and production in *Trichoderma reesei***
Bernard Seiboth

15.00-15.20 CS6.4 **The responses of *Aspergillus niger* to different lignocellulosic substrate**
Paul Daly

15.20-15.40 Break

15.40-16.00 CS6.5 **The first ribosomal peptide synthase pathway in filamentous fungi**
Myco Umemura

16.00-16.20 CS6.6 **Comparative genome-scale reconstruction of gapless metabolic networks**
Mikko Arvas

16.20-16.40 CS6.7 ***Yarrowia lipolytica* as a host for carotenoid production**
John Royer

16.40-17.00 CS6.8 ***Streptomyces*: the beauty of a filamentous industrial bacterium**
Gilles van Wezel

17.00-20.30 Sightseeing tour

Wednesday, March 26

09.00-12.30 **The *Scazzocchio* plenary session** (Lecture Hall Velázquez)
Chairs: Paul Tudzynski and Merja Penttilä

09.00-09.30 PS3.1 **Sensory perception in the mammalian host: Guiding invasive growth and rational therapeutic design**
Elaine Bignell. *The BMS lecture*

09.30-10.00 PS3.2 **Transposable elements reshaping genomes and favoring the evolutionary and adaptive potential of fungal phytopathogens**
Thierry Rouxel

10.00-10.30 PS3.3 **Septin-mediated plant tissue invasion by the rice blast fungus *Magnaporthe oryzae***
Nick Talbot. *The EMBO Lecture*

10.30-11.00 Break

11.00-11.30 PS3.4 **Genomic analysis in the search for oxidoreductases of industrial interest**
Ángel Martínez

11.30-12.00 PS3.5 **The evolution of fungal chemodiversity**
Antonis Rokas. *The FGB Lecture*

12.00-12.15 PS3.6 **Fungal genomics resources of the US Department of Energy Joint Genome Institute**
Igor Grigoriev

12.30-14.00 Lunch

13.30-14.00 **JGI Orientation** (Lecture Hall Aleixandre)

14.00-16.00 **Poster session 2** (Manolo “Caracol” Hall)
posters 038-074, 122-168, 211-252, 289-323, 367-410, 428-443, 468-493

16.00-19.00 **Concurrent sessions 7-9**

Inside the fungal cell (Lecture Hall Velázquez)
Chairs: Meritxell Riquelme and Steve Osmani

16.00-16.20 CS7.1 **The ordered accumulation of vesicles at the spitzenkörper is regulated by the action of distinct RAB GTPases and the exocyst in *Neurospora crassa***
Meritxell Riquelme

16.20-16.40 CS7.2 **Phosphatidylinositol phosphate gradients during fungal filamentous growth**
Robert Arkowitz

16.40-17.00 CS7.3 **Dual targeting of peroxisomal proteins**
Michael Bölker

17.00-17.20 CS7.4 **Inside an *A. gossypii* hypha: combining high-resolution electron tomography, video microscopy and proteomics**
Peter Philippsen

17.20-17.40 Break

17.40-18.00 CS7.5 **Mitotic regulation within a multicellular fungus**
Steve Osmani

18.00-18.20 CS7.6 **Autophagy controls nuclear dynamics during vegetative hyphal growth and fusion of *Fusarium oxysporum***
Carmen Ruiz Roldán

18.20-18.40 CS7.7 **Interplay of phosphatases and kinases: STRIPAK and MAP kinases regulate cell differentiation in *Sordaria macrospora***
Ines Teichert

18.40-19.00 CS7.8 **Cisternal maturation within the *Aspergillus nidulans* golgi visualized in vivo**
Areti Pantazopoulou

Fungal genomes: now what? (Lecture Hall Aleixandre)
Sponsored by *Pacific Biosciences*
Chairs: Hanna Johannesson and Toni Gabaldón

16.00-16.20 CS8.1 **Adaptive introgression slows down molecular degeneration of the mating-type chromosome in *Neurospora tetrasperma***
Hanna Johannesson

16.20-16.40 CS8.2 **Data-driven comparative functional genomics in yeast**
Maitreya J. Dunham

16.40-17.00 CS8.3 **Insights into the evolution of the mycorrhizal symbiosis**
Francis Martin

17.00-17.20 CS8.4 **The genomic architecture of ectomycorrhizal symbiosis in the genus *Amanita***
Jaqueline Hess

17.20-17.40 Break

17.40-18.00 CS8.5 **Got a genome? Get a phylome!: Fungi through the evolutionary lens**
Toni Gabaldón

18.00-18.20 CS8.6 **Early origins of the fungal cell wall and multicellularity in fungi**
Jason E Stajich

18.20-18.40 CS8.7 **Combining population genomics, RNA-seq and miniature transposable element (MITE) presence to identify the *AVR2* gene of the melon pathogenic fungus *Fusarium oxysporum* f. sp. *melonis***
Sarah M. Schmidt

18.40-19.00 CS8.8 **Insights on the evolution of mycoparasitism from the genome of *Clonostachys rosea***
Magnus Karlsson

DNA/RNA/protein interplay (Lecture Hall Turina)
Chairs: Ane Sesma and Michael Feldbrügge

16.00-16.20 CS9.1 **Multiple layers of regulation of fungal cleavage factor I proteins**
Ane Sesma

16.20-16.40 CS9.2 **The *FgPRP4* kinase is important for RNA processing, growth, and pathogenesis in *Fusarium graminearum***
Jin-Rong Xu

16.40-17.00 CS9.3 **Epigenetic control of effector gene expression in the plant pathogenic fungus *Leptosphaeria maculans***
Isabelle Fudal

17.00-17.20 CS9.4 **Regulatory crosslinks of the unfolded protein response control fungal development and pathogenicity**
Kai Heimel

17.20-17.40 Break

17.40-18.00 CS9.5 **mRNA transport meets membrane trafficking**
Michael Feldbrügge

18.00-18.20 CS9.6 **The coordination of mRNA degradation and translational repression**
Mark Caddick

18.20-18.40 CS9.7 **The *spf27*-homologue *num1* connects splicing and cytoplasmic trafficking processes in *Ustilago maydis***
Jörg Kämper

18.40-19.00 CS9.8 **Laser microdissection and transcriptomics of infection cushion development of *Fusarium graminearum***
Schäfer Willi

19.00-19.30 **Poster awards** (Lecture Hall Velázquez)

19.30-20.30 **Closing lecture** (Lecture Hall Velázquez)
Chair: Santiago Torres-Martínez

KL3 **Life and sex in the lab and in the field**
Enrique Cerdá-Olmedo

21.00 Conference banquet

Keynote Lecture Abstracts

KL1 γ-TUBULIN: A MULTITASKING CELL ORGANIZER

BERL OAKLEY

UNIVERSITY OF KANSAS, UNITED STATES

Microtubule organizing centers (MTOCs) such as spindle-pole bodies and centrosomes play an important role in nucleating microtubule assembly and establishing the structure of the mitotic apparatus. For many years the identities of the MTOC proteins responsible for microtubule nucleation were a mystery. We developed a genetic screen in Aspergillus nidulans for genes important to microtubule function and this led to our discovery of γ-tubulin. Work from our lab and others has led to the demonstration that complexes containing γ-tubulin and at least five other evolutionarily conserved proteins nucleate microtubule assembly. We are now beginning to understand the structures, functions and interactions of these γ-tubulin complex subunits. Analyses of γ-tubulin mutants, in our lab and in others, indicate that γ-tubulin has additional important functions. Intragenic complementation studies of A. nidulans γ-tubulin mutations, for example, indicate that A. nidulans γ-tubulin has at least three distinct, growth-limiting functions. Detailed analyses of one γ-tubulin mutant reveal that γ-tubulin plays an important role in inactivating the anaphase promoting complex/ cyclosome complexed with the activator protein Cdh1 at the end of the G1 phase of the cell cycle. Failure of inactivation leads to a constitutively active anaphase promoting complex/cyclosome which, in turn, results in a failure of cyclin B to accumulate. Since cyclin B is required for the S phase of the cell cycle in A. nidulans, nuclei lacking cyclin B do not progress into S phase and are removed from the cell cycle. γ-tubulin is, thus, a multifunctional protein with roles in mitotic spindle formation and interphase cell cycle regulation. It follows that spindle-pole bodies are multifunctional and accumulating data from animal cells reveal that centrosomes have critical functions in cell cycle regulation as well as in many forms of sensing and signaling. I suggest that the roles of MTOCs in microtubule nucleation, sensing and regulation are ancient and intimately related and that there is considerable validity to the suggestion by Boveri more than 100 years ago that polar MTOCs function as the “dynamic center” of the cell.

KL2 CHROMATIN STRUCTURE AS A MEDIATOR OF TRANSCRIPTION- AND R-LOOP-ASSOCIATED GENOME INSTABILITY

ANDRÉS AGUILERA

UNIVERSIDAD DE SEVILLA, SPAIN

Coordination of DNA replication with DNA-damage sensing, repair and cell cycle progression ensures with high probability genome integrity during cell divisions, thus preventing mutations and DNA rearrangements. Such events are usually associated with pathological disorders, including premature aging, various cancer predispositions and inherited diseases. One important type of genome instability is that associated with transcription. Transcription of a DNA sequence increases its frequency of recombination, a phenomenon referred to as transcription-associated recombination (TAR). We will provide data on the analysis of protein factors involved in transcription and RNA export that provide evidence that TAR is mediated by replication impairment and that it can be further enhanced by dysfunction of replication and repair factors in yeast cells and other eukaryotes via R-loops. Notably, we will provide evidence for a connection among R-loops and chromatin structure. We will disucss a general model for a role of RNA in chromatin structure, gene regulation and genome dynamics.

KL3 LIFE AND SEX IN THE LAB AND IN THE FIELD

ENRIQUE CERDÁ-OLMEDO

UNIVERSITY OF SEVILLE, SPAIN

Rarely seen in nature, Phycomyces grows out of the feces of various mammals and adapts easily to the laboratories, where it has provided many fascinating results over the last century and a half. It has been at the frontline of research on cellular morphology, fungal development, nutrition, carotene production and other aspects of metabolism. Growth, development, and metabolism are modified by environmental stimuli, such as light, wind, mechanical pressure, aerial and waterborne chemicals, and a misterious signal that allows it to locate nearby objects. Some of these responses are very sensitive; blue-light phototropism has been characterized particulary well.

The early discovery of the sexual process, spontaneous mutants, and mutagenesis made Phycomyces into a pioneer genetic organism, source, for example, of the concept and the name of heterokaryosis. Asexual genetic analysis is a practical tool and the heterokaryons offer unique methods to study gene function in vivo and the causes of cell death. The interaction between the two sexes provided the first evidence of pheromones. The sexual genetic analysis of Phycomyces is the only one developed in the Mucorales, but it remains harder and slower than those of the best fungi.

Interest in Phycomyces declined with the lack of stable transformation by exogenous DNA, but the application of other techniques of molecular genetics and genomics to existing knowledge and materials is becoming very fruitful. Some of the results can be complemented by work in Mucor circinelloides, with its effective transformation.

New results on the ecology of Phycomyces explain the often perplexing laboratory results as evolutionary adaptations required by its habitat, its role in nature and its dispersion mechanisms.

Plenary Lecture Abstracts

We have named the three plenary sessions of ECFG12 after three prominent fungal geneticists. Their contributions to fungal genetics are described below.

Charles Yanofsky

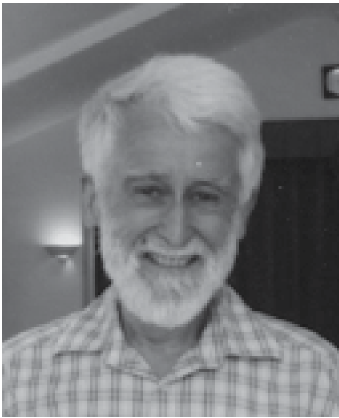
Charles Yanofsky was born in 1925 in New York City (USA). He received his undergraduate education in biochemistry at the City College of New York (1948) and obtained a Ph.D. in microbiology in 1951 from Yale University. Charles Yanofsky was an Assistant Professor at Western Reserve University Medical School (1954-1958) before moving to Stanford University in Palo Alto, California, where he is now Professor Emeritus.

Charles Yanofsky's interests in science started while attending the Bronx High School of Science, but his undergraduate education in New York was interrupted to serve in the Army during World War II. A visit to the Pasteur Institute while he was stationed in France renewed his interest to pursue a career in science.

During his senior year at the City College of New York he was impressed by the "one gene, one protein" hypothesis put forward by Beadle and Tatum. His main motivation to attend graduate school at Yale University was to work under the supervision of Ed Tatum. Unfortunately, Tatum had left Yale when Yanofsky arrived, but he decided to join the laboratory of David Bonner, Tatum's research associate, as a graduate student. Under Bonner's supervision Yanofsky characterized *Neurospora crassa* mutants altered in the metabolism of tryptophan, and put forward the idea that suppressor mutations involved missreading the genetic template (mRNA had not been discovered yet!). In his attempts to understand gene-enzyme relationships, he decided to use *Escherichia coli* and *Bacillus subtilis* as these organisms offered superior technological advantages to *Neurospora*. Later, he would return to use *Neurospora* as an experimental organism, and during several years his laboratory investigated the mechanisms of gene regulation using *E. coli*, *B. subtilis*, and *Neurospora* as models.

The most relevant scientific contributions from the laboratory of Charles Yanofsky have been the demonstration of gene-protein colinearity, and the elucidation of the features of operon regulation by transcription attenuation. In addition, his laboratory played a key role in the discovery of the nature of suppressor mutations due to changes in the sequences of tRNAs. He has played a major role in the development of *Neurospora crassa* as an experimental organism for modern molecular biology research. His *Neurospora* work included the development of methods for molecular manipulations, and the development of vectors for DNA transformation and gene libraries. In addition, his laboratory has made seminal contributions to the understanding of gene regulation during fungal development.

Charles Yanofsky has received numerous awards, including the Lasker Foundation award for medical research and the Louisa Gross Horwitz Prize for outstanding biology research. Charles Yanofsky is a member of the National Academy of Sciences (USA) and a foreign member of the Royal Society (UK). In 2003, he was awarded the National Medal of Science, the highest scientific honor in USA. However, his greatest reward, as he wrote, "was the feeling that I was helping young scientists experience the pleasure of performing creative research".



John Clutterbuck

John Clutterbuck graduated with a BSc in Botany (1960) and obtained his PhD at the University of Sheffield under the supervision of Alan Roper in 1964. He then moved to the Glasgow Genetics Dept., headed by Guido Pontecorvo, whose research focused on the use of microorganisms to probe fundamental mechanisms of heredity, especially the structure and function of the gene. As a young lecturer in Pontecorvo's lab, he actively contributed in the establishment of *Aspergillus nidulans* as an accepted tool for genetic analysis. He later became Senior Lecturer at the College of Medical, Veterinery and Life Sciences in Glasgow. In 2003, he became an Honorary Fellow of the University of Glasgow.

John's work laid the foundations of what is currently known of the developmental biology of Aspergillus. For example, the ground breaking discoveries by Bill Timberlake's lab and his collaborators the 1980's on the molecular biology development were founded on Clutterbuck's previous meticulous and thorough classical genetic work. In 1985, he was among the first groups in cloning an *Aspergillus nidulans* gene by complementation. In the 1990's John's laboratory described the autonomously replicating AMA1 plasmid, markedly facilitating the procedure to clone genes in this way.

His contributions to the Aspergillus community have been enormous through his mutation selection, gene mapping, stock collection and genome annotation work. Moreover, he combined his early ground-breaking work with that of others, in the ***Aspergillus nidulans* linkage map**. This openly available resource, which remains available today, became an invaluable reference in *Aspergillus nidulans* genetics, and set the trend for the online Aspergillus Sequence Database.

In recent years, John actively contributed in the sequencing of the *Aspergillus nidulans* genome and genomic studies on the incidence of repeat-induced point mutations and mapping of centromere-proximal regions of chromosome IV using state of the art genomic and molecular techniques.

A constant feature of John's career is the generous transmission of technical and conceptual advances in available form for non-expert scientists. His numerous book chapters on asexual development, parasexual recombination, stability and instability of fungal genomes, among others, are household references to postgraduate students and lecturers worldwide.

Claudio Sccazocchio

Claudio Scazzocchio was born in Rome in 1938. The wave of fascism that swept Europe at that time pushed his family from Italy to Montevideo, Uruguay, where he got his primary and secondary education and, in 1956, started his University studies, which he finished in his native Rome in 1961. In 1963 he moved to the Cambridge Department of Genetics, where he obtained his Ph.D. degree in 1966 with a Thesis entitled "Studies on the genetic control of purine oxidation in Aspergillus nidulans", supervised by John Pateman. This work, and the many papers from it derived, became classics of biochemical genetics and, with also ground-breaking work of his lab mate Herbert N. Arst on nitrogen metabolite repression, set the foundations for extending the nascent field of gene regulation from the bacterial operons to the regulation of catabolic pathways encoded by genes dispersed all over eukaryotic genomes. Between 1967 and 1969 he spent post-doctoral periods at the Weizmann Institute in Israel and at the Centre de Génétique Moléculaire CNRS in Gif-sur-Yvette, southwest of Paris. In 1969 he joined the ranks of the University of Essex, where he stayed until 1983, which included a sabbatical year at the MRC Laboratory of Molecular Biology in Cambridge in 1978, before moving back to France in 1983 and being appointed Professor of Microbiology by the University of Paris-Sud at Orsay, where he stayed until his formal retirement in 2006. In 2006 he joined the Section of Microbiology of Imperial College London, where he is actively pursuing research on the molecular genetics of Aspergillus nidulans. Claudio Scazzocchio is, among a long list of prominent distinctions, an EMBO Member and Honorary Senior Member of the Institut Universitaire de France and Honorary Fellow of the British Mycological Society. He holds ad honorem doctorates from the Universities of Montevideo (Uruguay) and Athens (Greece).

Prof. Scazzocchio combines his endless 'biological enthusiasm', focused on but certainly not restricted to fungal genetics, with an overwhelmingly humanistic background, a combination facilitated by his fluency in four different languages (Italian, Spanish, French and English). This unusual combination, his deep historical perspective and the breath of his biological knowledge are illustrated, for example, by his membership of the Editorial Board of Trends in Genetics and his participation in an uncountable score of evaluation committees and boards worldwide.

Amongst his major scientific contributions are the biochemical genetics of nitrogenous compound utilization by fungi [EFG12 attendees are strongly recommended to read his paper In praise of erroneous hypotheses, recently published in FGB], the discovery of the mechanism of splicing of introns in fungal mitochondria, the report, in close competition with Geoff Turner's and Bill Timberlake's' labs, of a procedure to transform *Aspergillus* (with his then Ph.D. student Joan Tilburn), the molecular definition of the regulation of catabolic pathways by wide-domain and pathway specific transcription factors (in close collaboration with Herb Arst and Béatrice Felenbok). More recently he has focused his interests on the cell biology of transporters, the mechanisms of chromatin organization and on the use of heterologous transposons as tools to study genome structure.

PS1.1 GENE SILENCING, HETEROCHROMATIN FORMATION AND DNA METHYLATION IN NEUROSPORA

ERIC SELKER

UNIVERSITY OF OREGON, UNITED STATES

Most methylated regions of Neurospora are relics of transposons inactivated by RIP (repeat-induced point mutation), a premeiotic homology-based genome defense system that litters duplicated sequences with C:G to T:A mutations. Detailed analyses of the distribution of DNA methylation in the Neurospora genome revealed that it is most concentrated at centromeric regions, subtelomeric regions and dispersed relics of RIP. Our genetic and biochemical studies on the control of DNA methylation revealed clear ties between DNA methylation and chromatin modifications. In vegetative cells, the DIM-2 DNA methyltransferase is directed by heterochromatin protein 1 (HP1), which in turn recognizes trimethyl-lysine 9 on histone H3, placed by the DIM-5 histone H3 methyltransferase. DIM-5 is sensitive to modifications of histones including methylation and phosphorylation and is found in a complex with several other proteins that are essential for DNA methylation: DIM-7, DIM-8 (DDB1), DIM-9 and CUL4. DNA methylation is modulated by a variety of additional factors. For example, mutants with defects in the DMM complex (DNA methylation modulator; comprised of DMM-1, DMM-2 and HP1) show aberrant methylation of DNA and histone H3K9, with both frequently spreading into genes adjacent to inactivated transposable elements. Mutants defective in dmm-1 grow poorly but growth can be restored by reduction or elimination of DNA methylation. Another HP1 complex, HCHC (HP1, CDP-2, HDA-1 and CHAP) is also important for the normal distribution and control of DNA methylation in Neurospora and is responsible for silencing independently of DNA methylation. HCHC defects cause hyperacetylation of centromeric histones, greater accessibility of DIM-2 and hypermethylation of centromeric DNA. Loss of HCHC also causes mislocalization of the DIM-5 H3K9 methyltransferase at a subset of interstitial methylated regions, leading to selective DNA hypomethylation. Thus Neurospora forms distinct DNA methylation and histone deacetylation complexes that work in parallel to assemble silent chromatin in N. crassa. I will summarize and discuss our recent progress towards the elucidation of mechanisms controlling DNA methylation in Neurospora.

PS1.2 EARLY ENDOSOME MOTILITY SPATIALLY ORGANIZES POLYSOME DISTRIBUTION

GERO STEINBERG, YUJIRO HIGUCHI, PETER ASHWIN, YVONNE ROGER

In fungi, early endosomes (EEs) undergo long-range microtubule-based motility. The reason for their rapid and continuous motility is not known. Here, we report an unexpected role of EE motility in distributing the translation machinery. We show that ribosomes diffused slowly throughout the cytoplasm (Dc, 60S=0.311 μm2s-1), whereas translationally active polysomes underwent long-range motility along microtubules as passive cargo on kinesin-3 and dynein-driven EEs. Mathematical modeling indicates that this transport is required for even distribution of newly-formed ribosomes within the cell. Indeed, impaired EE motility in motor mutants, or the inability of ribosomes to bind EEs, induced ribosome clustering near the nucleus. As a consequence, fungal tip growth was impaired. These results indicate that polysomes bind to moving EEs and that their 'off- and re-loading' distributes them for efficient protein translation.

PS1.3 HOOKA IS A NOVEL DYNEIN-EARLY ENDOSOME LINKER CRITICAL FOR ENDOSOME MOVEMENT IN VIVO

JUN ZHANG, RONGDE QIU, HERBERT ARST, MIGUEL PEÑALVA, XI**N XIANG**

UNIFORMED SERVICES UNIVERSITY, UNITED STATES

Cytoplasmic dynein transports membranous cargoes along microtubules, but the mechanism of dynein-cargo interaction is unclear. From a genetic screen, we identified a homolog of human Hook proteins, HookA, as a factor required for dynein-mediated early endosome movement in the filamentous fungus Aspergillus nidulans. HookA contains a putative N-terminal microtubule-binding domain followed by coiled-coil domains and a C-terminal cargo-binding domain, an organization reminiscent of "cytoplasmic linker proteins". HookA-early endosome interaction occurs independently of dynein-early endosome interaction and requires the C-terminal domain. Importantly, HookA interacts with dynein and dynactin independently of HookA-early endosome interaction but dependent upon the N-terminal part of HookA. Both dynein and the p25 subunit of dynactin are required for the interaction between HookA and dynein-dynactin, and loss of HookA significantly weakens dynein-early endosome interaction, causing a virtually complete absence of early endosome movement. Thus, HookA is a novel linker important for dynein-early endosome interaction in vivo.

PS1.4 PHOTOBIOLOGY IN MODEL AND CLINICAL FUNGI

JENNIFER LOROS, ARKO DASGUPTA, KEVIN FULLER, CHEN HUI CHEN, JAY DUNLAP

DARTMOUTH MEDICAL SCHOOL, UNITED STATES

Light is an indispensable energy source for life and also serves as influential environmental information of daily and seasonal time to organisms across kingdoms. Within the fungi, molecular mechanisms of light responsiveness are best understood in the model system Neurospora crassa. 6% of the Neurospora genome is light inducible at the level of gene expression, controlled largely by the LOV domain transcriptional activator White Collar-1 (WC-1) in complex with White Collar-2 (WC-2). This acute response to light results in cascades of transcriptional activators and repressors that eventually control biological outputs including development and general metabolism. An additional LOV containing photoreceptor, Vivid (VVD) permits the organism to repress the initial light response and then respond to increasing amounts of light, a process called photoadaptation, and also facilitates accurate entrainment of circadian rhythms. We have begun the molecular characterization underpinning a complex response to light in the clinically important fungus Aspergillus fumigatus. It responds to both red and blue light, with melanin pigmentation, conidial germination and stress responses under control of multiple photoreceptors including the WC-1 homolog LreA and a phytochrome, FphA playing unique and overlapping roles. Both the light responsive physiology of this organism and its photobiology are complex and involves input from additional, as yet uncharacterized, photosensory pathways.

PS1.5 LIGHT AND TIME IN ASPERGILLUS NIDULANS

REINHARD FISCHER

KIT, MICROBIOLOGY, GERMANY

Light serves as an important environmental signal to regulate development and metabolism in many fungi and has been studied to some detail in Neurospora crassa, Trichoderma harcianum and Aspergillus nidulans[1]. A. nidulans develops mainly asexually in light and mainly sexually in the dark. The red-light sensor phytochrome (FphA) and the WC-1 homologue blue-light receptor LreA have been shown to mediate the light response in A. nidulans [2]. There is evidence that both proteins form a light regulator complex or at interact transiently. LreB (WC-2) and VeA are probably also components of this complex [3].

Using Chromatin-Immunoprecipitation (ChIP) and quantitative Real Time PCR we show that HA-tagged FphA and LreA bind to the promoters of the A. nidulans homologues of N. crassa con-10 (conJ) and ccg-1 (ccgA). In A. nidulans conJ and ccgA are both induced during development but are also strongly upregulated in hyphae after short exposure to light. Surprisingly LreA bound to the conJ and ccgA promoter in the dark and were released upon illumination. This suggests a repressor function for LreA. In contrast, FphA was recruited to the promoters after short illumination and seems to function as activator of transcription. These results suggest that light induction depends on derepression followed by induction through FphA. We have evidence that light regulation depends on chromatin remodelling and that the light regulators interact with chromatin remodelling enzymes.

In N. crassa one important function of light is the entrainment of the circadian clock. Besides the WC complex, the Frq protein is an essential component of the clock system. In A. nidulans and several other fungi, however, no Frq homologue can be identified in their genomes. Nevertheless, in A. nidulans the expression of conJ and ccgA are controlled in a circadian way. Entrainment of the clock can be achieved with light or temperature.

[1] RODRIGUEZ-ROMERO ET AL., (2010) ANN. REV. MICROBIOL. 64:585-610.
[2] Blumenstein A. et al., (2005) Curr. Biol. 15(20):1833-1838.
[3] Purschwitz et al., (2008) Curr. Biol. 18(4):255-259.

PS2.1 THE INITIATION OF ASEXUAL DEVELOPMENT IN ASPERGILLUS NIDULANS

UNAI UGALDE

UNIVERSITY OF THE BASQUE COUNT, SPAIN

Fungal cells are endowed with the ability to undergo different developmental programmes in response to changing environments. This requires robust control mechanisms to articulate the transition between programmes and also to prevent mixed scheduling.
Cells of the filamentous ascomycete Aspergilllus nidulans are capable of undergoing three alternative developmental programmes: vegetative growth, conidiation and cleistothecium formation, directed at substrate colonisation, dispersal through conidiospores or long-term survival as ascospores. Moreover, all three programmes can be simultaneously in place at different regions of the same colony.
The initiation of conidiospore production involves regulatory factors which are expressed in vegetative cells, called “upstream developmental activators” (UDAs). They coordinate the early stages of development and activate conidiation-specific factors, which direct the formation of phialides and conidia.
In this presentation, new details on environmental change-mediated UDA response will be presented. In addition, recent findings of their direct involvement in the suppression of alternative developmental programmes, as sexual development and vegetative growth will be discussed.

PS2.2 ASPERGILLUS NIDULANS SEPTINS IN MULTICELLULAR DEVELOPMENT

MICHELLE MOMANY

UNIVERSITY OF GEORGIA, UNITED STATES

Septins are cytoskeletal elements with important roles in many processes including cytokinesis, nuclear division, secretion, and restricting diffusion of cellular components. They have been associated with fungal pathogenesis and human diseases including cancer and Alzheimer’s. Septins are classified into five orthologous groups and have been found in all fungi and animals and in some ciliates, chlorophyte algae and brown algae. Septin monomers from different groups associate to form nonpolar heteropolymeric rods that in turn assemble into a variety of higher-order structures. These higher-order structures include the rings and filaments visualized by fluorescent microscopy of GFP-tagged septins and are thought to be the biologically active form. The mechanisms driving septin heteropolymer and higher-order structure assembly are not understood.
Aspergillus nidulans has one septin from each phylogenetic group. Four of the A. nidulans septins are orthologs of the core septins in S. cerevisiae and the fifth septin, AspE, is lacking in unicellular yeasts. We have examined septins from defined stages of A. nidulans development using GFP and affinity purification tags, deletions, fluorescence microscopy, immunoprecipitation and LC-MS/MS. Our results suggest that the four core septins form heteropolymeric complexes. In contrast, AspE interacts with only one of the core septins, and only during multicellular growth. AspE is required for proper localization of three of the core septins, and requires this same subset of core septins for its own unique cortical localization. Our results show that at least two distinct septin heteropolymer populations co-exist in A. nidulans, and that while AspE is not a subunit of either heteropolymer, it is required for assembly of septin higher-order structures found in multicellular development.

PS2.3 MORPHOLOGICAL AND METABOLIC ADAPTATION TO ENVIRONMENTAL CONDITIONS BY PENICILLIUM MARNEFFEI AND ITS ROLE IN THE HOST

ALEX ANDRIANOPOULOS

UNIVERSITY OF MELBOURNE, AUSTRALIA

Penicillium marneffei (Talaromyces marneffei) is an important fungal pathogen of humans, in particular those who are immunocompromised. P. marneffei has the capacity to alternate between a hyphal and a yeast growth form, a process known as dimorphic switching. The strongest extrinsic trigger for dimorphic switching is in response to temperature. P. marneffei grows in the hyphal form at 25°C and in the yeast form at 37°C. The hyphal form produces conidia which are likely to be the infectious agent and believed to establish infection after inhalation. The yeast growth form is the pathogenic form found in infected patients. These yeast cells exist intracellularly in the mononuclear phagocyte system of the host.

P. marneffei is the only true pathogen in a genus comprising a large number of species and is also the only dimorphic fungus in this group. Yet there are a number of other fungi in more distantly related orders which also exhibit the capacity to alternate between hyphal and yeast growth forms. Many of these are also pathogens of animal or plants. As an intracellular pathogen, P. marneffei must be able to utilise the available nutrient sources in order to grow while evading or tolerating the host's defence systems. The results from a number of lines of investigation into the molecular control of the dimorphic switch and the events which establish and maintain the morphological states in P. marneffei, both of which are central to understanding pathogenicity, will be presented.

PS2.4 EVOLUTION OF SEXUAL REPRODUCTION: A VIEW FROM THE FUNGAL KINGDOM

JOSEPH HEITMAN

DUKE UNIVERSITY MEDICAL CENTER, UNITED STATES

Sex is ubiquitous in eukaryotes, and thought to have evolved once. Sex promotes genetic diversity and evolution, yet also confers costs that must be counterbalanced to explain its pervasiveness. Mechanisms of sex determination and mechanics of sexual reproduction are diverse. Fungi are robust models to analyze sex, and their study reveals surprising insights. We study how mating-type identity is specified and modes and roles of sexual reproduction in generating phenotypic/genotypic diversity. Many fungi are bipolar with two opposite mating types and a biallelic mating type locus. In the Basidiomycota many species have a more complex tetrapolar system with two unlinked multi-allelic mating type loci, yielding thousands of mating types and enhanced outcrossing but restricted inbreeding. Our studies reveal how transitions from ancestral tetrapolar to derived bipolar systems occur in pathogenic species embedded within saprobic sibling taxa. This includes molecular phylogenomics, discovery of extant sexual cycles, and analysis of engineered tetrapolar isolates. The tetrapolar-bipolar transition has occurred repeatedly in pathogens of plants and animals, suggesting it may promote host adaptation. Studies of Malassezia reveal an example of a possible pseudobipolar transitional state. Pathogenic Cryptococcus species have taken this transition further to a unipolar sexual cycle. These human pathogenic species are global and have largely unisexual populations involving reproduction via an unusual homothallic unipolar sexual cycle involving only one mating type (same-sex mating, unisexual reproduction). Like a-α opposite sex, α-α unisexual reproduction can admix parental diversity in the progeny. However, in other cases solo α-α unisex involves selfing of identical genomes with no genetic diversity to exchange. Why organisms engage in selfing challenges conventional views on roles of sex. We find unisex generates genetic diversity de novo, preserving well-adapted genomic configurations while generating more limited genetic diversity for selection to act upon. Discovery that other fungi and eukaryotic parasite pathogens also reproduce unisexually generalizes these findings, and suggests unisex may have evolved because it mitigates costs associated with sex. Studies of fungal sex and its evolution and impact illustrate general principles by which diversity is generated and maintained with implications for model and pathogenic microbes and multicellular eukaryotes.

PS2.5

FRUITING-BODY DEVELOPMENT IN SORDARIA MACROSPORA – A MATTER OF RECYCLING

STEFANIE PÖGGELER, OLIVER VOIGT, ANTONIA JAKOBSHAGEN, STEFAN FREY

GEORG-AUGUST UNIVERSITY, GERMANY

The homothallic filamentous ascomycete Sordaria macrospora is used as a model to study fruiting-body development. S. macrospora produces fruiting bodies, which are composed of many different cell types. Since supply and homeostasis of nutrients are important issues for the development of complex fungal structures, we analyzed the involvement of autophagy in the fruiting-body development. Autophagy is a tightly controlled degradation process in which eukaryotic cells digest their own surplus, aberrant or defective cell constituents. During autophagy the cytoplasm is randomly engulfed into double-membraned vesicles called autophagosomes and delivered to the vacuole for degradation of the cargo. Two ubiquitin-like-conjugation systems are required for autophagosome formation and expansion. In the first conjugation system, the ubiquitin-like protein Atg12 is covalently attached to Atg5. In the second conjugation system, the ubiquitin-like protein Atg8 is first C-terminally processed by the cysteine protease Atg4, then activated by the E1-like Atg7 and transferred to the E2-like enzyme Atg3. Finally, a conjugate of Atg8 and the lipid phosphatidylethanolamine (PE) is formed, which is a structural component of the outer and inner autophagosomal membrane. To analyze whether autophagy is necessary for vegetative growth and fruiting-body development in S. macrospora, we have characterized Smatg7, encoding the common E1-like enzyme of the Atg12 and Atg8 conjugation system. We were not able to generate a homokaryotic ΔSmatg7 mutant in S. macrospora, suggesting that Smatg7 is required for viability. In contrast to Smatg7, we were able to generate the homokaryotic deletion mutants ΔSmatg8, ΔSmatg4 and ΔSmatg12. All three mutants are impaired in vegetative growth and arrest fruiting-body development at early stages. Furthermore, we demonstrated that the protease SmATG4 was capable of processing the SmATG8 precursor. SmATG8 was localized to autophagosomes, whereas SmATG4 was distributed throughout the cytoplasm of S. macrospora. We could also show that Smatg8 and Smatg4 are not only required for non-selective 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 non-starvation conditions.

PS3.1 SENSORY PERCEPTION IN THE MAMMALIAN HOST: GUIDING INVASIVE GROWTH AND RATIONAL THERAPEUTIC DESIGN

ELAINE BIGNELL

UNIVERSITY OF MANCHESTER, UNITED KINGDOM

A growing body of transcriptome studies have revealed key stresses imposed by mammalian hosts upon infecting fungal pathogens. Among such stresses, those imposed by extremes of pH are relevant to multiple fungal pathogens of man, and have repeatedly been shown to influence the growth of infecting fungi in various host niches including the bloodstream, lung, vagina, cornea and phagolysosome of innate immune cells. Mutations which subvert the, usually robust, tolerance of these organisms to extremes of extracellular pH invariably result in modulation of virulence, most often severely abrogating infectious growth. We have found that the A. fumigatus pH-responsive transcription factor PacC governs expression of secreted proteases during invasive lung infections and is required for epithelial invasion and pathogenicity. Critically, mutants defective in PacC-mediated signalling are unable to breach the pulmonary epithelium. In- and ex-vivo analyses of infected epithelia revealed that A. fumigatus elicits a series of distinct, and sequentially implemented assaults upon epithelial integrity, consisting of (I) epithelial entry (II) cell wall-mediated epithelial decay and (III) protease-mediated damage, all of which are deficient in the ΔpacC mutant.
The molecular basis of pH responses mounted by diverse fungal pathogens is highly conserved. This, coupled with the absence of such signaling pathways in mammalian cells, suggests that this crucial fungal survival mechanism might provide a useful means of limiting a broad spectrum of infectious fungal growth. pH sensing likely involves a GPCR-like seven transmembrane domain (7-TMD) pH sensor, PalH/Rim21p, and a fungal arrrestin-like protein, PalF/Rim8p. The interactions between 7-TMD proteins and their cognate arrestins represent the most common class of target for existing pharmaceutical drugs. Accordingly, PalH/Rim21p and PalF/Rim8 present putative targets for development of novel antifungal therapies.

PS3.2 TRANSPOSABLE ELEMENTS RESHAPING GENOMES AND FAVORING THE EVOLUTIONARY AND ADAPTIVE POTENTIAL OF FUNGAL PHYTOPATHOGENS

THIERRY ROUXEL, JONATHAN GRANDAUBERT, MARIE-HÉLÈNE BALESDENT

INRA, FRANCE

Transposable Elements (TEs) have been considered for long as “junk” DNA in the genome of complex eukaryotes. However, massive sequencing efforts coupled with phylogenetic analyses suggest TEs can act as genome shapers and be a source of gene innovation and genome plasticity, eventually contributing to genome divergence. Fungi are simple and easy to manipulate eukaryote organisms, for which the ever-growing genome information indicates that many plant-associated fungi have a tendency towards genome size expansion. This increase in genome size is mostly driven by TE expansion that eventually shapes adaptive regions of the genome. Such genome regions host genes involved in niche adaptation and favor accelerated evolutionary dynamics of these genes. Focusing on the Leptosphaeria maculans-Leptosphaeria biglobosa species complex of closely related plant pathogenic fungi, I will discuss the link between TE invasion/TE bursts in genomes and (i) speciation, (ii) the rise of two-speed genomes, shaping plastic genome environments, (iii) gene diversification that contributed to adaptation to new hosts, (iv) heterochromatine-based regulation of expression of effector genes, (v) accelerated adaptation to resistance gene pressure in gene-for-gene systems.

PS3.3 SEPTIN-MEDIATED PLANT TISSUE INVASION BY THE RICE BLAST FUNGUS MAGNAPORTHE ORYZAE

NICHOLAS TALBOT, YASIN DAGDAS, LAUREN RYDER, MICHAEL KERSHAW, MIRIAM OSES-RUIZ, YOGESH GUPTA, GEORGE LITTLEJOHN

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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 to 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 and focal exocyst-mediated secretion. 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 necessary for development of a penetration peg to rupture 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. Cell cycle and pressure-mediated checkpoints, are necessary for initiation of septin activation and re-orientation of the cortical F-actin cytoskeleton to facilitate plant tissue invasion and secretion of fungal proteins from the penetration peg.

PS3.4 GENOMIC ANALYSIS IN THE SEARCH FOR OXIDOREDUCTASES OF INDUSTRIAL INTEREST

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In the Peroxicats project (www.peroxicats.org) we are involved in the analysis of basidiomycete genomes to identify new peroxidases of interest as industrial biocatalysts, in collaboration with JGI. The immediate aim is to understand the mechanisms of wood transformation by these fungi, taking advantage from genomic information on ligninolytic peroxidases and related oxidoreductases. These enzymes are the biocatalysts of choice for overcoming the lignin barrier in the sustainable production of chemicals and biofuels, as well as in other oxidation/oxygenation biotransformations. With the availability of massive sequencing, the number of basidiomycete genomes that we analyzed increased from the first brown-rot [1] and selective lignin-degrader [2] fungal genomes to the most recent project [3] where more than 30 genomes were analyzed to establish the origin and evolutionary history of ligninolytic peroxidases (in the superfamily of plant-fungal-prokaryotic peroxidases). Our contribution to these genomic studies focused on the in silico analysis of all the heme peroxidase genes (> 400 models) after their structural-functional classification, based on homology modeling and identification of catalytic sites. We also coordinated the annotation and analysis of other oxidoreductases (oxidases, laccases, P450s, etc) genes (> 5000 models). In addition to ligninolytic peroxidase genes, the analysis of basidiomycete genomes is revealing an unexpectedly high number of genes of two other interesting peroxidase superfamilies (with monooxygenase and dye-decolorizing activities) that were previously known for only a few purified proteins and cloned genes. Moreover, in some intriguing cases (such as ligninolytic basidiomycetes where no lignin-degrading peroxidases had been described) the above analyses were continued by the heterologous expression of all the peroxidase genes identified followed by biochemical characterization (together with crystallographic and stability studies) and interesting results were obtained. Finally, we combined the information obtained from the analysis of a variety of genes to obtain peroxidases with improved (tailored) catalytic and stability properties, showing that the motifs identified in the genomic analysis can be satisfactorily translated to the design of improved industrial biocatalysts.

1. Martinez D. Proc Natl Acad Sci USA 2009, 106:1954
2. Fernández-Fueyo E. Proc Natl Acad Sci USA 2012, 109:5458
3. Floudas D. Science 2012, 336:1715

PS3.5 THE EVOLUTION OF FUNGAL CHEMODIVERSITY

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Fungal species are typically saprobes, embedded in their food sources and required to digest their food externally in the presence of competitors. To survive in such a hostile environment, fungi have evolved a bewildering diversity of metabolic capabilities. Importantly, this phenotypic diversity is reflected in their genomes. Thus, by examining the fungal DNA record we can gain valuable insights into the evolution of their metabolic lifestyles. One conspicuous characteristic of fungal metabolic pathways is that genes participating the same metabolic pathway are frequently physically linked. Both ecologically specialized (accessory) metabolic pathways as well as ones that are essential for growth form such gene clusters have been shown to occasionally undergo horizontal transfer. However, several outstanding questions remain. What percentage of metabolic genes is involved in the formation of such clusters? What is the relative distribution of such clusters among pathways that are accessory or essential? Moreover, how this distribution influences the degree to which gene clusters undergo horizontal transfer? To address these questions, I will be presenting the results of our recent examination of the structure of synteny, degree of evolutionary conservation, and evolutionary history of ~230,000 genes participating in hundreds of metabolic pathways in 207 genomes that span the diversity of the fungal kingdom. I will also be presenting the results of our RNA-Seq studies to better understand the evolution of regulation of fungal metabolic pathways across fungal species and environmental conditions, which we are performing in collaboration with Ana Calvo's lab at Northern Illinois University.

PS3.6 FUNGAL GENOMICS RESOURCES OF THE US DEPARTMENT OF ENERGY JOINT GENOME INSTITUTE

IGOR GRIGORIEV

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Genomes of fungi relevant to energy and environment are in the focus of the Fungal Genomic Program at the US Department of Energy Joint Genome Institute (JGI). There are several ways how any researcher from any country can initiate new genomics projects with JGI . First, all year around, you can nominate individual species of fungi for sequencing via the 1000 Fungal Genomes project, which aims to build a reference genome for every family of fungi (1.usa.gov/JGI-1000-Fungi). Second, once a year, you can propose large scale genomics projects combining genome and transcriptome sequencing, with functional genomics studies and gene synthesis in response to the JGI Community Science Program call (1.usa.gov/JGI-CSP). Among other things, JGI is interested to explore effects of diverse fungi on global carbon cycling, study roles of fungi in soil and terrestrial environments, understand plant-fungal interactions, and develop new fungal models for bioenergy and bioremediation. All the data become assembled, annotated and publicly available in JGI fungal genomics portal MycoCosm (jgi.doe.gov/fungi).

Concurrent session abstracts

CS1.1 HISTONE DE-METHYLASES REGULATE PRIMARY AND SECONDARY METABOLISM IN ASPERGILLUS NIDULANS

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Gene expression is locus-dependent mainly because the chromatin structure defines accessibility of the transcriptional machinery. Changes in environmental conditions, nutritional status or developmental stages usually signal to pathway-specific or to broad-domain transcriptional regulators which, subsequently, recruit multi-subunit complexes able to modulate chromatin structure and change the histone code. Silenced, facultatively heterochromatic regions – typical for secondary metabolite gene clusters under primary metabolite conditions – undergo drastic chromatin rearrangements during their activation. We have studied the activation cycle and found that specific histone de-methylases are needed to first remove repressive marks (e.g. HP1, methylated K9 at H3) before activating marks, such as histone acetylation, phosphorylation or H3K4 methylation, can be positioned. Moreover, genetic and molecular data suggest that LaeA/Lae1, a conserved general activator of fungal SM, has a chromatin-related function and, in Aspergillus nidulans, is required to facilitate replacement of repressing by activating marks. A.nidulans contains two genes coding for a predicted function in histone H3 de-methylation. We have characterized both enzymes genetically and biochemically in vitro and in vivo under a variety of conditions. Our data suggest that both genes encode functional, iron- and alpha-ketoglutarate-dependent histone H3 de-methylases, although the enzymes show different specificities towards histone methyl-lysine substrates. Deletion of the enzymes individually and in combination produced a developmental phenotype and altered secondary metabolite profiles. Transcriptome and ChIP analysis provided evidence that, in vivo, the enzymes regulate different gene sets but are both required to fully activate the tested secondary metabolite gene clusters.

CS1.2 REGULATORY NETWORKS AND REGULATORS OF CHROMATIN STRUCTURE GOVERNING GLOBAL RESPONSES TO CHANGES IN LIGHT AND TIME

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Most fungi are highly responsive to their immediate environment, having developed sophisticated means to respond acutely to changes in their photic environment as well as circadian clocks to anticipate repeating environmental changes. Neurospora has proven to be a tractable model for understanding the proteins and networks underlying these responses. In this organism, blue light is detected by FAD stably bound by the transcription factor WC-1, eliciting photochemistry that drives a conformational change in the complex of WC-1 and WC-2 (WCC) resulting in activation of gene expression from promoters bound by the WCC. The circadian system allows anticipation of recurring environmental changes, and comprises a negative feedback loop wherein the WCC, in the dark, drives expression of frq. FRQ, an intrinsically disordered protein, stably interacts with casein kinase 1 and with FRH (a putative RNA helicase that does not function enzymatically in the clock; Hurley et al., Molecular Cell, 2013), and after phosphorylation-mediated delays, the complex downregulates the WCC (Baker, Loros, & Dunlap, FEMS Microbiol. Rev.36: 95-106, 2012). Using the tools of next generation sequencing, recombineering, and luciferase reporters, the molecular details of the clock itself as well as network ramifying from the clock out to primary, secondary, and tertiary targets of light and clock control can now be described. In a case study of regulation, structure/function analysis of WC-1 identified a region of 100 amino acids essential for frq circadian expression. A proteomics-based search for coactivators interacting with the WCC via this region of WC-1 uncovered the SWI/SNF (SWItch/Sucrose NonFermentable) complex: SWI/SNF interacts with WCC in vivo and in vitro, binds to the Clock box in the frq promoter, and is required both for circadian remodeling of nucleosomes at frq and for rhythmic frq expression. These data suggest a model in which WC-1 recruits SWI/SNF to remodel and loop chromatin at frq thereby activating frq expression to initiate the circadian cycle. These data, considered in the context of the genome, allow us to compare and contrast light-regulation of gene expression and clock-regulation of gene expression.

CS1.3 HETEROCHROMATIN CONTROLS γH2A LOCALIZATION AND GENOME STABILITY IN NEUROSPORA CRASSA

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The histones H2A in fungi and H2A.X in animals are phosphorylated by ATR and ATM kinase on a C-terminal serine to yield γH2A. γH2A recruits chromatin-binding proteins that recognize this phosphorylated serine and function to stabilize stalled replication forks or promote proper DNA repair. We performed ChIP-seq experiments to identify genomic regions that associate with γH2A, reasoning that these sites might interfere with normal genome integrity. We found that γH2A is localized to heterochromatin domains in Neurospora crassa. H3K9 methylation is required for normal γH2A localization, but γH2A is not required for H3K9 methylation or DNA methylation. γH2A localization is partially dependent on Heterochromatin Protein-1, but is independent of the DNA methyltransferase, DIM-2. We found that γH2A is massively induced in heterochromatin-defective mutants, suggesting that proper heterochromatin formation is important for normal DNA replication or repair.

CS1.4 THE FUNCTIONAL CHARACTERIZATION OF THE NEUROSPORA CRASSA HAC-1 TRANSCRIPTION FACTOR REVEALS A CRUCIAL ROLE FOR THE UNFOLDED PROTEIN RESPONSE IN PLANT CELL WALL DECONSTRUCTION

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High secretion capacity in filamentous fungi requires an extremely efficient system for protein synthesis, folding and transport. When the folding capacity of the endoplasmic reticulum (ER) is exceeded, a pathway known as the unfolded protein response (UPR) is triggered allowing cells to mitigate and cope with this stress. In yeast, this pathway relies on the transcription factor HAC1, which mediates the up-regulation of several genes required under these stressful conditions. In this work, we identify and characterize the HAC1 orthologue in the filamentous fungus Neurospora crassa. We show that hac-1 mRNA undergoes an ER stress-dependent unconventional splicing reaction, which in Neurospora removes a 23 nt intron, leading to a change in the open reading frame and the production of a functional transcription factor. By disrupting hac-1, we determined this gene to be crucial for activating UPR and for proper growth in the presence of ER stress-inducing chemical agents. Neurospora is naturally found growing on dead plant material and it has become a model organism for plant cell wall deconstruction studies. Notably, we found that Hac-1 is necessary for growth on cellulose or Avicel (crystalline cellulose). Further characterization of this phenomenon revealed that it is due, in part, to a drastic reduction in the levels of secreted proteins. Unexpectedly, however, we also observed that the expression of cellulolytic genes is partly impaired in the hac-1 KO strain, which correlates with poor induction of key transcription factors (CLR) required for deconstruction of cellulose. Nevertheless, growth of hac-1 deficient strains on xylan, cellobiose or glucose is not impaired, which can be partially explained by less challenging protein secretion demands. The characterization of this signaling pathway in Neurospora will help in the study of fungal plant cell wall deconstruction, highlighting UPR as relevant process that can be further manipulated with important biotechnological applications. FONDECYT1131030, MN-FISB NC120043.

CS1.5 REGULATION OF ENDOGENOUS FUNCTIONS BY SMALL RNAS IN THE PATHOGENIC FUNGUS MUCOR CIRCINELLOIDES

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The increasing knowledge on the functional relevance of endogenous small RNAs as riboregulators has stimulated the identification and characterization of these molecules in many eukaryotes and the analysis of their regulatory roles. While the function of small RNAs in viral defense, transposon silencing and heterochromatin formation is well known, their role in regulation of endogenous gene expression in fungal models has been barely described. The small RNA molecules suppress gene expression through RNA interference (RNAi) pathways, which may differ among organisms. In Mucor circinelloides, an opportunistic human pathogen evolutionary distant from other fungal model organisms, four different classes of small RNAs derived from exonic sequences (ex-siRNAs) have been identified. The four ex-siRNA classes have been classified based on their structural characteristics and the differential RNAi components involved in their biogenesis. These ex-siRNAs are functional, since they regulate the expression of the protein coding genes from which they were produced. Phenotypic alterations shown by RNAi mutants, who are affected in the response to environmental signals, support the regulatory role of these ex-siRNAs. To better understand the role of the RNAi machinery in the regulation of endogenous functions, we have performed whole-genome transcriptional analysis of wild-type and silencing mutant strains of M. circinelloides. We have identified a significant number of genes that were differentially expressed in one or more silencing mutant relative to the wild type. Some of those genes were up-regulated in the mutants, as it should be expected from direct regulation through ex-siRNAs, whereas others were down-regulated. A relevant proportion of the differentially expressed genes showed the same pattern of expression in the different silencing mutants, suggesting that they are directly or indirectly regulated by the canonical RNAi mechanism. A number of those genes are presumably involved in growth, cell wall integrity, stress responses and autophagy, which may explain the phenotypic changes shown by mutants affected in the RNAi machinery. These results reveal new roles for small RNAs in fungi and highlight their implications in a wide variety of functions. This work was funded by the Spanish MICINN (BFU2009-07220) and MINECO (BFU2012-32246) co-financed by FEDER.

CS1.6 MECHANISMS OF SMALL NON-CODING RNA PATHWAYS IN NEUROSPORA

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A variety of small non-coding RNAs, including miRNAs and Piwi-interacting RNAs, associate with Argonaute family proteins to regulate gene expression in diverse cellular processes. By studying small RNA that are associated with the Neurospora crassa Argonaute protein, we identified several types of small RNAs in this filamentous fungus, including the DNA damage-induced qiRNA, microRNAs and Dicer-independent small interfering RNAs. Our results showed that small RNA biogenesis is surprisingly complex and diverse in Neurospora; Different small RNAs are processed by a distinct combination of factors. Our studies uncovered several novel small RNA production pathways and shed light on the diversity and evolutional origins of eukaryotic small RNAs.

CS1.7 RNAI-DEPENDENT EPIMUTATIONS EVOKE ANTIFUNGAL DRUG RESISTANCE IN THE ZYGOMYCETE FUNGAL PATHOGEN MUCOR

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Microorganisms evolve via a panoply of mechanisms spanning aneuploidy, sexual/parasexual reproduction, mutators, Hsp90, and even prions. The pathogenic fungus Mucor circinelloides grows as a hyphae aerobically, but as a yeast in anaerobic conditions or in the presence of the immunosuppressive drug FK506. The antifungal activity of FK506 is exerted in complex with FKBP12, a protein folding enzyme conserved throughout eukaryotes. The FKBP12- FK506 complex inhibits the protein phosphatase calcineurin and thereby blocks hyphal growth of M. circinelloides. Continued exposure to FK506 yields resistant isolates, which exhibit hyphal growth emerging from the yeast colony. Some isolates harbor a variety of Mendelian mutations in the fkbA gene that encodes FKBP12 or the calcineurin genes, conferring stable drug resistance. However, other isolates harbor no mutations in the target genes. These unusual epimutant isolates also revert frequently within several generations of vegetative growth in drug-free media and are restored to wild-type (yeast growth in the presence of FK506). Northern and Western analyses revealed a loss of fkbA mRNA and FKBP12 protein in the epimutants. High-throughput sequencing and Northern blot also detected sRNA generated from fkbA in the epimutant strains, revealing a new role for RNAi in the development of transient, reversible resistance to an antifungal drug treatment. RNAi activation involves generation of a double-stranded RNA trigger intermediate using the fkbA mature mRNA as template. Our results reveal a novel epigenetic RNAi-based epimutation mechanism controlling phenotypic plasticity in fungi.

CS1.8 SPLICEOSOMAL TWIN INTRONS IN FUNGAL NUCLEAR TRANSCRIPTS: STRUCTURE AND EVOLUTION

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Introns are stretches of noncoding RNA within primary transcripts that need to be removed to produce a mature mRNA that translates into the correct product. Introns offer the possibility to diversify a part of the encoded peptide. Alternative intron splicing could lead to frame-shifted ORFs so that peptides with different terminal extensions can be produced from one gene, for instance, enzymes that are targeted to different subcellular locations. Moreover, introns in the untranslated regions can have regulatory functions. Such introns could, e.g., convey feed back inhibition at the post-transcriptional level by means of a riboswitch. Here, binding of a specific compound by the intron RNA determines its exact excision and thereby, the availability of the appropriate start codon. Other introns near the 5' end of the coding sequence could allow a gene's expression to be controlled by two, physically distinct promoters. In filamentous fungi, regulation at the level of transcript splicing has attracted more attention with the availability of high throughput RNA sequencing technology. Recently, we have predicted instances of spliceosomal twin introns (stwintrons) in fungal nuclear transcripts [1]. Typical of stwintrons is that excision of an external intron, and thus proper mRNA maturation, necessitates prior removal of an internal intron, which interrupts crucial sequences of the former. We have shown that two of these features, both having the donor of the external intron disrupted, are removed by sequential splicing reactions. They were the first examples of such complex intervening sequences in eukaryotic nuclear transcripts. Here, we show the structure of stwintrons and discuss how these features could be generated, maintained and (eventually) disappear. The stwintron may be the precursor from which alternatively spliced introns featuring dual acceptor or donor sequences have derived, and could be relevant to hypotheses of evolutionary recent (late) de novo intron generation from an endogenous origin.

[1] Flipphi et al., Fungal Genet. Biol. (2013) 57: 48-57.

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CS2.1 COMPARATIVE GENOMICS AND TRANSCRIPTOMICS TO ANALYZE FRUITING BODY DEVELOPMENT

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Filamentous ascomycetes develop complex, multicellular fruiting bodies for the differentiation and dispersal of sexual spores; however, the genetic control of this developmental process is not well understood. Major types of fruiting bodies (apothecia, perithecia, and cleistothecia) share a common ancestor; and thus, it is likely that a group of core genes is involved in fruiting body morphogenesis. One way to identify such genes is to search for evolutionary conserved expression patterns. We have compared microarray and RNA-seq data from Sordariomycetes (Sordaria macrospora, Fusarium graminearum) and Pezizomycetes (Pyronema confluens) to identify genes that are transcriptionally upregulated during sexual development. Among the genes that we identified were the histone chaperone gene asf1 and the transcription factor gene pro44, both of which were shown to be essential for fruiting body development in S. macrospora. Furthermore, the P. confluens orthologs of asf1 and pro44 can complement the corresponding S. macrospora mutants, showing that the genes have an evolutionary conserved function. In addition to the identification of target genes, comparative studies can be used to determine genome-wide transcription patterns during development. Comparative analysis of microarray data from S. macrospora and F. graminearum indicated that genes with predicted functions in metabolism were overrepresented among genes downregulated during fruiting body morphogenesis, whereas genes with predicted functions in transcription, protein activity regulation, cell fate, and cell wall biogenesis were overrepresented among the upregulated genes. These patterns support the long-standing hypothesis that fruiting body differentiation starts only when the mycelium has reached a stage of metabolic competence after which it is able to supply the developing fruiting bodies with nutrients. In a second approach, based on sequencing the genome and several development-dependent transcriptomes of P. confluens, we analyzed whether genes with different levels of evolutionary conservation differ in their transcription patterns. Interestingly, the highest percentage of genes upregulated during sexual development was found among the P. confluens orphan genes and Pezizales-specific genes (20 and 15 %, respectively), whereas it was less than 2 % in more conserved gene groups. This finding is consistent with the idea of rapid evolution of sex-associated genes.

CS2.2 TRACKING THE EVOLUTION OF PERITHECIUM MORPHOLOGY THROUGH TRANSCRIPTOMICS

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Recent investigations have shown that orders within the perithecium forming Sordariomycetes represent ancient lineages, with Sordariomycete lineages diverging before mammals appeared. We are investigating how the diversity in fruiting body forms among these orders evolved. To this end, we have performed transcriptional profiling of five species of Neurospora and Fusarium during six stages of perithecium development. We estimated the ancestral transcriptional shifts during the developmental process among the species and identified genes whose transcription had substantially and significantly shifted during the evolutionary process. We examined genes whose expression greatly increased in Fusarium graminearum perithecium development. Functional studies through gene disruption resulted in substantial changes in perithecium morphology in the mutants of many of these genes. These genes were not previously identified as candidates for function in perithecium development, illustrating the utility of this method for identification of genes associated with specific functional processes. In addition, the genes were frequently associated with morphologies that distinguished Fusarium from Neurospora.

CS2.3 SEXUAL DEVELOPMENT AND FEMALE FERTILITY IN TRICHODERMA REESEI

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Trichoderma reesei (syn. Hypocrea jecorina) is one of the most important cellulase producers in industry. Both research and industrial strains of T. reesei are derivatives of the original isolate QM6a. However, after achievement of sexual development with T. reesei, this strain was found to be female sterile. We therefore aimed to identify the mutations leading to this defect. After several crosses and selection for fertility we obtained three independent lines of sexually competent T. reesei strains with a phenotype and genetic background similar to QM6a. One strain of every independent line was sequenced and mapped to the published genome of QM6a followed by genome wide analysis of SNPs. We detected three genomic regions which had been consistently retained from the sexually competent parent in all three lines. In depth analysis of the roughly 100 genes in these areas revealed that the presence of 6 genes consistently correlated with female fertility i. e. rescue of the sexual defect of QM6a. Deletion of one of these 6 genes in the now fertile strains again caused female sterility. Transcriptome analysis upon growth under conditions favoring sexual development revealed significant differences in gene regulation between female fertile and female sterile strains, but hardly any regulation of the genes associated with female fertility in T. reesei. Hence the defect in female fertility of QM6a is likely to be caused by perturbed function of the encoded proteins rather than misregulation of these 6 genes. Knowledge of the gene(s) responsible for female fertility in QM6a and their function(s) can be used to accelerate strain improvement by sexual crossing in T. reesei.

CS2.4 A RETINOIC-ACID BIOSYNTHETIC ENZYME INVOLVED IN MORPHOLOGY AND SEXUAL DEVELOPMENT IN FUSARIUM VERTICILLIOIDES

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Aldehyde dehydrogenases (ALDHs) comprise a large family of enzymes that catalyse the oxidation of a wide range of aldehydes to the corresponding acid forms. Particularly relevant members from this family are retinaldehyde dehydrogenases (RALDHs), which convert retinal to retinoic acid, an important morphogen involved in development and embryogenesis in chordates. We formerly described the genes carRA, carB, carT and carD, encoding the enzymes needed for neurosporaxanthin biosynthesis in F. fujikuroi. As a lateral branch of the pathway, cyclization of gamma-carotene produces beta-carotene, which is cleaved by the CarX oxygenase to produce retinal, the presumed chromophore of the two rhodopsins encoded in the genome of this fungus, CarO and OpsA. The ability of Fusarium to produce retinal led us to consider the occurrence in this fungus of a RALDH enzyme able to convert this apocarotenoid into retinoic acid. Because of its higher amenability for sexual crosses and its close taxonomic relation to F. fujikuroi, we chose for this study Fusarium verticillioides. A search for genes encoding putative RALDH enzymes in the F. verticillioides genome identified several candidate genes, which were heterologously expressed in E. coli, and checked for activity on retinal as a substrate. One of them, that we called CarY, exhibited the higher similarity to mammal RALDHs and was able to convert efficiently retinal into retinoic acid. Targeted mutation of the gene carY revealed morphological alterations of the colonies, specially marked under certain culture conditions, and a decreased ability to produce perithecia when the mutant acted as a female in sexual crosses. The phenotypic alterations of the mutant were reverted upon introduction of a wild type carY gene, indicating that retinoic acid formation may play a relevant morphogenetic role in F. verticillioides. This species contains the same car genes for carotenoid metabolism and has a similar photoinduction of the pathway than F. fujikuroi, explained by the transcriptional photoinduction of the gene carRA, responsible for the first enzymatic step. However, a less efficient photoinduction was found for the genes carB, carT and carX. Under the same experimental conditions, transcript levels of the gene carY were hardly affected by light in both Fusarium species.

CS2.5 DECIPHERING THE ROLE OF THE FLB-APICAL COMPLEX IN ASEXUAL DEVELOPMENT OF ASPERGILLUS

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An intricate signalling pathway mainly involving transcription factors is behind the regulation of brlA expression, the central developmental activator of conidiation. In Aspergilli, members of this pathway were identified and called upstream developmental activators, UDAs. Along years, our work has shown that the bZIP transcription factor FlbB is a key element in the UDA pathway and its activity is modulated by a precise cellular location and interaction with other Flb (UDA) partners. UDA is not a linear pathway as initially modelled, in this talk we will learn about the molecular mechanism modelling the apical complex formed by FlbE and FlbB, and its role in cellular distribution and transcriptional activity of FlbB. The regulatory and functional relationship between FlbB and FlbB, a bZIP/cMyb connection, being a common regulatory mechanism of development found from fungi to higher eukaryotes. Finally, RNA sequencing has revealed a number of possible targets for FlbB regulation that extends its role above brlA regulation.

CS2.6 REGULATION OF MORPHOGENESIS DURING DEVELOPMENT IN THE FILAMENTOUS FUNGUS ASPERGILLUS NIDULANS

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In the filamentous fungus Aspergillus nidulans, the transition from hyphal growth to asexual development is associated with dramatic changes in patterns of cellular morphogenesis and division. These changes enable the formation of airborne conidiophores that culminate in chains of uninucleate spores generated by repeated budding of phialides. Our objective is to characterize the regulatory modules that mediate these morphological changes and to determine how their evolution could account for the differences in conidiophore morphology observed within the Trichocomaceae. In yeast, the transcription factor Ace2 regulates the expression of functions required for cytokinesis and entry into the next cell cycle. Previous studies also report that Ace2 is required for the formation of phialides in A. fumigatus. Here, we characterize the A. nidulans Ace2 homologue AN4873. We show that it is required for the transition from hyphal to budding growth during conidiophore development. In particular, the absence of AN4873 results in disordered cell cycle progression within phialides and the production of multinucleate cells. We also describe strong genetic interactions that implicate AN4873 and the Cdc42/RacA GTPases as parallel regulators of conidiophore morphology. Lastly, we present preliminary results from RNA-Seq experiments designed to identify potential targets of Ace2. Collectively, our results provide new insight into the regulatory mechanisms that differentially regulate cellular morphogenesis during growth and development in A. nidulans.

CS2.7 INVESTIGATING THE ROLE OF THE EXOCYST COMPLEX IN APPRESSORIUM-MEDIATED TISSUE INVASION BY RICE BLAST FUNGUS, MAGNAPORTHE ORYZAE

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Magnaporthe oryzae is a widespread and devastating plant pathogenic fungus, which causes blast disease in a broad range of cereals and grasses. M. oryzae develops a specialized infection structure called an appressorium which generates huge turgor to breach the leaf cuticle. The fungus than colonizes host epidermal cells and proliferates in plant cells. At the initial stages of infection, the fungus grows biotrophically and after 5 days, necrotrophic lesions appear on the leaf surface. During host invasion fungus secretes a repertoire of effector proteins which allow the fungus to evade the host immune response. These effectors have been shown to localize at the appressorium pore prior to plant infection, at the tips of primary invasive hyphae, and in a specialized plant-derived, membrane-rich structure called the Biotrophic Interfacial Complex (BIC). However the underlying mechanism controlling polarized secretion of effectors is not well defined in M. oryzae. The exocyst is an octameric protein complex (composed of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84) that appears to be evolutionary conserved in fungi and to play a crucial role in vesicle tethering to the plasma-membrane. The exocyst thus plays an important role in polarized exocytosis and interacts with signaling pathways at the apex of fungal cells controlling polarity. We are currently characterizing components of the exocyst complex during infection-related development of M. oryzae. In M. oryzae all of the exocyst components localize to the tip of vegetative hyphae as in other fungi. Interestingly, exocyst components also localize around the appressorium pore, which suggests the pore is an active site for secretion at the point of plant infection. We have shown that Exo70 and Sec5 are involved in secretion of cytoplasmic effectors but not apoplastic effectors. Furthermore, we have also shown that organization of the appressorium pore requires a hetero-polymeric septin GTPase network and here we show that localization of the exocyst at the appressorium pore is septin-dependent. Temperature-sensitive mutation of Sec6 completely disrupts exocyst assembly at the appressorium pore which suggests that Sec6 is an important component of this complex. Targeted gene deletion of exocyst components Exo70 and Sec5 and temperature sensitive mutation of Sec6 also cause significant loss of virulence. We will present new information on the role of the exocyst during invasive growth of M. oryzae.

CS2.8 LIGHT-RESPONSIVE TRANSCRIPTION FACTORS (LTFS) REGULATE DIFFERENTIATION AND VIRULENCE IN THE GRAY MOLD FUNGUS BOTRYTIS CINEREA

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Botrytis cinerea 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. 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; 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 TF in virulence, and discovered furthermore its 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 in B. cinerea B05.10 including five further TF-encoding genes (BcLTF2-6), and that the expression levels of the majority of these genes (66%) are modulated by BcLTF1. Bcltf2 encodes a C2H2-TF which is the homologue of N. crassa SAH-1 (short-aerial-hyphae-1). Expression levels are increased in mutants exhibiting a hyper-conidiation phenotype such as deletion mutants of BcLTF1, the VELVET protein BcVEL1 and the WHITE COLLAR-like TF BcWCL1, suggesting a role of BcLTF2 in regulation of conidiation. Indeed, Δbcltf2 mutants do not produce conidia in axenic culture and in planta. Notably, mutants form sclerotia under all illumination conditions indicating that the suppression of sclerotial development by light is likely due to the induction of conidiation rather than due to a direct suppression of the sclerotium differentiation program.

CS3.1 DEFINING THE LINE BETWEEN MUTUALISM AND PARASITISM

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Arbuscular mycorrhizal fungi form a mutualistic symbiosis with roots of most terrestrial plants. In this symbiosis, the fungus improves plant nutrition and health by supplying the host plant with minerals such as phosphate, nitrogen and sulfur. In reward, the plant meets the fungal demand for carbohydrates what imposes an increase in the sink strength of roots. In this delicate situation, an exquisite balance between giving and taking has to be achieved in order to stabilize this mutualism and avoiding parasitism. It is to be predicted that to this end a molecular communication between fungus and plant has to exist to guarantee proper symbiosis development at all stages. We recently showed that part of this communication is based on the secretion of fungal effector proteins that modulate the plant cell program. This is a common mechanism exerted by pathogenic microbes when interacting with their hosts, and thus our finding reveals that the line between mutualism and parasitism is thinner than previously expected. To investigate this aspect in more detail we are analyzing the mechanisms by which fungal effectors of mutualistic and parasitic fungi highjack common plant targets.

CS3.2 DLD1, A NOVEL FUNGAL HISTIDINE-RICH EFFECTOR-PROTEIN THAT BINDS TO METAL IONS TO PERTURB PLANT IMMUNITY

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Plant-colonizing fungi often use small secreted proteins targeted to the apoplast to promote colonization by suppressing host immunity. One effector, Dld1, expressed by the plant mutualistic root endophyte Piriformospora indica, belongs to a large paralogous family whose members show a regular distribution of histidines. Dld1 tightly binds to divalent metal ions at different pH and to iron III at pH lower than 7. Upon P. indica colonization of barley roots the host reacts with targeted redistribution of iron III at the site of attempted penetration where production of cell wall apposition, accumulation of glycoproteins and redox activity is observed. The bulk secretion of iron III and its accumulation at fungal attack sites mediate the oxidative burst and regulate cereal defenses. We demonstrate here that Dld1 distribution coincides with that of iron III at the host cell wall appositions and that this effector is able to suppress the iron III dependent burst, suggesting a role in perturbation of cereal host immunity.

CS3.3 THE EFFECTOR PROTEIN MISSP7 OF THE MUTUALISTIC ECTOMYCORRHIZAL FUNGUS LACCARIA BICOLOR INTERACTS WITH POPULUS JAZ PROTEINS

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Roots of most trees form a nutrient-acquiring symbiosis with mutualistic fungi living in soils. We have previously shown that the effector protein MiSSP7 encoded by the ectomycorrhizal fungus L. bicolor is necessary for the establishment of symbiosis with Populus trees (Plett et al., 2011), although the mechanistic reasoning behind this role was unknown. We demonstrate here that MiSSP7 interacts with the Populus protein PtJAZ6, a putative negative regulator of jasmonic acid signaling. In presence of the JA-Ile mimick coronatine, PtJAZ6 is interacting with PtCOI1. In planta, MiSSP7 is able to stabilize PtJAZ6 from methyl-jasmonate induced degradation. Further, loss of MiSSP7 expression in L. bicolor can be complemented by transgenically varying the transcription of PtJAZ6 or through inhibiting jasmonic acid signaling in poplar roots. We conclude that L. bicolor, in contrast to arbuscular mycorrhizal fungi and biotrophic pathogens, promotes mutualism by blocking JA action through the interaction of MiSSP7 with PtJAZ6.

Plett JM, et al. (2011) Curr Biol . 21:1197-1203

CS3.4 TROJAN HORSE STRATEGY AND FAIR TRADE AMONG SYMBIOSES: HOW ONE FUNGAL SPECIES CAN INVADE THOUSANDS OF PLANT SPECIES

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Arbuscular Mycorrhizal (AM) symbiosis, the oldest mutualistic plant-fungal symbiosis, concerns 80% of land plants. AM fungi (Glomeromycota) can indeed associate with non-vascular plants (liverworts, hornworts), early diverging vascular plants (ferns) or seed plants. Following the presymbiotic molecular dialog, the mycelium invades roots/thallus and grows in the cortical/parenchymal tissues where it develops intercellular highly branched hyphae - arbuscules – allowing bidirectional nutrient exchanges. The fungus forms a network of extraradical mycelium (ERM) in the soil that recruits water, nitrogen and phosphorus. Nutrients are efficiently taken up by high and low affinity transporters, then translocated to the Intra Radical Mycelium (IRM) where they will be exchanged in arbuscules against plant hexoses. The recent release of the gene repertoire of the AM fungal model species Rhizophagus irregularis (Tisserant et al., 2012; Tisserant, Malbreil et al., 2013) opens the way toward the description of the fungal processes involved in the establishment of the AM symbiosis. We addressed the question of host invasion by Rhizophagus irregularis. Like other AMF, R. irregularis has the ability to invade all mycotrophic plant species, implying different tissues to colonize (either thallus or true roots). Based on RNAseq approaches, we compare the fungal transcriptomes in symbiotic tissues from three host species (one liverwort and two flowering plants, a legume and a grass). Comparing IRM/ERM gene expression, we defined a set of specifically or highly up-regulated genes shared in the different interactions that can be hypothesized as the common fungal symbiotic toolkit. It includes nutrient transporters, metabolic genes and a panel of secreted proteins potentially involved in host manipulation. A part of those symbiosis-specific genes has been chosen for qPCR validation in an extended number of hosts, covering the green lineage. It will give the first glimpse of the gene set involved in symbiosis establishment regardless of the host colonized.

CS3.5 CELL FUSION IS REQUIRED TO MAINTAIN AN EPICHLOË FESTUCAE SYMBIOTIC HYPHAL NETWORK IN LOLIUM PERENNE

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Epichloë festucae is a filamentous fungus that forms a mutually beneficial symbiotic association with Lolium perenne. This biotrophic fungus systemically colonises the intercellular spaces of leaves to form a highly structured and interconnected symbiotic hyphal network. Establishment and maintenance of this hyphal network is dependent on a number of important fungal signaling pathways including ROS signaling from the NADPH oxidase (Nox) complex. Disruption of noxA and additional components of the Nox complex leads to a breakdown in the symbiosis. Disruption of the transcription factor ProA also leads to a breakdown in the symbiotic interaction. ΔnoxA and ΔproA share a defect in hyphal cell fusion, which we hypothesize, is responsible for triggering the dramatic host interaction phenotype of these mutants. Using a forward genetic screen we isolated a mutant of the cell wall integrity MAP kinase kinase (mkkB) that shows a very similar symbiotic interaction phenotype to the ΔnoxA and ΔproA mutants. Plants infected with ΔmkkB were severely stunted and underwent premature senescence. Hyphae of ΔmkkB had a proliferative pattern of growth within the leaves, formed hyphal bundles within the mesophyll tissue and colonized the vascular bundles. In addition, we observed the formation of intra-hyphal hyphae, a phenotype indicative of hyphal stress within the plant. A mutant defective in the downstream MAP kinase, mpkA, showed a very similar host-interaction phenotype. When grown on water agar both ΔmkkB and ΔmpkA were shown to be defective in cell fusion, a phenotype confirmed using GFP and mRFP marked strains, and underwent hyperconidiation. Taken together these results support the symbiotic hyphal fusion hypothesis. To garner further support for this hypothesis we have embarked on a reverse genetics strategy to test whether genes known to be defective in hyphal anastomosis in N. crassa (ham mutants), S. macrospora (pro) and P. anserina (IDC) also have a symbiosis interaction phenotype. E. festucae mutants of the N. crassa homologues, ham-5 (Pa IDC1) and ham-7, were shown to have very similar culture and plant-interaction phenotypes to the ΔmkkB and ΔmpkA mutants, providing further support for our hypothesis. These results highlight the importance of hyphal anastomosis for establishing a symbiotic hyphal network in the mutualistic symbiotic interaction between E. festucae and L. perenne.

CS3.6 GENOMIC ANALYSES OF MORTIERELLA ELONGATA AND ASSOCIATED BACTERIAL ENDOSYMBIONT (CANDIDATUS GLOMERIBACTER SP.)

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Collaborative efforts to characterize the Populus root microbiome have resulted in numerous isolates and genome sequences of its beneficial rhizospheric fungi and bacteria. One of these fungi is Mortierella elongata (Mortierellales, Zygomycota), a rapidly growing coenocytic, multinucleate fungus with an interesting yet cryptic ecology. Traditionally considered a soil dwelling saprotroph, M. elongata can also be isolated from the rhizosphere of plants. In fact, greenhouse studies show that plants inoculated with Mortierella grow faster and tolerate greater heat stress than non-inoculated control plants. Mortierella species are unique in their high production of unsaturated fatty acids, making them relevant to commercial and biological applications. Recent sequencing of the M. elongata genome revealed the presence of a bacterial endosymbiont, identified as a relative of the genus Burkholderia. Further analyses, including full genome sequencing of the Mortierella endosymbiont, indicate its close relationship with other fungal endosymbionts, namely the Candidatus Glomeribacter lineage associated with arbuscular mycorrhizal fungi in the genera Gigaspora and Scutellospora. Genomic signatures of the fungal endosymbiosis mirror those documented in other Eurkaryotic-endosymbiont systems, including genomic reduction when compared to facultative and free-living relatives, degradation of select metabolic pathways, increased interdependence between bacteria and host, and control of reproductive fitness. By clearing the endosymobiont from several M. elongata strains we observed fitness costs to the host under specific conditions. Several lineages of fungi and bacteria presented here exhibit congruent cophylogenies suggesting an ancient origin for this co-evolved symbiosis and begging the question; what, if any benefit do endosymbiotic bacteria offer their fungal host, and in turn, plant associates? Aspects of the Mortierella endosymbiont genome and functional host-endosymbiont relationship hypotheses will be discussed further.

CS3.7

VERTICILLIUM TRANSCRIPTION ACTIVATOR OF ADHESION VTA2 SUPPRESSES MICROSCLEROTIA FORMATION AND IS REQUIRED FOR SYSTEMIC INFECTION OF PLANT ROOTS

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Six transcription regulatory genes of the Verticillium plant pathogen, which reprogrammed non-adherent budding yeasts for adhesion, were isolated by a genetic screen to discover control elements for early plant infection. Verticillium transcription activator of adhesion Vta2 is highly conserved in filamentous fungi but not present in yeasts. The Magnaporthe grisea ortholog Con7 controls the formation of appressoria which are absent in Verticillium species. Vta2 was analyzed by using genetics, cell biology, transcriptomics, secretome proteomics and plant pathogenicity assays. Nuclear Vta2 activates the expression of the adhesin encoding yeast genes FLO1 and FLO11. Vta2 is required for fungal growth of Verticillium where it is a positive regulator of conidiation. Vta2 is mandatory for accurate timing and suppression of microsclerotia as resting structures. Vta2 controls expression of 270 transcripts including 10 putative genes for adhesins and 57 for secreted proteins. Vta2 controls the level of 125 secreted proteins including putative adhesins or effector molecules and a secreted catalase-peroxidase. Vta2 is a major regulator of fungal pathogenesis, controls host-plant root infection and H2O2 detoxification. Verticillium impaired in Vta2 is unable to colonize plants and induce disease symptoms. Vta2 represents an interesting target to control growth and development of these vascular pathogens.

CS3.8

PHYLOGENOMICS OF HYPOCREALES AND THE EVOLUTION OF SECONDARY METABOLISM

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Hypocreales comprises one of the largest and ecologically diverse orders of filamentous ascomycetes. Ecologies of the order include saprobes, pathogens and endophytes of plants, pathogens of arthropods, and parasites of other fungi. These ecologies are not randomly distributed across the phylogeny of the order, however. Early diverging lineages of the order mainly consist of saprobes, pathogens and endophytes of plants while pathogens of arthropods and parasites of other fungi characterize more derived families of the order. One exception to this pattern is the grass endophytes of the family Claviciptaceae, which represents a reversal from pathogens of arthropods to endophytes and pathogens of plants. The ecologies of these fungi and their interactions with other organisms are partially mediated by the production of biologically active secondary metabolites including peptides, polyketides, alkaloids and terpenes. We have initiated a phylogenomic study of the order that entails sequencing of genomes across the seven families of Hypocreales. The phylogeny of the order is inferred from genome-scale data and is used as a phylogenetic framework to investigate the evolution of secondary metabolism. Here we present data and analyses on modular nonribosomal peptide synthetases (NRPS) and the homology of their adenylation domains and biosynthetic gene clusters. These analyses reveal complex evolutionary patterns of homologous modules that are distributed across NRPSs not previously suspected of sharing common ancestry and in fungi not previously known to harbor these enzymes. This is especially true among the derived families of the order that include closely related species that have undergone more recent interkingdom host jumps among insect, fungal and plant hosts. The evolutionary processes that function in diversification of these enzymes include duplication and divergence of closely related modules and fusion of distantly related modules that collectively result in chemically distinct metabolites. While horizontal gene transfer has been demonstrated to explain the distribution of peptides among distantly related taxa, we show that deep coalescence is also a common mechanism that results in the patchy phylogenetic distribution of NRPSs among closely related but ecologically distinct taxa.

CS4.1 A SECRETED USTILAGO MAYDIS EFFECTOR PROMOTES VIRULENCE BY TARGETING ANTHOCYANIN BIOSYNTHESIS IN MAIZE

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The biotrophic fungus Ustilago maydis causes smut disease in maize with characteristic tumor formation and anthocyanin induction. Here we show that anthocyanin biosynthesis is induced by the virulence promoting secreted effector protein Tin2. Tin2 protein functions inside plant cells where it interacts with maize protein kinase ZmTTK1. Tin2 masks a ubiquitin-proteasome degradation motif in ZmTTK1, thus stabilizing the active kinase. Active ZmTTK1 controls activation of genes in the anthocyanin biosynthesis pathway. Without Tin2, enhanced lignin biosynthesis is observed in infected tissue and vascular bundles show strong lignification. This is presumably limiting access of fungal hyphae to nutrients needed for massive proliferation. Consistent with this assertion, we observe that maize brown midrib mutants affected in lignin biosynthesis are hypersensitive to U. maydis infection. We speculate that Tin2 rewires metabolites into the anthocyanin pathway to lower their availability for other defense responses.

CS4.2 EFFECTOR SPECIALIZATION IN A LINEAGE OF THE IRISH POTATO FAMINE PATHOGEN

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Accelerated gene evolution is a hallmark of pathogen adaptation following a host jump. However, the biochemical basis of adaptation and specialization to new hosts remains largely unknown. We describe functional specialization of a plant pathogen effector following a host jump. Orthologous protease inhibitor effectors from the Irish potato famine pathogen Phytophthora infestans and its sister species Phytophthora mirabilis are better adapted to protease targets from their respective host plants potato and Mirabilis jalapa. Single amino acid polymorphisms in the inhibitors and their target proteases largely underpin biochemical specialization. These results provide a molecular framework for how antagonistic pleiotropy drives effector specialization in a plant pathogen ultimately resulting in diversification and speciation.

CS4.3 HOTSPOTS OF RECOMBINATION SHAPE THE EVOLUTION OF VIRULENCE IN THE WHEAT PATHOGEN ZYMOSEPTORIA TRITICI

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The co-evolutionary arms race between host and pathogen selects for virulence genes to emerge in rapidly evolving compartments of the genome. Most known plant pathogenic fungi reproduce sexually at least sporadically. High recombination rates may lead to increased mutation rates and enable novel gene variants to penetrate and sweep in different genomic backgrounds. Hence, local variation in recombination rates can be a major determinant of rapid evolution. We generated a fine-scale recombination map for the wheat pathogen Zymoseptoria tritici (formerly Mycosphaerella graminicola) based on restriction-site associated DNA sequencing (RADseq). We obtained a dense map containing 19,355 SNP markers in 214 and 227 progeny from two different crosses. We found that recombination rates were highly heterogeneous both within and among chromosomes. We localized a series of recombination hotspots showing strongly elevated recombination rates that were enriched in specific GC-rich oligonucleotide sequences. High recombination rates were found preferentially in gene-dense and sub-telomeric regions. Using polymorphism data from a resequenced pathogen population, we found that chromosomal regions of high recombination rates co-localized with regions of weak linkage disequilibria. This suggests that locally high recombination rates were effective in reshuffling gene variants in a population. We applied the knowledge of recombination hot- and cold-spots to understand the evolutionary trajectory of secondary metabolite gene clusters. Secondary metabolite production is an important contribution to virulence in necrotrophic Dothideomycetes. We identified the transcriptional profiles of polyketide synthase (PKS) gene clusters during infection of wheat leaves. We found that transcriptional co-regulation within clusters correlates with low recombination rates within gene clusters. However, we found hotspots of recombination in close proximity to PKS clusters. The proximity of recombination hotspots and genes underlying virulence may play a major role shaping the genetic basis of virulence in Z. tritici.

CS4.4 CHARACTERIZATION OF A CIRCADIAN CLOCK IN BOTRYTIS CINEREA AND ITS ROLE IN PATHOGENESIS USING ARABIDOPSIS THALIANA AS A PLANT MODEL

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Botrytis cinerea is a necrotrophic fungus that infects over 200 plant species, ranking as the second most important phytopathogen according to its scientific-economic importance. Although, it has been suggested that the outcome of a plant-pathogen interaction could have daily oscillations, the importance of a circadian clock has only been addressed in the plant, but not in the pathogen. Therefore, we have started to characterize the B. cinerea clock, which is composed of the BcFRQ1 protein and a transcriptional complex formed by BcWCL1 and BcWCL2. Our results indicate that bcfrq1 mRNA presents daily oscillations in a light-dark cycle and in constant darkness (DD), rhythms which are lost in a bcwcl1 KO strain. We have observed oscillatory levels of the BcFRQ1 protein under temperature cycles and DD. Both the bcfrq1 mRNA and BcFRQ1 protein anticipate cyclical-environmental changes, a key characteristic of circadian behavior. Importantly, we have observed an impaired infection process using bcfrq1 and bcwcl1 KO strains. Moreover, we demonstrate that the outcome of the plant- fungal pathogen interaction using Arabidopsis thaliana and B. cinerea as working model varies with the time of day. These results provide the first evidence indicating the existence of a circadian clock in this necrotrophic pathogen, putting forward the concept that fungal clocks can synchronize key elements of pathogenesis.
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CS4.5 CHEMOTROPIC SENSING IN THE FUNGAL PATHOGEN FUSARIUM OXYSPORUM

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Chemotropism, the ability to re-orient the growth axis in response to chemical cues, plays a critical role in key aspects of the fungal lifestyle, including colony establishment, foraging for nutrients or location of host organisms. Plant pathogenic fungi have been known for more than 100 years to orient hyphal growth towards chemical stimuli from the host. However, the nature of the chemoattractants as well as the mechanisms underlying chemotropism have remained obscure. 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 chemotropic sensing of the plant signal by Fusarium requires a functional homolog of the yeast α-pheromone receptor Ste2 and conserved elements of the cell integrity mitogen-activated protein kinase (MAPK) cascade. By contrast, chemotropism towards nutrients is governed by the funcionally distinct invasive growth MAPK pathway. These findings suggest that root-colonizing fungi exploit the highly sensitive sex pheromone perception system to locate signals from the plant host in a complex environment such as the soil.

CS4.6 MODULATION OF PATHOGENICITY BY PH REGULATION IN THE HOST

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The ability to thrive over a wide range of ambient pH levels is one aspect of a complex set of characteristics of a fungus that govern its interaction with the host. Although a steady intracellular pH must be maintained, to provide enzymes with their optimal conditions for their activity, proteins that come in contact with the external host environment need to be selectively expressed at pH values conducive to their functioning. Several phytopathogenic fungi as Colletotrichum, Alternaria have been shown to alkalize their ambient pH environment during pathogenesis while others as Penicillium, Botrytis, Sclerotinia to acidify it. Ambient pH levels or the factors that determine host pH are important in that they determine the capability of the pathogen to successfully colonize, invade, and kill the targeted host, with the aid of secreted pathogenicity factors. Since pH is a critical consideration in its attack strategy, the pathogen cannot simply adapt to any pH determined by its host; therefore, it has developed environmental sensing mechanisms that enable it to modify the ambient conditions to best suit its offensive arsenal and, at the same time, to compromise the host defense. In filamentous fungi, the expression of genes in alkaline conditions is governed by a complex ambient-pH-sensing, signal transduction pathway regulated by the zinc finger transcription factor pacC at alkaline pH. Thus, under alkaline pH conditions, pacC serves as a positive regulator promoting transcription of alkaline-expressed. Our hypothesis is that the impact of pacC on virulence is due to its regulation of pathogenicity genes under alkalizing pH conditions. We have analyzed global aspects of pacC regulation by transcriptome and functional analysis. Based on high-throughput RNA-sequencing we demonstrate that pacC up-regulates and down-regulates genes, some of which appear to be involved in the development of necrotrophic pathogenicity by modulation of the production, secretion and uptake of ammonia as well as many cell wall degrading enzymes that contribute to the pathogenicity process. Transcriptome and functional analysis of pacC mutants and pacC regulated genes revealed an arsenal of pathogenicity factors, transporters, and antioxidants to control virulence and homeostasis under changing ambient pH conditions. Comparison of diverse fungal genomes showed a similar strategy of control by pacC, indicating a conserved role in regulating fungal genes.

CS4.7 INVESTIGATING THE ROLE OF TYROSINE CATABOLISM AND PYOMELANIN PRODUCTION DURING IN VIVO GROWTH IN THE HUMAN PATHOGEN PENICILLIUM MARNEFFEI

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For pathogens to successfully infect a host, two equally important events must be achieved; the pathogen must be able to evade or tolerate the host's defence systems and it must be able to acquire and utilise the available nutrient sources within the host in order to grow. These factors are significant hurdles for most pathogens, but especially so for intracellular pathogens which have to evade or tolerate the cytotoxic machinery of innate immune cells and scavenge nutrients from this relatively nutrient poor environment. In a screen aimed at identifying genes which are important determinants of pathogenicity in the dimorphic fungus Penicillium marneffei, a number of metabolic genes were found which are specifically expressed in the pathogenic yeast cells and not in the saprophytic hyphal cells. Preliminary characterisation showed that one of these genes, designated hpdA, encodes 4-hydroxyphenlypyruvate dioxgenase (4HPPD) which catalyses the conversion of 4-hydroxyphenlypyruvate to 2,5-dihydroxyphenylacetate (homogentisate), a step in the tyrosine catabolic pathway. Tyrosine is metabolized via a conserved pathway to provide the fungus with both nitrogen and carbon. In addition, the oxidation and polymerization of a tyrosine metabolic intermediate, homogentisate, can generate the brown pigment pyomelanin which can protect against oxidative stress and is therefore an important survival and pathogenicity determinant. Genes required for the catabolism of tyrosine are located in a conserved gene cluster. This study describes the deletion of genes of the tyrosine catabolism cluster and the characterization of their role in growth, pyomelanin production and pathogenicity in P. marneffei.

CS4.8 PATHOGENICITY CHROMOSOMES IN HOST-SPECIFIC TOXIN-PRODUCING ALTERNARIA SPECIES

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Alternaria alternata plant pathogens consist of seven variants (pathotypes), all of which produce host-specific (selective) toxins (HSTs); all cause necrotic diseases on different plants. We have shown that all strains of A. alternata pathotypes harbour small and extra chromosomes, whereas nonpathogenic isolates do not have these small chromosomes. Based on biological and pathological observations, those small chromosomes were termed conditionally dispensable chromosomes (CDCs) and pathogenicity chromosomes. HST biosynthetic genes have been isolated from five pathotypes (apple, Japanese pear, strawberry, tangerine, and tomato) of A. alternata and found to be clustered on the CDCs. Sequencing of the entire CDCs of the apple, strawberry and tomato pathotypes which produce AM-, AF- and AAL-toxins, respectively, revealed that the CDC of each consists of CDC-specific and repetitive sequences related to the HST production and pathogenicity. The CDC in the tomato pathotype strains from different geographical origins was identical although the genetic backgrounds of the strains differed. The results imply that CDCs have a different evolutionary history from the essential or core chromosomes in the same genome. A hybrid strain between two different pathotypes was shown to harbour the CDCs from both parental strains and had an expanded pathogenicity range, indicating that CDCs could be transmitted from one strain to another and stably maintained in the new genome. We propose a hypothesis whereby the ability to produce HSTs and to infect a plant is distributed among A. alternata strains by horizontal transfer of an entire pathogenicity chromosome (CDC). This could provide a possible mechanism by which new pathogens arise in nature. The chemical structure of the AAL-toxin resembles to that of a mycotoxin fumonisin produced by Fusarium (Gibberella) spp., and both toxins are classified as sphinganine-analog mycotoxins due to their structural similarity to sphinganine. The AAL-toxin biosynthetic (ALT) gene cluster consists of at least 13 genes homologous to the fumonisin biosynthetic (FUM) genes in F. verticillioides. The FUM (ALT) cluster homologues are found in Fusarium spp., Aspergillus niger and Cochliobolus heterostrophus. On the other hand, only the tomato pathotype of A. alternata has the cluster among Alternaria spp.. The ALT cluster in the tomato pathotype also might be acquired by horizontal transfer of the entire cluster genes from those pathogenic fungi.

CS5.1 AN INJURY RESPONSE MECHANISM CONSERVED ACROSS KINGDOMS

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Species belonging to the genus Trichoderma are free-living fungi common in soil and root ecosystems, and are used broadly in industry and agricultural biotechnology. In order to survive and spread, Trichoderma switches from vegetative to reproductive development, and has evolved with several sophisticated molecular mechanisms to this end. Asexual development (conidiation) is induced by light and mechanical injury. Although light responses in fungi have been well studied, information on their response to injury is extremely limited. We have used functional genomics approaches to study light and injury induced conidiation in Trichoderma. Using high-throughput RNAseq, we identified genes responsive to both stimuli, and determine the set of genes that are shared during the response. 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. Mutant analyses indicate that upon mechanical injury NADPH oxidase 1 (Nox1) dependent ROS production is detected, and Nox1 activity is essential for entry into asexual development in response to damage. We have further determined that the MAPKs Tmk1 and Tmk3 are involved in injury-induced conidiation. In particular, Tmk3 appears to play a key role since it is phosphorylated very rapidly upon injury, and its phosphorylation is both NoxR and Nox1 dependent. Our data suggest that as in plants and animals H2O2 and oxylipins may act as signaling molecules in injury responses in fungi, and follow similar signal transduction cascades. Thus, the molecular responses upon injury are a highly conserved mechanism present across three kingdoms.

CS5.2 INTERPLAY BETWEEN SELF AND NONSELF RECOGNITION MECHANISMS REGULATE CHEMOTROPIC INTERACTIONS AND CELL FUSION IN NEUROSPORA CRASSA

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Cell fusion between genetically identical germlings is a highly regulated process associated with the early colony establishment and formation of an interconnected network in filamentous fungi. In the ascomycete fungus, Neurospora crassa, cell fusion occurs between germinated conidia (germlings) via specialized structures termed "conidial anastomosis tubes" (CATs) and between fusion hyphae within a mature colony. In N. crassa, both CAT and hyphal fusion are under the regulation of a conserved MAP kinase cascade, NRC1, MEK2, MAK2 and a Ste12-like transcription factor, PP1. By utilizing phosphoproteomics and profiling approaches, we identified new targets of the cell fusion pathway that oscillate with MAK2/MEK2/NRC1 during chemotropic interactions and others that, when mutated, showed defects in communication and oscillation, but which were restored when mutant germlings were matched with wild type partner germlings. Using population genomics approaches and genome-wide association studies (GWAS), we identified new components associated with regulated secretion that regulate chemotropic interactions and communication during fusion between genetically identical germlings. However, using this same population, we determined that cell fusion between genetically non-identical germlings is associated with communication interference, whereby chemotropic interactions and cell fusion are inhibited. Populations of N. crassa have three mutually exclusive communication groups (CGs). By bulked segregant analysis and high throughput sequencing, we identified a 100 kbp region that showed segregation of SNPs at 100 % frequency in CG1 vs. CG2 progeny. Two genes encoding large hypothetical proteins with two predicted transmembrane domains are associated with CG group phenotype. Strains carrying a deletion of one of the two hypothetical proteins lost the ability to discriminate between CGs as well as showing a reduced fusion frequency. These data indicate that self and nonself interactions during germling fusion are linked and that an interplay between these two systems is important in the regulation of chemotropic interactions and cell fusion.

CS5.3 CLASS III PEROXIDASES SECRETED BY TOMATO ROOTS TRIGGER HYPHAL CHEMOTROPISM IN FUSARIUM OXYSPORUM

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Soil-inhabiting fungal pathogens and symbionts exhibit directed hyphal growth towards plant roots, but the chemoattractant signals are currently unknown. Using a plate assay, we found that germ tubes of Fusarium oxysporum display a significant chemotropic response towards root exudates from the host plant tomato (Solanum lycopersicum). Fractionation of root exudate by size exclusion and anion-exchange chromatography followed by liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) analysis, identified class III peroxidases as the chemoattractant compounds secreted by tomato roots. Class III peroxidases exist as large multigene families in plants and have been associated with a variety of processes such as cell elongation, cell wall construction and differentiation, and pathogen defense. Importantly, commercial class III peroxidase from horseradish also triggered a robust chemotropic response in F. oxysporum, while specific peroxidase inhibitors abolished the chemoattractant activity of tomato root exudates and horseradish peroxidase. Collectively, these results suggest that secreted class III peroxidases may represent a general means for chemotropic sensing of plant roots by soil-inhabiting fungi. Knowledge of the mechanism through which the peroxidase signal is sensed by the fungal cell will advance our understanding of the molecular events triggering fungus-root interactions.

CS5.4 COMPOSITION OF THE MAK-2 MAP KINASE CASCADE IN NEUROSPORA CRASSA

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The mechanistic understanding of oscillatory MAK-2 signaling during fungal self-signaling is hampered by the fact that most components of the signaling machinery, including the postulated secreted signal and its cognate receptor, regulators of the MAP kinase cascade as well as most MAK-2 targets, are unknown. In order to identify additional components involved in MAK-2 MAP kinase signaling we performed GFP-trap affinity purification experiments coupled to mass spectrometry with strains expressing functional GFP-fusion proteins of the MAP kinase cascade. Subsequent yeast two-hybrid tests were used to confirm many of the identified interactions and to map interacting domains. This approach identified two scaffold/adapter proteins of the kinase cascade as well as several upstream regulatory elements and putative MAK-2 targets, whose characterization will be presented.

CS5.5 COOPERATION OF THE GATA TYPE TRANSCRIPTION FACTORS WCC AND SUB1 IN LIGHT-INDUCED TRANSCRIPTION

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The GATA type transcription factor White Collar Complex (WCC is a blue-light photo receptor required for all light induced transcription in Neurospora. Here we show that the GATA type transcription factor SUB1 and WCC share many overlapping binding sites in the Neurospora genome. Binding of SUB1 to WCC sites augments light-activated transcription of target genes by the WCC. However, SUB1 cannot activate transcription of light-induced genes in the absence of the WCC. SUB1 interacts with a putative acetyl transferase. Time-resolved nucleosome mapping after light induction revealed SUB1- and WCC-dependent genome-wide chromatin changes in promoters. Our data suggests that SUB1 is an amplifier of gene transcription.

CS5.6 LIGHT SENSING IN PHYCOMYCES BLAKESLEEANUS

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Phycomyces species have long been of interest for photobiology research because they produce large asexual sporangiophores that are highly light-sensitive. Mutants impaired in the phototropic response were isolated from the 1960s onwards, yet without a system for stable transformation many of these mutations and the genes affected are unknown. A genetic map was constructed using PCR-RFLP markers between two wild type strains. This map was a starting point to use traditional map-based identification with crosses with light-insensitive mutants, as well as genome resequencing, to establish the identity of these unknown mutations. For instance, the madI mutants are heterozygotes that have mutations in a white collar 1 homolog that affects a highly conserved residue. The madI phenotype demonstrates that the efficiency of phototropism is dependent on the dosage of the photosensor. The madC gene was also mapped, to mutations in a regulator of Ras signaling. The protein currently has no role in photobiology when tested in a basidiomycete Cryptococcus neoformans. However, mutation of the homologous affects the circadian clock output in the model ascomycete Neurospora crassa, having the same banding phenotype as the classic band allele of Ras used in circadian studies. Thus, despite experimental limitations, analysis of Phycomyces can shed light onto the processes of light-sensing in the fungi.

CS5.7 THE NOVEL SENSOR-GLOBIN FUNGOGLOBIN IS INVOLVED IN LOW OXYGEN ADAPTATION OF ASPERGILLUS FUMIGATUS

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Infection with conidia of the pathogenic fungus Aspergillus fumigatus is a frequent health threat for immunocompromised human individuals. While classic virulence factors have not been identified, it has become increasingly clear that its physiological versatility as a saprophyte may largely contribute to the establishment of invasive growth. Low oxygen partial pressures is a property which transiently occurs in most natural growth environments, but also defines deep layers of infected human tissue. A. fumigatus survives and prospers in such hypoxic areas and, as other fungi, exploits ergosterol biosynthesis as an essential measure for oxygen. However, the direct metabolic and energetic consequences of low O2 availability are less understood, and we hypothesized that the fungus could also sense and react to O2 directly. In a first approach, we used Next Generation Sequencing to study the dynamic and short term response to a transient exposure to low O2. Deprivation of O2 triggered a more than threefold induction of 680 genes after only 15 min while 420 genes were down regulated at the same time point. Among the highest upregulated genes we identified a gene encoding a hypothetical protein which appears to be conserved in filamentous fungi. In sharp contrast, reoxygenation of the growth medium resulted in the complete repression of its mRNA. This transcriptional dynamics was verified by Northern Hybridization, and interestingly, hypoxic induction was also observed in an srbA deletion mutant. Low iron also induced its expression, but in a HapX-independent mode, indicating that this gene is not under direct control of either of these two regulators. The encoded protein comprised a globin-like N-terminal domain and was identified as a member of the large protein family of sensor globins. The function of these proteins is largely unknown but heterologous expression and purification gave evidence for a functional heme binding site. Furthermore, the deletion of the gene led to an impaired growth of A. fumigatus in low oxygen atmospheres and hence, the putative role of this "fungoglobin" during such conditions will be discussed.

CS5.8 GENOME-WIDE TRANSCRIPTIONAL RESPONSE TO AMBIENT PH CHANGES IN FUSARIUM GRAMINEARUM: A LARGE METABOLIC REORGANIZATION CONTROLLED BY PAC1

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The pathogenic fungus Fusarium graminearum produces type B trichothecene mycotoxins during wheat infection that are now recognized as virulence factor. These toxins accumulate in cereal grains and represent a threat for health of animal and human consumers. Early steps of infection and induction of toxin biosynthesis can respond to various ambient signals, which can be determinant for the establishment of infection and the levels of contamination with toxins. During the initial stages of infection, the fungus can be subjected to drastic pH changes. Expression studies of the Tri genes implicated in trichothecene biosynthesis have demonstrated that acidic extracellular pH is a determinant inducer. It was shown that the pH regulatory factor FgPac1 negatively regulates the expression of Tri genes under neutral or basic pH. Beside their role in regulating secondary metabolites in fungi, Pac transcription factors are also known to regulate various classes of genes, especially the genes whose roles involve adaptation to the environment. In this study, a genome-wide transcriptional analysis conducted in two different pH conditions using a strain deleted for FgPac1 and a strain expressing a constitutively active form of FgPac1 was carried out to investigate global regulation by the pH in F. graminearum. Our data identifies a set of genes related to various functions which expression levels are affected by pH change. As expected, our results enlighten a general switch from basic to acidic metabolic activities upon acidification. In addition, our results further point towards a potential role of a calcium-mediated regulation in response to ambient pH, and identify the specific activation of the GABA shunt by acidic pH. Finally, a clustering approach followed by cis-regulatory motifs search highlight the presence of complex stress-response regulatory circuits impacted by the change in external pH.

CS6.1 SYSTEMS BIOLOGY APPROACHES FOR ORGANIC ACID PRODUCTION IN FILAMENTOUS FUNGI

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Among filamentous fungi Aspergillus niger is a well known production host for a wide variety of enzymes (amylase, cellulose, protease) and metabolites (organic acids). Based its performance in these more traditional fermentation processes A. niger is already used for the production of novel proteins. But even more recently this fungus is now also considered for the production of new so-called platform or building-block chemicals for the chemical industry. These chemicals, currently produced based on petrochemistry, are the starting point for the production of a wide variety of materials, such as resins, plastics, etc. Production of these compounds via biobased routes will be a major contribution towards a Biobased Economy. For the production of these bulk compounds robust host organisms are required, suitable for using low cost lignocellulose-based feedstocks, resistant against adverse conditions due to inhibitory feedstock compounds and capable of coping with high product concentrations. A. niger was shown to fulfill most of these prerequisites. Based on the extended molecular genetic toolkit systems biology approaches were developed for A. niger and other fungi. These approaches may be followed to produce several of these platform chemicals in A. niger, as will be demonstrated by the example of itaconic acid

CS6.2 GENETIC CHARACTERIZATION OF ITACONIC ACID BIOSYNTHESIS IN USTILAGO MAYDIS

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The unsaturated dicarboxylic acid itaconate is widely used for the production of plastics, paints and resins. Currently, it is produced in large amounts by fermentation of Aspergillus terreus. Itaconic acid production has been observed also in other fungi including some basidiomycetes. The phytopathogenic fungus Ustilago maydis and some related Pzeudozyma strains also have the ability to produce itaconic acid. While in A. terreus the key enzyme cis-aconitate decarboxylase (CAD) is solely responsible for itaconic acid production, the biosynthetic route of itaconic acid in U. maydis has not been characterized, yet. Here, we present data that U. maydis uses an alternative biosynthesis pathway for itaconic acid production. All genes required for itaconate biosynthesis are organized in a gene cluster, which also contains a pathway-specific transcription factor. We were able to reconstitute itaconic acid production in the yeast Saccharomyces cerevisiae by introducing the respective U. maydis genes. In addition, the key enzymes in itaconic acid production were heterologously expressed in E. coli. Biochemical characterization of purified proteins allowed us to confirm the catalytic activities in vitro. The detailed characterization of enzymes critical for itaconic acid production in U. maydis approves this fungus as alternative organism for biotechnological production of itaconic acid. The yeast-like growth of U. maydis in liquid culture may confer some advantages in fermentation, since filamentous fungi tend to clog in liquid culture.

CS6.3 CELLULASE AND HEMICELLULASE REGULATION AND PRODUCTION IN TRICHODERMA REESEI

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The ascomycete Trichoderma reesei is a paradigm for commercial scale production of cellulolytic and hemicellulolytic enzyme cocktails and serves as a model system for the biochemistry and regulation of the respective enzymes. Today, these enzyme mixtures are applied in the conversion of lignocellulosic plant biomass to simple sugars which can then be fermented to second generation biofuels or other biorefinery products. To make the saccharification process commercial viable one of the strategies is the reduction of enzyme costs by designing appropriate production hosts improved in their enzyme expression and yield. In my talk I will outline different approaches ranging from the identification of single components essential for (hemi)cellulase overproduction to a systems biological view of the process of (hemi) cellulase regulation and production.

CS6.4 THE RESPONSES OF ASPERGILLUS NIGER TO DIFFERENT LIGNOCELLULOSIC SUBSTRATES HIGHLIGHT BOTH SIMILARITIES AND SUBSTRATE-SPECIFIC DIFFERENCES

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Fungi are major degraders of lignocellulose in nature and are the main sources of enzymes used to saccharify lignocellulose in the production of second generation biofuels. The cost of producing these enzyme cocktails is a major barrier to cheaper biofuels. One path to reducing costs is through a better understanding of the response of fungi to lignocellulose. Aspergillus niger has an extensive repertoire of genes encoding enzymes that hydrolyse the components of lignocellulose (cellulose, hemicelluloses and pectin) as well as various accessory proteins. This repertoire of genes makes A. niger an excellent model to investigate fungal response to lignocellulosic substrates over time. Previously we investigated the global transcriptional response of A. niger to wheat straw (Delmas et al., 2012) and more recently have compared this response with the response to stems from a willow tree at a single time point. At the transcriptional level, there are many similarities in the responses to the two substrates such as the large increase in carbohydrate active enzyme (CAZy) transcripts from glycosyl hydrolase (GH) families. Some of the differences in the responses can in part be explained by the differences in composition of the substrates. At the biochemical level, enzymes prepared from A. niger exposed to wheat straw were used in a saccharification assay and were more effective at saccharification of wheat straw than willow. In further work at the transcriptional level, we are investigating with the Joint Bioenergy Institute (JBEI) and the Joint Genome Institute (JGI) the responses of A. niger to untreated and pre-treated (ionic liquid or hydrothermal) Miscanthus stems and wheat straw at nine time points from three hours until five days after transfer from a simple carbon source. Here we aim to elucidate the transcriptional response of A. niger as a function of time and lignocellulosic substrate and we will present some preliminary results from our study. In terms of fungal biology, our work shows that A. niger has some ability to distinguish between different lignocellulosic substrates and that is likely to be related to the differences in inducing molecules derived from these different substrates and the evolutionary selective advantage to the fungus of preferentially synthesising appropriate enzymes for available carbon sources. In terms of applications,our work could improve functionality of enzyme cocktails and reduce the costs of their production.

CS6.5 THE FIRST RIBOSOMAL PEPTIDE SYNTHASE PATHWAY IN FILAMENTOUS FUNGI

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Ustiloxin B, a fungal secondary metabolite, was found to be produced by Aspergillus flavus, and the entire biosynthetic gene cluster was identified by the disruption of genes predicted from DNA microarray data and the subsequent analysis of the ustiloxin B-deficient deletants using LC-MS. The gene cluster was composed of at least 16 genes, including those encoding a fungal type C6 transcription factor, a cytochrome P450, and a major facilitator superfamily transporter. We constructed an overexpression strain of the gene encoding the C6 transcription factor (ustR), which led to a five-fold overproduction of ustiloxin B in the transformant. The following sequence analysis of the cluster revealed that the translated product of ustA (AFLA_094980), UstA, contains a 16-fold repeated peptide containing a tetrapeptide, Tyr-Ala-Ile-Gly, that is converted into the cyclic moiety of ustiloxin B. This result strongly suggests that ustiloxin B is biosynthesized through a ribosomal peptide synthase (RiPS) pathway, and that UstA provides the precursor peptide of the compound. This pathway is the first example of RiPS in Ascomycetes. Moreover, this is the first report of a complete RiPS gene cluster in fungi. Our finding indicates the possibility that a number of unidentified RiPS exist in Ascomycetes as the biosynthetic genes of secondary metabolites, and that the feature of a highly repeated peptide sequence in UstA will greatly contribute to the discovery of additional RiPS.

CS6.6 COMPARATIVE GENOME-SCALE RECONSTRUCTION OF GAPLESS METABOLIC NETWORKS FOR PRESENT AND ANCESTRAL SPECIES

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We introduce a novel computational approach CoReCo for comparative metabolic re-construction and provide genome-scale metabolic network models for 49 important fungal species. Leveraging on the exponential growth in sequenced genome availability, our method reconstructs genome-scale gapless metabolic networks simultaneously for a large number of species by integrating sequence data in a probabilistic framework. High reconstruction accuracy is demonstrated by comparisons to the well-curated Saccharomyces cerevisiae consensus model and large-scale knock-out experiments. Our comparative approach is particularly useful in scenarios where the quality of available sequence data is lacking, and when reconstructing evolutionary distant species. Moreover, the reconstructed networks are fully carbon mapped, allowing their use in 13C flux analysis. We demonstrate the functionality and usability of the reconstructed fungal models with computational steady-state biomass production experiment, as these fungi include some of the most important production organisms in industrial biotechnology. In contrast to many existing reconstruction techniques, only minimal manual effort is required before the reconstructed models are usable in flux balance experiments. CoReCo is available at http://esaskar.github.io/CoReCo/. Originally we used KEGG as the compound and reaction database in our reconstruction pipeline. To further improve our reconstructions a new improved database of compounds and electron and atom balanced reactions was created to replace KEGG. Compounds and reactions from YMDB, HMDB, ChEBI, KEGG, MetaCyc and Rhea databases and from metabolic models of several microorganisms were used. Results of fungal metabolic model reconstruction using the combined database will be presented.

CS6.7 YARROWIA LIPOLYTICA AS A HOST FOR CAROTENOID PRODUCTION

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Yarrowia lipolytica is an oleaginous yeast that can accumulate greater than 40% of its dry cell weight as lipids in a large central lipid body. It has a genetic system with molecular biology tools, as well as a genome sequence in the public domain. It is non-pathogenic, cannot grow at greater than 34 C, and has a history of commercial use. It is also safe for human consumption and is considered GRAS for specific applications by the FDA. These attributes make Y. lipolytica an attractive host for expression of lipophilic compounds. Carotenoids are lipophilic, naturally occurring pigments that have a commercial value approaching $1 billion. Most carotenoids are currently produced by synthetic processes. At DSM-Lexington (Microbia), we have investigated the use of Y. lipolytica for industrial production of carotenoids. We will present data that shows that highly lipophilic carotenoids selectively partition into the lipid body. The lipid body appears to serve as a sink for these lipophilic compounds, and potentially reduces negative impacts that the overproduction of these molecules may have on the host organism during fermentation. We will describe strategies that we have utilized to overexpress the isoprenoid pathway and carotenoid biosynthetic genes that have led to enhanced titers of a number of commercially relevant carotenoids.

CS6.8 STREPTOMYCES: THE BEAUTY OF A FILAMENTOUS INDUSTRIAL BACTERIUM

GILLES VAN WEZEL

LEIDEN UNIVERSITY, THE NETHERLANDS

Central in this talk is Streptomyces, a complex filamentous bacterium that produces half of all known antibiotics. The beauty lies in the organism itself, with its wonderful development, and in the often colourful natural products it produces. Their identification and exploitation may well be our final resource in the fight against the rapidly emerging multi-drug resistant pathogens. Yet streptomycetes are not a preferred industrial host, primarily due to their adverse growth properties resulting from complex mycelial growth and the increasing difficulty to find new lead compounds. Mycelial morphology and production are highly coordinated, and we aim to understand where and when antibiotics and enzymes are produced, and to adapt morphology such as to optimise productivity. We seek to improve streptomycetes through rational strain engineering approaches, whereby detailed fundamental insights go hand in hand with modelling and fermentations. We have shown that enhanced cell division by manipulation of the cell division activator SsgA enhances growth rate and enzyme production during industrial fermentations, thus improving yield, but the effect on antibiotic production is less straightforward. By means of dual-axis cryo-tomography on vegetative hyphae we visualise large intracellular molecular structures in three-dimensions, which control growth and division. This revealed among others the complexity of the hyphal tip organising complex and membrane structures that control cell division. The combined information gained from modelling, imaging and fermentation is used to develop novel approaches to further improve growth and production of Streptomyces for industrial applications.

CS7.1 THE ORDERED ACCUMULATION OF VESICLES AT THE SPITZENKÖRPER IS REGULATED BY THE ACTION OF DISTINCT RAB GTPASES AND THE EXOCYST IN NEUROSPORA CRASSA

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Neurospora crassa hyphae exhibit at their apex a prominent Spitzenkörper (Spk), a multi-vesicular complex to which cargo-carrying vesicles arrive before being directed to specific cell sites. In N. crassa we have found that vesicles carrying the biosynthetic nanomachines for the fibrillar components of the cell wall concentrate at the Spk in an orderly manner. Microvesicles (chitosomes) at the core of the Spk contain chitin synthases, whereas macrovesicles at the outer layer carry glucan synthase. The mechanisms that ensure the directionality of the secretory vesicles that reach the Spk are still unknown. Nevertheless, it is well-known that secretion mechanisms in eukaryotic organisms require the coordinated action of small Rab GTPases, which interact with the membrane of vesicles and promote the subsequent fusion of the vesicles with a target membrane. Hence, we have analyzed the N. crassa Rab GTPase YPT-1 (Rab1), a key regulator of the secretory pathway involved in the ER-Golgi and Late Endosome-Golgi traffic in Saccharomyces cerevisiae. Co-expression of YPT-1 and the post-Golgi Rab GTPases SEC-4 (Rab8) or YPT-3 (Rab11) showed that YPT-1 was confined at the Spk microvesicular (chitosomal) core, while SEC-4 and YPT-3 occupied the Spk peripheral macrovesicular layer, suggesting that trafficking of the macro and microvesicles is regulated by distinct Rabs. On sucrose density gradients, YPT-1-associated particles sedimented mainly in fractions with a density, which coincides with the density of chitosomes as previously shown. Secretory vesicles are presumably tethered to their target acceptor membrane, in this case the plasma membrane, in a process mediated by the exocyst. In N. crassa an intact exocyst complex is required for formation of a functional Spk and for maintenance of regular hyphal growth. Two distinct localization patterns of the exocyst subunits suggest the dynamic formation of two exocyst assemblies. The EXO-70/EXO-84 subunits associate with secretory vesicles at the peripheral part of the Spk, which partially coincides with the outer macrovesicular layer. The remaining exocyst components form a delimited crescent at the apical plasma membrane. In summary, we propose that the highly organized arrival, accumulation, and departure of micro and macrovesicles at the Spk involves the participation of different Rab GTPases and the exocyst.

CS7.2 PHOSPHATIDYLINOSITOL PHOSPHATE GRADIENTS DURING FUNGAL FILAMENTOUS GROWTH

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Membrane phospholipids, such as phosphatidylinositol phosphates are required for cytoskeleton organization, G-protein signaling, cell polarity and morphogenesis in a range of organisms. As in many fungi, neither PI(3,4,5)P3 nor PI-3-kinase homologs are present in the opportunistic fungal pathogen Candida albicans, however there is a single PI(4)P-5-kinase (Mss4) and three PI-4-kinases (Lsb6, Stt4 and Pik1). In S. cerevisiae Mss4, Stt4 and Pik1 are required for viability, actin cytoskeleton organization and membrane traffic, with the two former kinases localized to the plasma membrane and the latter to the Golgi. In this yeast, PI(4,5)P2 is important for mating pheromone response and invasive growth (1,2). Previously, we showed that PI(4,5)P2 is critical for C. albicans filamentous growth and that this lipid is highly polarized in budding and filamentous cells, with a long-range PI(4,5)P2 gradient emanating from the filament tip [(3)]. Here we examined the function, levels and distribution of PI(4)P during the C. albicans yeast to filamentous growth transition, that is critical for virulence. We generated mutant strains in which the levels of Pik1 and Golgi PI(4)P can be reduced. When Golgi PI(4)P levels are reduced, strains are viable and grow via budding, yet are defective for filamentous growth. Using fluorescent lipid associated reporters we analyzed the distribution of PI(4)P at the Golgi and the plasma membrane during the yeast to filamentous growth transition. In hyphal filaments, we observed a dramatic PI(4)P polarization with a cap-like distribution, in contrast to the long-range gradient of PI(4,5)P2 [(3)]. We show that in hyphae the actin cytoskeleton is required for both PI(4)P and PI(4,5)P2 asymmetries. Previously, it has been demonstrated that a sec3 mutant, which lacks an exocyst subunit, is still able to initiate filamentous growth and form short germ tubes (4). Strikingly the pik1 mutant, that we show is defective in membrane traffic and exocytosis, is unable to even initiate filamentous growth. In the pik1 mutant, we also observed that the late Golgi and its dynamics were altered. Together, our results indicate that membrane traffic is critical for the yeast to filamentous growth transition and suggest that the Golgi and plasma membrane pools of PI(4)P are functionally distinct.

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CS7.3 DUAL TARGETING OF PEROXISOMAL PROTEINS

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Peroxisomes are ubiquitous eukaryotic organelles, which contain enzymes for the breakdown of fatty acids. We recently discovered that fungi have developed different mechanisms to accomplish dual peroxisomal and cytosolic localization of several enzymes. In the plant pathogenic fungus Ustilago maydis, several genes show hallmarks of differential splicing and alternative polyadenylation. We detected that this process leads to generation of an C-terminally extended glyceraldehyde-3-phosphate dehydrogenase GAPDH isoform harboring a C-terminal peroxisomal targeting sequence (PTS1). We could also detect peroxisomal isoforms of two further glycolytic enzymes, phosphoglycerate kinase (PGK) and triosephosphate isomerase (TPI). Remarkably, for these enzymes the peroxisomal isoforms are generated by translational read-through. Further analysis revealed that dual targeting of glycolytic enzymes to peroxisomes and the cytoplasm is not restricted to U. maydis but occurs in a variety of fungal species. Interestingly, in these species different mechanisms to generate extended peroxisomal isoforms of glycolytic enzymes are operating. In the ascomycete Aspergillus nidulans the PTS1-motif of PGK is derived from alternative splicing and polyadenylation, while translational read-through is used to generate a peroxisomal isoform of GAPDH. We also detected that for some enzymes weak peroxisomal targeting signals result in partial peroxisomal localization. Dual localization of enzymes to peroxisomes and the cytosol appears to be widespread not only in fungi but also in higher eukaryotes. This indicates that eukaryotic peroxisomes are endowed with a more complex metabolism than previously assumed. Thus, future proteomic and metabolomic studies of organelles have to consider the impact of alternative splicing and translational read-through for subcellular distribution of proteins.

CS7.4 INSIDE AN A. GOSSYPII HYPHA: COMBINING HIGH-RESOLUTION ELECTRON TOMOGRAPHY, VIDEO MICROSCOPY AND PROTEOMICS

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It is common practice to visualize the localization of fluorescently labeled proteins in filamentous fungi. Such studies have also revealed the morphology and dynamic of nuclei, mitochondria and other organelles in growing hyphae but have so far not generated a comprehensive view of the interior of a hypha. We therefore employed electron tomography to establish for the first time a high-resolution, three-dimensional map of nuclei, mitochondria, vacuoles, endosomes, multivesicular bodies, peroxisomes and vesicles within a hypha. In a first step, we reconstructed serial 300 nm thick sections of hyphal tip segments and stacked them to obtain a complete view of a hypha. We then tracked the membranes of organelles and vesicles to determine their density and 3D organization. We found that nuclei frequently assume irregular shapes with nucleolar protrusions and that mitochondria form an abundant and highly branched network with a tip-polarized spherical fraction. Endosomes, multivesicular bodies and vacuoles aggregate in distinct clusters while peroxisomes are randomly distributed. Coated and uncoated vesicles show a polarized distribution towards the hyphal tip. Presently, we try to relate the organelle- and vesicle-specific volumes calculated from the 3D model with the fractions of organelle- and vesicle-specific proteins in the recently finished high-resolution A. gossypii proteome.
The electron tomography images also revealed the lengths and numbers of microtubules (MTs) which were exclusively emanating from spindle pole bodies. We observed that each nucleus forms an autonomous cytoplasmic MT cytoskeleton, consisting on average of 3 cMTs for nuclei with one spindle pole body and 6 cMTs for nuclei with duplicated spindle pole bodies. Once cMTs have reached a critical length they are mainly growing as concluded from the structures at their ends. Some cMTs grow past several nuclei and, when sliding along the hyphal cortex, exert pulling forces (shown by video microscopy) which can last long enough to pull nuclei past adjacent nuclei, thus achieving nuclear mixing.

CS7.5 MITOTIC REGULATION WITHIN A MULTICELLULAR FUNGUS

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Filamentous fungi display a remarkable variability in how mitosis is integrated with cell growth and cell division. Even within the cell types of individual species the level of integration between mitosis and cell division can display considerable variability. How mitotic regulation is integrated with cell growth and divisions is poorly understood in filamentous fungi. Our research of mitotic regulation in Aspergillus nidulans has led to studies aimed at further understanding how mitosis is coordinately regulated in relationship to cell growth and division. The findings have provided new insights to how cells with physically connected cytoplasm maintain mitotic autonomy through the cell cycle regulation of septal pore permeability.

CS7.6 AUTOPHAGY CONTROLS NUCLEAR DYNAMICS DURING VEGETATIVE HYPHAL GROWTH AND FUSION OF FUSARIUM OXYSPORUM

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In the fungal pathogen Fusarium oxysporum, vegetative hyphal fusion triggers a series of nuclear events including mitosis in the invading hypha, nuclear migration into the receptor hypha and subsequent degradation of the resident nucleus. Here we examined the role of autophagy in fusion-induced nuclear degradation. A search of the F. oxysporum genome database for autophagy pathway (Atg) components identified putative orthologues of 16 core ATG genes in yeast, including the ubiquitin-like protein ATG8 which is required for the formation of autophagosomal membranes. F. oxysporum Δatg8 mutants were generated in a strain harbouring H1::ChFP-labelled nuclei to facilitate analysis of nuclear dynamics. The Δatg8 mutants failed to develop autophagic compartments in contrast to the wild type strain, suggesting that ATG8 is required for autophagy in F. oxysporum. The Δatg8 strains displayed reduced rates of hyphal growth and fusion, and were significantly attenuated in virulence on tomato plants and on the non-vertebrate animal host Galleria mellonella. Whereas wild type hyphae were almost exclusively composed of uninucleated cells, the hyphae of the Δatg8 mutant contained a significant fraction of cells with two or more nuclei. The increase in the number of nuclei per cell was particularly evident after hyphal fusion events between Δatg8 hyphae, or between hyphae of the Δatg8 and wild type strains. Furthermore, time-lapse microscopy analyses revealed abnormal mitotic patterns during vegetative growth in the Δatg8 mutants. Our results suggest that autophagy mediates nuclear degradation after vegetative hyphal fusion, and may function as a general mechanism to control the number of nuclei per cell in F. oxysporum.

CS7.7 INTERPLAY OF PHOSPHATASES AND KINASES: STRIPAK AND MAP KINASES REGULATE CELL DIFFERENTIATION IN SORDARIA MACROSPORA

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Phosphorylation and dephosphorylation are crucial for signal transduction and are carried out by kinases and phosphatases. Both play a role in fruiting body formation in the filamentous ascomycete Sordaria macrospora. This fungus has extensively been used as a model system for fungal cell differentiation, since developmental mutants are easily recognizable due to Sordaria`s homothallic lifestyle [1, 2]. We analyzed sterile mutants by complementation and next-generation sequencing and identified a number of proteins essential for fruiting body formation. Among these proteins were PRO22, PRO40 and PRO45 [3, 4]. Using affinity purification and MudPIT (multi-dimensional protein identification technology) mass spectrometry (AP-MS) with PRO22 and PRO40 as bait, we detected phosphatases and kinases as interaction partners. Strikingly, we identified the highly conserved striatin-interacting phosphatases and kinases (STRIPAK) complex as master regulator of fruiting body formation, containing PRO22, striatin homolog PRO11, SmMOB3, and protein phosphatase 2A subunits. We further characterized STRIPAK subunit PRO45, homologous to Neurospora crassa HAM4 and human sarcolemmal membrane-associated protein (SLMAP). Using AP-MS and co-immunoprecipitation, we confirmed that PRO45 is part of fungal STRIPAK. Super-resolution structured-illumination microscopy showed PRO45 localization to the nuclear envelope and to mitochondria. Our studies now focus on the significance of PRO45 localization to different compartments. Experimental evidence suggests interaction of STRIPAK with the cell wall integrity (CWI) MAP kinase cascade via developmental protein PRO40, a homolog of N. crassa SOFT. PRO40 binds to protein kinase C, MAPKKK MIK1, and MAPKK MEK1 via unstructured and WW domain-containing regions. We generated shared interaction networks of PRO40 and MEK1 and integrated data from recent transcriptomics analyses [5]. Our results strengthen our hypothesis that STRIPAK and the CWI pathway are interconnected and reveal a number of candidate proteins for future studies. Due to the evolutionary conservation of the protein complexes under investigation, our data are significant for invertebrate and vetebrate systems.

[1] Kück et al. 2009 The Mycota XV: 17-39 // [2] Engh et al. 2010 J Cell Biol 89:864-872 // [3] Bloemendal et al. 2012 Mol Microbiol 84:310-323 // [4] Engh et al. 2007 Eukaryot Cell 6:831-843 // [5] Teichert et al. 2012 BMC Genomics 13:511

CS7.8 CISTERNAL MATURATION WITHIN THE ASPERGILLUS NIDULANS GOLGI VISUALIZED IN VIVO

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115 years after Camillo Golgi´s description of the homonymous organelle, the mechanism by which proteins and lipids traffic in the secretory pathway, undergoing ordered modifications before being distributed to their target organelles, remains elusive and debated. Over the last few years, we have established that Golgi cisternae of the filamentous fungus Aspergillus nidulans are not stacked and are thus optically resolvable, as opposed to the mammalian Golgi, which is organized in stacks of sub-resolution cisternal distance. In hyphal cells, Golgi cisternae display polarized distribution towards the growing apex; however, the late Golgi is absent from a ≈3μm region immediately below the apex, where secretion predominates. This region is populated by both microtubules and the actin mesh emerging from the Spitzenkörper, where secretory membranes accumulate, awaiting fusion with the plasma membrane. According to the cisternal maturation model for cargo transport, acute impairment of traffic in the ER-Golgi interface would lead to rapid disorganization of both the early and the late Golgi cisternae, while the vesicular transport model anticipates that stable Golgi cisternae would not be affected under these conditions, at least not promptly. We have constructed appropriate conditional mutants and, using in vivo fluorescence microscopy, we observed that a reversible block in the ER-Golgi traffic results in the reversible disorganization of both the early and late Golgi cisternae within minutes, as predicted by the cisternal maturation model. Indeed, we have found that Golgi cisternae in growing hyphae are transient entities. By employing multidimensional microscopy, we are able to directly observe cisternal maturation; that is the de novo formation of an early Golgi compartment and its subsequent enrichment in a late Golgi marker with concomitant loss of the early Golgi marker, until the formation of a late Golgi compartment. In turn, the late Golgi eventually diminishes.

CS8.1 ADAPTIVE INTROGRESSION SLOWS DOWN MOLECULAR DEGENERATION OF THE MATING-TYPE CHROMOSOME IN NEUROSPORA TETRASPERMA

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In this study, we used a population genomics approach to understand the roles that selection, recombination and introgression have played in shaping the evolutionary history of the filamentous ascomycete Neurospora tetrasperma. We sequenced the genomes of 92 strains of N. tetrasperma and the genomes of two heterothallic species of Neurospora (N. hispaniola and N. sitophila). With this data we resolved the relationship of N. tetrasperma to other Neurospora species and the relationship of lineages within the N. tetrasperma clade. Comparisons of N. tetrasperma genomes revealed large regions of suppressed recombination on the mating-type chromosomes of all lineages of N. tetrasperma, that cover over 80% of the chromosome in some lineages. We used population genomic analyses to compare the evolutionary history of the recombining and the recently evolved non-recombining regions and found that suppressed recombination of the mating-type chromosome in N. tetrasperma has resulted in a decrease in genetic diversity and a higher rate of molecular degeneration relative to recombining regions of the genome. We also found that introgression has been a common occurrence in the region of suppressed recombination in multiple lineages of N. tetrasperma. These introgressed regions on the mating-type chromosome have become fixed in N. tetrasperma lineages and have likely been driven to fixation by selective sweeps. We hypothesize that the fixation of these introgressed regions from heterothallic species has resulted in a temporary regeneration of degenerated regions of the mating-type chromosomes in N. tetrasperma.

CS8.2 DATA-DRIVEN COMPARATIVE FUNCTIONAL GENOMICS IN YEASTS

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With genome sequencing becoming ever easier and cheaper, functional annotation has become the bottleneck in understanding fungal genes and genomes. I will discuss our efforts to leverage experiments in the workhorse model budding yeast, S. cerevisiae, to design experiments to efficiently annotate newly sequenced species. Using the classic literature as a prioritization tool, we have performed specific high information content, genome-scale experiments to survey gene expression, mutant phenotypes, and origins of DNA replication across a wide range of budding yeasts. Through these efforts, we have identified many aspects of genome biology that are resistant to study by DNA sequence analysis alone, including orthologs with large changes in their gene expression network, species-specific essential genes, and fast-evolving DNA replication elements.

CS8.3 INSIGHTS INTO THE EVOLUTION OF THE MYCORRHIZAL SYMBIOSIS

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Mutualistic mycorrhizal symbioses—the alliance of roots and soil fungi—are universal in terrestrial ecosystems and may have been fundamental to land colonization by plants. Boreal and temperate forests all depend on ectomycorrhizal (ECM) symbioses. ECM fungi may have evolved as many as 60 times from saprotrophic or parasitic ancestors, with the vast majority of ECM lineages found in the Agaricomycetes (Basidiomycotina), which also includes orchid mycorrhizae and root endophytes. Identification of the primary genetic factors that regulate symbiotic development and metabolism will therefore open the door to understanding the role of mycorrhizal symbioses in plant and fungal biology, allowing the full ecological significance of these mutualistic associations to be explored. The Mycorrhizal Genomics Initiative has introduced an important new dimension into mycorrhizal research by establishing genomic data for >30 mycorrhizal fungi genomes to serve as a fundamental resource for understanding symbiosis formation. As of today, we analyzed 20 new basidiomycete genomes, including ten ECM species, an orchid symbiont, Tulasnella calospora, as well as the ericoid symbiont Oidiodendron majus, and we performed transcriptome profiling in ECM root tips of five species representing independent origins of ECM. With this genomic view, a possible scenario suggests that [1] irreversible losses of lignocellulose decomposition pathways play a key role in the evolutionary stability of the ECM mutualisms and [2] that each major ECM fungal clades have subsequently and independently designed symbiotic molecular toolboxes to control the plant development and immunity. The polyphyletic ECM guild is unified by the loss of the plesiomorphic mechanisms for lignocellulose decay, but each lineage has retained a unique suite of enzymes associated with decay and may have unique capabilities to act as saprotrophs. In contrast to ECM symbionts, it appears that ericoid and orchid symbionts retained a full set of decay enzymes. Several mycorrhizal symbionts are using mycorrhiza-induced small secreted proteins (MiSSPs) to manipulate their host during the interaction. They likely use secreted effectors to target plant host hormone pathways to foster fungal colonization. Convergent evolution between mutualistic and biotrophic pathogenic fungi is striking and consolidate the idea that ECM fungi fall within the saprotrophism–mutualism–parasitism continuum.

CS8.4 THE GENOMIC ARCHITECTURE OF ECTOMYCORRHIZAL SYMBIOSIS IN THE GENUS AMANITA

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The ectomycorrhizal (ECM) symbiosis of fungi and trees is a successful evolutionary strategy, and has evolved repeatedly and independently, spawning many species-rich lineages among the fungi. Comparative genomics of two unrelated ECM fungi, the black truffle Tuber melanosporum, an ascomycete, and Laccaria bicolor, a basidiomycete, reveal commonalities in the gene repertoires of the two fungi, for example loss of plant cell wall degrading enzymes (PCWDEs). Nevertheless, the genomic architectures of the two species are dramatically different. The truffle has a very large, yet gene poor genome, densely populated by transposable elements (TEs) and with few gene families. In contrast, L. bicolor contains a large repertoire of genes, the majority of which are part of abundant gene families. Obviously, different gene sets and different genomic architectures can lead to the same kind of symbiosis, raising interesting questions about its evolvability. The genus Amanita encompasses approximately 500 species, the majority of which are ectomycorrhizal. However, a basal clade within the genus is asymbiotic and the single origin of symbiosis within the Amanita allows for a close investigation of the differences between free-living and ECM niches. We have sequenced the genomes of five species of Amanita, three ECM species and two asymbiotic species; as well as the asymbiotic outgroup Volvariella volvacea. We will discuss differences in gene content among the species, the origin of different gene families on the phylogeny, changes in overall genome architecture and possible evolutionary mechanisms. ECM Amanita are characterized by increased gene content compared to asymbiotic species. Patterns are similar to those discovered in L. bicolor: expansions in gene families involved in signaling, protein-protein interactions and small secreted proteins, but losses of PCWDEs and other secreted enzymes. It appears that loss of PCWDEs was an important early step towards the ECM symbiosis in this clade, but the asymbiotic species A. inopinata also shows significant reductions in PCWDEs. We find abundant numbers of TEs in two of the three ECM species and will highlight their potential influence on genome architectures.

CS8.5 GOT A GENOME? GET A PHYLOME!: FUNGI THROUGH THE EVOLUTIONARY LENS

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Genomes are shaped in many ways during the course of evolution. Phylogenomics provides us with the tools to study such events and interrogate their possible impacts. In this context, the use of genome-wide collections of gene evolutionary histories have been proved useful to study several processes. In this talk I will provide an overview of several of our findings related to the genome evolution of fungal organisms, for which hundreds of genomes are now available. Contrary to general expectations for eukaryotes and for a group of organisms with a cell wall and no phagocitosis, we found that horizontal gene transfer is common in fungi. This process has mediated the acquisition of important phenotypic innovations and has often involved the transfer of entire gene clusters between phylogenetically distant lineages. Gene duplication in the form of family expansions, or duplication of entire genomes has also played a major role in the evolution of fungi, and I will show several examples of relevance for pathogenic species. Finally, hybridization between different species is emerging as a common evolutionary mechanism that readily generates new species and innovative phenotypes through the combination of entire genetic complements. Altogether the emerging picture is that fungi present highly plastic genomes prone to rapid adaptations through the duplication of existing genetic material or the lateral acquisition of genes.

CS8.6 EARLY ORIGINS OF THE FUNGAL CELL WALL AND MULTICELLULARITY IN FUNGI

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Major innovations in fungal evolution include the acquisition of complex multicellular tissues in the form of hyphae, fruiting bodies, and specialized structures for spore discharge. The evolutionary transition from a single celled fungal ancestor to multicellular growth forms required changes in gene regulation and partitioning, the structure of cell walls and polar growth. The sampling of fungal genomes from across the phylogeny and from lineages that precede or follow transitions in growth forms permits the comparison of gene content and genome organization and identify candidate pathways or genome properties that correlate with phenotypic changes. We have sequenced the genome of Neolecta irregularis, a multicellular fungus that is evolutionary positioned within the Taphrinamyoctina, may represent an independent gain of multicellularity. Comparison of Taphrinamycotina genomes including N. irregularis and species from the sister group of Pezizomycotina can test the hypotheses about properties of multicellular growth. Our analyses identified changes in genome structure and content that suggests an increase in genome complexity in the fungi with hyphal growth and also reductions in the yeast lineages such as Schizosaccharomyces. Expansions of gene family copy number were observed for those involved in signaling pathways including kinase families. Microscopy has identified a septal plug in Neolecta analogous to Woronin bodies but the genome lacks an identifiable homolog to the HexA gene found in Neurospora suggesting independent evolution of this important component of hyphal growth in the lineage. A second genome comparison of early diverging fungi with the Dikarya fungi identified of gene families and pathways shared among early fungi and Animals but lost in Dikarya. These losses appear to be correlated with changes in growth forms including the emergence of hyphae. Gene families found only in the early lineages include those for flagellum, specific subfamilies of light sensing and cell wall genes. Further reconstruction of these genome changes in context of changes in fungal morphology will provide a framework for studying the evolution of growth forms within the Fungi.

CS8.7 COMBINING POPULATION GENOMICS, RNA-SEQ AND MINIATURE TRANSPOSABLE ELEMENT (MITE) PRESENCE TO IDENTIFY THE AVR2 GENE OF THE MELON PATHOGENIC FUNGUS FUSARIUM OXYSPORUM F. SP. MELONIS

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Fusarium oxysporum (Fo) is a soilborne fungus that causes Fusarium wilt disease in many plant species by colonizing and eventually blocking the host xylem vessels. The F. oxysporum species complex is a collection of apparently asexual non-pathogenic and pathogenic clonal lineages. Many lineages harbour unique genomic sequences residing mostly on extra chromosomes. One class of lineage-specific genes encodes effector proteins that are crucial determinants of virulence on a given host species. Some of these effectors, called avirulence proteins are recognized by plant resistance proteins. Effector recognition makes the plants resistant to the pathogen expressing the so-called avirulence (AVR) gene. To identify the Fom effector gene AVR2, we have sequenced several Fom isolates that differ in their recognition by the melon Fom-2 resistance protein. All effector proteins in the tomato wilt strain Fo f. sp. lycopersici harbor a miniature transposable elements (MITE) in their promoter. We have exploited the presence of the MITE to predict the effector complement of each sequenced Fom isolate. We combined this analysis with RNA sequencing of Fom-infected melon roots to pinpoint those effector candidates that are expressed during melon infection. Currently, we are testing the recognition by the melon Fom-2 gene of an AVR2 candidate from our combined analyses.

CS8.8 INSIGHTS ON THE EVOLUTION OF MYCOPARASITISM FROM THE GENOME OF CLONOSTACHYS ROSEA

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The mycoparasitic fungus Clonostachys rosea is an efficient biological control agent (BCA) under field conditions for a variety of plant diseases on agricultural crops. C. rosea belongs to the same order (Hypocreales), but to a different family (Bionectriaceae), than the more studied Trichoderma spp. BCAs. Comparative studies between C. rosea and Trichoderma spp. BCAs may thus improve our understanding of critical components of the mycoparasitic lifestyle. We sequenced the genome of C. rosea strain IK726 using Illumina/SOLiD technology, and transcriptomes from C. rosea interacting with Botrytis cinerea and Fusarium graminearum. Comparative genomics revealed a significant ($P \leq 0.05$) increase in the number of ABC-transporters, polyketide synthases, cytochrome P450 monooxygenases, pectin lyases and GMC oxidoreductases compared with other filamentous ascomycetes, including T. atroviride and T. virens. Interestingly, the increase of ABC-transporter gene number in C. rosea was associated with phylogenetic subgroup G (pleiotropic drug resistance transporters), while ABC-transporter gene number changes in Trichoderma spp. involved subgroup C that is putatively involved in secondary metabolite export. Gene expression data indicated that certain C. rosea subgroup G ABC-transporter genes were induced by exposure to the Fusarium mycotoxin zearalenone (ZEA), and deletion of a single ABC-transporter gene (abcG5) resulted in reduced growth rate ($P=0.001$) on ZEA-containing media and on fungicide-containing media. Deletion of the abcG5 ABC-transporter gene or the zearalenone lactonohydrolase gene zhd101, previously shown to encode an enzyme that detoxifies ZEA, resulted in mutants that failed to protect wheat and barley seedlings against F. graminearum foot rot disease in growth chamber tests. The comparative genomics study between C. rosea and Trichoderma spp. suggests that these BCAs rely on different mechanisms for attack. T. atroviride and T. virens contain many genes encoding hydrolytic enzymes that degrade fungal cell walls, and proteins involved in secondary metabolite biosynthesis, but few membrane transporters. This is in sharp contrast to the situation in C. rosea that contains only few genes encoding fungal cell wall degrading enzymes, but high numbers of proteins involved in secondary metabolite biosynthesis and membrane transport. In summary, our data suggest that mycotoxin tolerance/detoxification is an important component of the biocontrol ability of C. rosea.

CS9.1 MULTIPLE LAYERS OF REGULATION OF FUNGAL CLEAVAGE FACTOR I PROTEINS

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Polyadenylation is a key step required for maturation of pre-mRNAs. It is a nuclear process, tightly coupled to transcription and splicing. During this process, the 3' end of the pre-mRNA undergoes a specific endonucleolytic cleavage. This first reaction regulates the 3' UTR length of the transcript depending on exactly where processing has occurred. Cleavage can also happen within 5' UTRs, introns and exons giving rise to regulatory transcripts and mRNA isoforms with different exons and cis elements. The addition of a polyadenosine tail by poly(A) polymerases completes the 3' end processing.
It is frequent to find multiple potential poly(A) sites within pre-mRNAs. Several proteins of the polyadenylation machinery have been shown to regulate alternative polyadenylation, including Cleavage Factor I (CFI) in metazoans, and Hrp1 in yeast. CFIm68 and CFIm25 are the two components of metazoan CFI, which are both absent in yeast. Filamentous fungi lack a clear orthologue of CFIm68.
In the rice blast fungus Magnaporthe oryzae, Rbp35 interacts in vivo with CFIf25. This protein presents multiple layers of regulation, which have been investigated in more detail. Rbp35 undergoes a proteolytic cleavage at the C-terminus generating two Rbp35 isoforms in the cell. Several constructs of Rbp35 allowed us to identify the cut site and regulatory regions within the protein. The C-terminus of Rbp35 tightly regulates its own protein levels. Cellular content of Rbp35 isoforms relies on the 5' UTR intron and nutrient conditions.
In addition to the Rbp35/CFI25 complex, filamentous fungi also have a homologue of Hrp1. The role of M. oryzae Hrp1 has been analysed to understand why filamentous fungi have maintained proteins with apparently redundant functions. In contrast to S. cerevisae, the M. oryzae mutant Δhrp1 is not lethal. However, Δhrp1 shows more severe developmental defects compared to Δrbp35, and is also unable to infect rice plants. Our results suggest the existence of both common and specific pre-mRNA processing pathways regulated by Hrp1 and Rbp35/CFIf25, both of which are essential for fungal development and plant pathogenicity.

CS9.2 THE FGPRP4 KINASE IS IMPORTANT FOR RNA PROCESSING, GROWTH, AND PATHOGENESIS IN FUSARIUM GRAMINEARUM

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Pre-mRNA splicing is an important gene expression regulation mechanism in eukaryotic cells. Among all the spliceosome components that have been identified, Prp4 is the only protein kinase and likely plays an important role in spliceosome function. In the fission yeast and mammals, it is an essential gene but the budding yeast lacks a distince Prp4 ortholog. However, PRP4 is not an essential gene in Fusarium graminearum, the causal agent of wheat scab although the prp4 mutant had severe defects in growth and conidiation and was non-pathogenic and female sterile. RNA-sequence analysis showed that 28% introns had over 8-fold reduction in splicing efficiency in the prp4 mutant but complete splicing of 2591 introns was not affected. Reduced RNA splicing efficiency and defects in alternative splicing were verified by RT-PCR analysis. Interestingly, the prp4 mutant was unstable and a total of 49 spontaneous fast-growing suppressors were isolated. The majority of the suppressor mutants could grow as fast as the wild type but RNA-seq analysis revealed that splicing defects were not fully rescued in two suppressor mutants analyzed. Moreover, we identified nine 10 mutations in four genes orthologous to PRP1, PRP31, BRR2, and PRP8. The effects of these mutations on the interactions among components of the spliceosome were further investigated. Nevertheless, none of these mutants are recovered in sexual reproduction or plant infection. We also failed to identify mutations in 20 other suppressor mutants subjected to sequencing analysis. These results indicate that PRP4 may interact with various spliceosome components and regulate the activation of spliceosome and alternative splicing in F. graminearum and other eukaryotic organisms.

CS9.3 EPIGENETIC CONTROL OF EFFECTOR GENE EXPRESSION IN THE PLANT PATHOGENIC FUNGUS LEPTOSPHAERIA MACULANS

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Plant pathogens secrete an arsenal of small secreted proteins (SSPs) acting as effectors that modulate host immunity to facilitate infection. In Eukaryotic phytopathogens, SSP-encoding genes are often located in particular genomic environments and show waves of concerted expression at diverse stages of plant infection. To date, little is known about the regulation of their expression. Leptosphaeria maculans is an ascomycete fungus responsible for the most devastating disease of oilseed rape (Brassica napus). The sequencing of its genome revealed a bipartite structure only described so far in higher Eukaryotes, alternating gene rich GC-equilibrated isochores and gene poor AT-isochores made up of mosaics of transposable elements. The AT-isochores encompass one third of the genome and are enriched in putative effector genes that present the same expression pattern (no or a low expression level during in vitro growth and a strong over-expression during primary infection). Here, we investigated the involvement of one histone modification, histone H3 lysine 9 methylation (H3K9me3), in epigenetic regulation of concerted effector gene expression in L. maculans. For this purpose, we silenced expression of two key players in heterochromatin assembly and maintenance, HP1 and DIM5, by RNAi. By using HP1-GFP as a heterochromatin marker, we observed that almost no chromatin condensation is visible in a silenced-dim5 background. Whole genome oligoarrays performed on silenced-hp1 and silenced-dim5 transformants background revealed an over-expression of pathogenicity-related genes during in vitro growth, with a favored influence on SSP-encoding genes in AT-isochores. That increase of expression during in vitro growth was associated with a reduction of H3K9 trimethylation at two SSP-encoding gene loci. The ectopic integration of four effector genes in GC-isochores led to their overexpression during growth in axenic culture. These data strongly suggest that an epigenetic control, mediated by HP1 and DIM5, represses the expression of at least part of the effector genes located in AT-isochores during growth in axenic culture. Our hypothesis is that changes of lifestyle and a switch toward pathogenesis lift chromatin-mediated repression, allowing a rapid response to new environmental conditions.

CS9.4 REGULATORY CROSSLINKS OF THE UNFOLDED PROTEIN RESPONSE CONTROL FUNGAL DEVELOPMENT AND PATHOGENICITY

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The Unfolded Protein Response (UPR) represents a conserved mechanism of eukaryotes to cope with misfolded proteins in the endoplasmic reticulum (ER) that accumulate during situations of high secretory activity. In addition to this recognized function, we observed that in the smut fungus Ustilago maydis the UPR is closely connect to the regulatory network that controls pathogenic development. While the transition from saprophytic sporidial growth to the infectious biotrophic filament is suppressed on transcriptional level by an active UPR, fungal proliferation after penetration of the host plant is mediated via protein-protein interaction between the central UPR regulator, Cib1 (homologue of Hac1) and the developmental regulator Clp1. A functional UPR is crucial for pathogenicity and detailed analysis revealed that this phenotype is likely caused by a combination of (I) the reduced ability for effector secretion and (II) a developmental block after host penetration. We observe a multi-layered interplay of the different regulatory networks that is based on unconventional splicing of cib1 mRNA, transcriptional regulation and modification of protein functionality and abundance of key regulatory components. We anticipate that this network allows for the establishment of developmental checkpoints that incorporate fungal physiology and by that ensures efficient adaptation and developmental progression during biotrophy.

CS9.5 mRNA TRANSPORT MEETS MEMBRANE TRAFFICKING

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Endosomes transport cellular macromolecules such as lipids and proteins that mainly receive their cargo from other membrane compartments. Particularly in highly polarised cells like neurons and fungal hyphae, endosomes shuttle along microtubules and carry out sophisticated functions in long-distance transport. Evidence is accumulating that these membranous carriers also transport mRNAs as unconventional cargo. However, little is known about the precise molecular function of this endosomal mRNA transport. Here, we present data on a novel cell biological mechanism: endosome-coupled translation functions during loading of endosome with protein cargo.

CS9.6 THE COORDINATION OF mRNA DEGRADATION AND TRANSLATIONAL REPRESSION

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Within eukaryotes there are various mechanisms that coordinate the silencing and degradation of transcripts. Generally these mechanisms promoted translational repression, decaping and rapid transcript degradation. For most transcripts a key determinant is poly(A) tail length. Shortening to a critical length leads to what is termed, deadenylation dependent transcript degradation. Utilising this phenomena regulatory mechanisms which act at the level of transcript stability often modulate the rate of deadenylation. However, in the case of cell cycle regulated stability of the histone mRNAs the trigger for degradation appears to be independent from deadenylation. Another system which leads to deadenylation independent transcript degradation is nonsense mediate decay (NMD), a major quality control mechanism which triggers degradation of transcripts that contain a premature termination codon. In A. nidulans these three apparently distinct mechanisms, deadenylation dependent transcript degradation, NMD and cell cycle regulated histone mRNA stability, all induce mRNA 3' tagging and involve components of the NMD pathway. The tagging process results in the addition of a short run of pyrimidine nucleotides (C and/or U) at the mRNA 3' end which is mediated by two terminal transferases CutA and CutB. The addition of a 3' pyrimidine tag has been observed in a range of eukaryotes and is likely to act by recruiting the Lsm-Pat1 complex to the transcript, which then initiates a cascade of events including translational repression, dissociation of the termination complex, decapping and both 5' and 3' mRNA degradation. Utilising molecular genetic techniques we are investigating the role of these various components and addressing the question as to why they should all involve Upf1, which is known as a central component within NMD.

CS9.7 THE SPF27-HOMOLOGUE NUM1 CONNECTS SPLICING AND CYTOPLASMIC TRAFFICKING PROCESSES IN USTILAGO MAYDIS

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The conserved NineTeen protein complex (NTC) is an integral subunit of the spliceosome and required for intron removal. The NTC mediates conformational changes of core spliceosomal components, stabilizing RNA-RNA- as well as RNA-protein interactions. In addition, the NTC is involved in cell cycle checkpoint control, response to DNA damage, as well as formation and export of mRNP-particles. In the basideomycetous fungus Ustilago maydis, we have identified the Num1 protein as the homologue of SPF27, one of NTC core components. Num1 is required for polarized growth of the fungal hyphae, and, in line with the described NTC functions, affects the cell cycle and cell division. RNA-Seq analysis revealed that in num1 deletion strains splicing is affected on a global scale. In a screen for Num1 interacting proteins, not only NTC core components as Prp19 and Cef1 were identified, but several proteins with putative functions during vesicle-mediated transport processes. Among others, Num1 interacts with the motor protein Kin1 in the cytoplasm. Similar phenotypes with respect to filamentous and polar growth, vacuolar morphology, as well as the motility of early endosomes corroborate the genetic interaction between Num1 and Kin1. Our data implicate a previously unidentified connection between a component of the splicing machinery and cytoplasmic transport 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 proteins functions in the coordination of pre-mRNA splicing with nuclear-pore complex dependent export of mRNP-particles and microtubule-based mRNA-transport.

CS9.8 LASER MICRODISSECTION AND TRANSCRIPTOMICS OF INFECTION CUSHION DEVELOPMENT OF FUSARIUM GRAMINEARUM

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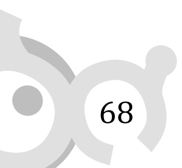
The fungal plant pathogen Fusarium graminearum Schwabe (teleomorph Gibberella zeae (Schwein) Petch) is the causal agent of Fusarium head blight (FHB) of small grain cereals and cob rot of maize worldwide. Trichothecene toxins produced by the fungus e.g. nivalenol (NIV) and deoxynivalenol (DON) contaminate 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). 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. 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 separately epiphytically growing runner hyphae and infection cushions. Several hundred runner hyphae and infection cushions grown on wheat glumes were isolated and RNAseq perfomed. Quantitative expression analysis show marked differences in gene expression patterns between runner hyphae and infection cushions. We will discuss specifically differences in carbohydrate active enzymes including cell wall degrading enzymes, putative effector proteins, and secondary metabolites. 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.



Poster abstracts

001 A NOVEL SIALIDASE IN THE OPPORTUNISTIC FUNGAL PATHOGEN, ASPERGILLUS FUMIGATUS

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We have studied the role of sialic acids in the opportunistic fungal pathogen, Aspergillus fumigatus, the most common cause of airborne mould infections worldwide. The pathogenesis of many bacteria and viruses is known to involve sialic acids, negatively charged monosaccharides present on cell surface glycans. We have shown that unsubstituted N-acetylneuraminic acid (Neu5Ac) is present on A. fumigatus spores and that the removal of spore Neu5Ac decreases their uptake by murine macrophages and human lung epithelial cells. How A. fumigatus presents sialic acids on its cell surface is unknown; to date, no sialic acid biosynthetic genes have been found in the genome. However, a sialidase gene was identified and the protein was expressed in bacteria and a crystal structure obtained. Further analysis of the A. fumigatus sialidase revealed an unexpected finding: rather than Neu5Ac, the enzyme prefers KDN as a substrate, an uncommon member of the sialic acid family. KDN is found in almost all types of glycoconjugates in place of Neu5Ac. The crystal structure of the recombinant A. fumigatus KDNase enzyme showed that the arginine pocket can accommodate the smaller substitution at the fifth carbon on KDN. To investigate the mechanism of the A. fumigatus sialidase (KDNase), we used site-directed mutagenesis to create mutant recombinant enzymes modified at the active site including the catalytic nucleophile (Y358H), the general acid/ base catalyst (D84A), and an enlargement of the binding pocket to attempt to accommodate the N-acetyl group of Neu5Ac (R171L). Crystal structures for all three mutant enzymes were determined. The D84A mutant had a greater effect on decreasing the activity of AfKDNase compared to the same mutation in the structurally similar sialidase from the bacterium, Micromonospora viridifaciens. Removal of the catalytic nucleophile (Y358H) significantly lowered the activity of the enzyme but this mutant remained a retaining glycosidase as demonstrated by NMR spectroscopic analysis. This is a novel finding that has not been shown with other sialidases. Kinetic analysis revealed that R171L had higher activity on a Neu5Ac-based substrate compared to wild type KDNase; hence, leucine in place of arginine in the binding pocket improved catalysis towards Neu5Ac substrates. Hence, whether a sialidase is primarily a KDNase or a neuraminidase is due in part to the presence of an amino acid that creates a steric clash with the N-acetyl group.

002 A RETINOIC-ACID BIOSYNTHETIC ENZYME INVOLVED IN MORPHOLOGY AND SEXUAL DEVELOPMENT IN FUSARIUM VERTICILLIOIDES

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Aldehyde dehydrogenases (ALDHs) comprise a large family of enzymes that catalyse the oxidation of a wide range of aldehydes to the corresponding acid forms. Particularly relevant members from this family are retinaldehyde dehydrogenases (RALDHs), which convert retinal to retinoic acid, an important morphogen involved in development and embryogenesis in chordates. We formerly described the genes carRA, carB, carT and carD, encoding the enzymes needed for neurosporaxanthin biosynthesis in F. fujikuroi. As a lateral branch of the pathway, cyclization of gamma-carotene produces beta-carotene, which is cleaved by the CarX oxygenase to produce retinal, the presumed chromophore of the two rhodopsins encoded in the genome of this fungus, CarO and OpsA. The ability of Fusarium to produce retinal led us to consider the occurrence in this fungus of a RALDH enzyme able to convert this apocarotenoid into retinoic acid. Because of its higher amenability for sexual crosses and its close taxonomic relation to F. fujikuroi, we chose for this study Fusarium verticillioides. A search for genes encoding putative RALDH enzymes in the F. verticillioides genome identified several candidate genes, which were heterologously expressed in E. coli, and checked for activity on retinal as a substrate. One of them, that we called CarY, exhibited the higher similarity to mammal RALDHs and was able to convert efficiently retinal into retinoic acid. Targeted mutation of the gene carY revealed morphological alterations of the colonies, specially marked under certain culture conditions, and a decreased ability to produce perithecia when the mutant acted as a female in sexual crosses. The phenotypic alterations of the mutant were reverted upon introduction of a wild type carY gene, indicating that retinoic acid formation may play a relevant morphogenetic role in F. verticillioides. This species contains the same car genes for carotenoid metabolism and has a similar photoinduction of the pathway than F. fujikuroi, explained by the transcriptional photoinduction of the gene carRA, responsible for the first enzymatic step. However, a less efficient photoinduction was found for the genes carB, carT and carX. Under the same experimental conditions, transcript levels of the gene carY were hardly affected by light in both Fusarium species.

003 AEGEROLYSINS AND PROTEINS WITH MACPF DOMAIN IN FILAMENTOUS FUNGUS ASPERGILLUS NIGER

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Aegerolysins and MACPF domain containing proteins (Pfam06355 and 01823 protein families, respectively) comprise of more than 350 and 500 proteins found in various kingdoms of life. While biological roles of MACPF domain containing proteins are well-known (plant and animal defence mechanism, virulent factors, development...), biological roles of aegerolysins remain to be elucidated. So far it has been shown that they play an important role during sporulation of bacteria and development of primordia and fruiting bodies of fungi from Basidiomycota phylum. They were also suggested to act as virulence factors in filamentous fungi. Various members of both protein families display hemolytic activity and have been shown to form pores in biological and artificial lipid membranes, either sole or in combination with one another (aegerolysin - MACPF domain containing protein). While organisms containing only aegerolysins or only proteins with MACPF domain are abundant, those containing members of both protein families are scarce.
Aspergillus niger is a saprophytic, filamentous fungus found throughout the world. When screening various strains of genus Aspergillus for hemolytic activity, we found that two Aspergillus niger strains displayed hemolytic activity on sheep blood agar plates and their mycelial ethanolic extracts lysed bovine erythrocytes in suspension. This fungus is also one of a few organisms whose genome contains both aegerolysin and MACPF domain containing homologues. In order to determine the biological role of these proteins in filamentous fungi and to find the possible link between hemolytical activity and the presence of these proteins we are now using various approaches, such as QPCR for monitoring the expression of the target proteins in different time points and different conditions of growth, deletion mutants for target proteins and their combinations for morphological and transcriptome studies. We are also trying to obtain recombinant proteins for studies of hemolytical activity, membrane interactions and production of antibodies that will be used further for immunolocalization studies.

004 APOCAROTENOIDS OF THE MUCORALES

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The sexual interaction in the Mucorales involves complex morphological and metabolic changes, including an increased accumulation of β-carotene that is exploited by industry. The process is initiated by an exchange of sex-specific signals between mycelia of opposite sex (Burgeff, 1924). These signals and the inducers of sexual carotenogenesis derive from β-carotene (Caglioti et al. 1966, Sutter 1975). Cleavage of β-carotene, a C40 compound, produces three fragments, heads, respectively, of three families of apocarotenoids: the trisporoids (C18), the cyclofarnesoids (C15), and the methylhexanoids (C7). This process was discovered in Phycomyces blakesleeanus (Polaino et al. 2010, Medina et al. 2011) and extended to Blakeslea trispora, Mucor mucedo and M. circinelloides (Barrero et al. 2011, Sahadevan et al. 2013, on line, and our unpublished work).
We have extended previous work to the structural identification and quantitative determination of the major apocarotenoids of wild-type Phycomyces and Blakeslea in single and sexually mixed cultures. The compounds vary not only with the species, but between strains of the same species. We arranged the apocarotenoids in a network or periodic system that helps classify them, predict new compounds and establish tentative biosynthetic pathways. We did not find evidence of a metabolic complementation between strains of different sexes to produce trisporoids.
Phycomyces transforms β-apo-12-carotenol (very similar to the first cyclofarnesoid) to several more elaborate cyclofarnesoids, thus confirming our proposed pathway. The two sexes differ in their biotransformation of β-apo-13-carotenone (the first trisporoid); the (+) wild-type removes three carbon atoms and diverts part of the C18 flow to 4-dihydrocyclofarnesine S and its derivatives. Thus, the cyclofarnesoids derive directly from the initial C15 fragment of β-carotene and, in the (+) strain, indirectly through a shunt between the two apocarotenoid pathways.

005 ASPERGILLUS NIDULANS SIRTUIN A REGULATES SECONDARY METABOLISM PRODUCTIONS

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Sirtuins are member of the NAD(+)-dependent histone deacetylase (HDAC) family and are ubiquitous in eukaryotes although their physiological role in fungi is unknown especially that in gene regulation. We found Aspergillus nidulans gene encoding a sirtuin isozyme (SirA) with an amino acid identity of 47% to yeast Sir2p. Recombinant SirA exhibited nicotinamide sensitive NAD(+)-dependent HDAC activity, and deacetylated lysine 16 residue of histone H4 (H4K16), indicating that SirA is a fungal sirtuin counterpart. Gene disruptant of sirA (⊿SirA) accumulated more acetylated H4K16 at the gene promoters of ipnA and aflR and their transcripts and produced more penicillin G and sterigmatocystin, indicating that SirA removes H4K16 acetylation and represses the expression of these secondary metabolite genes. The increased cellular NAD(+) and the decreased secondary metabolite production induced by adding nicotinamide riboside accompanied decreased levels of H4K16 acetylation at the ipnA and aflR gene promoters, which was not evident in ⊿SirA. These results indicate that cellular NAD(+) modulates the HDAC reaction by SirA. DNA microarray analyses indicated that genes for synthesizing emericellamide, aspernidine A, xanthone, austinol, and siderophores are up-regulated in ⊿SirA, in addition to those for penicillin G and sterigmatocystin. HPLC analyses showed more peaks of secondary metabolites in mycelial extracts of ⊿SirA comparing to wild type, indicating that SirA repressed those secondary metabolite production. Adding nicotinamide to the wild-type and ⊿SirA cultures increased production of sterigmatocystin and other compounds, indicating that other sirtuin isozyme is involved in the secondary metabolite production.

006 BIOCHEMICAL CHARACTERIZATION AND CLONING OF A Β-XYLOSIDASE FROM TALAROMYCES AMESTOLKIAE

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The main carbon sources of biosphere are cellulose and hemicelluloses, two plant cell wall components. Xylan is the predominant hemicellulosic material in hardwood and grass, and hence industrial 2G bioethanol production strongly depends of its explotation to reach profitable yields. In nature, xylan conversion to xylose (fermentable sugar) is catalyzed by xylanases, members of glycosyl hydrolase superfamily. Endo-β-1,4-xylanases and β-xylosidases are responsible for the main hydrolytic steps. The first enzymes cut the xylan polymer into oligosaccharides that are quickly converted to xylose by the action of β-xylosidases. A huge interest in the identification and characterization of new β-xylosidases is rising since robust enzymes are needed in commercial cocktails for lignocellulose biomass applications. In addition, fungal β-xylosidases usually show transxylosylation capacity, attaching xylose units to alcohols, monosaccharides and disaccharides. By this mechanism new xylooligosaccharides can be synthesized with prebiotic potential and interest for pharmacological uses. In the present work, a β-xylosidase of the ascomycete Talaromyces amestolkiae has been purified and named as BxyTW. BxyTW was biochemically characterized: physicochemical properties, kinetic parameters and substrate specificity were determined. BxTW had a molecular mass of 102.275 kDa and a a basic pI (7.6). Maximal activity was found at pH 3 and 70 °C. Its kinetic study using 4-Nitrophenyl β-D-xylopyranoside as the substrate, gave values of Km and Vmax around 0.17 mM and 19.8 mU/µg, respectively. The enzyme was also active against xylobiose and 4-Nitrophenyl α-L-arabinofuranoside. Preliminary results show that it is able to carry out transxylosylation reaction successfully. The transxylosylation capacity of BxyTW combined with good values of substrate affinity and stability comparing to other similar fungal β-xylosidases, suggest that it could be an attractive biotechnological tool. The coding sequence of BxyTW has been amplified by using PCR primers designed according to its peptide mass fingerprinting. Its comparison with other β-xylosidases suggests that it belongs to Glycoside Hydrolase Family 3. Currently BxyTW has been successfully expressed in Pichia pastoris and the recombinant protein is being studied.

007 CARBON METABOLISM OF ASPERGILLUS NIDULANS DURING GROWTH ON PLANT BIOMASS

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Plant biomass is the most abundant and renewable carbon source for many fungal species and it consist of polysaccharides, lignin and proteins. Plant polysaccharides come from a variety of plants, and the composition depends not only on the plant, but also on the growth conditions, on the part of the plant, and on the season. The average composition is 40-45% cellulose, 20-30% hemicellulose, and 15-25% lignin. Monosaccharides 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.

Several carbon metabolism mutants of Aspergillus nidulans were generated that were (partially) impaired in glycolysis and therefore are reduced in their ability to use hexoses as carbon source. In addition, strains were generated in which these mutations were combined with a mutation in CreA, the major regulatory system involved in carbon catabolism. Growth of these mutants on plant biomass was performed and compared to the wild type, while enzyme assays were performed to study the production of extracellular enzymes involved in degradation of polysaccharides. The results highlight the interplay between hexose release and catabolism and the central role CreA plays.

008 CATALYTIC PROPERTIES OF LACCASE-LIKE MULTICOPPER OXIDASES FROM ASPERGILLUS NIGER

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Multicopper oxidases (MCOs) form a family of enzymes that is widely distributed in nature. These enzymes play different biological roles in e.g. lignification and delignification processes, transport of ions or cell division and morphogenesis. This family of enzymes includes laccases, ascorbate oxidases, metal oxidases and bilirubin oxidases, which are evolutionary and structurally related but have different substrate affinity and specificity. The characteristic of these enzymes to catalyze reactions having water as the only by-product, has drawn the interest of the industry for their use as 'green' catalysts. Their importance is reflected in the broad spectrum of reported applications, that range from pulp delignification, textile dye bleaching and water or soil detoxification, to the formation of pigments, the development of clinical tests and applications in the field of biosensors, bioreactors, and biofuel cells. The Aspergillus niger ATCC 1015 genome (Aspergillus comparative database, www.broadinstitute.org) contains 13 MCO coding genes. Their phylogenetic relationships were determined and those genes identified as good candidates to produce functional MCOs with laccase activity (mcoA, mcoB, mcoC, mcoD, mcoE, mcoF, mcoG, mcoI, mcoJ and mcoM) were cloned and overexpressed in A. niger. The overexpressed MCOs showed, in plate assays, different patterns of activity towards a number of laccase substrates, suggesting that they have different specificity and therefore different biochemical characteristics. McoA, McoB and McoG enzymes were selected for their purification and characterization. The newly obtained oxidases displayed strongly different activities towards aromatic compounds and synthetic dyes. McoB exhibited high catalytic efficiency with N,N-dimethyl-p-phenylenediamine (DMPPDA) and 2,2 azino-di(3-ethylbenzthiazoline) sulfonic acid (ABTS), and appeared to be a promising biocatalyst. Besides oxidizing a variety of phenolic compounds, McoB catalyzed successfully the decolorization and detoxification of the widely used textile dye malachite green. Furthermore, the crystal structure of McoG was solved, showing the presence of a histidine (His253), substituting a carboxylate residue present in all the structures of basidiomycetous laccases (Asp) and in the ascomyceteous laccases from Thielavia arenaria (Asp236) and Melanocarpus albomyces (Glu235). Site directed mutagenesis of His253 revealed that it is a critical residue in McoG's substrate specificity.

009 CHARACTERIZATION OF THE PR-TOXIN GENE CLUSTER IN PENICILLIUM ROQUEFORTI

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The PR-toxin is a bicyclic sesquiterpene belonging to the eremophilane class. This compound is a mycotoxin that is produced by several strains of Penicillium roqueforti in moulded grains and grass silages and may contaminate blue-veined cheese. We have cloned and sequenced four PR-toxin genes, which were named prx1 (ari1), prx2, prx3 and prx4. Gene silencing of the four genes resulted in a drastic reduction in the PR-toxin production and in an overproduction of mycophenolic acid. Mycophenolic acid is an antitumor compound formed by an unrelated pathway suggesting a cross-talk of PR-toxin and mycophenolic acid production. In the genome of Penicillium chrysogenum an eleven-gene cluster was found, which includes the above mentioned four prx genes and a 14-TMS drug/H+ antiporter. These genes are poorly expressed in P. chrysogenum under penicillin production conditions. In our research, we found that this apparently silent gene cluster is able to produce PR-toxin in different strains of P. chrysogenum on hydrated rice medium and under static culture conditions. Based on all available evidence, a detailed PR-toxin biosynthesis pathway is proposed

010 CITRIC ACID PRODUCTION IN 24-WELL PLATES IS AN EFFICIENT SCREENING PLATFORM FOR ASPERGILLUS CARBONARIUS

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Conventional citric acid batch-fermentation by filamentous fungi is often linked with large variations in production levels. The most used citric acid batch-fermentation method described in literature is based on Erlenmeyer flasks with cotton-stoppers in a heated shaker. Our preliminary studies using Erlenmeyer flasks in sextuplicates showed a relative standard sample deviation of 54 % citric acid produced with the filamentous fungi Aspergillus carbonarius, thereby confirming these large variations. The objective of this study was to propose a different and more reliable screening method for citric acid production from filamentous fungi, using A. carbonarius as test organism in a 24 well plate system. The two systems where run simultaneously with only the mechanical parameters as difference between them. All experiments were performed in sextuplicates to be able to give a higher statistical impact than the conventional triplicates. After 5 days fermentation, the citric acid produced in the Erlenmeyer system had a relative standard deviation of 48% compared to the 24-well system that gave a relative standard deviation of 6%. The statistical analysis showed that the difference between the replicates in the 24 well plate system was significantly smaller than in the conventional Erlenmeyer flask setup. This allows for a more precise determination of effects caused by for example changing fermentation parameters or making genetic alterations. We therefore conclude that fermentation in 24-well plates is a more reliable screening method for citric acid production by filamentous fungi, and likely for screening in general. Furthermore by screening in a 24 well plate system, it is possible to screen the same amount of samples as in a conventional flask system, using less space and less materials.

011 CONTRIBUTION OF PEROXISOMES TO GLYCOLIPID BIOSYNTHESIS IN USTILAGO MAYDIS

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Glycolipids synthesized by a variety of bacteria and fungi are surface-active secondary metabolites, which basically consist of a sugar backbone linked to acyl groups. The basidiomycete Ustilago maydis produces two different classes of glycolipids, ustilagic acids (UA) and mannosylerythritol lipids (MEL). The biosynthesis of both compounds is induced upon nitrogen starvation.
We could show that the two acyltransferases (Mac1 and Mac2) required for MEL production in U. maydis both contain a type 1 peroxisomal targeting signal (PTS1). Mac1 and Mac2 exclusively localize in peroxisomes, while the other enzymes involved in MEL production reside in the cytosol or at the plasma membrane. Orthologs of Mac1 and Mac2 in other fungal species were also found to contain PTS1 motifs indicating that peroxisomal localization of these enzymes is conserved among fungi.
Mis-targeting of both enzymes to the cytosol but did not block MEL synthesis. Cytosolic localization of Mac1 and Mac2 promotes production of MEL species with altered acylation pattern. Interestingly, omega hydroxylated fatty acids appear in MEL indicating cross reaction between both glycolipid biosynthesis pathways. In addition, we could show that cytosolic targeting of Mac1 and Mac2 significantly reduces the amount of the cellobiose lipid UA presumably via competition for the substrate palmitoyl-CoA. Therefore, peroxisomal localization of MEL biosynthesis is not only prerequisite for generation of the natural spectrum of MELs, but also to allow simultaneous assembly of different glycolipids in a single cell.

012 DEGRADATION OF PLANT POLYSACCHARIDES THROUGHOUT THE LIFE CYCLE OF AGARICUS BISPORUS

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The common edible mushroom Agaricus bisporus is a basidiomycete that thrives on decaying plant material in the forests and grasslands of North America and Europe. It is adapted to forest litter and contributes to global carbon recycling, degrading cellulose, hemicellulose and lignin in plant biomass to oligomers and monomers. A. bisporus is also an edible mushroom that is widely cultivated and economically important. But the process of growing A. bisporus in compost and utilization of this substrate is poorly understood.
In this project, a broad range of genes encoding plant biomass degrading enzymes and a wide array of enzymatic activities were studied during development of A. bisporus under commercial cultivation to understand the carbon nutritive needs of the fungus and its capabilities to degrade plant biomass.
Clear correlations were observed between the activity of extracellular polysaccharide degrading enzymes, the expression of the corresponding genes and the composition of compost, which is reach in plant material such as cellulose and hemicellulose. Differences in the expression of genes from different stages of development were detected between spawning, pinning and harvesting stages, suggesting that as soon as the monosaccharides that were released during composting are depleted, A. bisporus starts producing enzymes to degrade plant biomass components to satisfy its nutritive needs.

013 DIVERSITY IN PLANT BIOMASS DEGRADATION BY THE WHITE ROT BASIDIOMYCETE DICHOMITUS SQUALENS

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The white rot basidiomycete Dichomitus squalens is capable of efficient cellulose and lignin degradation. It is mainly found on softwood but can also degrade hardwood. In combination with the availability of a genome sequence this makes this species a very interesting model organism to study the molecular basis of plant biomass degradation in white rot fungi.
D. squalens is able to grow on a large variety of plant biomass components, but appears to prefer residues of the main chains of plant cell wall polysaccharides over side chain residues, suggesting that it is likely a secondary coloniser of woody biomass.
The CBS collection contains a set of monokaryotic strains of this species and these have been compared to each other and several dikaryotic strains to study the diversity within this species. This demonstrated very strong difference between the monokaryons to use various plant cell wall polysaccharides as sole carbon source. Significant differences were also found between the different dikaryons and between the dikaryons and the monokaryons. Based on these data selected strains have been studied in more detail using liquid cultures and enzyme assays and sexual crosses. Highlights of this study will be presented.

014 ELUCIDATION OF THE BIOSYNTHETIC PATHWAY OF THE ANTIBIOTIC YANUTHONE D IN ASPERGILLUS NIGER

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In this study we elucidated the genetic and biosynthetic pathway of meroterpenoid yanuthone D originating from Aspergillus niger. Feeding A. niger labeled 6-methylsalicylic acid (6-MSA), we showed that this building block is the precursor of yanuthone D. Moreover, we identified 10 genes, involved in the production of yanuthone D, including yanA, the gene encoding 6-MSA synthase. The deletion of all genes within this cluster and analyses of the constructed strains allowed for the identification of three intermediates in the yanuthone biosynthesis pathway. Additionally, we associated the gene yanI with a novel enzymatic activity, O-mevalon transferase. In the proposed biosynthetic pathway we identified several branching points that led to the production of five novel yanuthones (F-J), which might be the result of detoxification. The chemical analysis revealed another novel compound, yanuthone X1, with a structure similar to the other yanuthones. Adding to the complexity of yanuthones, this product, despite requiring several genes from the yan cluster, is not based on 6-MSA, but rather on a different and still unknown precursor.

015 ENHANCED ORGANIC ACID PRODUCTION OF ASPERGILLUS CARBONARIUS BY GENETIC ENGINEERING

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The black fungus Aspergillus carbonarius has potential as a good cell factory for production of organic acids. The attempts have been made to improve the conversion of the renewable carbon sources and the flux into selected organic acids by engineering of the central carbon metabolism. The present work is to evaluate the effect of deleting the gluconic acid producing pathway and inserting an alternative cytosolic pathway on organic acid production. The gox mutant was constructed by deleting the gox gene, which is assumed to encode the glucose oxidase in A. carbonarius, as there is a very high accumulation of gluconic acid as the main byproduct during organic acid fermentation from glucose. It was shown that the glucose consumption and efficiency of citric acid production were improved compared with the wildtype strain, and no gluconic acid could be detected from gox mutants. The pepck and ppc mutant was constructed separately by inserting the gene pepck and ppc, which encode the phosphoenolpyruvate carboxykinase in Actinobacillus succinogenes and phosphoenolpyruvate carboxylase in Escherichia coli, into A. carbonarius, in order to create a new bypass for shunting the carbon flux into the reductive pathway for stimulating the organic acid production. An improvement on both malic acid and citric acid production was observed which may result from the competition on the phosphoenolpyruvate with the original pathway. This may further lead to the enhanced carbon flux towards the cytosolic reductive pathway. Combining the two mutations in one strain shall demonstrate whether the production of organic acids will further increase.

016 ENVIRONMENTAL STRESSES AFFECT AFLATOXIN SYNTHESIS AND CONIDIOGENESIS IN A. FLAVUS THROUGH SIRTUIN PATHWAY INVOLVEMENT

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Aflatoxins are health hazardous secondary metabolites produced by the cosmopolitan fungus A. flavus. The ability of this fungus to exploit several substrates is also related to its psicrophylicity and enormous production of conidia. In relation to this, some ongoing climate changes favor plant susceptibility to the attack by this fungus with a consequent, dangerous, increase of aflatoxins into previously unexploited feed- and foodstuff.
In this study we analyze how some environmental stresses to which A. flavus may undergo also during host exploitation – namely, hypoxia, pH alteration, carbon starvation and oxidative stress – affect aflatoxin biosynthesis, fungal growth and conidiogenesis. By multiple analytical approaches (mass spectrometry, transcriptional analysis, fluorimetric assays) we check the trend of specific molecular pathways related to metabolism reprogramming in consequence of environmental signals. Results indicate that sirtuins– a class of deacetylase enzymes – could represent the interface between signal transduction pathways and transcriptional reprogramming into A. flavus.Nevertheless the results obtained, further study to clearly define the role of sirtuins in shaping transcriptome in A. flavus are required.

017 ERGOTHIONEINE IS INVOLVED IN ANTIOXIDATIVE DEFENSE IN ASPERGILLUS FUMIGATUS

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Ergothioneine (EGT) is a naturally occurring thiourea derivative of histidine, which contains a sulphur atom in the imidazole ring. While Saccharomycotina species lack the EGT biosynthetic pathway, it is produced by filamentous fungi, Actinomycetales and cyanobacteria. Furthermore, it is present in higher eukaryotes due to nutritional uptake via a specific EGT-transporter. Although various human diseases, such as inflammatory processes, rheumatoid arthritis and Morbus Crohn are linked to raised EGT-levels, the function of EGT is still elusive. Similar to thiols such as glutathione, neuroprotective effects in mouse models and oxidative stress resistance in fungi have been found to be associated with EGT.
The airborne human-pathogenic saprophytic mould Aspergillus fumigatus causes life-threatening diseases in immunocompromized patients and the protection against oxidative stress is crucial for virulence of A. fumigatus. To examine the function of EGT in A. fumigatus, we generated a mutant strain (ΔegtA) lacking the putative EGT biosynthetic enzyme AFUA_2G15650, termed EgtA. Consistent with the proposed function, inactivation of EgtA eliminated EGT production. EGT-deficiency was phenotypically inconspicuous and did not affect resistance against oxidative or metal stress. To circumvent the redundancy of oxidative stress defense, we next generated a mutant strain (ΔegtAΔyap1) lacking EgtA and additionally Yap1, a transcription factor that orchestrates oxidative stress defense. In this background, EGT-deficiency decreased resistance to superoxide (e.g. hydrogen peroxide, menadione) and metals (zinc, copper and cobalt) demonstrating that EGT is involved in protection against oxidative stress in its producer. EGT-deficiency decreased the accumulation of the intracellular siderophore ferricrocin (FC) in ΔegtA. Interestingly, in the Yap1-lacking background, EGT-depletion affected production of extracellular siderophores: it increased fusarinine C (FusC) but decreased triactylfusarinine C (TAFC) biosynthesis. The effects of EGT-deficiency on siderophore production indicate a link between the cellular redox system and iron homeostasis.

018 EXPLORATION OF DIMORPHIC SWITCHES FROM FILAMENTOUS GROWTH TO SINGLE HÜLLE CELLS AS TOOL FOR SYNTHETIC BIOLOGY

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Different secondary metabolites of Hülle cells have crucial nursing function during the fruiting body development in Aspergillus nidulans. Therefore the production and the producers of the secondary metabolism of these specialized cells have to be investigated to understand sexual development in this filamentous fungus.
To explore the dimorphic switch of Hülle cells they have to be first isolated to allow the extraction of proteins, metabolites and RNAs. Within these data one can not only learn about the function of Hülle cells during the sexual development, but one can also identify gene clusters for the production of secondary metabolites. The identified gene clusters will be then expressed in order fungal species such as Saccharomyces cerevisiae. Over all this research question will shed light on the usability of Hülle cells for the biotechnology industry.

019 EXPLORING THE ENZYMATIC MECHANISM AND BIOLOGICAL FUNCTION OF GLIOTOXIN S-METHYLATION IN ASPERGILLUS FUMIGATUS

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Gliotoxin is a redox active molecule secreted by Aspergillus fumigatus. It is an epipolythiodioxopiperazine class fungal toxin containing a disulphide bridge which is essential to its biological activity. GliT, an oxidoreductase, has been shown to be essential for self-protection against gliotoxin. A. fumigatus also produces an inactive bis-S-methylated form of gliotoxin. This modification of the active dithiol form has been hypothesised by others as a backup plan to protect the producer from dithiol end-products/toxic biosynthetic intermediates. Despite the identification of S-methylated forms for the majority of ETPs, to date, no enzyme has been demonstrated to catalyse this modification. Bioinformatic analysis of the A. fumigatus genome identified three methyltransferase encoding genes which may be responsible for this enzymatic conversion: the gli cluster genes gliN, gliM and a previously unclassified non-gli cluster encoded methyltransferase termed gliotoxin thiomethyltransferase A (gtmA). Disruption of gtmA completely abrogated organismal ability to biosynthesize and secrete bis-methyl gliotoxin (BmGT), while gliotoxin production and secretion were unaffected. This result also excludes a role for either GliM or GliN in gliotoxin thiomethylation. Surprisingly, exposure of A. fumigatus ΔgtmA to exogenous gliotoxin or H2O2 did not reveal the acquisition of a sensitive phenotype compared to wild-type. Thus, GtmA-mediated gliotoxin bismethylation is not essential for self-protection. Subsequent recombinant expression of GtmA as a GST fusion protein in Escherichia coli, led to the assessment of activity of the purified enzyme. Recombinant GtmA was shown to bismethylate dithiol gliotoxin using S-adenosyl methionine as methyl donor, via a novel LC-MS enabled activity assay. GtmA activity was detectable in A. fumigatus protein lysates obtained only under conditions permissive for BmGT formation. The S-methylation of endogenous and exogenous bioactive natural products was originally proposed as a host strategy for detoxification, shared amongst multiple species of bacteria and fungi. Our data unambiguously identify the first enzyme involved in ETP S-methylation and demonstrate that this mechanism is not essential for self-protection in A. fumigatus. Additionally, the location of this enzyme outside of the gliotoxin biosynthetic cluster is an exception to the rule that genes involved in the production of a particular metabolite are contiguously aligned.

020 EXPLORING THE FUNGAL BIODIVERSITY FOR ORGANIC ACID PRODUCTION: A STUDY OF 66 FUNGAL STRAINS

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Filamentous fungi are well known for their ability to degrade lignocellulosic biomass. The CIRM-CF, curated by our laboratory, contains more than 1600 strains of filamentous fungi, mainly basidiomycetes and ascomycetes. The natural biodiversity found in this collection is wide, with strains collected from around the world in different climatic conditions. This collection is mainly studied to unravel the arsenal of secreted lignocellulolytic enzymes available to the fungi in order to enhance biomass degradation. Filamentous fungi have a natural ability to convert certain products of biomass degradation, for example glucose, into various organic acids. Organic acids are suggested to give a competitive advantage to filamentous fungi over other organisms by decreasing the ambient pH. They also have an impact on the ecosystem by enhancing weathering, and metal detoxification. Commercially, organic acids can be used as chemical intermediates or as synthons for the production of biodegradable polymers which could replace petroleum-based or synthetic chemicals. One of the advantages of filamentous fungi as biotechnological production platforms is their ability to degrade vegetal biomass, which is a promising feedstock for the biotechnological production of organic acids. Indeed, for wood-decaying and saprotrophic fungi, organic acid have been suggested to be involved in the lignocellulolysis process. In this study, we selected 40 strains of Ascomycota and 26 strains of Basidiomycota representing the CIRM-CF in order to determine their basal organic acid and ethanol production profiles in glucose liquid medium. We observed that most of the filamentous fungi are able to acidify the medium and grow at low pH. We were also able to discriminate groups of filamentous fungi considering their basal organic acid production. A wider description of this ability within the fungal kingdom may help to understand the physiological role of these compounds and contribute to a greater biotechnological exploitation of filamentous fungi.

021 FATTY ACIDS, PHOSPHOLIPIDS AND TRIGLYCERIDES OF THE WILD TYPE AND CAROTENE-OVERPRODUCING MUTANT STRAINS OF PHYCOMYCES BLAKESLEEANUS

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Phycomyces accumulates large amounts of lipids in the form of oil droplets visible in the mycelium and sporangiophores. The yellow color of the mycelia and other structures is due to β-carotene, an effective provitamin A. Mutants with various colors have been isolated that carry modified genes for carotene biosynthesis or its regulation. The "superyellow" color mutants are richer in β-carotene than the wild type. There is a positive correlation between carotene overproduction and total lipid content in the mutants. The main fatty acids in the wild type are oleic (C18:1) and palmitic (C16:0) acids, each about one quarter of the total, and linoleic (C18:2), and γ-linolenic (C18:3) acids, each about one sixth of the total. Stearic acid (C18:0) is present at about one tenth and arachidic (C20:0) and behenic (C22:0) acids as minor compounds. The fatty acids and their proportions are conserved in the mutants, with the most remarkable exception of the carS carF double mutant, which differs in the C18 desaturations: oleic acid is present as in the wild type; linoleic acid, more abundant; stearic acid, very scarce; and γ–linolenic acid, completely absent. The phospholipids of the wild type and the mutants are similar in amount but different in fatty acid composition. In the wild type, saturated and unsaturated fatty acids are about even in phosphatidylinositol (PI) and lysophosphatidic acid (LPA); unsaturated fatty acids are the vast majority in phosphatidylethanolamine (PE), phosphatidylcholine (PC) and phosphatidic acid (PA). The main change in the mutants is that the vast majority of the fatty acids in PI of the double mutant carS carF are saturated. The fatty acids of LPA present smaller and opposite changes in the carS and the carF mutants. The increased lipid content of the carotene–overproducing strains is due essentially to the triacylglycerides. We detected 22 different triglycerides. The most abundant are POP and POO. All the mutants have one triglyceride, OLL, absent in the wild-type. Triglyceride EOL is found only in the carF mutant strains and LLL only in the carS carF double mutants. Changes in triglycerides and phospholipids, the two major classes of lipids, may be responsible for some of the phenotypic changes found in the color mutants. Phycomyces strains are good sources of β-carotene and γ-linolenic acid and efficient at converting carbohydrates into fat.

022 FEEDBACK LOOPS CONNECTING NON-RIBOSOMAL PEPTIDE SYNTHESIS WITH TRANSITION METAL HOMEOSTASIS IN ASPERGILLUS FUMIGATUS

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Aspergillus fumigatus, a soil dwelling opportunistic pathogen, produces many secondary metabolites thought to be essential for survival in environmental milieus and/or host tissues. Iron plays a critical role in fungal survival and virulence and two transcription factors, the GATA-factor SreA and the bZip-factor HapX closely monitor iron homeostasis. Both factors regulate production of specialized iron chelating non-ribosomal peptides known as siderophores, with HapX inducing siderophore production during iron starvation and SreA repressing siderophore production during iron sufficiency. Recently, another non-ribosomal peptide, hexadehydroastechrome (HAS; a tryptophan-derived iron (III) complexing compound), has been found important in A. fumigatus virulence. Here we find that manipulating expression of the HAS cluster has several consequences on metal homeostasis and other secondary metabolites in A. fumigatus. Our collective data suggests that HAS chelation of transition metals impacts their accessibility to the fungus and is part of a metabolic feedback circuitry designed to balance metal pools in the fungus, thereby affecting production of other non-ribosomal peptides and virulence.

023 FUNGAL GLUTATHIONE TRANSFERASES: TARGETS FOR EVOLUTIONARY INNOVATIONS

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While many studies concern the extracellular system developed by fungi to degrade plant biomass, few concern the intracellular mechanisms associated to this process. Glutathione transferases (GST) are intracellular phase II detoxifying enzymes, which accept a broad range of substrates with low specificity. They are key enzymes in catabolic/ detoxification pathways and redox signalisation. The complexity of this superfamily in soil microorganisms makes these proteins promising models to connect evolutionary analyses with environmental constraints. In particular, two microbial specific GST classes (GSTFuA and Ure2p) present a large copy number variation in genomes of wood degrading fungi. By combining genomic, biochemical and structural data, we have highlighted new functions for some isoforms of both classes in Phanerochaete chrysosporium, demonstrating that these enzymes are highly versatile and adaptable. As an example, GSTFuA could have evolved in parallel to classical GSTs, losing the capacity to transfer glutathione onto aromatic compounds but rather gaining the ability to function as ligandin for wood-derived compounds. Despite the fact that fungal GST globally share common features in terms of structure, activities and substrate recognition, they exhibit fine specificities, which could have be driven by local environmental pressure.

024 GENETIC CHARACTERIZATION OF ITACONIC ACID BIOSYNTHESIS IN USTILAGO MAYDIS

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The unsaturated dicarboxylic acid itaconate is widely used for the production of plastics, paints and resins. Currently, it is produced in large amounts by fermentation of Aspergillus terreus. Itaconic acid production has been observed also in other fungi including some basidiomycetes. The phytopathogenic fungus Ustilago maydis and some related Pzeudozyma strains also have the ability to produce itaconic acid. While in A. terreus the key enzyme cis-aconitate decarboxylase (CAD) is solely responsible for itaconic acid production, the biosynthetic route of itaconic acid in U. maydis has not been characterized, yet.
Here, we present data that U. maydis uses an alternative biosynthesis pathway for itaconic acid production. All genes required for itaconate biosynthesis are organized in a gene cluster, which also contains a pathway-specific transcription factor. We were able to reconstitute itaconic acid production in the yeast Saccharomyces cerevisiae by introducing the respective U. maydis genes. In addition, the key enzymes in itaconic acid production were heterologously expressed in E. coli. Biochemical characterization of purified proteins allowed us to confirm the catalytic activities in vitro.
The detailed characterization of enzymes critical for itaconic acid production in U. maydis approves this fungus as alternative organism for biotechnological production of itaconic acid. The yeast-like growth of U. maydis in liquid culture may confer some advantages in fermentation, since filamentous fungi tend to clog in liquid culture.

025 GENETIC MODIFICATION OF CARBON CATABOLITE REPRESSION LEVELS IN ASPERGILLUS NIGER FOR IMPROVED HEMICELLULASE PRODUCTION

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Aspergillus niger is known worldwide for its ability to produce an extensive range of extracellular glycohydrolases, including xylanases, pectinases, and β-glucosidase. Cultivation and media optimization is one way to improve enzyme production in microorganisms. However, bioprocess approaches have limitations since fungal metabolism involves a complex network control. In A. niger the synthesis of hemicellulolytic enzymes is controlled at the transcriptional level mainly by a carbon catabolite repressor protein (CreA) and the activator XlnR, which directs the expression of the hemicellulolytic genes. In this way, this study aimed to improve hemicellulases production by genetic modifications of CreA an XlnR genes in the carbon catabolic repression (CCR) of A. niger. Submerged fermentations (SmF) were performed in agro waste residues and pure substrates and several cellulolytic and hemicellulolytic enzymes were measured. The kinetics studies revealed that the genetic tools associated with biochemical engineering increased enzyme production. As so far, these modifications could be the key to develop of an economic process in hemicellulase production applied to biomass saccharification.

026 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, which have roles in a range of cellular processes such as transcription, development and intercellular communication. Despite the large number of known bioactive compounds produced by fungi, the biosynthetic potential of these microorganisms is greatly underestimated due to many of these SM gene clusters are silent under standard laboratory conditions. In our case, we are interested in Ustilago maydis, the causative agent of the maize smut. Despite the genome size of U. maydis (20.5Mb), just few secondary metabolites have been described: (Mannosylerythritol Lipids (MELs), Ustilagic Acid (UA) and the cyclic peptides ferrichrome and ferrichrome A).Therefore, we strongly believe that there might be other SM´s waiting to be discovered, especially those that could be involved in plant-pathogen interaction. For the identification of potential SM gene clusters two strategies were followed: the first one was focused on the search of central biosynthesis genes encoding polyketide synthases (PKS's), non-ribosomal peptide synthetases (NRPS´s), tryptophan dimethylallyltransferase (DMAT's) and terpene cyclases (TC's) by taking advantage of bioinformatic tools (SMURF, antiSMASH and FungiFun) and the well annotated U. maydis genome sequence. The second strategy was to seek groups of co-regulated gene clusters in the microarrays publicly available of U. maydis. The overexpression of the transcription factors (TFs) associated with the potential biosynthetic gene clusters identified in both strategies allowed the identification of one gene cluster that triggers the production of a green-brownish pigment when is activated. The SM gene cluster denominated as "A" cluster contains genes encoding 3 PKS´s, 2 transcription factors, 1 cytochrome P450, and 5 secreted proteins. By northern blot was possible determine the co-regulation of the majority of the genes upon the induction of one of the transcription factors (Um04101). Single knockout mutants of the cluster genes have been generated in the overexpressing strain background to analyze the fungal extracts and determine the role of each gene in the production of the metabolite by LC-MS. In addition, deletion mutants in the strain SG200 background will be tested in order to study the role in pathogenicity of the gene cluster in maize plants.

027 GENOME-WIDE TRANSCRIPTIONAL PROFILING OF THE LICHEN-FORMING FUNGUS CLADONIA METACORALLIFERA DURING PRODUCTION OF CRISTAZARIN

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Lichens are symbiotic organisms composed of lichen-forming fungus and algae, cyanobacterium, or both. Lichens produce a number of potentially valuable chemical compounds as secondary metabolites. These metabolites are also produced in the culture of the isolated fungus without the photosymbiont. We found that the isolated fungus Cladonia metacorallifera strain KoLRI002260 produces cristazarin which has antibacterial and antitumor activity on culture media supplemented with 1% fructose, but not glucose or other carbon sources. The results indicate that fructose may induce the cristazarin biosynthetic pathway in the C. metacorallifera mycobiont. RNA-Seq was carried out to determine the effect of fructose on the C. metacorallifera transcriptome. Go term analysis revealed that genes involved in metabolic processes and catalytic activity were differentially expressed. Furthermore, a polyketide synthase (PKS) gene is up-regulated (>2 fold) when grown on fructose compared to when grown on glucose. These results may contribute to revealing a molecular switch for the cristazarin biosynthetic pathway under induction by fructose.

028 HETEROLOGOUS EXPRESSION AND MODIFICATION OF A 30-KB GENE CLUSTER IN ASPERGILLUS NIDULANS FOR THE PRODUCTION OF NOVEL SECONDARY METABOLITES

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Fungal secondary metabolism is the source of a large number of structurally diverse natural products that possess a wide variety of biological activities. With the continuous increase in the number of filamentous fungi that have their genome sequenced, it has been become clear that the full potential of fungi as a producer of natural products has yet to be explored. The genes responsible for the synthesis of a given metabolite are most commonly collected in clusters that, in many cases, also encode transcription factors that will specifically induce expression of the genes in the cluster. However, clusters are not always expressed under standard laboratory conditions, thereby preventing metabolite production and pathway characterization. Characterization of unexplored biosynthetic pathways is also hampered by the lack of genetic tools for many fungi. These problems can be addressed by the heterologous expression of single genes or entire gene clusters in your favorite fungal hosts. Recently, we developed a novel approach for heterologous production of secondary metabolites, involving the two-step transfer of a 25-kb gene cluster from Aspergillus terreus to A. nidulans enabling synthesis of the polyketide geodin (Nielsen et al., 2013). Inspired by this success, we apply this strategy for heterologous expression of the 30-kb ccs gene cluster from A. clavatus for characterization of the pathway. Subsequently, we will use this cluster as a platform for engineering in A. nidulans with the aim of producing an array of related products with differentiated bioactivities.

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029 HIGH-LEVEL EXPRESSION OF MANGANESE PEROXIDASE, LIGNIN PEROXIDASE, VERSATILE PEROXIDASE AND LACCASE IN LIGNINOLYTIC FUNGUS PHANEROCHAETE CHRYSOSPORIUM

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Lignin is a complex and heterogeneous aromatic polymer, very abundant in nature and highly recalcitrant to degradation. White rot fungi are the only organisms able to mineralize completely this compound employing an efficient enzymatic machinery. Four types of extracellular ligninolytic enzymes have been identified and characterized in different species of these fungi: manganese peroxidase (MnP), lignin peroxidase (LiP), versatile peroxidase (VP) and laccase (Lac). The white rot fungus Phanerochaete chrysosporium is a valuable species in lignin degradation. This species produces extracellular oxidative enzymes secreted during secondary metabolism, but lacks VP and Lac, which are important for ligninolysis and diverse biotechnology processes. VP and Lac degrade and modify both phenolic and non-phenolic aromatic compounds, which cannot be oxidized directly by peroxidases from P. chrysosporium. Availability of fungal peroxidases has been limited due to the slow growth of native producers and low productivity of the enzymes secreted. In addition, the lack of an efficient genetic transformation method for expression of multiple enzymes into a single fungal strain has been an obstacle. In this study, we report the constitutive co-expression in Phanerochaete chrysosporium of endogenous MnP and LiP, and, for the first time, the heterologous expression of the vpl2 gen from Pleurotus eryngii and the lacIIIb gene from Trametes versicolor. For this purpose we employed a new and highly efficient transformation method based in the use of shock waves recently developed by our group. The expression of recombinant proteins was verified by PCR, Southern blot and qRT-PCR. Transcriptional analysis confirmed the increased expression of rMnP and rLiP in the transformants. Assays of enzymatic activity in early stages showed that the degradation of phenolic and non-phenolic substrates was several times higher in comparison with wild type strain. Also, an apparent synergic effect between endogenous enzymes from P. chrysosporium and recombinant proteins was observed when the cells were grown on minimal medium. To the best of our knowledge, this is the first report in co-expression of four enzymes in basidiomycetes. These results could be applied in various industrial processes like biofuels, paper industry and bioremediation.

030 HISTONE ACETYLTRANSFERASE GCN5 HAS A STRONG IMPACT ON SECONDARY METABOLISM AND GROWTH IN FUSARIUM FUJIKUROI

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Histone modification was demonstrated to have a crucial influence on secondary metabolism in various filamentous fungi. Recent functional studies on histone deacetylases (HDACs) of the plant pathogen Fusarium fujikuroi revealed a strong impact of FfHda1 and FfHda2 on the regulation of secondary metabolism. Deletion of the corresponding genes led to deregulation of several known secondary metabolites (SMs), including activation as well as silencing. In the present work we focused on histone acetyltransferases (HATs) and their role in SM regulation in F. fujikuroi.
In Aspergillus nidulans 36 of the 40 acetyltransferases were deleted. One of them, the HAT GcnE belonging to the Saga/Ada complex was shown to be essential for the Streptomyces rapamycinicus induced activation of the orsellinic acid gene cluster and for H3K9 and H3K14 acetylation and thereby the activation of several other secondary metabolite clusters. The GcnE homolog in F. fujikuroi, Gcn5, is localized in the nucleus. Compared to the wild type gcn5 deletion mutants are strongly impaired in growth on complete medium as well as in submerse culture. Furthermore, the mutants revealed severe alteration in production of known SM. In correlation to the expression level of key cluster genes the production of gibberellic acids is inhibited and bikaverin production is enhanced under low nitrogen concentration. Surprisingly, bikaverin production is also detectable under bikaverin repressing conditions (high nitrogen, alkaline pH) in the deletion mutants. However, the second group of PKS-derived red pigments, the perithecia pigments fusarubins, is neither produced under inducing nor repressing conditions. In contrast to the deletion the gcn5 overexpression had no distinct effect on secondary metabolism suggesting other complex partners to be essential for its function.
According to genome wide expression studies of the recently sequenced F. fujikuroi genome many of the 45 putative SM gene clusters are silent under all conditions tested. The manipulation of Gcn5 might be a proper tool to activate such cluster genes. To check for these putative further regulatory effects of Gcn5, microarray analysis of the deletion mutant and the wild type grown under low and high nitrogen concentration were performed and the results will be discussed.

031 HMG-COA REDUCTASE GENES OF A MUCOR RACEMOSUS STRAIN ISOLATED FROM CHEESE RIPENING PROCESS

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Several species in the zygomycete order Mucorales are reported as carotene producing organism. Moreover, some species, such as Phycomyces blakesleeanus, Blakeslea trispora and Mucor circinelloides, are involved in the study of the fungal carotenoid production as model organisms. Other members of this fungal group are used in certain food industrial procedures. Until to date, mucoral fungi used in food processing are poorly isolated and characterized, especially at a molecular level. Recently, the genome sequence of a Mucor racemosus strain isolated from cheese processing was determined. The fungus produces beta-carotene in an amount, which is comparable to that of measured previously in Mucor circinelloides. Based on the genome sequence, we have started to analyse its genes possibly participating in the carotenoid and the terpenoid biosynthesis. In the frame of these studies, presence of three HMG-CoA reductase genes was detected in the genome. HMG-CoA reductase is one of the key enzymes of the terpenoid pathway and its activity highly affects not only the carotenoid level, but the synthesis of ergosterol or prenyl groups of different proteins. The three isogenes were isolated and cloned; their nucleotide and the proposed amino acid sequences were analysed and compared to those of other known mucoral HMG-CoA genes. Reverse transcription PCR revealed that all of them are transcribed raising their functionality. Their transcription was analysed by quantitative real-time reverse transcription PCR after cultivating the fungus under various conditions (such as temperature or aerobic and anaerobic growth). The three genes showed different patterns of relative transcript levels.
This research was supported by the grant TÁMOP-4.1.1.C-12/1/KONV-2012-0014 and OTKA NN106394. For Cs.V. this research was realized in the frames of TÁMOP 4.2.4. A/2-11-1-2012-0001 „National Excellence Program – Elaborating and operating an inland student and researcher personal support system" The project was subsidized by the European Union and co-financed by the European Social Fund.

032 HOW DOES GLIOTOXIN INHIBIT THE GROWTH OF ASPERGILLUS NIGER?

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Aspergillus fumigatus makes an epipolythiodioxopiperazine-type toxin, called gliotoxin (GT), which has deleterious effects in immunocompromised humans. GT contains a characteristic disulphide bridge, which it employs in two damaging activities; (i) cross-linking with proteins via thiol residues and (ii) generation of reactive oxygen species (ROS) through redox cycling. The mechanism of GT cytotoxicity in fungi has not been fully characterised. Previously, identification of genes associated with increased resistance and sensitivity to gliotoxin have been performed in Saccharomyces cerevisiae in order to elucidate the molecular mechanisms of GT action in humans, however little has been done to understand GT affects in fungi. Our objectives were (i) to investigate A. niger as a model to explore GT sensitivity, as it does not produce gliotoxin and (ii) to improve our understanding of GT cytotoxicity and reveal new metabolic systems interactions in filamentous fungi. Inhibition assays in A. niger showed significantly ($p < 0.001$) impaired growth in a dose-dependent manner when exposed to gliotoxin. Exposure of A. niger to exogenous gliotoxin (2.5 μg/ml), for 3 h followed by LC-MS enabled comparative proteomic analysis, resulted in the identification of 30 proteins that were differentially regulated ($p < 0.05$) in response to exogenous gliotoxin. Specifically, proteins involved in the methyl/methionine cycle were differentially expressed in A. niger in response to gliotoxin. Expression of S-adenosylmethionine (SAM) synthetase was down-regulated (3.1 fold) and homoserine dehydrogenase was up-regulated (1.5 fold). Furthermore, SAM levels were decreased when A. niger was exposed to gliotoxin in accordance with the aforementioned proteomic results. Interestingly, we have also found that exogenous gliotoxin (2.5 μg/ml) is taken up by A. niger and is converted to bis-methyl gliotoxin (BmGT) over a 3 h period. Our current hypothesis is that S-methylation of gliotoxin may result in the production of S-adenosyl homocysteine (SAH), and homocysteine, both are toxic towards A. niger, which could explain why the organism is sensitive to gliotoxin. Further investigation will be focused on elucidating the mechanism of gliotoxin methylation and determination of cellular homocysteine levels in A. niger.

033 HOW TRANSPORTER SPECIFICITY IS DETERMINED: AN EMERGING ROLE OF CHANNEL-LIKE GATING DOMAINS (LESSONS FROM ASPERGILLUS NIDULANS)

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Transporters are proteins mediating the translocation of solutes and drugs across the cell membrane of all cells, a biological process implicated in nutrition, signaling and cell communication with its environment. The biological importance of transporters is reflected in several genetic diseases and phenomena of drug resistance or sensitivity, neurotransmission or metabolite redistribution in heterotrophic tissues. Similarly to enzymes, transporters have a major substrate binding site interacting specifically with a single molecule of the substrate in each transport cycle. Consequently they are characterized, in most cases, with Michaelis-Menten kinetics. Substrate binding elicits consequential conformational changes which eventually produce a transporter conformer open to the opposite side from that from where the substrate originally bound the transporter. This rocking-switch mechanism, implicating alternate outward- and inward-facing transporter conformers, has gained significant support not only from structural studies but also from a plethora of genetic, biochemical or biophysical approaches. Most transporters are rather specific for a given substrate or a group of substrates with similar chemical structure (e.g. amino acids, purines), but their degree of substrate specificity and affinity can vary dramatically, even among phylogenetically related members of a transport family, showing high overall similarity. How then substrate affinities and specificities are determined or evolve? The current dogma is that transporter specificity is determined by the interactions a given solute can make within a specific binding site. However, genetic, biochemical and in silico modeling approaches from our lab, using the A. nidulans purine transporter UapA, have challenged this dogma. We are going to highlight results leading to a novel concept stating that substrate specificity and transport kinetics are determined by subtle intramolecular interactions between a major substrate binding site and independent outward- and cytoplasmically-facing, gating domains, analogous to those present in channels. The significance of this finding will be discussed in relationship to transporter turnover regulation and the development of novel transporter-specific antifungals.

034 IDENTIFICATION OF THE INDUCER OF THE BGAD (BETA-GALACTOSIDASE-ENCODING) GENE IN ASPERGILLUS NIDULANS UPON GROWTH ON D-GALACTOSE

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Fungal beta-D-galactosidase is an elementary enzyme in biotech industry on account of its ability to hydrolyse lactose to produce galactose and glucose. Fungal beta-galactosidases can be distinguished into extracellular enzymes characterised by an acidic pH optimum, and intracellular ones, that function optimally at neutral pH. In Aspergillus nidulans, only a lactose- and D-galactose inducible intracellular activity with a neutral pH optimum has been described. Functionally characterised intracellular (pH-neutral) b-galactosidases belong to the Glycosyl Hydrolase family 2 (GH2). Genome annotation predicts nine genes for GH2 enzymes, out of which four were mapped to chromosome VI, but only one (AN3201) appeared to specify a gene big enough to produce a peptide of 120 kDal, the experimentally estimated molecular weight of the b-galactosidase subunit. This gene was assigned the abbreviation bgaD. In this study we report on the expression characteristics of the A. nidulans bgaD gene upon growth on D-galactose. The potential inductors can be identified by testing loss-of-function mutants defective in a single defined step of D-galactose catabolism. In case of a galactokinase (galE) mutant, bgaD is strongly induced by D-galactose, indicating that the intermediates of the Leloir pathway are dispensible for the induction. The expression profile of bgaD was similar in L-arabitol dehydrogenase (araA1) and hexose kinase (frA1) negative backgrounds, indicating that intermediates of the oxido-reductive pathway downstream of galactitol are not necessary for bgaD induction either. An frA1/galeE double mutant (which cannot grow on D-galactose) still produced bgaD transcript upon transfer onto D-galactose. Together, these results suggested that the true inducer of bgaD upon D-galactose induction is either D-galactose itself or its poliol form (galactitol). To test this hypothesis, we grew the wild-type as well as the galE mutants on galactitol as a sole carbon source. No bgaD induction was observed at any time-points tested, e.g. galactitol is not an inducer of the major intracellular beta-galactosidase in A. nidulans. We therefore concluded that the true inducer of bgaD in A. nidulans upon growth on D-galactose is the sugar itself, and its catabolism is not needed to achieve sufficient level of transcript formation. The research was supported by the EU and co-financed by the European Social Fund under the project ENVIKUT (TÁMOP-4.2.2.A-11/1/KONV-2012-0043).

035 IMPROVING PH-STABILITY OF MODEL VERSATILE PEROXIDASE THROUGH THE SEARCH FOR STRUCTURAL MOTIFS IN STABLE PEROXIDASES FROM GENOMES

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Versatile peroxidase (VP) from the white-rot fungus Pleurotus eryngii is a model high-redox potential peroxidase of biotechnological interest due to its ability to oxidize a wide range of recalcitrant substrates, including phenolic and non-phenolic aromatic compounds and dyes. However, its relatively low pH stability is a drawback for industrial application. During screening of the sequenced Pleurotus ostreatus genome (Fernández-Fueyo et al, this congress) the highly pH-stable manganese peroxidase (MnP) isoenzyme-4 was identified and crystallized. The analysis of the MnP4 crystal structure revealed the presence of several H-bonds, salt bridges and solvent exposed charged residues that could have a stabilizing effect and contribute to the high pH stability of this enzyme. With the aim of improving the pH-stability of versatile peroxidase, the possible stabilizing motifs found in MnP4 were introduced in the model VP through site-directed mutagenesis, giving rise to three VP variants. The variant VP150 included eight residues responsible for creating several H-bonds and salt bridges that would reinforce the MnP4 structure. VP150 showed a wider range of pH in which it is stable, compared with native VP, both at acid and alkaline pH. In parallel, seven basic residues exposed to the solvent and putatively contributing to stabilize MnP4 at acidic pH were also identified. We introduced these basic residues in VP150, obtaining the VP153 variant. Finally, the VP154 variant was designed by introducing the double A49C/A61C mutation in VP150. These two new cysteines should form a new disulfide bridge near de distal calcium stabilizing this region. The VP153 and VP154 variants exhibited significantly improved stability at acidic pH, retaining 85% of the initial activity at pH 3.5 after 25 h incubation at 25ºC, whereas VP150 retained 60%, and native VP was fully inactivated. The motifs found in MnP4, such as H-bonds and salt bridges, as well as the extra disulfide bridge introduced in VP reinforced the structure of the enzyme improving its pH-stability. Moreover, the introduction in VP of the solvent exposed basic residues found in MnP4 also enhanced the acidic stability of the resulting enzyme. These results show the possibility of using structural motifs of highly pH-stable peroxidases identified in large genomic analyses for rational design of biocatalysts of interest.

036 INCREASED PRODUCTION OF FATTY ACIDS BY DELETION OF ONE OF THE SIX PREDICTED ACYL-COA SYNTHETASES IN ASPERGILLUS ORYZAE

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Fatty acids are attractive molecules as source materials for biodiesel. Previously we attained a 2.4-fold increase in fatty acid production by increasing expression of fatty acid synthesis-related genes in Aspergillus oryzae. In this study, we show an additional increase in the fatty acid production by disrupting a predicted acyl-CoA synthetase gene in A. oryzae. The A. oryzae genome is predicted to encode six acyl-CoA synthetase genes and disruption of ACS1, one of the six genes, showed a 9.1-fold increase in intracellular fatty acid accumulation compared with that of the wild-type strain. The analysis of the composition of the major intracellular fatty acids and successive comparison with the composition of the wild-type strain showed increase of stearic acid (5% to 20%), decrease of linoleic acid (50% to 25%) and no significant change of palmitic acid and oleic acid by the disruption of ACS1. On the other hand, another predicted acyl-CoA synthetase gene, ACS2, was indicated to work on catabolizing fatty acids predominantly in this study because the disruptant showed retarded growth compared with the other acyl-CoA synthetase gene disruptants on minimal agar medium supplemented with a fatty acid as a sole carbon source. Disruptants of the other four acyl-CoA synthetase genes did not show any phenotypic differences from the wild-type strain, resulting in their unclear characteristics in the cell growth.

037 INDUCTION OF LACCASES IN FUNGI FOR MEDIATORLESS CATHODIC DIOXYGEN REDUCTION

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Laccases are multicopper enzymes that are widely distributed in bacteria, fungi and plants. They catalyze oxidation of aromatic and nonaromatic compounds and use molecular oxygen as final electron acceptor. Because of their broad substrate specificity and catalytic properties, laccases are especially interesting for applied research. Fungal laccases are exoenzymes that are secreted into the culture medium and can be harvested from the culture supernatant. In biotechnology - among other applications - laccases can be used in enzymatic biofuel cells for the cathodic oxygen reduction to improve cathode performance. Essential in this case is the ability of laccases to mediate direct electron transfer between a copper atom in its catalytic center and the cathode, which eliminates the need for mediators. Currently, a laccase from T. versicolor is mostly used in biofuel cells. Recent studies even show that crude laccase-containing culture supernatant is similarly efficient as purified enzymes in improving cathode performance, which eliminates the need for expensive enzyme purification. However, various other fungi possess laccases that can potentially be used for the same purposes and can perform cathodic oxygen reduction under various conditions. For the production of large quantities of the required enzyme in a short time period, cultivation conditions should be optimized. Many fungal species need inducers, such as copper or aromatic compounds, to trigger increased laccase production. Here we show, that natural substrates like wood, hay and straw enhance laccase activity in ligninolytic fungi Rhizoctonia solani and Aurantiporus fissilis up to 149 U/L and 290 U/L respectively, which is approximately two times higher than after induction with 1 mM copper sulfate. In addition, we discovered that co-cultivation of A. fissilis with Aspergillus nidulans crude culture supernatant caused laccase activity of 1585 U/L compared to the 41 U/L in non-induced culture. The A. nidulans culture supernatant has also induced laccase production in R. solani and P. sanguineus cultures. Using crude culture supernatant of induced cultures in a biofuel cell reveals new laccases suitable for direct electron transfer and oxygen reduction on the cathode.

038 INTERACTION OF ASPERGILLUS ORYZAE HYDROPHOBIN ROLA WITH SOLID SURFACES

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Hydrophobins are amphipathic proteins secreted by filamentous fungi, and play several important roles in fungal physiology, for example, in fungal adhesion to hydrophobic surfaces, in the formation of a protective surface coating, and in the reduction of water surface tension; these roles support the growth of fungal aerial structures such as hyphae and conidiospores. Furthermore, when the industrial fungus Aspergillus oryzae is grown in a liquid medium containing the polyester polybutylene succinate co-adipate (PBSA), the fungus produces RolA, a hydrophobin, and CutL1, a PBSA-degrading cutinase. Secreted RolA attaches to the surface of the PBSA-particles and recruits CutL1, which then condenses on the particles and stimulates the hydrolysis of PBSA. Because the enzyme recruitment requires RolA adsorption onto the solid surfaces precedent to the recruitment, kinetic properties of RolA-adsorption to solid surfaces are important. In order to examine kinetic properties of RolA-adosorption onto solid surfaces, we constructed self-assembly monolayers (SAM) of 1-undcanethiol (UD), 11-amino-1-undecanethiol (AUD, and 10-carboxy-1-undecanethiol (CUD) on the electrodes of Quartz Crystal Microbalance (QCM) and analyzed kinetics of RolA-adsorption to the SAM-electrodes. The Kd values of RolA to AUD were larger than Kd values to UD at pH 4 and 7, and was decreased at pH 10. The amounts of adsorbed RolA to CUD were significantly smaller than those to UD or AUD at the three pH points. RolA indicates pI=4.8 and large negative zeta-potentials at pH 7 and 10. These results suggest that RolA-adsorption to SAMs depends on pH and electrical properties of SAMs. Overall, hydrophobicity and positive charges of solid surfaces contribute to adsorption of RolA to the surface and negative charges of solid surfaces leads to repulsion between the surface and RolA.

039 INVESTIGATING DITERPENE BIOSYNTHESIS IN BASIDIOMYCETE FUNGI

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With the increasing rise in the evolution of antibiotic resistance, there is the pressing need to find alternative antibiotics to combat this problem. One such source of antibiotics may be natural products produced by basidiomycete fungi which until recently have been largely neglected as a source of novel bioactive compounds. Recently, the diterpene antibiotic pleuromutilin, which is active against bacteria such as methicillin-resistant Staphylococcus aureus (MRSA), has been isolated from the basidiomycete Clitopilus passeckerianus. Basidiomycetes are known to be prolific producers of terpenoids, an extensive class of bioactive compounds, the genes coding for which are commonly found to be located in biosynthetic gene clusters.

This work focusses primarily on two basidiomycete species, Lepista sordida and Coprinopsis strossmayeri. These species have been identified as producing antimicrobial compounds preventing the growth of Bacillus subtilis, Escherichia coli and Saccharomyces cerevisiae. Genes involved with the biosynthesis of diterpenes (from the terpenoid class of compounds) have also been identified in these fungal species through degenerate PCR and genome walking by inverse PCR. This project is focussing on locating diterpene gene clusters through genome analysis; establishing transformation systems to facilitate the manipulation of production pathways; and analysing the chemical profile of secondary metabolites produced by these species. The progress on each of these will be presented.

040 INVESTIGATING PROGRAMMING OF FUNGAL PKS-NRPS MEGASYNTHASES VIA CONSTRUCTION OF HYBRIDS

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Polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS) catalyse production of an extreme diversity of natural products exhibiting many beneficial biological activities. PKS-NRPS hybrids combine the acetate-derived polyketide chains with a range of proteinogenic and nonproteinogenic amino acids. Fungal highly-reducing PKS and PKS-NRPS hybrids work in iterative manner and consist of one set of catalytic domains controlling every extension cycle and degree of reduction and dehydration. However, the programming of these iterative enzymes is cryptic and poorly understood. The objective of my work is to investigate the programming of fungal iterative PKS-NRPS by conducting domain swaps between closely related enzymes and heterologous expression of these hybrids. I also explore the potential for production of novel compounds via construction of non-natural hybrids. My work focuses on the biosynthesis of tenellin, a dimethylated pentaketide, and desmethylbassianin (DMB), a monomethylated hexaketide from the insect pathogen Beauveria bassiana. The structures of the tenellin and DMB biosynthetic gene clusters are identical and genes have 90% identity at the nucleotide level. Previous work has shown that the only differences in programming map to the β-keto reduction (KR) and C-methylation (C-MeT) domains controlling chain length and methylation pattern respectively. I performed swaps between the C-MeT and KR domains of the tenellin synthase (tenS) and DMB synthase genes (dmbS), by excising fragments of dmbS and replacing them with equivalent fragments from tenS. Substituting the C-MeT sequence resulted in production of prebassianin, a dimethylated hexaketide, as the major compound with minor amounts of monomethylated hexaketide. This confirmed that the C-MeT domain controls methylation pattern but lacks full fidelity when placed in a heterologous environment. It also appears that the tenS C-MeT can accept longer substrates. Addition of the KR sequence of tenS led to the production of small amounts of the dimethylated pentaketide pretenellin A. Extending the sequence to contain the entire C-MeT-KR region, however, did not lead to the observation of any compounds. Similarly the KR-only swap did not result in the production of any compounds. Swapping the domains between closely related synthases proved less straightforward than initially presumed; however, the results show that rational manipulation is feasible.

041 INVESTIGATING THE METABOLIC SHIFT AT THE PYRUVATE BRANCH POINT OF RHIZOPUS ORYZAE GROWN UNDER AEROBIC AND ANAEROBIC CONDITIONS BY GLOBAL TRANSCRIPTOMICS ANALYSIS

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The pyruvate branch point is an established important cross-road in the metabolism of Rhizopus oryzae. The pathways taken at this branch point under aerobic and anaerobic conditions determine the yield of the main metabolites. Under aerobic conditions, mainly lactic acid (type I strains) or malic acid and fumaric acid (type II strains) are produced. Under anaerobic conditions the fermentation product is mainly ethanol. Analysis of the pyruvate branch point on gene expression level can enhance the understanding of the metabolic processes underlying this shift from organic acid production to ethanol formation. To this end, we cultivated a type II R. oryzae strain under aerobic and anaerobic conditions and performed global transcriptomics analysis of RNA sequence data obtained from the different growth conditions. Differential expression analysis confirms the main branches of the cross road and shows comparatively high activity in anabolic reactions in the anaerobic condition as compared to the aerobic condition.

042 INVOLVEMENT OF THIAMINE TRANSPORT SYSTEM IN THE RESPONSE OF BUDDING YEAST SACCHAROMYCES CEREVISIAE TO OXIDATIVE STRESS

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Thiamine (vitamin B1) is an essential compound for all living organisms, mainly because of the universal role of thiamine diphosphate (TDP) as a cofactor in cellular metabolism. However, it has also been hypothesized that thiamine is involved in responses to stress conditions, decreasing the generation of reactive species or serving as a stress-signaling molecule. These functions have been investigated in different organisms but none of the studies addressed a possible significance of thiamine transport system under unfavorable conditions. This issue is particularly important because the disruptions in thiamine transport can lead to severe disorders in humans, including mainly the TMRA syndrome that combines symptoms of megaloblastic anemia, diabetes mellitus and hearing loss. In the current study we investigated the role of thiamine transporters in the stress response of S. cerevisiae as a model microorganism which is able to synthesize thiamine de novo and, thus, the effects dependent on both thiamine biosynthesis and uptake can be simultaneously assessed. Yeasts possess two main thiamine transporters: (i) Thi7, the plasma membrane transporter for thiamine, and (ii) Tpc1, the mitochondrial carrier for TDP. Although we observed an elevated thiamine and TDP accumulation in cells under oxidative stress, the expression of genes encoding both transport proteins decreased, suggesting that their regulation can occur on other than transcriptional level. The analysis of mutant strains, devoid of functional Thi7 or Tpc1 proteins, revealed an altered growth rate comparing to the wild type, as well as different gene expression pattern for major TDP-dependent enzymes. In particular, for tpc1- strain cultured in thiamine-free medium we observed a significant growth impairment, correlated with an increased production of free radicals and higher expression of mitochondrial superoxide dismutase. We also showed an interplay between the transporters, as thi7- strain had higher expression of Tpc1 carrier under both control and stress conditions. Taken together, our results show the new aspect of thiamine action under stress conditions, confirming the key role of TDP in metabolic pathways but linking it also with stress response mechanisms. However, it seems clear that thiamine uptake is also important, contributing to the other, non-cofactor protective functions of thiamine.

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043 LACTOSE CATABOLISM IN PENICILLIUM CHRYSOGENUM: PHYLOGENETIC AND EXPRESSION ANALYSIS OF THE PUTATIVE PERMEASE AND HYDROLASE GENES

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Strains of Penicillium chrysogenum (sensu lato) are used as industrial producers of penicillin. We investigated its catabolism of lactose, an abundant component of the diary residue whey that has been used extensively in penicillin fermentation, comparing the type strain NRRL 1951 with the producer AS-P-78. Both strains grew similarly on lactose as the sole carbon source under batch conditions, exhibiting almost identical time-profiles of sugar depletion. In silico analysis of the genome sequences revealed that P. chrysogenum features at least five putative bGal-encoding genes at the annotated loci Pc22g14540, Pc12g11750, Pc16g12750, Pc14g01510 and Pc06g00600. The first two proteins appear to be orthologs of two Aspergillus nidulans intracellular family 2 glycosyl hydrolases expressed on lactose. The latter three P. chrysogenum proteins appear distinct paralogs of the extracellular bGal from Aspergillus niger, LacA, a family 35 glycosyl hydrolase. The P. chrysogenum genome also specifies two putative lactose transporter genes at the annotated loci Pc16g06850 and Pc13g08630. These are orthologgs of the paralogs of the gene encoding the high-affinity lactose permease (lacpA) in A. nidulans for which P. chrysogenum appears to lack the ortholog. Transcript analysis of Pc22g14540 showed that it was expressed exclusively in response to lactose, while Pc12g11750 was weakly expressed on all carbon sources tested, including D-glucose. Pc16g12750 was co-expressed with the two putative intracellular bGal genes on lactose and also on L-arabinose, but not on any other carbon sources, while its two paralog genes were apparently not transcribed under any condition tested. Pc13g08630 transcript was also formed exclusively on lactose, suggesting that it may indeed function as a lactose permease. The data strongly suggest that P. chrysogenum exhibits a dual assimilation strategy for lactose, simultaneously employing extracellular and intracellular hydrolysis, without any correlation to the penicillin-producing potential of the fungus.
Acknowledgement: The research was supported by the EU and co-financed by the European Social Fund under the project ENVIKUT (TÁMOP-4.2.2.A-11/1/KONV-2012-0043), and also by the Hungarian Scientific Research Fund (OTKA Grant K1006600 to Dr. Erzsébet Fekete).

044 LACTOSE INDUCTION OF STERIGMATOCYSTIN FORMATION IN ASPERGILLUS NIDULANS

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Seed contamination with polyketide mycotoxins such as aflatoxin (AF) and sterigmatocystin (ST) produced by Aspergillus spp., is an agricultural, economic, and medical issue worldwide. ST is the penultimate intermediate in the biosynthesis of AF; moreover, in several fungi including the model fungus A. nidulans, it is the end product of the AF pathway. The AF/ST biosynthetic pathway is well-characterized in A. nidulans, but many of the regulatory aspects including those related to the carbon source available for the fungus are still enigmatic. For example, A. nidulans mutants unable to produce ST on glucose medium have been observed to produce ST on lactose as a carbon source. One of the genes important for ST production is the so-called "velvet" gene (veA). The wild type allele is veA while veA1 is the mutation. In A. nidulans, VeA has been shown to control the AF/ST regulatory gene aflR and, subsequently, ST production. To address the relative importance of VeA and its interaction with carbon source, we initiated a project of lactose metabolism vs. ST production in A. nidulans in both a veA and veA1 background. Since environmental factors influence ST formation, we employed well-controlled submerged cultivation methods where temperature, pH and DO levels could be kept at preset values, and light intrusion could be prevented. To isolate and detect ST from fungal cultures, we developed an extraction protocol suitable to extract ST with an efficiency of 98.5%, and a rapid, reliable HPLC–UV method. Under these conditions, independently of the carbon source available, A. nidulans was not able to produce any ST in a veA1 background. In contrast, veA strain was capable of producing ST on both glucose and lactose in a concentration of up to 0.5 mg per liter. Time-profiles of ST formation were markedly different, however: on D-glucose, ST could be detected only after glucose was depleted from the medium, while on lactose, ST appeared in the early stages of the rapid growth phase. We concluded that ST-formation in A. nidulans may either be mediated by a carbon catabolite regulatory mechanism prominent on D-glucose, or induced by the low specific growth rate attainable on lactose. We are currently testing this hypothesis employing chemostat-type continuous cultures.
Acknowledgement: The research was supported by the EU and co-financed by the European Social Fund under the project ENVIKUT (TÁMOP-4.2.2.A-11/1/KONV-2012-0043), and by the OTKA (Grant No. K1006600).

045 METABOLIC CHANGES CAUSED BY DIFFERENT MUTATIONS IN THE SDHB SUBUNIT AND THEIR INFLUENCE ON THE PATHOGENICITY OF THE GREY MOULD PATHOGEN BOTRYTIS CINEREA

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Botrytis cinerea Pers.:Fr. is a worldwide occurring plant pathogenic ascomycete with a wide host range, including fruits, vegetables and ornamental flowers. Resistance development against fungicides used to control grey mould disease of valuable crops has been reported for many years. Therefore, an efficient disease management is mandatory to maintain the efficiency of botryticides in the field. Syngenta's resistance monitoring includes B. cinerea samples received from different European countries; most isolates originate from vineyards and strawberry fields. Mutations reported to be responsible for reduced sensitivity to SDH inhibitors (SDHIs, targeting the mitochondrial complex II) are monitored by pyrosequencing of the SDHB, SDHC and SDHD encoding genes of field strains. In addition, forward (UV mutagenesis) and reverse genetics (transformation) techniques are used to identify and confirm mutations associated with resistance to current and new SDHI chemistries. SDHI resistance frequencies increased significantly both in samples from grape and strawberry fields over the past six years. Resistance could be ascribed to published mutations in SDHB, while cross resistance tests showed that the sensitivity levels to different SDHI subclasses varied between the different types of mutations. Mutant genotypes carrying B_H272R and B_H272Y amino acid substitutions are currently dominating in the field (>95% of the SDH mutants), whereas isolates carrying the B_P225L substitution are still rarely detected (~1% of the SDH mutants). These field results are in contradiction with in vitro assessments, such as frequency of occurrence in UV screenings or measurements of resistance factors with isogenic lines, which suggests a differential impact of these mutations on fitness parameters. To assess putative impacts of SDHB subunit mutations on fitness parameters of the respective isolates, we used a combination of approaches:
-Competitive pathogenicity assay conducted in planta, using various isogenic SDHB mutant lines.
-Spore germination assays.
-Cellular respiration efficiency assays in different media.
-Full metabolomics profiling of the mycelium and the growth medium.
This study enabled us to suggest metabolic markers and phenotyping methods for the prediction of fitness penalty associated with SDHI resistance in fungi.

046 MOLECULAR CHARACTERIZATION OF THE CYTOCHROME P450 BENZOATE PARA-HYDROXYLASE GENE IN AUREOBASIDIUM PULLULANS ATCC 20524

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Enzymes exhibiting the benzoate para-hydroxylase activity are found in several ascomycetes and basidiomycetes and crucial in the detoxification of plant-released phenolic compounds. Benzoate para-hydroxylase (EC 1.14.13.12) belongs to the CYP53 family of the cytochrome P450 superfamily and only a few fungal genes encoding the enzyme have been characterized so far. To date, there have been no reports of cytochrome P450 enzymes in the saprophytic fungus Aureobasidium pullulans. Recently, we have cloned the 5-kb DNA fragment containing the complete open reading frame (ORF) of the alpha-L-arabinofuranosidase gene (abfB) from the A. pullulans strain ATCC 20524 (K. Ohta et al., J. Biosci. Bioeng., 116, 287–292, 2013). In this study, database searches fortuitously found the presence of a predicted ORF with similarity to benzoate para-hydroxylase gene that was located 555-bp downstream of the previously reported abfB gene in the same direction of transcription within the 5.0-kb DNA fragment. The cDNA encoding benzoate para-hydroxylase was cloned and sequenced. The ORF (1512 bp) of the benzoate para-hydroxylase gene bphA was interrupted by a single intron of 54 bp. The bphA 5′-noncoding region had a putative TATA box (TATAA) resembling the canonical TATAAA sequence at nucleotide (nt) position −96 from the start codon, but no sequence equivalent to a CCAAT box. Five consensus binding sites for the CreA repressor (5′-SYGGRG-3′), which mediates carbon catabolite repression, were located at nt positions –420, –352, –341, –239, and –204 from the start codon. A transcription start point of the bphA gene was present at nt position −63 (A) from the start codon. The bphA gene encoded a protein of 504 residues with a calculated Mr of 56,729 Da and a theoretical isoelectric point of 7.33. The deduced amino acid sequence showed a similarity to that of the cytochrome P450 superfamily; the protein possessed a cytochrome P450 cysteine heme-iron ligand signature FSYGPRACVG at residues 444–453 in the C-terminal region. The transmembrane helices of the A. pullulans BphA appear to lie within the noncleavable hydrophobic N-terminal region at residues 7–29, and are proposed to serve as an anchor sequence in the membrane of the endoplasmic reticulum. The deduced amino acid sequence of the bphA gene product was 74% identical with that of a cytochrome P450 benzoate para-hydroxylase CYP53A15 from the pathogenic filamentous ascomycete Cochliobolus lunatus, and designated as CYP53A37.

047 NEW INSIGHTS INTO GLIOTOXIN SECRETION FROM ASPERGILLUS FUMIGATUS

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Aspergillus fumigatus is an opportunistic pathogen which produces gliotoxin, an epipolythiodioxopiperazine (ETP) which is characterised by an intramolecular disulphide bridge. The gli gene cluster is comprised of thirteen genes which are involved in aspects of gliotoxin biosynthesis and self-protection. The cluster includes gliA (CADRE locus identifier: AFUA_ 6G09710), a gene which is predicted to encode a transmembrane gliotoxin efflux pump, which is a member of the Major Facilitator Superfamily (MFS). The role of gliA in the biosynthesis and secretion of gliotoxin by A. fumigatus is unknown, however a previous study in which the sirA gene, an ortholog of gliA, from Leptosphaeria maculans was deleted, resulted in increased sensitivity to both gliotoxin and sirodesmin, and also an increase the secretion of sirodesmin from L. maculans. Deletion of gliA was undertaken in A. fumigatus ATCC26933, previously shown to produce gliotoxin at high levels, using a split marker strategy and acquisition of pyrithiamine resistance. It was also observed that A. fumigatus ATCC26933 secretes bis-methyl gliotoxin (BmGT), a derivative of gliotoxin where both thiols are methylated. Both Southern and qRT-PCR analysis confirmed deletion of gliA and absence of gliA expression in A. fumigatus gliA26933, respectively. Deletion of gliA completely abolished gliotoxin secretion, as determined by both RP-HPLC and LC-MS analysis, compared to that from A. fumigatus ATCC26933. Interestingly, secretion of the gliotoxin derivative, BmGT was not inhibited, indeed, there was a significant increase in the levels of BmGT secreted by A. fumigatus gliA26933 compared to wild type between 48-96 h growth ($p < 0.001$). A. fumigatus gliA26933 also exhibited significant sensitivity to 5 ¼g/ml exogenous gliotoxin at both 24 ($p < 0.005$) and 48 h ($p < 0.001$) growth. However, determination of the gliotoxin uptake rate revealed no significant difference between that of ATCC26933 and �gliA ($p = 0.3$). These results strongly suggest a role for gliA in the secretion of endogenously produced gliotoxin, but not bis-methyl gliotoxin, and that gliA functionality is necessary to protect against exogenous gliotoxin. Finally, we speculate that gliotoxin may undergo bis-methylation, if the secretion pathway is inhibited, but that this process does not facilitate self-protection.

048 NOVEL FUNGAL NITROSOTHIONEIN PEPTIDE INVOLVED IN TOLERANCE TO NITRIC OXIDE

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Nitric oxide (NO) is one of the best-characterized reactive nitrogen species (RNS), and it is ubiquitous in biological systems. The production of NO at the pathophysiological level, like reactive oxygen species, damages cellular transition metals, lipids, thiols and other cellular compounds, interferes with their functions, and thus causes nitrosative stress in cells. Fungi can persist in both naturally and artificially high RNS concentrations. However, the foundation of the fungal response mechanisms to RNS remains unknown. Here, we identified a novel ntpA gene that conferred growth tolerance upon A.nidulans against exogenous NO. The gene encoded a cysteine-rich 23-amino acid peptide that reacted with NO and S-nitrosoglutathione to generate an S-nitrosated peptide. Disrupting ntpA increased cellular S-nitrosothiol levels and NO susceptibility. Thioredoxin and its reductase (Trx/TrxR) denitrosated the S-nitrosated peptide back to the thiolate-form in vitro. Studies using gene disruptants indicated that Trx/TrxR decreased cellular S-nitrosothiol, and conferred tolerance against NO. These results indicated peptide-mediated catalytic NO removal. The peptide is similar to N-terminal beta domain of metallothioneins and binds copper(I) in vitro, but is dispensable for metal tolerance unlike metallothionein. Nitric oxide but not metal ions induced production of the peptide and ntpA transcripts. We discovered that the thionein family of peptides has NO-related functions and propose that the novel peptide be named NO-inducible nitrosothionein (iNT). The ubiquitous distribution of iNT-like polypeptides constitutes a potent NO-detoxifying mechanism that is conserved among various organisms.

049 NOVEL TREHALOSE-BASED OLIGOSACCHARIDES FROM EXTREME STRESS-TOLERANT ASCOSPORES OF NEOSARTORYA FISCHERI (ASPERGILLUS FISCHERI)

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Ascospores of Neosartorya, Byssochlamys and Talaromyces can be regarded as the most stress-resistant eukaryotic cells. For example, they can survive exposure at temperatures as high as 85 C for 100 min or more. Here we describe the identification and characterization of novel trehalose-based oligosaccharides (TOS) as compatible solutes that are accumulated to high levels in ascospores of the fungus Neosartorya fischeri. These compounds are also found in other members belonging to the genus Neosartorya and occur in other genera within the order Eurotiales that also include Byssochlamys and Talaromyces. These oligosaccharides consist of a trehalose backbone with one, two or three glucose molecules attached via an α-1,6 linkage. The tetra- and pentasaccharide, dubbed neosartose and fischerose, respectively, have not been reported in nature before. Neosartorya fischeri ascospores that contain TOS and trehalose are more viscous and more resistant to the combined stress of heat and desiccation than the ascospores of T. macrosporus that contain predominantly trehalose. TOS glasses have a higher glass transition temperature (Tg) than trehalose, and they form a more stable glass with crystallizing molecules, such as mannitol. Our data indicate that TOS are important for prolonged stabilization of cells against stress.

050 ON THE BIOSYNTHESIS AND FUNCTION OF THE ANTIOXIDANT ERGOTHIONEINE IN ASPERGILLUS FUMIGATUS

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Ergothionine (EGT; 2-mercaptohistidine trimethylbetaine) is a trimethylated and sulphurised histidine derivative which exhibits antioxidant properties due to its ability to exist in both thiol and thione forms. EGT production has been recently demonstrated in Aspergillus fumigatus and its biosynthesis was also found to be significantly elevated in a mutant (ΔgliK), deficient in gliotoxin biosynthesis. It was therefore hypothesised that the production of these two molecules may be related. Indeed, our analysis has revealed that EGT production in A. fumigatus is inversely proportional to that of gliotoxin in A. fumigatus strains ATCC26933 and ATCC46645, where high gliotoxin production coincides with low ergothionine levels, and vice versa. Label-free quantitative proteomic analysis has further revealed a log2 2.25-fold increase in abundance of a putative EGT biosynthetic enzyme (11 % sequence coverage) in A. fumigatus ΔgliK, compared to ATCC46645. To further investigate EGT biosynthesis, the predicted ergothionine biosynthesis gene, egt1, (2.9 kb; containing 6 introns) was identified by bioinformatic analysis, and deleted from A. fumigatus ATCC26933 via a split marker strategy. Deletion was confirmed by Southern analysis and absence of expression demonstrated via RT-PCR. Pre-column derivatisation of EGT using 5'iodoacetamidofluorescein (5-IAF) and subsequent RP-HPLC, plus high resolution LC-MS analysis confirmed that A. fumigatus Δegt1, was unable to produce ergothionine, confirming its role in ergothionine biosynthesis. A. fumigatus Δegt1 could still synthesise gliotoxin, however the level of gliotoxin production was found to be significantly reduced ($p > 0.001$), suggesting that high level gliotoxin production is dependent on EGT presence. A. fumigatus Δegt1 was also found to be sensitive to 3 mM hydrogen peroxide pared to wild type ($p > 0.001$), however no sensitivity was observed at lower hydrogen peroxide concentrations. This suggests that EGT may act as an auxiliary antioxidant, the presence of which is necessary to adapt to growth at higher levels of oxidative stress. As EGT appears to be involved in protecting against oxidative damage, we speculate if gliotoxin production is dependent on cellular redox homeostasis, whereby absence of EGT could result in sub-optimal redox conditions for gliotoxin production and secretion.

051 OXYLIPINS QUANTIFICATION IN FUSARIUM VERTICILLIOIDES IN VITRO AND DURING ITS INTERACTION WITH MAIZE KERNELS

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Fusarium verticillioides is one of the most important fungal pathogens to cause ear and stalk rot in maize, even if frequently asymptomatic and producing an harmful series of compounds named fumonisins. Plant and fungal oxylipins play a crucial role in determining the outcome of the interaction between the pathogen and its host. Moreover, as known, oxylipins result as signals able to modulate the secondary metabolism in fungi.
In relation to this, we design a novel quantitative LC-MS/MS method for quantifying up to 25 different oxylipins and their own precursors linoleic and linolenic acid produced by F. verticillioides and Zea mays kernels during their interaction. By applying this method we were able to analyze the trend of oxylipin production both in vitro – F. verticillioides grown into Czapek-Dox/Yeast extract medium amended with 0.2% of cracked maize – and in vivo - F. verticillioides inoculated in maize ears.
Results pinpoint the role played by oxylipins during plant-pathogen interaction and, further, this method, setting up an important pathosystem - the mycotoxins producer F. verticillioides with one of the largest cereal crop, i.e. maize -, could represent a novel tool for uncovering the eventual role of specific oxylipins in plant-pathogen interactions.

052 POLYGALACTURONASE PROFILE OF THE TOMATO WILT PATHOGEN F. OXYSPORUM F.SP. LYCOPERSICI

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Fungal plant pathogens secrete an array of enzymes capable of degrading the different components of the plant cell wall. Particular attention has focused on enzymes that depolymerize pectin, a major component of the primary plant cell wall. Among these, endo-α 1,4-polygalacturonases (endoPGs) have received most of the attention, due to their massive induction by pectic substrates and their ability to macerate plant tissues. In most cases, however, inactivation of individual endoPG genes by targeted disruption had no apparent effect on virulence, probably due to the presence of additional PG genes. In addition to endoPGs, exo-α 1,4-polygalacturonases (exoPGs) have also been reported from plant-pathogenic fungi since they degrade elicitor-active oligogalacturonides released by endoPGs and are generally not inhibited by plant polygalacturonase-inhibiting proteins (PGIPs). The tomato vascular wilt pathogen, Fusarium oxysporum f.sp. lycopersici, secretes a wide number of pectinolytic enzymes. In order to determine their role in virulence, in vitro and in planta transcription profile of ten different F. oxysporum predicted PG encoding genes was studied by qRT-PCR. Assays after induction with pectin or during tomato plant colonization determined that all identified PGs showed basal levels of expression, but only pg1 and pg5 (endo-) and pgx4 and pgx6 (exo-) showed significant relative expression, over 10% of actin. The in planta expression pattern differed between the different endo- and exo-PGs throughout the colonization of the host plant. We have generated newly targeted pg1 and pgx6 null mutants whose characterization would provide further information on the role of these genes during infection of tomato plants by F. oxysporum.

053 PRNB, THE PROLINE TRANSPORTER OF ASPERGILLUS NIDULANS: A PARADIGM OF THE SUBSTRATE TRANSLOCATION MECHANISM OF FUNGAL AMINO ACID TRANSPORTERS

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The PrnB proline transporter of the filamentous fungus Aspergillus nidulans is a well characterized member of the YAT family and the APC superfamily. PrnB has been previously used as a model for the regulation of gene expression by carbon and nitrogen availability. Regarding the structure-function relationships of amino acid transporters, a sensitive homology threading approach, using the crystal structure of LeuT (a bacterial leucine transporter), has produced a 3D model of the PrnB structure. This structure, consisting of two intertwined, antiparallel V-shaped repeating units (TMDs1-5 and TMDs 6-10) connected by a relatively long loop (21-29 residues long), followed by two additional helices (TMDs11 and 12), is commonly found in many transporter families, including APC and NCS1. In the crystal structures of LeuT and AdiC, TMDs 1, 3, 6 and 8 seem to contribute to substrate binding. Mutations in TMDs1 and 6 of PrnB have previously suggested their involvement in substrate translocation. In this study, in order to elucidate the mechanism of YAT function, using in vitro site-directed mutagenesis, direct radiolabelled proline uptake measurements and competition assays of selected proline analogues, we confirm that residues G56, T57 and G58 of TMD1 contribute to substrate binding and also identify residues in TMD 3 (E138), TMD 6 (F248, F250, F252, S253, E255) and TMD 8 (W351) that are necessary for the functionality of PrnB and/or the determination of its kinetic characteristics and specificity profile. Moreover, in a fully functional PrnB allele devoid of Cys residues, we apply cysteine scanning mutagenesis in selected residues, in order to further confirm their proximity to the substrate binding pocket. These studies, currently being combined with substrate docking and molecular dynamics simulations, will lead to a better understanding of the translocation mechanism of eukaryotic amino acid transporters.
Acknowledgment This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) – Research Funding Program: THALES, Investing in knowledge society through the European Social Fund.

054 PROPERTIES OF PROTEIN LAYERS OF THE CERATO-PLATANIN PROTEIN EPL1 AND ANALYSIS OF CERATO-PLATANIN GENE KNOCKOUT STRAINS IN TRICHODERMA SPP

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Members of the cerato-platanin family are small, secreted proteins, with four conserved cysteines. These proteins are exclusively found in filamentous fungi. They appear to be easily recognized by other organisms and therefore they are associated with the induction of defence responses in plants and allergic reactions in humans. However, these proteins are also found in non-pathogenic fungi and are strongly conserved throughout the fungal kingdom. Proteins from the cerato-platanin family are important for the Trichoderma-plant interaction, but the primary function of this protein family in fungal growth and development has not been elucidated yet.
We studied the transcriptional profiles of three genes encoding cerato-platanin proteins (epl1/2/3 and sm1/2/3, respectively) in the mycoparasites and plant-beneficial fungi Trichoderma atroviride and T. virens. Further, the phenotypes of gene knockout strains were analyzed with respect to fungal growth and development as well as in Trichoderma-plant interactions, revealing different alterations of the induction plant-defense responses in sm1/epl1 and sm2/epl2 knockout strains.
Biochemical analysis of T. atroviride EPL1 showed that it has a dual functionality. On one hand it is a chitin-binding protein, as we could show previously for EPL1 and has also already been reported for other cerato-platanin proteins, but on the other hand EPL1 self-assembles into protein layers at air/water interfaces. High resolution imaging of the protein layers with AFM (atomic force microscopy) on surfaces with different hydrophobicity showed that at EPL1 forms not only protein layers at air/water interfaces but also biofilms with a highly regular pattern at solid/liquid interfaces. Further, it interferes with hydrophobin layers, strongly altering their hydrophobic/hydrophilic properties.

055 RECONSTRUCTION OF THE TR OPOLONE STIPITATIC ACID BIOSYNTHETIC PATHWAY IN ASPERGILLUS ORYZAE

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Tropolones are a group of chemicals that are defined as containing a ring of seven carbon atoms with an adjoined ketone group. How the seven-membered ring is synthesised remained unknown for over 70 years from its first description; the puzzle was eventually solved by the Polyketide Group at the University of Bristol, starting from the discovery of the mechanism by which Acremonium strictum produces 3-methylorcinaldehyde, which was expected to be the main precursor of tropolones. This compound is produced by methylorcinaldehyde synthase (MOS), a non-reducing polyketide synthase. The function of a putative mos gene identified in the genome sequence of the stipitatic acid producer Talaromyces stipitatus was confirmed by gene knock out and the gene was named tropA. Knock out of the genes surrounding tropA indicated the next steps in the stipitatic acid biosynthetic pathway, and these genes (trop B, C and D) were then expressed in E. coli for in vitro feeding experiments. While tropD protein proved to be insoluble and refractory to study in vitro, the results showed that tropA, B and C were needed to produce stipitaldehyde, the first effective tropolone in the biosynthetic pathway.
The approach adopted to elucidate the whole biosynthetic pathway of stipitatic acid in T. stipitatus involves heterologous expression of the genes in the cluster in Aspergillus oryzae. A multigene expression vector was constructed for co-expression of tropA, B, C and D in A. oryzae, but no tropolone was detected in organic extracts of fungal transformants. The precursors 3-methylorcinaldehyde (product of tropA activity) and anthranilic acid (product of tropA plus tropB activity) were found, indicating that tropC was not functioning as expected. qRT-PCR confirmed that tropC was transcribed at a high level and DNA sequencing confirmed the integrity of the gene. The possibility that the tropC enzyme and its anthranilic acid substrate are located in separate intracellular compartments is being investigated by co-expression with tsR2, which encodes a membrane transporter protein.

056 SECONDARY METABOLITE GENE CLUSTERS IN CLAVICEPS PURPUREA

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The phytopathogenic ascomycete Claviceps purpurea is an important food contaminant as it infects a broad range of grasses including economically important cereal crop plants. The toxic ergot alkaloids produced in the sclerotia, the survival structure of the fungus, are the best characterized secondary metabolites of C. purpurea (Haarmann et al. 2009). Apart from that, little is known about the secondary metabolism of the fungus and not all toxic substances going along with the food contamination with Claviceps are known yet.
The availability of the C. purpurea genome sequence allowed a bioinformatical screening approach for typical secondary metabolite key enzymes which revealed that the genome encodes 9 polyketide synthases (PKSs) and 18 nonribosomal peptide synthetases (NRPSs) (Schardl et al. 2013). This shows the great potential of C. purpurea for producing secondary metabolites. However, many of the NRPSs and PKSs genes are not expressed under the tested standard laboratory conditions or in planta in the late stages of infection (sclerotia) on rye. As an exception one PKS gene is highly expressed in the sclerotia and first results indicate that this cluster is responsible for formation of the red anthraquinone ergot pigments. Another interesting cluster is probably responsible for formation of an epipolythiodioxopiperazine (ETP) toxin, characterized by an internal disulphide bridge. Overexpression of the cluster specific transcription factor leads to an increased expression of the other cluster genes. Product analysis of this mutant strain using mass spectrometry is ongoing and preliminary results reveal that this cluster is responsible for the synthesis of a so far unknown ETP.

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057 SECONDARY METABOLITES AND THEIR REGULATION IN TRICHODERMA REESEI

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Changing light conditions, caused by the rotation of earth resulting in day and night or growth on the surface or within a substrate, result in considerably altered physiological processes in fungi. For the biotechnological workhorse Trichoderma reesei (syn. Hypocrea jecorina), regulation of glycoside hydrolase gene expression, especially cellulase expression was shown to be a target of light dependent gene regulation. Investigation of genes regulated in response to light in T. reesei and their distribution within the genome revealed several genomic clusters. As the transcription factor within one of these clusters (the LCS cluster) is assumed to play a role in its activation, we investigated this gene now designated msm1 (modulator of secondary metabolism 1) in more detail. Microarray analysis of T. reesei wild-type and recombinant strains under different conditions in light and darkness indicates that msm1 is regulated in response to light and nutrient signals as mediated by the respective signaling cascades.
High performance thin layer chromatography of supernatants of several strains in light and darkness clearly supported this hypothesis. In order to confirm the regulation of secondary metabolite production in dependence of light and by MSM1, a mass spectrometric screening analysis for known mycotoxins was performed. This analysis showed several compounds to be regulated by light and by MSM1, including already known secondary metabolites of T. reesei. We conclude that MSM1 is an important light dependent regulator of secondary metabolism.

058 SECRETION PATHWAY ENGINEERING OF ASPERGILLUS NIDULANS

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Recombinant protein production is a multi-billion dollar market and most biopharmaceuticals today are produced from a recombinant host. Therefore, there is a huge demand for optimizing productivity and yields in producing bioactive compounds such as enzymes, antibodies and other biopharmaceuticals from recombinant hosts. To facilitate downstream processing, products are preferably secreted out of the cell. The Aspergillus spp. are well known for their ability to secrete proteins at high levels and are therefore commonly applied as cell factories for heterologous protein production. Research in filamentous fungi is moving in to a systems biology based mode, where more genome sequences are becoming available and transcriptome analyses are more frequently pursued in these hosts. Together with better tools for genetic engineering, this enables exciting new studies that previously have been predestined to be performed in easier model organisms such as S. cerevisiae. For example, enzyme secretion is now being extensively studied in Aspergillus spp., with detailed characterization of how protein secretion is affecting the host cell. Studies have mainly focussed on assigning function to genes involved in the secretory pathway by investigating deletion mutants. Overexpressing genes involved in the secretion pathway of filamentous fungi has not yet been extensively tested. Thus, metabolic engineering of the secretory pathway in filamentous fungi is a new and interesting way of engineering protein secretion in these organisms. The aim of this project is to investigate the protein secretion machinery in Aspergillus spp., with Aspergillus nidulans as a model organism, through manipulation of key genes in order to increase the capacity of the secretory pathway. Overexpression of secretory transport genes has been performed in order to increase the transport of three recombinant model proteins: Beta-glucosidase and cellobiohydrolase from Trichoderma reesei, and red fluorescent protein mRFP1. Targets were based on a number of transcriptomic studies performed in fungal hosts under secretion stress. The mutants were analysed with respect to recombinant protein secretion, total amount of protein secreted, biomass generation and phenotype on plates. This will give new insights into how Aspergillus can be further improved for production of recombinant proteins.

059 STUDIES ON FUNCTIONAL DIVERGENCE BETWEEN SACCHAROMYCES CEREVISIAE ALT1 AND ALT2 USING KLUYVEROMYCES LACTIS KLALT1 AND LACHANCEA KLUYVERI LKALT1 AS "ANCESTRAL TYPE YEAST"

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Gene duplication has a relevant role in evolution, since diversification of paralogous genes allow the emergence of new or specialized functions from the preexisting ones. Saccharomyces cerevisiae experimented whole genome duplication (WGD) about 100 millon years ago. Comparisons between Saccharomyces and Kluyveromyces linages suggest that the last linage diverged after whole genome duplication of Saccharomyces. It is thus possible to consider that Kluyveroyces physiology its more similar to the ancestor which did not underwent duplication and gave rise Kluyveromyces lactis and Lachancea kluyveri linages, these is why both are considered as “ancestral type yeasts”. Aminotransferases constitute an interesting model to study diversification of paralogous genes since aminotransferases constitute biosynthetic and catabolic pathways whose opposed action relies on a single catalytic site. ALT1 and ALT2 are two paralogous genes present in S. cerevisiae genome. These paralogs encode 65% identical proteins. Previous results showed that only Alt1 displays alanine aminotransferase activity. Alt1 its localized in the mitochondria and Alt2 in the citosol . ALT1 is alanine-induced showing an expression profile of a gene encoding an enzyme involved in amino acid catabolism, conversely, ALT2 expression is alanine-repressed, indicating a role in alanine biosynthesis, although the encoded-protein has no alanine aminotransferase enzymatic activity. Since a double alt1Δ alt2Δ mutant is not an alanine auxotroph it can be concluded that there exists a yet unidentified alternative pathway for the alanine biosynthesis. K. lactis and L. kluyveri only have one ALT1-ALT2 ortholog, identified by sequence homology and sinteny: KlALT1 and LkALT1 respectively. Through the characterization of the null mutant of the orthologous genes, we discovered that KlALT1 and SkALT1 are the principal pathways of alanine biosynthesis and catabolism, in each one of these yeasts, although in both cases an alternative alanine biosynthetic and catabolic pathway is present, although it has not been identified yet. Purification and kinetic analysis of KlAlt1 and LkALt1 shows that both display alanine aminotransferase activity, suggesting that the biosynthetic and catabolic capacity displayed by alanine aminotransferases has been exclusively delegated to Alt1. The characterization of KlALT1 and LkALT1 and their encoded products will allow the proposition of a model for the divergence of ALT1 and ALT2.

060 THE ASPERGILLUS NIGER CHRA PROTEIN CONFERS CHROMATE SENSITIVITY PARTICIPATING AS A CHROMATE TRANSPORTER

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The widespread use of chromium in industrial processes has become a serious pollution problem, which has caused contamination of air, soil and water. Hexavalent Cr, in the form of chromate (CrO42-) or dichromate (Cr2O72-) oxyanions is considered the most toxic form of chromium, which actively enters biological membranes by means of sulfate uptake pathways in a variety of cells, reflecting the chemical analogy between these two oxyanions. The ChrA membrane protein belongs to the CHR superfamily of chromate ion transporters, which includes homologues from bacteria, archaea and eukaryotes. Bacterial ChrA homologues confer chromate resistance by exporting chromate ions from the cell’s cytoplasm. It has been shown that in the genome of filamentous fungi, but not in yeast genomes, there are homologous genes that encode ChrA proteins. In this work we demonstrated the absence of the chrA gene in the chromate-tolerant environmental strain Ed8 of Aspergillus tubingensis, isolated from a Cr(VI)-contaminated site; reference strains FGSC A732 of A. niger and NRRL593 of A. tubingensis showed the presence of the chrA gene and it was observed they show increased expression of the gene in the presence of Cr(VI). With the purpose to determine the function of the ChrA protein in A. niger, the chrA gene from the reference strain FGSC A732 of A. niger was cloned in an expression vector and the construct was introduced into the genome of strain Ed8 of A. tubingensis. Transformant LIVG6, expressing the chrA gene, was further characterized; LIVG6 showed decreased chromate tolerance, exhibiting an oxyanion sensitivity similar to that observed in the reference strains of A. niger and A. tubingensis. In addition, Cr uptake assays in cultures incubated with Cr(VI) revealed that chromium incorporation in the biomass was higher in transformant strain LIVG6 and in the A. niger and A. tubingensis reference strains, as that observed in strain Ed8 of A. tubingensis. These results indicate that the ChrA protein from Aspergillus strains functions as a cromate transporter, incorporating chromium into the cells.

061 THE EHRLICH PATHWAY IS RESPONSIBLE FOR 2-PHENYLETHANOL PRODUCTION IN ASHBYA GOSSYPII

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Fermented food and beverages are defined by unique flavor fingerprints. Most of the volatiles are derived from fungal amino acids catabolism via the Ehrlich pathway. This enzymatic pathway catalyzes the conversion of amino acids into fusel alcohols or fusel acids. In this work we focused our attention on two Eremothecium species, the riboflavin overproducer Ashbya gossypii, which also produces a very aromatic flavor and its close relative Eremothecium cymbalariae, which appears to be much less aromatic. These closely related species showed different aroma profiles by GC/MS. Both produce large amounts of isoamyl alcohol while A. gossypii also produces high levels of 2-phenyl ethanol. Comparative genomic analysis showed the absence of key genes involved in the aromatic amino acid catabolism in E. cymbalariae. We present the functional analysis of key genes of the Ehrlich pathway in A. gossypii, namely ARO8a, ARO8b, ARO10 and ARO80. Deletion of any one component resulted in a strong reduction of flavor production as determined by GC/MS but no growth defects. Additionally, the overexpression of ARO80, the main transcriptional regulator of the pathway, showed a 50% increase in flavor production, especially in isoamyl alcohol, a banana-like flavor. Our data indicate the potential of strains within the yeast biodiversity as novel producers of natural flavors.

062 THE FIRST RIBOSOMAL PEPTIDE SYNTHASE PATHWAY IN FILAMENTOUS FUNGI

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Ustiloxin B, a fungal secondary metabolite, was found to be produced by Aspergillus flavus, and the entire biosynthetic gene cluster was identified by the disruption of genes predicted from DNA microarray data and the subsequent analysis of the ustiloxin B-deficient deletants using LC-MS. The gene cluster was composed of at least 16 genes, including those encoding a fungal type C6 transcription factor, a cytochrome P450, and a major facilitator superfamily transporter. We constructed an overexpression strain of the gene encoding the C6 transcription factor (ustR), which led to a five-fold overproduction of ustiloxin B in the transformant. The following sequence analysis of the cluster revealed that the translated product of ustA (AFLA_094980), UstA, contains a 16-fold repeated peptide containing a tetrapeptide, Tyr-Ala-Ile-Gly, that is converted into the cyclic moiety of ustiloxin B. This result strongly suggests that ustiloxin B is biosynthesized through a ribosomal peptide synthase (RiPS) pathway, and that UstA provides the precursor peptide of the compound. This pathway is the first example of RiPS in Ascomycetes. Moreover, this is the first report of a complete RiPS gene cluster in fungi. Our finding indicates the possibility that a number of unidentified RiPS exist in Ascomycetes as the biosynthetic genes of secondary metabolites, and that the feature of a highly repeated peptide sequence in UstA will greatly contribute to the discovery of additional RiPS.

063 THE INFLUENCE OF REGULATORY AND METABOLIC MUTATIONS ON GROWTH OF TRICHODERMA REESEI ON PLANT BIOMASS RELATED CARBON SOURCES

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Many regulators and pathways are involved in fungal growth on plant biomass. In this study we have analysed regulatory and metabolic mutants of Trichoderma reesei (Hypocrea jecorina) with respect to growth on pure and complex carbon sources.
Growth and enzyme production of wild-type and knock-out strains were compared on 33 different plant biomass related carbon sources, ranging from monosaccharides to crude plant biomass. This data helps to understand the biotope specificity of this fungus. Differences in growth on carbon sources suggest which metabolic pathways and regulators are important for the utilization of the different substrates. Highlights from this study will be presented.

064 THE METAL TRANSPORTER FETD IS INVOLVED IN LOW-AFFINITY IRON UPTAKE IN A. FUMIGATUS

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Iron is an essential nutrient for all eukaryotes. Iron uptake has been investigated extensively in various fungi including the opportunistic human pathogen Aspergillus fumigatus. To satisfy its cellular iron demand, A. fumigatus employs three main iron uptake mechanisms. When iron is scarce iron import is mediated mainly by two high-affinity systems, reductive iron assimilation and siderophore-assisted transport. At high iron concentrations (a) so far uncharacterized low-affinity transport system contributes to supply the need for iron. In this study we identified a protein, termed FetD, which is part of the low-affinity system. fetD transcription is activated at harsh iron starvation and iron excess while lack of fetD in wt (ΔfetD) results in decreased cellular iron content. Nevertheless, fetD deletion had no effect on A. fumigatus growth rate, which demonstrates that the two high-affinity systems can compensate fetD deficiency. However, deletion of fetD in a strain lacking both high-affinity systems (ΔsidAΔftrAΔfetD) results in severe growth reduction, especially under hypoxic conditions. Taken together, our data show that FetD is involved in low-affinity uptake. Moreover, FetD activity is of major importance during hypoxic conditions.

065 THE NOVEL SENSOR-GLOBIN FUNGOGLOBIN IS INVOLVED IN LOW OXYGEN ADAPTATION OF ASPERGILLUS FUMIGATUS

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Infection with conidia of the pathogenic fungus Aspergillus fumigatus is a frequent health threat for immunocompromised human individuals. While classic virulence factors have not been identified, it has become increasingly clear that its physiological versatility as a saprophyte may largely contribute to the establishment of invasive growth. Low oxygen partial pressures is a property which transiently occurs in most natural growth environments, but also defines deep layers of infected human tissue. A. fumigatus survives and prospers in such hypoxic areas and, as other fungi, exploits ergosterol biosynthesis as an essential measure for oxygen. However, the direct metabolic and energetic consequences of low O2 availability are less understood, and we hypothesized that the fungus could also sense and react to O2 directly. In a first approach, we used Next Generation Sequencing to study the dynamic and short term response to a transient exposure to low O2. Deprivation of O2 triggered a more than threefold induction of 680 genes after only 15 min while 420 genes were down regulated at the same time point. Among the highest upregulated genes we identified a gene encoding a hypothetical protein which appears to be conserved in filamentous fungi. In sharp contrast, reoxygenation of the growth medium resulted in the complete repression of its mRNA. This transcriptional dynamics was verified by Northern Hybridization, and interestingly, hypoxic induction was also observed in an srbA deletion mutant. Low iron also induced its expression, but in a HapX-independent mode, indicating that this gene is not under direct control of either of these two regulators. The encoded protein comprised a globin-like N-terminal domain and was identified as a member of the large protein family of sensor globins. The function of these proteins is largely unknown but heterologous expression and purification gave evidence for a functional heme binding site. Furthermore, the deletion of the gene led to an impaired growth of A. fumigatus in low oxygen atmospheres and hence, the putative role of this "fungoglobin" during such conditions will be discussed.

066 THE TOXICOGENIC POTENTIAL OF FUSARIUM FUNGI SPECIES

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Research of fungi toxin-producing ability has both scientific and practical value for forecast and detection of micromycets and their metabolites effect.
The aim of our investigation was to study the toxicogenic capacity of Fusarium sporotrichioides species isolated in various regions.
In the research the isolates of Fusarium sporotrichioides species isolated in various years from Russian and Kazakhstan regions where mass mycotoxicoses and crop contamination with T-2 toxin at concentration from 0,063 to 0,814 mg/kg were previously reported. Toxin-producing rate was evaluated by inoculation into sterile substrate and stationary cultivation (various options on cultivation time and temperature).
Results. For active toxin production Fusarium sporotrichioides species isolated from areas with mycotoxicoses outbreak reports require lower temperature modes. For the only isolate from imported soya (USA) low temperature did not promoted toxin production during cultivation on the contrary, toxin active production was observed at quite high temperature rates, 260C. The highest activity was shown by an isolate from Kazakhstan where mass destruction of saiga antelopes (more than 1 million herds) because of mycotoxins was reported. This isolate produced T-2 toxin up to 422,70 mg/kg of substrate. The isolates selected as perspective toxin producers were mutated by irradiation to improve their metabolic activity. A series of irradiation was done along with colony selection, toxin production rate was managed to increase up to 7,2 g/kg substrate, however, mutants showed poor growth and somatic instability.
Therefore, the isolated species had high toxicogenic capacity and the revealed difference in toxin production rate depending on substrate and cultivation rate can explain the cases of mycotoxicoses spontaneous outbreaks. Defining of toxin production laws depending on fungi species and strains will allow an efficient prediction of mycotoxicoses cases and their detection.

067 TOWARDS RECONSTITUTION OF THE BIOSYNTHETIC PATHWAY FOR THE BASIDIOMYCETE ANTIBIOTIC PLEUROMUTILIN IN ASPERGILLUS ORYZAE

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Pleuromutilin is an antibiotic that is naturally produced as a secondary metabolite by the basidiomycete fungus Clitopilus passeckerianus. Pleuromutilin, a tricyclic diterpene, has been exploited as a precursor for many semi-synthetic antibiotics, some of which are already available on the market, with others under development or study in clinical trials. Key features for Pleuromutilin antibiotics are their recognized activity against the severe human pathogen MRSA (Methicillin-Resistant Staphylococcus aureus) and their specific mode of action, which slows development of resistance in bacteria. However Pleuromutilin is produced by C. passeckerianus in low amounts, strain improvement has been problematic, and this fungal species is difficult to cultivate in large-scale fermenters.
In order to overcome these issues reconstruction of the Pleuromutilin biosynthetic pathway in Aspergillus oryzae will be undertaken, through heterologous expression of the Pleuromutilin gene cluster from C. passeckerianus. Furthermore, a step-by-step transformation approach is being adopted and will help to shed light on the biosynthetic processes that lead to the production of Pleuromutilin, through isolation of the intermediate compounds produced along the pathway. All the genes of the Pleuromutilin gene cluster have been cloned in the form of their cDNA and specific clones selected in order to be introduced in A. oryzae. Multigene expression vectors have been built for these genes, by placing their coding sequences under control of promoters that can lead to high levels of expression in A. oryzae. Several genes of the Pleuromutilin gene cluster have been successfully introduced in A. oryzae and their expression has been confirmed by the mean of RT-qPCR. Metabolomic analyses, through LC-MS, are being conducted on these transformant strains in order to detect production of the predicted intermediates of the Pleuromutilin biosynthetic pathway. We will present data from this ongoing study linking the biosynthetic genes to their metabolites. The ultimate goal is to provide an alternative method for biosynthesis of the antibiotic and allow increased understanding of how Pleuromutilin is produced.

068 TRANCRIPTIONAL LANDSCAPE AT BREAKING OF DORMANCY AND EFFECT OF D-GLUCOSE ANALOGUES ON GERMINATION IN ASPERGILLUS NIGER

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Fungal conidia are reproductive structures that are important for both dispersal and survival within harsh environments. Conidial germination is a key factor in the infection of target organisms by pathogenic fungi as well as in the spoilage of food. It is initiated by conidial swelling and mobilization of internal carbon and energy stores, followed by polarization and emergence of a hyphal germ tube. Genome-wide analysis was performed to assess the transcriptional landscape of germinating Aspergillus niger conidia using both next generation RNA-sequencing and GeneChips. Conidial swelling requires a germination trigger, which is sensed by the conidium and leads to catabolism of D-trehalose and isotropic growth. The effects of D-glucose analogues on the germination of A. niger conidia were explored and metabolism of storage compounds was also examined. The transcriptome of dormant conidia was shown to be highly differentiated from that of germinating conidia and major changes in response to environmental shift occurred within the first hour of germination. Dormant conidia contained transcripts of genes involved in fermentation, gluconeogenesis and the glyoxylate cycle. The presence of such transcripts in dormant conidia may indicate the generation of energy from non-carbohydrate substrates during starvation-induced conidiation or for maintenance purposes during dormancy. The breaking of dormancy was associated with increased transcript levels of genes involved in the biosynthesis of proteins, RNA turnover and respiratory metabolism. The immediate onset of metabolism of internal storage compounds after the onset of germination, and the presence of transcripts of relevant genes, suggest that conidia are primed for the onset of germination. The transcriptome of dormant conidia contained a significant component of antisense transcripts and they were represented in higher abundance than in germinating conidia. Antisense transcription was also evident during early germination suggesting that antisense transcripts participate in the regulation of changing functionalities at this critical period of conidial outgrowth. Analysis of D-glucose analogues showed they are involved in two separate events in germination, triggering and subsequent outgrowth. Certain sugars triggered germination and outgrowth, other sugars triggered germination but did not support outgrowth, and certain sugars supported outgrowth if added in the presence of a complementary triggering sugar.

069 TRANSCRIPTION ANALYSIS AND OVEREXPRESSION OF THE TERPENOID BIOSYNTHESIS PATHWAY GENES IN MUCOR CIRCINELLOIDES

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Mucor circinelloides is a carotenoid producing filamentous fungus, which has been used as a model organism in various genetic, biochemical and molecular studies. Terpenoids, such as prenyl groups of several signal proteins, membrane components, pigments and hormones/pheromones are synthesised via the mevalonate-isoprenoid pathway in fungi. These metabolites play an important role in cell differentiation, morphogenesis, adaptation to environmental changes, signal transduction, apoptotic processes, etc.

To date, little is known about the function and regulation of the mevalonate-isoprenoid pathway genes in zygomycetes. Therefore, our aim is to reveal the genetic and biochemical background of the terpenoid biosynthesis in M. circinelloides. The transcription of six terpenoid pathway genes, encoding the 3-hydroxy-3-methylglutaryl coenzyme A synthase, mevalonate kinase, diphospho-mevalonate decarboxylase, isopentenyl-pyrophosphate isomerase, farnesyl-pyrophosphate synthase and geranylgeranyl-pyrophosphate synthase, was analysed under different culturing conditions. Effect of cultivation time, light, salt stress, media, temperature, oxygen tension, and statin treatment on the transcript levels was also investigated. Autonomously replicating vectors, harbouring these genes under the control of own and Mucor glyceraldehyde-3-phosphate dehydrogenase (gpd1) promoter and terminal sequences were constructed. This promoter is very effective and can be induced by glucose. PEG/CaCl2-mediated protoplast transformation was used to elevate the copy number of these genes in M. circinelloides. Investigation of germination of spores, morphology, growth intensity and terpene production (e.g. carotenoid and ergosterol) of the resulting transformants are in progress.

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070 TRANSPORT PROCESSES IN THE PRODUCTION OF ORGANIC ACIDS BY ASPERGILLUS NIGER

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Especially dicarboxylic acids like itaconic, succinic and fumaric acid have a good potential as chemical building blocks for polymer synthesis. At present, production of these acids via a fermentative approach, e.g. using Aspergillus niger, is considered as a feasible alternative for the current petroleum-based synthesis.

The main aim of this project is to obtain leads for further improvement of A. niger as a cell factory for dicarboxylic acid production. Thereby we will focus on the identification and characterization of substrate (sugars) import and product (acids) export systems, as well as resolution of the mechanisms of these systems and their energetic efficiencies. Finally, with the obtained knowledge we expect to be able to increase the efficiency of organic acid production.

A first challenge has to do with A. niger cultivation under well-defined chemostat conditions. Especially in continuous culture fermentations, the filamentous growth-form poses problems due to the tendency of the organism to accumulate on the walls and probes of the fermentor, as well as accumulation near the medium inlet and in the outflow system. Therefore we developed a dedicated chemostat system, which could be operated under such conditions that biomass accumulation was avoided and proper steady state growth could be achieved.

After succeeding in reaching well defined metabolic steady states, we aimed at acquiring meaningful quantification of the A. niger endo- and exometabolome, thereby specifically focusing on metabolites relevant for the identification and characterization of the above mentioned transport processes. Accurate metabolite quantification requires rapid sampling and fast quenching of all metabolic activity, preventing changes in metabolite levels after sampling. Different methanol quenching concentrations and quenching temperatures were compared, and a leakage free quenching/washing protocol for accurate metabolite quantification in mycelium samples was established.

071 UNLOCKING THE LIGNIN DEGRADING POTENTIAL OF ASCOMYCETE FUNGI

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Lignin degradation involves several classes of oxidative enzymes, such as peroxidases and laccases. Traditionally, lignin degradation is considered to be an ability of mainly basidiomycete fungi, in particular white rot fungi. Many oxidative enzymes from these fungi have been characterised and genome analysis of white rot fungi demonstrated the presence of gene families encoding these enzymes. In contrast, ascomycete fungi are generally considered to be incapable of degrading lignin and their genomes lack (most of) the traditional lignin-related oxidases. However, many ascomycete fungi live in biotopes that contain high amounts of lignin (e.g. forest soils, leaf litter), suggesting at least a tolerance to the presence of aromatic compounds.
We have analysed several ascomycete fungi for their ability to grown on medium with lignin as the sole carbon source. Some species displayed good growth even on lignins that were unable to support growth of most other species, suggesting a lignin degrading ability for these fungi. In contrast, other species, such as the industrially relevant fungus Aspergillus niger, were unable to grow on these media. Using a novel approach we have isolated mutants from A. niger that were able to grow on lignin as the sole carbon source. This growth was similar or possibly even better than growth of the basidiomycete Phanerochaete chrysosporium. The genome of A. niger contains several laccases as well as genes encoding other oxidative enzymes, which may explain this phenotype. Highlights of these studies will be presented.

072 UNRAVELLING THE IN VIVO KINETICS OF THE PENICILLIN BIOSYNTHESIS PATHWAY

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In this study we combined experimentation with mathematical modeling to unravel the in vivo kinetic properties of the enzymes and transporters of the penicillin biosynthesis pathway of a high producing strain of Penicillium chrysogenum, with the aim to identify possible bottlenecks. We performed stimulus response experiments whereby, in a steady state carbon limited chemostat cultivation of P. chrysogenum, the penicillin pathway was perturbed by a stepwise increase of the concentration of the side-chain precursor for penicillin-G production, Phenylacetic acid (PAA). Subsequently, the rapid dynamic responses of all relevant intra- and extracellular metabolites were captured, using state of the art rapid sampling and quenching procedures as well as highly accurate Isotope Dilution Mass Spectrometry (IDMS) for metabolite quantification. The thus obtained dynamic data provide information about the in-vivo metabolic regulation of the pathway as well as metabolite transport.
The datasets obtained from these experiments were used to construct a kinetic model of the pathway, based on mechanistic, Michaelis-Menten type rate equations. The model included the formation of several by-products as well as the transport of substrates, intermediates, the product penicillin-G and by-products over the cytoplasmic membrane. Parameter estimation was carried out by fitting the model to the obtained dynamic metabolite patterns.
The obtained full kinetic model is capable of describing the flux through the penicillin biosynthetic pathway and predicting changes in the levels of intra- and extracellular metabolite concentrations as well as long term changes in enzyme levels of the pathway. Application of the model for metabolic control analysis revealed that both in the absence and presence of PAA, ACVS, the first enzyme of the pathway, is the main flux controlling enzyme in this P. chrysogenum strain.

073 VARIATION IN THE FUMONISIN BIOSYNTHETIC GENE CLUSTER IN FUMONISIN-PRODUCING AND NONPRODUCING STRAINS OF ASPERGILLUS NIGER AND A. WELWITSCHIAE

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Aspergillus niger and A. welwitschiae strains isolated from different food crops cultived in Mediterranean basin and American Countries were examined for fumonisin B2 (FB2) production and presence/absence of sequences within the fumonisin biosynthetic gene (fum) cluster. Presence of 13 regions in the fum cluster was evaluated by PCR assays, and confirmed for some strains by Southern hybridization analysis. The results of our study indicate that some strains of both species produce FB2 while other strains do not. In A. welwitschiae, all FB2-nonproducing strains appear to have a large deletion within the fum cluster between fum15 and fum6; the putative deleted region includes eight fum genes. In contrast, in FB2-nonproducing strains of A. niger, there is no evidence for such a lesion, suggesting that the lack of production in strains of this species is caused by a small lesion in one or more cluster genes or by a lesion in a regulatory gene(s) outside the cluster. Maximum Parsimony analysis using DNA sequence of the calmodulin gene indicates that the phylogenetic relationships of A. welwitschiae and A. niger strains does not correlate with the lesion between fum15 and fum6. The phylogenetic data also indicate that relationships between fum–gene sequences are not correlated with the ability or inability to produce FB2. The results of this study suggest that both A. welwitschiae and A. niger are being maintained as mixed populations of FB2-producing and nonproducing individuals. However, the genetic basis of FB2 nonproduction differs in these two closely related aspergilli suggesting that nonproduction has arisen independently in each species.

074 XANTHOPHYLLOMYCES DENDRORHOUS, A PLATFORM FOR GENETIC ENGINEERING OF ASTAXANTHIN SYNTHESIS AND OTHER ACETYL-COA DERIVED METABOLITES

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Astaxanthin is a high value carotenoid commercially used as feed additive in fish cultures. Only very few organisms are able to synthesize this keto carotenoid among which is Xanthophyllomyces dendrorhous (its asexual state (anamorph) is named Phaffia rhodozyma) as the only fungus. In order develop X. dendrorhous as a biological system and to increase astaxanthin formation towards an economically interesting yield, an approach combining chemical mutagenesis and genetic pathway engineering has been pursued. High astaxanthin mutants were screened for astaxanthin accumulation and for limiting reactions in the biosynthesis pathway [1]. Tandem integration vectors were constructed with different selection markers for multi transformation of carotenogenic genes into the genome of X. dendrorhous high astaxanthin mutants. We targeted the over-expression of the enzymes of limiting steps in the mevalonate pathway supplying carotenoid precursors, of the gateway enzyme for the carotenoid pathway and a limiting enzyme for carotenoid intermediate conversion into the end product astaxanthin. With this approach, it was possible to increase astaxanthin formation totally by 90-fold, 16-fold from wild type to chemical mutant and additionally 5.6-fold by pathway engineering. Finally a concentration of astaxathin in X. dendrorhous of 9 mg/g dry weight was reached in non-optimized laboratory cultures . In addition to high astaxanthin mutants, other mutants are available for the production of different di- and tetraterpenoids and other acetyl-CoA related compounds.

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075 A GPI-ANCHORED PROTEIN INTERACTS WITH THE STRIPAK PROTEIN SMMOB3 IN SORDARIA MACROSPORA

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GPI anchors are complex glycolipids posttranslationally linked to a conserved omega side at the C-terminus of proteins. The synthesis as well as the covalent attachment of the glycolipid anchor to a special group of proteins takes place in the ER. Until now many GPI-anchored proteins have been identified various in structure and functionality. The core structure of the glycolipid anchor comprises 3 mannose residues, ethanolamine, glucosamine and phosphor-inositol bound to fatty acids. GPI-anchored proteins are known to play a role in various processes, such as cell signaling, pathogenicity, immune response, and cancer. Here, we report about the interplay of a putatively secreted and GPI-anchored protein SmGPI1 with the putative kinase activator SmMOB3, a prominent member of the intracellular localized Striatin interacting phosphatase and kinase (STRIPAK) complex in Sordaria macrospora. First identified in a Y2H screen, the physical interaction of SmGPI1 and SmMOB3 was further proved by means of co-immunoprecipitation studies. In vivo localization of different GFP-tagged versions of SmGPI1 suggest that the protein is predominately secreted and attached to the cell wall. Deletion of Smgpi1 led to a reduction of the number of fruiting-body and a growth inhibition on medium supplemented with 1% KOH. However, the phenotype could be partially complemented by N- and C-terminally truncated versions of SmGPI1. Beside these findings a genetic interaction between Smmob3 and Smgpi1 has been observed. Deletion of Smgpi1 in a sterile ΔSmmob3 background restores fertility as well as the hyphal fusion defect of ΔSmmob3. This suppression effect occurred neither in combination with other STRIPAK deletion strains (Δpro11/ΔSmgpi1 and Δpro22/ΔSmgpi1) nor in the combination of ΔSmmob3 with the deletion of another gene coding for a GPI-anchored protein. Our results are highly contrary to the dogma saying GPI-anchored proteins are invariably bound to the cell surface.

076 A MONOOXIGENASE INVOLVED IN THE NICOTINIC ACID DEGRADATION PATHWAY IN ASPERGILLUS NIDULANS

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A number of prokaryotic nicotinic acid degradation routes have been elucidated. However, with the exception of the first step, the pathway leading to nicotinate degradation in eukaryotes is unknown. We have identified three genes (hxnX, hxnV, hxnM) encoding enzymes, which participate in the degradation of nicotinic acid in A. nidulans, downstream from 6-hydroxynicotinate, the first product of nicotinate oxidation. Here we report the cloning, expression and functional analysis of HxnV, a FAD-dependent enzyme. HxnV shows significant similarity with bacterial phenol 2-monooxygenases in both the FAD binding and dimerisation domains. Thus it is likely that it could catalyse the further oxidation of an aromatic derivative of 6-hydroxynicotinate The coding sequence of HxnV was 6His-tagged, cloned into an integrative pANpantoB A. nidulans vector and transformed into a pantothenic acid auxotroph hxnV deletion mutant. Pantothenic acid prototroph transformants were selected and tested for the utilization of nicotinic acid as sole N-source. Purification of His-tagged HxnV was optimized, preliminary evidence indicate that the native enzyme is an oligomer.
This research was supported by the Hungarian Scientific Research Fund (OTKA-K101218).

077 A NOVEL ER/GOLGI-RESIDENT MECHANISM CONTROLS VACUOLAR TURNOVER OF PARTIALLY MISFOLDED TRANSPORTERS

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Using as a model cargo protein the extensively studied uric acid-xanthine transporter of the model filamentous fungus Aspergillus nidulans, we investigate the signals and mechanisms controlling transporter trafficking, endocytosis or direct sorting from the ER/Golgi to the vacuole. Here we show that specific mutations in UapA lead to problematic ER exit and dramatically increased rates of vacuolar degradation, a process that depends on HulARsp5-dependent ubiquitination, but independent of arrestin-like adaptors and endocytosis. We subsequently identify an ER-localized, transmembrane adaptor, named BsdA, as essential for ubiquitination and vacuolar turnover of the UapA mutant proteins. Our results reveal a novel ER/Golgi-resident protein quality control system which recognizes partially misfolded plasma membrane proteins that have escaped ERAD. We are going to present novel findings related to the mechanisms and molecular factors involved in this cellular system operating on transporter turnover.

078 A NOVEL PROTEIN LINKS DYNEIN COMPLEX TO CONVENTIONAL KINESIN

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In filamentous fungi, the kinesins and cytoplasmic dynein are motor molecules that are required for the proper growth and development of the mycelium. Great advances have been made in our understanding of these fascinating proteins and their multiple functions within growing hyphae. Their ability to transport cargoes to and from hyphal tips is one of the most studied functions; however, the mechanism of interaction between cytoplasmic dynein and kinesins remains elusive. In this study we employed genetic, molecular, and fluorescence microscopy techniques to isolate and analyze mutants affected in hyphal growth and the localization of cytoplasmic dynein in the model organism Neurospora crassa. Our efforts led to the identification of a novel protein involved in the interaction of cytoplasmic dynein with the conventional kinesin, NcKin. This protein is specific to the Ascomycetes and is highly conserved among the Pezizomycetes. It is required for proper hyphal growth and is transported to the hyphal tips through NcKin. Interestingly, this protein is required for maintaining a high concentration of dynein at growing hyphal tips of N. crassa. Our current focus is to understand the molecular mechanisms controlling the actions of this highly conserved novel protein. Our work should shed light on the evolution and the mechanisms of regulation of microtubule-dependent transport in filamentous fungi and other Eukaryotes.

079 A ROLE FOR THE PROTON PUMPS DURING AUXIN-INDUCED MORPHOGENETIC TRANSITION IN YARROWIA LIPOLYTICA

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The effect of auxin (indole-3-acetic acid, IAA) on dimorphic fungi Y. lipolytica was investigated. Very low auxin concentrations (pM or fM) promoted morphological development stimulating yeast-to-hypha transition, a concentration-dependent phenomenon. Furthermore, Y. lipolytica cells produced IAA in culture medium. The H+ extrusion from Y. lipolytica cells was assessed by visualization of the pH changes in solid medium using pH indicator. Y. lipolytica colonies exhibited an increase in the external medium pH, which was further stimulated in the presence of auxin. Vacuolar and plasmalemma H+-ATPases were examined as components of auxin-signaling cascade. H+ transport mediated by both pumps was induced at transition point and after yeast-hypha transition. There was a correlation between morphogenetic transition, auxin production and H+ pumps activities and expression. Notably, auxin inhibitors, PCIB and TIBA, remarkably reduced morphogenetic transition, H+-pump activity, ambient pH changes and affected colony morphology. The data indicate an existence of a possible auxin-dependent concerted activation of H+ pumps underlying cell elongation and hyphal development. The results of this study suggest that auxin may have functions related to signaling of the morphogenesis and its regulatory effects on the proton pumps may represent an acid growth mechanism previously described only for plant, but that seems to be conserved in fungi and governs filamentation.
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080 A ROLE FOR VE-1 IN LIGHT SENSING AND CONIDAL DEVELOPMENT IN NEUROSPORA CRASSA

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Neurospora crassa perceives light through a light-dependent transcription factor complex, the WCC. In addition, the N. crassa genome contains genes for secondary photoreceptors: two phytochromes genes (phy-1 and phy-2), one cryptochrome gene (cry-1), and one opsin gene (nop-1). In addition, the N. crassa genome contains a homolog of the Aspergillus nidulans veA gene, ve-1. In A. nidulans mutations in veA results in constitutive conidiation, and VeA forms a complex with blue and red photoreceptors. The N. crassa ve-1 mutant has defects in aerial hyphal growth and increased conidiation. We have characterized the light-dependent accumulation of carotenoids in strains with deletions in these genes. The threshold for photocarotenogenesis in the wild-type strain is 10 J/m2, and this sensitivity is not altered in strains with mutations in phy-1. However, a reduction in the maximum accumulation of carotenoids and a small reduction in sensitivity to light was observed in phy-2, nop-1 and cry-1 mutants, suggesting that the corresponding proteins play a minor role in light sensing. A ten-fold reduction in sensitivity was observed in the ve-1 mutant, an indication that VE-1 participates in the mechanism of photoreception in N. crassa. We have characterized the expression of ve-1 and the accumulation of VE-1 after illumination and during asexual development. We observed a minor increase in the accumulation of ve-1 mRNA after light exposure in vegetative mycelia (30 min), that did not lead to changes in VE-1 accumulation. The mutation in ve-1 results in decreased light-dependent accumulation of mRNA for several genes, including the carotenogenesis genes (al-1, al-2, al-3, cao-2), wc-1, vvd, and frq. We have characterized the cellular localization of VE-1 under different light conditions and we have observed that VE-1 is preferentialy located in the nucleus under all conditions, but VE-1 was also detected in cytoplasm. We observed the accumulation of ve-1 mRNA in vegetative mycelia, and a reduction in mRNA accumulation after the induction of conidiation. VE-1 was not present during conidial development, but VE-1 was detected in vegetative mycelia. Our results suggest that the development of conidia requires a reduction in the amount of VE-1 in N. crassa.

081 ADAPTATION OF ENDOCYTOSIS TO FAST HYPHAL GROWTH IN ASHBYA GOSSYPII

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In a fast spreading mycelium of Ashbya gossypii, the surface expansion rate of a hypha is up to 40 times higher than that of a growing bud of its close relative Saccharomyces cerevisiae. To maintain polarity it is important to restrict the sites of surface growth to hyphal tips and emerging branches. Polarity factors and excessive membrane material have to be internalized subapically. This is achieved by endocytosis. In S. cerevisiae the major pathway for endocytosis is clathrin and actin dependent; this has already been well characterized. A. gossypii has homologues for almost all endocytic machinery components and is, therefore, especially suitable for the study of endocytosis during hyphal growth. To investigate the adaptation of the endocytic process to fast polar growth, we used Live-cell imaging together with Total Internal Reflection Fluorescence microscopy. Monitoring a set of typical endocytic proteins revealed that some steps in the process are up to 5 times faster than in budding yeast. Also, the number of endocytic events per square micrometer is significantly increased in A. gossypii. Another major difference in this process, when compared to that of S. cerevisiae, is the role of clathrin for endocytosis: Clathrin does not co-localize with any of the proteins we tested, suggesting that it does not contribute to the majority of endocytic events. These differences to the established model of clathrin dependent endocytosis in S. cerevisiae could be caused by a minimal divergence in the gene set of endocytic proteins in A. gossypii, which have finally led to a change in the Clc1 structure and function.

082 ANALYSIS OF APICAL MEMBRANE DOMAINS, MICROTUBULE AND ACTIN CYTOSKELETONS IN ASPERGILLUS NIDULANS BY PHOTOACTIVATED LOCALIZATION MICROSCOPY (PALM)

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The hyphae of filamentous fungi embody one of the most polarized structures in nature. The hyphae grow only by apical extension, through which the cell surface expansion is restricted to a defined region. This mode of growth is supported by the polarization of the cytoskeleton, which is responsible for the transport of vesicles that contain the material for the apical extension of the tip. Despite the fact that polarized growth is the defining feature of filamentous fungi, the molecular mechanisms underlying the hyphal morphogenesis are poorly understood. In recent years, super-resolution microscopy techniques have been improving and breaking the diffraction limit of conventional light microscopy of 250 nm. The resulting lateral image resolution as high as 20 nm will be a powerful tool for the investigation of membrane microdomains and components of the cytoskeletons. Our work aims to visualize the actin- and microtubule-cytoskeletons and exocytosis in the filamentous fungus Aspergillus nidulans with the help of several microscopy techniques including the super resolution microscopy technique Photoactivated Localization Microscopy (PALM). In addition to that we want to elucidate the interplay of the different factors that lead to the polarized growth of the hyphae by performing timing-experiments with exocytosis markers, actin- and microtubule markers as well as the growth-rate.

083 AUTOPHAGY CONTROLS NUCLEAR DYNAMICS DURING VEGETATIVE HYPHAL GROWTH AND FUSION OF FUSARIUM OXYSPORUM

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In the fungal pathogen Fusarium oxysporum, vegetative hyphal fusion triggers a series of nuclear events including mitosis in the invading hypha, nuclear migration into the receptor hypha and subsequent degradation of the resident nucleus. Here we examined the role of autophagy in fusion-induced nuclear degradation. A search of the F. oxysporum genome database for autophagy pathway (Atg) components identified putative orthologues of 16 core ATG genes in yeast, including the ubiquitin-like protein ATG8 which is required for the formation of autophagosomal membranes. F. oxysporum Δatg8 mutants were generated in a strain harbouring H1::ChFP-labelled nuclei to facilitate analysis of nuclear dynamics. The Δatg8 mutants failed to develop autophagic compartments in contrast to the wild type strain, suggesting that ATG8 is required for autophagy in F. oxysporum. The Δatg8 strains displayed reduced rates of hyphal growth and fusion, and were significantly attenuated in virulence on tomato plants and on the non-vertebrate animal host Galleria mellonella. Whereas wild type hyphae were almost exclusively composed of uninucleated cells, the hyphae of the Δatg8 mutant contained a significant fraction of cells with two or more nuclei. The increase in the number of nuclei per cell was particularly evident after hyphal fusion events between Δatg8 hyphae, or between hyphae of the Δatg8 and wild type strains. Furthermore, time-lapse microscopy analyses revealed abnormal mitotic patterns during vegetative growth in the Δatg8 mutants. Our results suggest that autophagy mediates nuclear degradation after vegetative hyphal fusion, and may function as a general mechanism to control the number of nuclei per cell in F. oxysporum.

084 AUTOPHAGY IN ASPERGILLUS NIDULANS. THE ER AS A POSSIBLE SOURCE OF MEMBRANES FOR AUTOPHAGY

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The genetic model Aspergillus nidulans, whose multinucleated hyphal cells are notably larger than those of Saccharomyces cerevisiae, is ideally suited for in vivo microscopy and intracellular trafficking studies. Therefore, we have exploited these advantages to investigate autophagy. A protein playing a central role in autophagy in yeast is the ubiquitin-like Atg8, which localizes to the phagophore assembly site (PAS). This location depends on the conjugation of phosphatidylethanolamine (PE) to this molecule, mediated by a set of proteins, including Atg4 cysteine protease and E1-like Atg7. This protein modification is necessary to anchor Atg8 to membranes and plays a key role in autophagosome biogenesis. In A. nidulans the localization of Atg8 to the PAS is independent of PE conjugation to the protein, since it is located in this structure in the absence of Atg4 and Atg7. Under nitrogen starvation conditions, GFP-Atg8 containing pre-autophagosomal puncta give rise to cup-shaped phagophores and circular (0.9-µm diameter) autophagosomes that disappear in the proximity of the vacuoles after their shape becomes irregular and their GFP-Atg8 fluorescence decays. Autophagy does not require endosomal maturation or ESCRTs, as autophagosomes fuse with the vacuole in a RabS (RAB7) / HOPS dependent manner. Also, does not require Golgi or post-Golgi traffic since mutations affecting known Golgi resident proteins, or mutations in proteins involved in the post-Golgi trafficking to the plasma membrane or endosomes do not affect the formation of autophagosomes and their fusion with the vacuole. By using a ts mutation in rabO, we have seen that autophagy it is dependent on this Rab protein. RabO (RAB1) localizes to phagophores and autophagosomes. Additionally TRAPPIII-specific factor Trs85 localizes to the PAS. The critical role of RabO (Rab1) in autophagy, combined with the fact that the traffic through the Golgi is not required for this process, suggest that the ER could be a potential source of autophagic membranes. In fact we have detected the presence of omegasome-like structures, similar to those described in mammalian cells, associated with fungal autophagosomes.

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085 CALCIUM IS ESSENTIAL IN MEMBRANE REMODELING ON NEUROSPORA CRASSA AFFECT BY CHITOSAN, MODULATION OF GENE EXPRESSION IN GERMINATING CONIDIA

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Chitosan is a natural compound able to permeabilize Neurospora crassa membranes, in an energy dependent manner. Plasma membrane permeabilization by chitosan depends on membrane fluidity, with FFA unsaturated membrane fungi (N. crassa) being chitosan sensitive. Plasma membrane permeabilization is also enhanced under glucose starvation. Chitosan, also causes intracellular ROS increases in N. crassa and is involved in cell division. Conidial germination is the most sensitive step to chitosan in filamentous fungi. We have used N. crassa conidia germinating with chitosan at a sub-lethal concentration for evaluating the transcriptomic response of the fungus. We determined chitosan IC50 for N.crassa (4, 8 and 16 hours after inoculation, hai) and analyzed the effect on gene expression over time. Using RNA-seq we have detected the genes involved in N. crassa response to chitosan. Chitosan modified expression of 22 N. crassa genes with significant differences, in the time course. These genes are involved mainly in programmed cell death (NCU05018) and heterokaryon incompatibility Pin c1. (NCU03494). Chitosan also regulated genes involved in plasma membrane signaling and response to oxidative stress (NCU02363; NCU05134). Chitosan early induced (4-8 hai) genes, involved in ROS protection (e.g. catalases, monooxigenase and SOD) but also transport and degradation of nitrogen compounds. We also detected late expression of genes involved in biosynthetic processes, nitrogen compounds (including proteins) metabolism and transport. We determine the role of Ca2+ in plasma membrane remodeling in presence of chitosan, since this cation is involved in restoring plasma membrane integrity. Our study will open new possibilities for the application of this versatile natural compound as an antifungal and gene modulator.

086 CHARACTERIZATION OF CRG1: A REGULATOR OF G-PROTEIN SIGNALING IN THE MUSHROOM COPRINOPSIS CINEREA

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Regulators of G-protein signaling (RGS) play a vital role in modulating the level of heterotrimeric G-protein signaling [1]. In the genome of Coprinopsis cinerea, a RGS encoding gene, referred as crg1, was identified which shares homology to Aspergillus nidulans flbA. Crg1 contains N-terminal two DEP (Dishevelled, Egl-10, and Pleckstrin) domains which play a role in subcellular targeting [2] and a C-terminal RGS domain. crg1 homologs have been studied before in several fungi and shown to participate in regulation of processes such as vegetative growth, asexual sporulation, mating, mycotoxin and pigment production and pathogenicity [3]. Genes crg1 and crg2 in the basidiomycetous yeast Cryptococcus neoformans and gene thn1 in the filamentous Schizophyllum commune are functionally linked to G-protein signaling in the pheromone- and the cAMP-response pathways [5,6]. To effectively examine the functions of crg1 in C. cinerea, homologous gene targeting is needed. Homologous gene targeting in Basidiomycetes has so far been difficult to achieve because of low frequency of homologous DNA integration, as the non-homologous end joining pathway (NHEJ) is the predominant pathway in DNA repairing, resulting in ecotopic DNA integration during transformation. Inactivation of genes such as ku70 and lig4 acting in the NHEJ pathway enhanced the frequency of specific gene targeting in a C. cinerea self-compatible Amut Bmut homokaryon [7]. We are currently on the way to elucidate the functions of crg1 in asexual sporulation, mating, vegetative growth of mono- and dikaryons, secondary metabolism and fruiting body formation using homologous gene targeting and overexpression of gene crg1 with the aid of a C. cinerea A5B6 delta-ku70 monokaryon obtained from crosses of a wildtype monokaryon with the self-compatible Amut Bmut delta-ku70 homokaryon generated by Kamada and coworkers [7].

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087 CHARACTERIZATION OF THE OSAA GENE ENCODING A WOPR BOX DOMAIN PROTEIN IN THE GENUS ASPERGILLUS

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The genus Aspergillus primarily reproduces asexually by forming conidia (asexual spores), which serve as the main mean of proliferation, infection and dispersal. Nonetheless, many members have a defined sexual cycle results in the production of ascospores. The reproductive process is under the control of various genetic components. Here, through a gain-of-function screen of negative regulators of asexual development in Aspergillus nidulans, we have identified osaA (Orchestrator of Sex and Asex A) predicted to encode a "WOPR box" domain protein. The "WOPR box" domain protein family is a newly identified DNA-binding class of proteins that are found in almost all sequenced fungal genomes. The osaA multi-copy strain is completely blocked in development showing a fluffy phenotype. Deletion of osaA results in an increased number of sexual fruiting bodies (cleistothecia) and decreased number of conidia in A. nidulans. Northern blot analyses have revealed OsaA negatively controls expression of veA (sexual activator), while promoting expression of brlA (asexual activator). Accordingly, we have concluded that OsaA functions in repressing sexual development in A. nidulans. Deletion mutants of osaA homologues in A. fumigatus and A. flavus show different aberrations in growth and development. These lead us to propose that OsaA is a conserved regulator that participates in controlling the process of development in Aspergillus.

088 CISTERNAL MATURATION WITHIN THE ASPERGILLUS NIDULANS GOLGI VISUALIZED IN VIVO

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115 years after Camillo Golgi´s description of the homonymous organelle, the mechanism by which proteins and lipids traffic in the secretory pathway, undergoing ordered modifications before being distributed to their target organelles, remains elusive and debated.
Over the last few years, we have established that Golgi cisternae of the filamentous fungus Aspergillus nidulans are not stacked and are thus optically resolvable, as opposed to the mammalian Golgi, which is organized in stacks of sub-resolution cisternal distance. In hyphal cells, Golgi cisternae display polarized distribution towards the growing apex; however, the late Golgi is absent from a ≈3µm region immediately below the apex, where secretion predominates. This region is populated by both microtubules and the actin mesh emerging from the Spitzenkörper, where secretory membranes accumulate, awaiting fusion with the plasma membrane. According to the cisternal maturation model for cargo transport, acute impairment of traffic in the ER-Golgi interface would lead to rapid disorganization of both the early and the late Golgi cisternae, while the vesicular transport model anticipates that stable Golgi cisternae would not be affected under these conditions, at least not promptly. We have constructed appropriate conditional mutants and, using in vivo fluorescence microscopy, we observed that a reversible block in the ER-Golgi traffic results in the reversible disorganization of both the early and late Golgi cisternae within minutes, as predicted by the cisternal maturation model. Indeed, we have found that Golgi cisternae in growing hyphae are transient entities. By employing multidimensional microscopy, we are able to directly observe cisternal maturation; that is the de novo formation of an early Golgi compartment and its subsequent enrichment in a late Golgi marker with concomitant loss of the early Golgi marker, until the formation of a late Golgi compartment. In turn, the late Golgi eventually diminishes.

089 COBUB2-COBFA1 COMPLEX IS REQUIRED FOR G1/S PHASE PROGRESSION AND PATHOGENESIS IN COLLETOTRICHUM ORBICULARE

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Morphogenesis in filamentous fungi depends on accurate cell cycle progression. To identify genes involved in infection related morphogenesis, we performed a forward-genetics mutant screening in cucumber anthracnose fungus, Colletotrichum orbiculare. From the mutant named coQ-1, we identified a gene CoBUB2, a homolog of Saccharomyces cerevisiae BUB2. In S. cerevisiae, BUB2 is a component of spindle position checkpoint (SPOC) which prolongs mitosis by inhibiting mitotic exit network (MEN) when the spindle fails to align along the mother-daughter axis. Bub2 forms GTPase activating protein (GAP) complex with Bfa1. Here, we analyzed functional roles of CoBUB2 and CoBFA1 by generating gene disruption mutants. Morphogenesis analysis of the cobub2 mutants and the cobfa1 mutants showed that appressoria had defects in forming penetration hyphae into the host plant cells. Consequently, these mutants failed to cause full disease lesions on cucumber leaves. Importantly, time course observations of histone H1-GFP introduced strains and by DAPI staining confirmed that CoBUB2 and CoBFA1 are involved in the timing of mitosis and proper assignment of nuclei during appressorium development. Furthermore, the experiments of cell cycle specific inhibitors revealed that the transition period from G1 phase to S phase of the cobub2 mutants and the cobfa1 mutants was accelerated about 2h than that of the wild type. From these analyses we concluded that CoBUB2 and CoBFA1 are required for G1/S phase progression during appressorium development. In S. cerevisiae, Tem1 is a GTP-binding protein and Bub2/Bfa1 GAP complex inhibits Tem1 activity by GTP hydrolysis. Tem1 constitutes MEN and regulates mitotic exit. Then we generated tem1 homolog disruption mutants in C. orbiculare. In order to reveal the downstream target of CoBub2/CoBfa1 regulating G1/S phase progression, the nuclear division of cotem1 mutants was investigated. The cotem1 mutants showed similar mitotic behavior to the wild type. We assumed that CoTEM1 could not be a direct downstream target of CoBub2/CoBfa1 complex. In conclusion, our findings suggest that the CoBub2-CoBfa1 complex plays critical roles in G1 phase to S phase progression and pathogenesis in C. orbiculare although the Bub2-Bfa1 complex of S. cerevisiae functions as a regulator of mitotic exit network.

090 COLLETOTRICHUM ORBICULARE COPAG1, A COMPONENT OF MOR PATHWAY, IS INVOLVED IN APPRESSORIUM DEVELOPMENT TRIGGERED BY PLANT-DERIVED SIGNALS

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In many plant pathogenic fungi, morphogenesis of infection structures is triggered by physical or chemical signals from 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 substrate but is dispensable for appressorium formation on host plant surface (Sakaguchi et al., 2009). Our results suggested that there could be a bypass pathway that transduces plant-derived signals for appressorium formation independent of CoKEL2. To determine specific components of the plant-derived signaling pathway for appressorium formation, we obtained six insertional mutants in cokel2Δ background that formed abnormal appressoria not only on artificial substrate but also on host plant surface. We identified candidate-mutated genes by whole genome sequencing of the six mutants. The predicted amino acid sequence encoded by the mutated gene of mutant kanI-9 had high homology to that of PAG1 (TAO3) in Saccharomyces cerevisiae. Pag1 is one of the components of RAM (regulation of Ace2p and morphogenesis), a signaling cascade that is involved in the maintenance of cell polarity and morphogenesis. Because Ace2p is not conserved in C. orbiculare, MOR [morphogenesis-related NDR (nuclear Dbf2-related) kinase network] is used in this study. To define the involvement of CoPag1 in appressorium formation, we analyzed the phenotypes of copag1 mutants. As expected, copag1Δcokel2Δ formed abnormal appressoria on both artificial substrate and host plant surface, indicating that CoPag1 is a key component of plant-derived signaling pathway. To further elucidate the cellular role of CoPag1, we investigated the localization of CoPag1::RFP during appressorium formation. CoPag1::RFP was observed at cytoplasm on glass slide, whereas CoPag1::RFP was localized to plasma membrane on cucumber cotyledon, suggesting that CoPag1 responds to biological signals during appressorium development. In S. cerevisiae, Pag1 facilitate normal activation of the NDR kinase Cbk1, the downstream module in MOR. To assess whether CoPag1 functions via a similar signaling cascade in C. orbiculare, we obtained the constitutively active strain of CoCbk1 in copag1Δ background by site directed mutagenesis. Expectedly, copag1Δcokel2Δ/CoCbk1CA formed normal appressoria on host plant surface, suggesting that CoPag1 functions via MOR pathway in C. orbiculare.

091 COMPLEMENTATION STUDIES OF ASPERGILLUS NIDULANS RPDA

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In eukaryotic organisms, DNA is compacted into an elaborate structure called chromatin, thus enabling regulation of transcription by controlling the accessibility of the genetic information for transcription factors. Among the key players involved in the regulation of chromatin structure are histone acetyltransferases and histone deacetylases (HDACs) – enzymes establishing distinct acetylation patterns in the N-terminal tails of core histones and other proteins. In filamentous fungi still little is known about the biological functions of these enzymes; nevertheless recent studies have shown that HDACs affect the regulation of genes involved in stress response and secondary metabolite production. We have recently shown that depletion of RpdA, a class 1 HDAC of Aspergillus nidulans, leads to a drastic reduction of growth and sporulation. Functional studies revealed that a short C-terminal motif unique for RpdA-type proteins of filamentous fungi is required for the biological activity and consequently cannot be deleted without affecting the viability of Aspergillus nidulans. Here we show, that deleterious effects of depleted RpdA can be complemented by expression of fungal but not of yeast or mammalian orthologs. Further, nuclear localization is crucial for RpdA function and most likely dependent on the presence of the essential motif. Thus, the C-terminal extension of RpdA-type proteins represents a promising target for fungal specific HDAC-inhibitors that might have potential as new antifungal compounds.

092 COMPOSITION OF THE MAK-2 MAP KINASE CASCADE IN NEUROSPORA CRASSA

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The mechanistic understanding of oscillatory MAK-2 signaling during fungal self-signaling is hampered by the fact that most components of the signaling machinery, including the postulated secreted signal and its cognate receptor, regulators of the MAP kinase cascade as well as most MAK-2 targets, are unknown. In order to identify additional components involved in MAK-2 MAP kinase signaling we performed GFP-trap affinity purification experiments coupled to mass spectrometry with strains expressing functional GFP-fusion proteins of the MAP kinase cascade. Subsequent yeast two-hybrid tests were used to confirm many of the identified interactions and to map interacting domains. This approach identified two scaffold/adapter proteins of the kinase cascade as well as several upstream regulatory elements and putative MAK-2 targets, whose characterization will be presented.

093 COORDINATION BETWEEN BRLA REGULATION AND SECRETION OF THE OXIDOREDUCTASE, FMQD, TO THE CELL WALL DIRECTS SELECTIVE ACCUMULATION OF FUMIQUINAZOLINE C TO THE SPORES OF ASPERGILLUS FUMIGATUS

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Aerial spores, crucial for propagation and dispersal of the Kingdom Fungi, are commonly the initial inoculum of pathogenic fungi. Natural products (secondary metabolites) have been correlated with fungal spore development and enhanced virulence in the human pathogen Aspergillus fumigatus but mechanism(s) for metabolite deposition in the spores is unknown. The fumiquinazolines (Fqs) comprise a related, sequentially generated family of cytotoxic peptidyl alkaloids that are signature metabolites from A. fumigatus. Metabolite profiling of clinical A. fumigatus isolates reveal that the first two products of the Fq cluster, FqF and FqA, are produced to comparable levels in all fungal tissues but the final enzymatically-derived product, FqC, selectively accumulates in the fungal spore. Loss of the sporulation-specific transcription factor, BrlA, yields a strain incapable of FqA and FqC production. However, loss of two sequentially downstream transcription factors involved in conidiophore development and maturation namely AbaA and WetA did not affect selective accumulation of FqC in the spores. In silico analysis of FmqD, the oxidoreductase required to generate FqC, predicted an N-terminal secretion signal on this enzyme. Fluorescence microscopy showed that FmqD is secreted via the Golgi apparatus to the cell wall in an actin-dependent manner. Removal of the signal peptide abolished FmqD cell wall localization and significantly reduced FqC production. In contrast, all other members of the Fq pathway including the putative transporter, FmqE – which had no effect on Fq biosynthesis – were internal to the fungal hyphae with varying subcellular localization. The coordination between BrlA-mediated tissue specificity with FmqD secretion to the cell wall presents a previously undescribed mechanism to direct localization of specific secondary metabolites to spores of the differentiating fungus.

094 DELETION OF RLMA ENCODING GENE IN ASPERGILLUS FUMIGATUS LEADS TO DEFECTS IN THE CELL WALL INTEGRITY MAINTENANCE AND ASEXUAL DEVELOPMENT

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Aspergillus fumigatus is a ubiquitous mold that causes a number of clinical diseases in humans including invasive pulmonary aspergillosis, the life-threatening form of infection. The CWIP (cell wall integrity pathway) signaling cascade is activated in fungal cells under stressing conditions and plays a role in the adaptation of several fungal pathogens to the human host. The Mpk1 MAP kinase of the Saccharomyces cerevisiae cell wall integrity signaling pathway phosphorylates and activates the Rlm1 transcription factor in response to cell wall stress. Signaling through Rlm1 regulates the expression of at least 25 genes, most of which have been implicated in cell wall biogenesis, maintenance and reinforcement. Here, we constructed a rlmA null mutant through DNA-mediated transformation in A. fumigatus. To test the involvement of rlmA in the CWIP, conidia from the wild type and delta rlmA strains were spotted on complete and minimal agar plates supplemented with different concentrations of agents disturbing/interfering the CWI or causing oxidative damage. The sensitivity of the delta rlmA strain to congo red and calcofluor white was increased and could be partially restored by osmotic stabilizer D-sorbitol. Decreased resistance to oxidative damage caused by hydrogen peroxide and paraquat was also observed. The rlmA transcript is induced upon cell wall stress caused by congo red exposure in the wild type strain and the levels of phosphorylated MpkA was constitutively increased in the delta rlmA strain. Vegetative growth was also affected mainly at 30°C and 45°C in the mutant strain. Interestingly, conidia production was also considerably decreased in the delta rlmA mutant mainly at 30°C. This reduction was coincident with a down-regulated expression of the transcription factor brlA gene which encodes the early conidiophore development regulator. In addition, the delta rlmA mutant strain presented attenuated virulence in comparison to the wild type and reconstituted strain in a mouse model of invasive pulmonary aspergilosis. These results indicate an important role of rlmA in the signaling system for the maintenance of cell wall integrity and also an undescribed role in the asexual development in A. fumigatus.
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095 DISRUPTION OF THE MYOSIN MOTOR DOMAIN-CONTAINING CHITIN SYNTHASE GENE PDCHSVII IN THE CITRUS POSTHARVEST PATHOGEN PENICILLIUM DIGITATUM

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An important challenge in fungal biology is the development of new alternatives to the fungicides currently used. Fungal cell wall (CW) is considered an excellent potential target for novel antifungals and is composed of chitin, glucans, mannans and glycoproteins. Chitin has been involved in the sensitivity to various antifungal peptides and proteins. In filamentous fungi, chitin is synthesized by a complex family of chitin synthase genes (chs) grouped into seven classes. We have characterized seven different chs in Penicillium digitatum, the main postharvest pathogen of citrus that causes important production losses. Comparative analyses grouped each PdChs in each one of the classes I to VII previously established, and support the classification of these into three divisions. Pdchs gene expression was analysed during different axenic growth conditions, antifungal peptide treatment and fruit infection. To determine functional relevance, we have obtained disruption mutants of the myosin motor domain-containing PdchsVII using Agrobacterium tumefaciens-mediated transformation. The resulting disruption strains (ΔPdchsVII) were viable, showed reduced growth and conidia production, alterations of hyphal morphology and higher sensitivity to fungicides and CW-interfering compounds such as calcofluor white or SDS, but not to distinct antifungal peptides. It also showed increased sensitivity to reactive oxygen species such as H2O2. Infection assays of citrus fruits with PdchsVII disruption strains showed reduced virulence, defects in the production of visible mycelium and, importantly, also of conidia production on the infected fruit.

096 DUAL MATING IN BOTRYTIS CINEREA

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B. cinerea is considered a typical example of a heterothallic fungus which requires two compatible mating types to complete the sexual cycle. Mating occurs between a strain carrying a MAT1-1 locus and a strain carrying a MAT1-2 locus. Having both loci initiates sexual development called apothecia. However, an unusual mating type behaviour was reported of certain B. cinerea strains, referred to as ‘dual mater’, in which a strain carrying a single MAT allele is able to mate both with a MAT1-1 ánd a MAT1-2 reference strain. In this study, we performed crosses of dual mater strain RS11 (carrying a MAT1-2 allele) with the two reference strains and studied the segregation of MAT loci in the progenies by PCR with primers specific for the MAT1-1 (α domain) and MAT1-2 (HMG box domain) locus. The result showed that dual mater strain RS11 is able to cross with the MAT1-2 reference strain (SAS405) and form apothecia. 50 single ascospore progeny were sampled from individual apothecia and all of the progeny contained the MAT1-2 allele. If the dual mating behavior is a monogenic trait, it would be expected to segregate 1:1, where 50% of the progeny would behave as dual mater and the other 50% would act as a standard heterothallic (MAT1-2) isolate. We performed a backcross of 30 single ascospore progeny (all carrying the MAT1-2 allele) to the MAT1-2 reference strain, and 10 out of 30 were able to form apothecia. One of the progeny was even homothallic, i.e. able to form apothecia without fertilization. The mechanism of this phenomenon remains unknown. We are considering sequencing dual mater strains and their progeny (that behave as a dual mater or a homothallic isolate), but we also consider the possibility that this trait is under epigenetic control.

097 ECOPHYSIOLOGICAL ROLES OF THE TWO NOVEL CLASS II HYDROPHOBINS (HFB4 AND HFB7) FROM TRICHODERMA VIRENS

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Hydrophobins are small (around 7-12kDa) surface active proteins that are exclusively produced by filamentous fungi. Due to their amphiphilic properties they play important roles in fungal growth and interactions with the environment. Their remarkable biophysical properties, such as high surface activity and the formation of self-assembled structures have also raised considerable interest in the industrial application for processes requiring surface modification. The highest diversity of class II hydrophobins was found in the genus Trichoderma (teleomorph Hypocrea, Hypocreales, Ascomycota), commonly isolated mycotrophic fungi with a broad range of environmental adaptations. In this work T. virens was selected as a model system for elucidating the function and amphiphilic properties of HFB4 and HFB7, which represent the most evolutionary derived and most conserved clade of class II hydrophobins in Trichoderma, respectively. We characterize the role of hfb4 and hfb7 genes in protein production, hyphal surface activity, mycoparasitic potential and several other biotrophic interactions of the fungus Physiological characterization of the mutants unraveled ecophysiological function of these two genes.

098 EFFECT OF CHITOSAN ON GERMINATION OF CHLAMYDOSPORES OF THE NEMATOPHAGOUS FUNGUS POCHONIA CHLAMYDOSPORIA

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P. chlamydosporia is a nematophagous fungus endophytic in tomato plants. The development of the fungus (e.g. conidiation) is favored by chitosan. The fungus forms dictyochlamydospores which remain dormant in soil and cause suppression to its target plant-parasitic nematods. We have analyzed the effect of chitosan on P. chlamydosporia chlamydospores of several strains of the fungus from wordwide origin. The effect varied with the strain with some strains resistant to chitosan. The effect of chitosan on chlamydospores al cell level is analyzed using viability (propidium iodide), membrane tracker (FM4-64) as well as membrane permeability (Sytox) strains. Also we determined the effect of chitosan on root colonization by chlamydospores from different P. chlamydosporia world wide isolates.The effect of chitosan on tomato seed germination (where P. chlamydosporia develops endophytically) has also been analyzed. Our study will open new possibilities for the application the P. chlamydosporia as a biocontrol agent against Meloidogyne javanica pest.

099 EFFECT OF SINGLE AND DOUBLE DELETION OF GENES INVOLVED IN CARBON CATABOLITE REPRESSION ON AMYLASE PRODUCTION IN ASPERGILLUS ORYZAE

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In filamentous fungi, the expression of secretory glycoside hydrolase encoding genes, such as those for amylases, cellulases, and xylanases, is generally repressed in the presence of glucose. The carbon catabolite repression (CCR) in filamentous fungi is regulated by the transcription factor CreA, and CreB and CreC have been also observed to be regulating factors for carbon catabolite repression. It has been shown that CreB and CreC form a complex in vivo and that the CreB protein is a ubiquitin processing protease. In this study, we generated single and double deletion mutants of creA, creB and/or creC in Aspergillus oryzae. The amylase activities of each strain were compared under various culture conditions. For the wild-type strain, mRNA levels of amylase were markedly decreased in the late stage of submerged culture under inducing conditions, whereas this reduced expression was not observed for single creA, double creA/creB, and double creA/creC deletion mutants. In addition, amylase activity of the wild-type strain was reduced in submerged culture containing high concentrations of inducing sugars, whereas all constructed mutants showed considerably higher amylase activities. Interestingly, the double creA/creB and creA/creC deletion mutants had higher amylase activity than the single creA, creB, and creC deletion mutants. In particular, the amylase activity of the double creA/creB deletion mutant in a medium containing 5% starch was >10-fold higher than that of the wild-type strain under the same culture conditions. In contrast, the amylase activity of double creB/creC mutant was comparable with that of the single creB deletion mutant. In solid-state cultures using wheat bran as a substrate, the amylase activities of single creA and double creA/creB deletion mutants were >2-fold higher than that of the wild-type strain. These results suggested that deleting both creA and creB resulted in dramatic improvements in the production of secretory glycoside hydrolases in filamentous fungi.
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100 ELUCIDATING THE ROLE OF THE HYPHAL TIP OF IN THE TRIGGERING MECHANISM OF CONIDIATION

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Contact of growing hyphae with the air is a prerequisite for the induction of the asexual development in Aspergillus nidulans. The induction process involves a group of proteins called Upstream Developmental Activators (UDAs). One of these UDAs is the bZIP-type transcription factor (TF) FlbB, which directly binds the promoter of the coniciation-specific transcription factor brlA. FlbB is located at the hyphal tip and the most apical nucleus. Constitutive expression of flbB under the gpdAmp promoter altered the nuclear accumulation of FlbB in vegetative hyphae, leading to an even distribution among nuclei. Addition of latrunculin B excluded constitutively expressed GFP::FlbB from the tip and provoked its accumulation at subapical regions. flbE is a second UDA factor that interacts with FlbB at the tip of hyphae. Through the analysis of a FlbE::GFP chimera constitutively expressed under gpdAmp, we observed that its localization and dynamics also depended on the actin cytoskeleton. Latrunculin B has the same effect on FlbE as it has on FlbB, leading to accumulation of both proteins at the subapical region. Stream acquisitions also showed that both proteins move together to the tip. The FlbB/E interaction is mediated by the bZIP transcriptional regulatory domain of the former, which is sufficient and necessary for its binding to FlbE. FlbE has a signal sequence at its N-terminus, which purportedly targets the protein to the secretory pathway. Deletion of this sequence or the N-terminal GFP tagging of a wild-type FlbE generate a fluffy phenotype in which FlbE movement is highly restricted. This suggests that the signal sequence of FlbE is necessary for the transport of both FlbE and FlbB. A new feature of FlbE is its ability to be imported to nuclei when constitutively expressed under gpdAmp. The nuclear accumulation of FlbE is increased in flbB mutants that inhibit the tip localization of the signalling complex: C382A and L104A;E105A (being the latter mutation located within the dimerization domain of the bZIP). Overall, these results suggest that the transport of the FlbB/E complex can be divided into two stages: initial delivery to the subapical region and a final transport to the apex. The second step depends on actin filaments. Furthermore, these findings emphasize the need of FlbE for the apical localization of FlbB, which is a pre-requisite for the correct induction of asexual development at nuclei.

101 FLOW CYTOMETRIC ANALYSIS OF ASPERGILLUS NIGER CONIDIA

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Aspergillus niger is an important host organism for the production of organic acids and proteins. The germination of conidia is the first step in the fermentation process and a highly relevant process, because the molecular mechanisms during germination directly influence the morphology of the culture. The germination process can be followed by flow cytometry until the germ tube is formed. Flow cytometry is a powerful single cell analysis tool, which amongst others allows to simultaneously monitor cell growth and fluorescent biomarkers. We set out to establish flow cytometry as a tool for the analysis of fungal conidia and the germination process. With this technique it is possible to analyze on a single cell level the underlying principles of germination and to investigate the dynamic behavior of conidia populations. In this work, we analyzed the impact of age and media composition on the size and swelling of Aspergillus niger conidia. Potato dextrose agar and malt extract agar - two commonly used sporulation media - were compared. Thereby, the media used for conidiation has a direct influence on the further development in the swelling/growth medium. The size distribution of the conidia populations between the two conidiation media differed significantly. Furthermore, the conidia showed different kinetics during swelling on the same media.

102 FUNCTION AND SUBCELLULAR LOCALIZATION OF MUCOR CIRCINELLOIDES HMG-COA REDUCTASE 2 AND 3

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3-Hydroxy-3-methylglutaril-coenzymeA reductase (HMGR) is a membrane anchored protein catalyzing the central step of the mevalonic acid pathway. This step is the conversion of the HMG-CoA to the mevalonic acid. The pathway possesses biotechnological importance through the formation of several terpene-type metabolites with a broad range of functions, e.g. ergosterol (the main sterol compound of the fungal cell membrane), carotenoids, functional groups of certain proteins (RAS), prenyl group of ubiquinone, etc. Saccharomyces cerevisiae has two HMG-CoA reductases with different regulation and subcellular localization. Genome of the zygomycete Mucor circinelloides contains three HMG-CoA reductase genes named as hmgR1, 2 and 3. In this study, function and subcellular localization of the Mucor hmgR2 and hmgR3 genes were analysed. To examine the function of the two genes, overexpression and gene silencing studies were performed. Morphology, growth dynamics and ergosterol and carotenoid content of the constructed transformants were analysed. Subcellular localization of the HMGR proteins was examined with fluorescence and confocal microscopy. Strains harboring the two hmgR genes fused with green fluorescent protein were constructed and different dyes were used to analyze their co-localization with the fusion proteins to the endoplasmic reticulum, nucleus and mitochondria. Our results suggest that both proteins co-localize to the endoplasmic reticulum. However, while HMGR2 plays role in the general isoprenoid biosynthesis, the HMGR3 is especially active under anaerobic growth condition. Moreover, silencing of hmgR3 with asRNA technique changed the macro- and micro morphology of the transformants.
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103 FUNCTIONAL ANALYSIS OF PROTEIN UBIQUITINATION IN THE RICE BLAST FUNGUS MAGNAPORTHE ORYZAE

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Rice blast is the most important disease of rice worldwide, and is caused by the filamentous ascomycete fungus, Magnaporthe oryzae. Protein ubiquitination, which is highly selective, regulates many important biological processes including cellular differentiation and pathogenesis in fungi. Gene expression analysis revealed that a number of genes associated with protein ubiquitination were developmentally regulated during spore germination and appressorium formation. We identified an E3 ubiquitin ligase, MGG_13065 is induced during appressorium formation. MGG_13065 is homologous to fungal F-box proteins including Saccharomyces cerevisiae Grr1, a component of the Skp1-Cullin-F-box protein (SCFGrr1) E3 ligase complex. Targeted gene deletion of MGG_13065 resulted in pleiotropic effects on M. oryzae including abnormal conidia morphology, reduced growth and sporulation, reduced germination and appressorium formation and the inability to cause disease. Our study suggests that MGG_13065 mediated ubiquitination of target proteins plays an important role in nutrient assimilation, morphogenesis and pathogenicity of M. oryzae.

104 FUNCTIONAL ANALYSIS OF STEROL TRANSPORTER IN THE FILAMENTOUS FUNGUS ASPERGILLUS NIDULANS

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Polarized growth needs a continuous flow of secretion vesicles from the hyphal cell body to the growing hyphal tip for the cell wall and cell membrane extension. One of the important membrane compounds in fungi is ergosterol. At the apical plasma membrane ergosterol accumulations, which are called sterol-rich plasma membrane domains (SRDs), could be found by using the sterol-binding fluorescent dye filipin and become more important in polarized growth of filamentous fungi. The exact roles and formation mechanism of the SRDs remain rather unclear. Transport of sterol to hyphal tips is thought to be important for the organization of the SRDs. Oxysterol binding proteins, which are conserved from yeast to human, are involved in non-vesicular sterol transport. In Saccharomyces cerevisiae seven oxysterol-binding protein homologues (OSH1-7) are thought to be involved in the sterol distribution between closely located membranes independently of vesicle transport. We found five homologous genes (OSHA-E) in filamentous fungus Aspergillus nidulans. To investigate their functions for the polarized growth and SRDs organization, gene deletion strains were constructed. Their localizations were analysed by fluorescent protein tagging. Their expression patterns were analysed via qRT-PCR in several conditions. The sensitivities against antifungal drugs such as Voriconazole and Amphotericin B were investigated in the gene deletion strains.

105 FUNCTIONAL ANALYSIS OF THE A-1,3-GLUCAN SYNTHASE GENES AGSA AND AGSB IN ASPERGILLUS NIDULANS

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Although α-1,3-glucan is one of the major cell wall polysaccharides in filamentous fungi, the physiological roles of α-1,3-glucan remain unclear. The model fungus Aspergillus nidulans possesses two α-1,3-glucan synthase (AGS) genes, agsA and agsB. For functional analysis of these genes, we constructed several mutant strains in A. nidulans: agsA disruption, agsB disruption, and double-disruption strains. The agsA disruption strains did not show markedly different phenotypes from those of the wild-type strain. The agsB disruption strains and the double-disruption strains showed increased sensitivity to congo red and lysing enzymes, which are a cell wall stress–inducing compound and a cell wall–degrading enzyme, respectively. In addition, the agsB disruption strains formed dispersed hyphal cells under liquid culture conditions, regardless of the agsA genetic background. The mycelial dry weight of the agsB disruption strains cultured in liquid medium was increased compared with that of the wild-type strain, suggesting that the dispersed hyphal cells observed in the agsB disruption strains are applicable to high-density cultivation to achieve higher-productivity of biomaterials. Fractionation of the cell wall based on the alkali solubility of its components, quantification of composed monosaccharides in each fraction, and 13C-NMR spectroscopic analysis revealed that α-1,3-glucan was the main component of the alkali-soluble fraction in the wild-type and agsA disruption strains, but almost no α-1,3-glucan was found in the alkali-soluble fraction derived from either the agsB disruption strain, regardless of the agsA genetic background. Taken together, our data demonstrate that the two AGS genes are dispensable in A. nidulans, but that AgsB is required for normal growth characteristics under liquid culture conditions and is the major AGS in this species.

106 FUNCTIONAL STUDY OF TREHALOSE METABOLISM IN USTILAGO MAYDIS REVEALS ITS IMPORTANCE FOR FITNESS AND VIRULENCE

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Trehalose is a glucose alfa 1,1-glycoside non-reducing disaccharide synthesized by bacteria, fungi, insects and plants, but not by vertebrates. Initially, this molecule was considered mainly as carbon source storage, given that its hydrolysis produces two glucose molecules. Recent evidence indicates additional roles, responding to general abiotic stress, as cell protectant against damage by heat, desiccation, oxidation, salinity and freezing. Trehalose protects the cells by avoiding protein denaturation and by scavenging reactive oxygen species. In fungi, trehalose is synthesized by trehalose phosphate synthase (TPS) and trehalose phosphate phosphatase (TPP). In the first reaction, TPS transfers one glucose molecule from the donor uridine diphosphate glucose (UDP-glucose) to glucose 6 phosphate, producing trehalose 6 phosphate (T6P). Next, TPP removes the phosphate from T6P producing trehalose and inorganic phosphate. After stress, intracellular trehalose is hydrolyzed by acid or neutral trehalases. There is evidence that mutants unable to synthesize trehalose are sensitive to several stresses, reducing virulence in pathogenic fungi. On the other hand, mutants unable to carry out trehalose hydrolysis are resistant to several challenges, and in some cases are able to avoid the defense mechanisms of the host. Ustilago maydis is a biotrophic basidiomycota fungus whose life cycle in nature involves the alternation of two stages: saprophytic haploid yeasts and a dikaryotic parasitic hyphal stage that infects maize plants causing corn-smut disease. Here we studied the role trehalose plays in U. maydis. We deleted the trehalose phosphate phosphatase (UmTPS2) or the neutral trehalase (UmNTH1) genes. According to our HPCL data the first kind of mutants do not produce trehalose, and the second ones accumulate more trehalose than the wild-type strain.In our initial phenotypic analysis we found that tps2 mutants are more sensitive than the wild type to oxidative, osmotic, saline, cell wall or UV light stresses, and virulence is highly reduced, interestingly mutants are resistant to the LiCl salt. Experiments to determine the phenotype of nth1 mutants are in progress.

107 HETEROLOGOUS EXPRESSION OF FERULOYL ESTERASE OF THE LITTER-DECOMPOSING FUNGUS AGROCYBE PRAECOX

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In the cell walls of gramineous plants, hemicelluloses are crosslinked to aromatic lignin polymer via hydroxycinnamic acids (ferulic acid and p-coumaric acid). Feruloyl esterases (ferulic acid esterases, EC 3.1.1.73), classified in CAZy family CE1 (www.cazy.org), are enzymes that catalyse the cleavage of covalent ester bonds between carbohydrate and lignin moieties in plant cell walls. Due to the ability to specifically cleave ester linkages, feruloyl esterases are promising biocatalysts for a broad range of biotechnological applications. These include e.g. pharmaceutical, agricultural and food industries, as well as the production of biofuel.
As a result of their unique ability to modify and mineralize the recalcitrant lignin polymer, the litter-decomposing fungi play a significant role in global carbon cycling and are important organisms for the decomposition of lignocellulose in forest and grassland ecosystems. Agrocybe praecox is a litter-decomposing, white-rot causing basidiomycete found in forests and open woodlands. It grows in leaf-litter and soil and is also able to colonize bark mulch and wood chips. A. praecox has been shown to mineralize synthetic lignin and degrade polycyclic aromatic hydrocarbons, which makes it an interesting species to study the plant biomass degrading machinery of litter-decomposing fungi.
In this study, a feruloyl esterase encoding gene from A. praecox was cloned and expressed heterologously in the methylotrophic yeast Pichia pastoris. In phylogenetic analysis, the A. praecox feruloyl esterase clustered with putative acetylxylan esterase of the soil-inhabiting, coprophilic basidiomycete Coprinopsis cinerea. Biochemical properties, including substrate specificity, of the recombinant A. praecox feruloyl esterase will be presented.

108 HOW A DNA DAMAGE RESPONSE PATHWAY IS ACTIVATED BY A TRANSCRIPTIONAL FACTOR DURING DIKARYON FORMATION IN USTILAGO MAYDIS?

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The corn smut fungus Ustilago maydis represents an excellent model to study the relationships between cell cycle, morphogenesis and pathogenicity The activation of the virulence program in U. maydis involves the mating of a pair of compatible haploid budding cells to produce an infectious dikaryotic hypha. A peculiar characteristic of the U. maydis dikaryotic filament is the sustained cell cycle arrest while growing on the plant surface. This cell cycle arrest cycle arrest is necessary for the virulence in U. maydis, since mutant strains unable to arrest the cell cycle were severely impaired in its ability to infect corn plants. Previous research from our group showed that elements from the DNA Damage Response (DDR) cascade such as the kinase Chk1 and its upstream activating kinase Atr1 were required for efficient cell cycle arrest during filament formation. The described new role of Atr1 and Chk1 during pathogenic development in U. maydis fits in the emerging view that elements from the DNA damage response cascade can be utilized to modulate developmental processes in virtue to their ability to interact with cell cycle machinery elements. A main question to be answered concerns how the b heterodimer, the transcriptional factor that controls the dikaryotic infective hyphae, activates the Atr1-Chk1 cascade, which in normal conditions responds to DNA damage. In this communication we will describe our attempts to define additional elements on this cascade, as well as approaches dedicated to determine whether DNA damage is associated to the process responsible of the infective filament formation.

109 HYPHAL FUSION IN ASPERGILLUS ORYZAE AS EVIDENCED BY A DETECTION SYSTEM FOR HETEROKARYON FORMATION

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Hyphal fusion is involved in the formation of an interconnected colony in filamentous fungi. It is the first process in sexual/parasexual reproduction, which is biotechnologically important for crossbreeding. In the industrial filamentous fungus Aspergillus oryzae, a parasexual cycle has been reported, and its potential sexuality was suggested. However, as A. oryzae possibly enters into hyphal fusion with a much lower frequency than Neurospora crassa, it was difficult to detect the hyphal fusion in A. oryzae. In order to evaluate the hyphal fusion ability in A. oryzae, we developed a detection system for heterokaryon formation by differentially labeling strains with auxotrophies and fluorescent proteins. With mixed culture, it was demonstrated that AoSO and AoFus3 are required for heterokaryon formation, and the efficiency of heterokaryon appearance was varied by media composition. In paired culture, we detected formation of heterokaryotic sclerotia in the hyphal contact region between two auxotrophic strains. Sclerotia were reported to be capable of acting as repositories for a sexual reproductive structure ascocarp in other Aspergilli, but A. oryzae strains have no or much lower ability to form sclerotia. Overexpression of the sclR gene, which encodes a transcription factor promoting sclerotial formation, enhanced the formation of heterokaryotic sclerotia. This enhancing effect for heterokaryotic sclerotia was observed independently of the mating-type pairing combinations. Collectively, these findings would help for understanding of the colonial physiology in industrial processes and for an efficient crossbreeding with sexual/parasexual reproduction in A. oryzae.

110 HYPHAL MORPHOGENESIS RELATED GENES ARE SUPPRESSED IN THE LENTINULA EDODES LACCASE (LCC1) KNOCK DOWN TRANSFORMANT

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Laccase (EC 1.10.3.2) is known as one of lignin degrading enzymes. Basidiomycetous fungi have multiple laccase in their genome. Enzymatic properties of laccase have been well studied, but there is little knowledge about biological function of laccases in basidiomycetous fungi. L. edodes has at least 11 laccases in their genome, and a laccase, Lcc1, is secreted most abundantly from vegetative mycelia. For investigation of biological function of the lcc1, a lcc1 down regulated transformant (pChG'-ivrL1#32) was constructed by RNAi method (Nakade et al. 2011 Microbiol. Res. 166:484-93). The pChG'-ivrL1#32 did not form thick mycelium mat because of lacking aerial hyphae. From detail observation by using scanning electron microscope and transmission electron microscope, pChG'- ivrL1#32 had abnormal short branched mycelia, and lacked distinguishable outer and inner layers in the cell wall. Lack of chitin in the cell wall of pChG'-ivrL1#32 was observed by calcofluor white staining. These suggest that expression of genes related to hyphal morphogenesis will be suppressed in the pChG'-ivrL1#32. To investigate genes related to hyphal morphogenesis, we carried out transcriptome analysis by using Super-SAGE method (Matsumura et al. 2010 PloS one 5: e12010). We compared transcriptomes between wild type strain SR-1 and pChG'-ivrL1#32 of 3weeks-cultivated mycelia on agar plate. This revealed that differences of transcription levels of genes related to hyphal morphogenesis. Two hydrophobin genes, hyd2 (Kwan et al., 2000 FEMS Microbiol Lett. 15:139-45) and novel hydorophobin (hyd3) were down-regulated in pChG'-ivrL1#32. These will be involved in aerial hyphal formation in the wild type strain. A putative septin gene was suppressed in the pChG'-ivrL1#32, and down-regulation of septin will cause abnormal short branched hyphe in pChG'-ivrL1#32. We found several chitin related genes (putative chitin synthases, chitinases) in wild type strain. Suppression of these chitin related gene will cause reduction of chitin in the pChG'-ivrL1#32 cell wall. A homologue of Utr2p (also known as crh2) was suppressed in pChG'-ivrL1#32. Utr2p has glycoside hydrolase family 16 domain and chitin binding domain. Utr2p is involved in regulation of morphogenesis in yeast. Utr2p is strongly transcribed in wild type strain but significantly suppressed in the pChG'-ivrL1#32. These suggest that laccase suppression will cause reduction of hyphal morphogenesis related gene expressions in L. edodes.

111 IDENTIFICATION AND ANALYSIS OF NOVEL ASPERGILLUS FUMIGATUS TRANSCRIPTION FACTORS INVOLVED IN RESISTANCE TO AZOLES

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Aspergillus fumigatus is responsible for several life threatening diseases in humans. Currently, the drugs of choice for treatment of diseases caused by this fungus belong to the class of azoles and the Aspergillus is usually susceptible to those compounds. However, in the recent years increasing resistance to azoles has been observed and since they are a main class of antifungals, the resistance is a major problem in the clinical settings. Azole resistance has been associated with the mutations in the cyp51A genes or its promoters, however more often resistant isolates without mutations in this gene have been identified and new resistance mechanisms have been proposed. The aim of this study was to identify novel mechanism of azole resistance in A. fumigatus. An impala160 transposon mediated mutagenesis system was utilised to create a library of 500,000 A. fumigatus mutants for further investigation. The library was screened for resistance to itraconazole and amphotericin B and a number of genes associated with azole resistance were identified including two CBF/NF-Y transcription factors AFUB_029870 and AFUB_045980. Directed mutagenesis confirmed that loss of AFUB_029870 or of AFUB_045980 resulted in increased resistance to itraconazole (from 0.5mg/L to >8mg/L) and amphotericin B (from 0.5 mg/L to 2mg/L). Differential transcriptomics (RNAseq) performed on null and wild-type isolates identified a large number of genes (1438 for AFUB_029870 and 1400 for AFUB_045980) that are either directly or indirectly regulated by these transcription factors (+/->2 fold change; FDR<0.05). Almost complete overlap was observed in the genes dysregulated in the null mutants including the gliatoxin biosynthetic cluster and genes associated with cell wall remodelling. Co-immunoprecipitation studies confirmed a direct interaction between AFUB_029870 and AFUB_045980 as well as the global transcriptional regulator Mot1. Further studies are required to fully understand the mechanism of resistance in this fungus.

112 IDENTIFICATION OF GENES ESSENTIAL FOR SEXUAL DEVELOPMENT IN AGARICOMYCETE COPRINOPSIS CINEREA

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We isolated a number of mutants defective in sexual development from a homokaryotic fruiting strain, 326 (Amut Bmut pab1-1), after restriction enzyme-mediated integration (REMI) using plasmid pPHT1. Genetic analysis suggested that 13 of the mutants isolated were due to direct results of insertion of the plasmid. We then performed plasmid rescues/ inverse PCR followed by complementation experiments to identify the genes disrupted in these mutants. Here we show six genes identified to date. Mutations in two genes encoding components of SWI/SNF chromatin remodeling complex, Cc.snf5 and Cc.arp9, are shown to affect asexual development as well as sexual development. Various Cc.snf5 disruptions by gene targeting show that this gene is also involved in dikaryosis via rp1 and rp2 subdomains. These show that SWI/ SNF5 complex is involved in multiple aspects of life cycle in C. cinerea. B28, in which Cc.ubc2 encoding a MAPKK kinase adaptor is mutated, is shown to be defective in A-regulated clamp cell morphogenesis and oidia production as well as B-regulated nuclear migration for dikaryosis, suggesting crosstalk between the A- and B- regulated development pathways. Apa56 and Sac29, in which the Cc.rmt1 gene encoding a putative protein arginine methyltransferase is mutated, are shown to alter developmental regulation in response to external signals such as light and carbon sources. A gene encoding a GATA transcription factor is mutated in A320, in which asexual development is not suppressed even when the A-regulated pathway is switched on. In strain Xba65, a mutation in Cc.sec61 encoding a component of endoplasmic reticulum (ER) membrane protein translocator is shown to be defective in development into fruiting body primordia from hyphal knots.

113 IMAGING AND ANALYSIS OF THE HETEROGENEITY OF INTRACELLULAR CALCIUM DYNAMICS IN ASPERGILLUS FUMIGATUS IN RESPONSE TO ENVIRONMENTAL STRESS

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Calcium signalling and homeostasis are essential for the growth, differentiation and virulence of filamentous fungi. During infection, Aspergillus fumigatus must balance concomitant demands to: (1) withstand toxic levels of exogenous calcium (3-5 mM) in the host environment which can be >100,000x that of the fungal cytosolic free calcium ([Ca2+] c) concentration; appropriately integrate homeostatic and stress-responsive adaptations; and (3) undergo normal calcium signalling. There is evidence for calcium signalling regulating numerous processes including spore germination and hyphal tip growth. The low resting level of [Ca2+]c (50-100 nM) is maintained by Ca2+-pumps and -antiporters, and cytoplasmic Ca2+-buffering. However, [Ca2+]c becomes an intracellular signal when its concentration is transiently increased. We have developed two methods for routinely measuring and imaging [Ca2+]c in A. fumigatus: (1) 96-well plate luminometry using the genetically encoded, bioluminescent aequorin; and fluorescence microscopy using the genetically encoded calcium-sensitive, fluorescent protein G-CaMP5G. Aequorin is ideally suited for quantitative measurements of calcium signatures in cell populations whereas fluorescence imaging of the G-CaMP5G is proving excellent for single cell and subcellular measurements of [Ca2+]c dynamics. Using the aequorin methodology we have found that transient increases in [Ca2+]c with specific, reproducible calcium signatures in A. fumigatus arise from exposure to stresses such as high external calcium, alkaline or oxidative stress. Imaging calcium dynamics with GCaMP5G is providing extraordinary insights into the temporal and spatial dynamics of [Ca2+]c. Pulses in [Ca2+]c occur within actively growing hyphal tips. Exposure of conidial germlings to high external calcium induces dramatic and very dynamic changes in [Ca2+]c with the generation of localized [Ca2+]c transients and waves. Furthermore, there is considerable heterogeneity in the [Ca2+]c responses of different germlings within the cell population. Calcium imaging and measurement using genetically encoded probes, particularly when combined with pharmacological and genetic analyses, is initiating a revolution in our understanding of calcium signalling in filamentous fungi.

114 IMPACT OF AUTOPHAGY AS A QUALITY CONTROL MECHANISM ON FUNGAL SENESCENCE

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Prevention of the accumulation of damaged molecules or even organelles 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, protein quality control via the proteasome and autophagy are active in keeping cells functional over time. In recent years, we have focused on mitochondrial quality control and have studied various mutants impaired at different stages of this pathway in the filamentous fungus Podospora anserina, a well-established aging model. From these studies it became clear that mitochondrial quality control pathways significantly contribute to fungal lifespan. However, selective autophagy, and especially mitophagy, the selective autophagic degradation of mitochondria, has not yet been studied with respect to P. anserina aging. Data from a genome-wide longitudinal transcriptome study which demonstrate a significant increase of autophagy-related transcripts during aging (Philipp et al. PLOS One, accepted) indicate the importance of this process. In order to experimentally address its role during aging of P. anserina, we developed tools to measure autophagy and mitophagy, which make use of the relative stability of GFP in the vacuole. In this assay, the autophagy-dependent degradation of GFP fusion proteins leads to the accumulation of GFP, which can be quantified in Western Blot analyses. In line with the transcriptome data, we found the autophagy-dependent degradation of PaSOD1::GFP to be nearly three times higher in 20 days old cultures than in 6 days old ones. Moreover, a strain lacking PaATG1, a central component of the autophagy machinery, has a significantly shorter lifespan than wild type. Experiments under nitrogen limitation revealed that autophagy is able to delay senescence: While lifespan of the wild type is strongly increased under these conditions, this effect is nearly completely lost in a PaAtg1 deletion strain. In addition, we demonstrated a compensatory upregulation of autophagy in strains impaired in different protein quality control pathways, a mechanism which explains unexpected effects on lifespan in these mutants. Taken together, our data demonstrate that autophagy acts as a pro-survival mechanism and is part of a molecular and cellular quality control system.

115 IMPACT OF G PROTEIN-COUPLED RECEPTORS ON SENSING AND REGULATION OF GROWTH IN THE HUMAN-PATHOGENIC FUNGUS ASPERGILLUS FUMIGATUS

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The asexual conidia of the ubiquitous fungus Aspergillus fumigatus are a major thread to immunocompromised patients(1). Until now, little is known about the pathophysiology of A. fumigatus, especially with regard to the function of the 15 G protein-coupled receptors (GPCR's) that are encoded in the genome. Therefore, we investigate the signalling function of GPCR's and their contribution to physiology and virulence of A. fumigatus. To characterise the function, we generated single knock-out mutant strains that were phenotypically characterised. Furthermore, we applied the BIOLOG-System to investigate putative ligands as well as the Split-Ubiquitin-Yeast-Two-Hybrid-System to verify protein-protein interaction with cytoplasmic signal transduction proteins. With the methods mentioned, we could show that the deletion of some GPCR-encoding genes leads to a decrease in spore formation, whereas the germination rate and the response towards reactive oxygen-inducing agents remained unaffected. The deletion of gprG additionally led to a significant delay in radial growth. Using the BIOLOG-System, we showed that the lack of certain GPCR's alters the growth of the respective mutant strains. Interestingly, addition of the identified metabolites in excess complemented the growth defect of the respective mutants, indicating the link between the ability to sense certain molecules via GPCR's and the effect of these molecules on the physiology of A. fumigatus. In summary, GPCR's of A. fumigatus are involved in sensing of nutrients, as it has been shown recently for A. nidulans. Furthermore, they contribute to the regulation of growth and spore formation as indicated by the changed phenotypes of single-ko strains. Current investigations focus on the link of GPCRs to signal transduction proteins and on their contribution to virulence of A. fumigatus.

[1] KWON-CHUNG, SUGUI; PLOS PATHOGENS, 2013 // DE SOUZA ET AL.; PLOS ONE, 2013

116 INTERPLAY OF PHOSPHATASES AND KINASES: STRIPAK AND MAP KINASES REGULATE CELL DIFFERENTIATION IN SORDARIA MACROSPORA

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Phosphorylation and dephosphorylation are crucial for signal transduction and are carried out by kinases and phosphatases. Both play a role in fruiting body formation in the filamentous ascomycete Sordaria macrospora. This fungus has extensively been used as a model system for fungal cell differentiation, since developmental mutants are easily recognizable due to Sordaria`s homothallic lifestyle [1, 2]. We analyzed sterile mutants by complementation and next-generation sequencing and identified a number of proteins essential for fruiting body formation. Among these proteins were PRO22, PRO40 and PRO45 [3, 4] Using affinity purification and MudPIT (multi-dimensional protein identification technology) mass spectrometry (AP-MS) with PRO22 and PRO40 as bait, we detected phosphatases and kinases as interaction partners. Strikingly, we identified the highly conserved striatin-interacting phosphatases and kinases (STRIPAK) complex as master regulator of fruiting body formation, containing PRO22, striatin homolog PRO11, SmMOB3, and protein phosphatase 2A subunits. We further characterized STRIPAK subunit PRO45, homologous to Neurospora crassa HAM4 and human sarcolemmal membrane-associated protein (SLMAP). Using AP-MS and co-immunoprecipitation, we confirmed that PRO45 is part of fungal STRIPAK. Super-resolution structured-illumination microscopy showed PRO45 localization to the nuclear envelope and to mitochondria. Our studies now focus on the significance of PRO45 localization to different compartments. Experimental evidence suggests interaction of STRIPAK with the cell wall integrity (CWI) MAP kinase cascade via developmental protein PRO40, a homolog of N. crassa SOFT. PRO40 binds to protein kinase C, MAPKKK MIK1, and MAPKK MEK1 via unstructured and WW domain-containing regions. We generated shared interaction networks of PRO40 and MEK1 and integrated data from recent transcriptomics analyses [5]. Our results strengthen our hypothesis that STRIPAK and the CWI pathway are interconnected and reveal a number of candidate proteins for future studies. Due to the evolutionary conservation of the protein complexes under investigation, our data are significant for invertebrate and vetebrate systems.

[1] Kück et al. 2009 The Mycota XV: 17-39 // [2] Engh et al. 2010 J Cell Biol 89:864-872
[3] Bloemendal et al. 2012 Mol Microbiol 84:310-323 // [4] Engh et al. 2007 Eukaryot Cell 6:831-843
[5] Teichert et al. 2012 BMC Genomics 13:511

117 INTRACELLULAR PH OF THE INDUSTRIALLY IMPORTANT CELLULOLYTIC FUNGUS TRICHODERMA REESEI

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The pH homeostasis of filamentous fungus Trichoderma reesei has not been studied before. We analyzed an impact of growth condition on intracellular pH of a T. reesei Rut-C30 strain and a mutant, M106, that accumulates L-galactonate as a consequence of L-galactonate dehydratase deletion. For live-cell measurements of intracellular pH we used the genetically encoded ratiometric pH-sensitive fluorescent protein RaVC and confocal laser scanning microscopy. D-xylose, glucose and lactose used as a carbon source had an important imprint on intracellular pH of T. reesei. Growth in lactose containing medium acidified cytosol most from the carbon sources studied, while intracellular pH of hyphae cultured in medium with xylose remained at higher level. The M106 maintained higher intracellular pH in the presence of D-galacturonic acid than its parental strain Rut-C30. We also studied the effect of extracellular pH to the intracellular pH and were able to show that more acidic external pH caused significant acidification of cytosol compared to near neutral growth conditions. It has been suggested that fungal hyphae have a cytoplasmic pH gradient that has a role in polarised growth form of the fungi. No clear cytoplasmic pH gradient was detected in the growing T. reesei hyphae. As cytoplasmic pH is an important factor that affects the physiology of the cells, it is important to have tools to monitor the pH homeostasis. We have shown that there are big variations in the intracellular pH of T. reesei in different growth conditions and having a tool to follow the chances in intracellular pH during fermentations may prove to be important in optimising the fermentation conditions to improve productivity.

118 INVESTIGATING THE ROLE OF THE EXOCYST COMPLEX IN APPRESSORIUM-MEDIATED TISSUE INVASION BY RICE BLAST FUNGUS, MAGNAPORTHE ORYZAE

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Magnaporthe oryzae is a widespread and devastating plant pathogenic fungus, which causes blast disease in a broad range of cereals and grasses. M. oryzae develops a specialized infection structure called an appressorium which generates huge turgor to breach the leaf cuticle. The fungus than colonizes host epidermal cells and proliferates in plant cells. At the initial stages of infection, the fungus grows biotrophically and after 5 days, necrotrophic lesions appear on the leaf surface. During host invasion fungus secretes a repertoire of effector proteins which allow the fungus to evade the host immune response. These effectors have been shown to localize at the appressorium pore prior to plant infection, at the tips of primary invasive hyphae, and in a specialized plant-derived, membrane-rich structure called the Biotrophic Interfacial Complex (BIC). However the underlying mechanism controlling polarized secretion of effectors is not well defined in M. oryzae. The exocyst is an octameric protein complex (composed of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84) that appears to be evolutionary conserved in fungi and to play a crucial role in vesicle tethering to the plasma-membrane. The exocyst thus plays an important role in polarized exocytosis and interacts with signaling pathways at the apex of fungal cells controlling polarity. We are currently characterizing components of the exocyst complex during infection-related development of M. oryzae. In M. oryzae all of the exocyst components localize to the tip of vegetative hyphae as in other fungi. Interestingly, exocyst components also localize around the appressorium pore, which suggests the pore is an active site for secretion at the point of plant infection. We have shown that Exo70 and Sec5 are involved in secretion of cytoplasmic effectors but not apoplastic effectors. Furthermore, we have also shown that organization of the appressorium pore requires a hetero-polymeric septin GTPase network and here we show that localization of the exocyst at the appressorium pore is septin-dependent. Temperature-sensitive mutation of Sec6 completely disrupts exocyst assembly at the appressorium pore which suggests that Sec6 is an important component of this complex. Targeted gene deletion of exocyst components Exo70 and Sec5 and temperature sensitive mutation of Sec6 also cause significant loss of virulence. We will present new information on the role of the exocyst during invasive growth of M. oryzae.

119 INVOLVEMENT OF A CLASS V MYOSIN IN INTRACELLULAR TRAFFIC IN NEUROSPORA CRASSA

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Polarized growth in filamentous fungi depends on vesicle secretion at the cell apex. Vesicle movement is propelled by motor proteins associated to either the microtubular or the actin cytoskeleton. Class V myosin is thought to be responsible for intracellular vesicle transport along actin filaments,. We studied the localization and dynamics of the class V myosin (MYO-5) in Neurospora crassa by tagging it with GFP and observing it by confocal and TIRF microscopy. Additionally, we deleted the myo-5 genes to observe its phenotypic consequences. MYO-5-GFP is present in the apex co-localizing with the Spitzenkörper (Spk) and forming a cloud of fluorescence around it. It is also possible to observe it very close to the plasmalema in the apical dome. In basal parts of the hypha, there is just a faint fluorescence. MYO-5-GFP is present during septum formation; it is recruited when the actomyosin ring starts constriction. The absence of MYO-5 produces alterations in growth and hyphal morphogenesis, although polarized growth is maintained. myo-5 mutant forms small and compact colonies compared with the wild type stain (WT). myo-5 mutant produces 22 % of the WT biomass. Conidiation is strongly affected (0.09% of WT). myo-5 mutant branches mainly apically and has a smaller hyphal diameter (less than 33.33 %) than the WT. The lack of MYO-5 has a strong effect in cell growth; it seems to participate in the secretion pathway and in the Spk organization.

120 INVOLVEMENT OF UBIQUITINATION MACHINERY IN ENDOCYTIC DEGRADATION OF MALTOSE TRANSPORTER (MALP) IN ASPERGILLUS ORYZAE

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Maltose transporter encoded by the malP gene in the MAL cluster involved in maltose utilization is essential for uptake of maltose that induces the amylolytic genes through the activation of the transcription factor AmyR in Aspergillus oryzae. Expression of the malP gene is induced by maltose and repressed by glucose [1]. On the other hand, we constructed the MalP-GFP fusion protein expressed by own promoter to examine how the MalP protein is regulated at the protein level in response to carbon sources, and found that MalP-GFP was promptly internalized and delivered to the vacuole by endocytosis after addition of glucose [2]. In yeast, endocytosis of several plasma membrane transporters is triggered by ubiquitination mediated by the HECT domain ubiquitin ligase Rsp5, for which an arrestin-like protein containing PY elements acts as an adaptor. One of such arrestin-like proteins Art4/Rod1 is known to be involved in glucose-induced endocytic degradation of lactate/pyruvate transporter Jen1. Because in Aspergillus, CreD that is likely involved in ubiquitination of the carbon catabolite repressor CreA is the arrestin-like protein most homologous to Art4, we investigated the involvement of CreD and Rsp5 ortholog HulA in glucose-induced endocytic degradation of MalP in A. oryzae. When MalP-GFP was expressed in a creD deletion mutant, GFP-derived fluorescence was observed at the plasma membrane in the presence of maltose and the fusion protein was not targeted to the vacuole after addition of glucose. Similarly, MalP-GFP was not delivered to the vacuole in the presence of glucose when expressed in a conditionally null hulA mutant. These results suggested that both CreD and HulA are required for glucose-induced endocytosis of MalP. In addition, pull-down analysis using CreD-3FLAG and WW domains of HulA fused to GST indicated that CreD could interact with HulA via WW domains. Because it has been reported that binding of the ubiquitin ligase and its adaptor, arrestin-like protein, is mediated by PY elements in the carboxy terminus of the adaptor, we mutated a PPXY and three PXY motifs of CreD and examined their effect on glucose-induced endocytosis of MalP. A mutation of PPLY to PPLA resulted in a significant delay in internalization of MalP-GFP after addition of glucose, suggesting that the PPXY motif plays an important role in the CreD-HulA interaction.

[1] Hasegawa et al., Fungal Genet. Biol., 47, 1–9 (2010)
[2] Hiramoto et al., ECFG11, Marburg, Germany (2012)

121 KIPA OF NO RETURN: REGULATION OF THE KINESIN-7 TURNOVER BY THE F-BOX PROTEIN RCYA IN ASPERGILLUS NIDULANS

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Filamentous growth needs to the continuous delivery of proteins, mRNAs and secretory vesicles to the growing tip. The growing tip is determined by specific proteins, the cell-end markers, and differentiated plasma membrane domains. To keep intact the specific PM-domain is necessary that the secretion is coupled with endocytosis. We present the characterization of RcyA, the A. nidulans homolog of Rcy1 in yeast. RcyA plays a role in coupling endo- and exocytosis. The cell-end markers TeaA and TeaR and the endocytic ring are spatially disturbed in the absence of rcyA. In addition, RcyA is necessary for the degradation of the kinesin motor protein KipA to keep it under physiological levels suggesting that RcyA is the substrate adaptor for KipA in a SCF-ubiquitin ligase complex.

122 LABELING HISTONE H2B WITH DIFFERENT FLUORESCENT PROTEINS FOR NUCLEAR VISUALIZATION IN VEGETATIVE AND MATING HYPHAE OF HOMOBASIDIOMYCETE SCHIZOPHYLLUM COMMUNE

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In filamentous fungi, vegetative hyphae are able to fuse and form networks. Fusions also initiate the mating interaction, and make possible the reciprocal nuclear exchange and migration leading to fertilization of both mates and the formation of dikaryotic mycelium. Until now, the nuclear behavior of S. commune hyphae has been realized using fixed hyphae and DAPI staining of the nuclei. In order to follow the fate of the reciprocal exchange and migration of nuclei in living hyphae with different mating-type genes, the histone encoded by one of the four histone H2B genes of S. commune genome was cloned together with its native promoter. This histone encoding gene (Schco_3: PID2605148) was selected due to its high expression both in haploid and dikaryotic hyphae1 and because it contains an intron in the 5´-upstream region. The gene with its promoter (1612 bp) was produced by PCR from genomic DNA of the sequenced S. commune strain and cloned into the pCR2.1 TOPO vector (Invitrogen). The carboxyl terminus was tagged with a 30 bp linker, egfp gene and the Sccdc42 gene terminator. The plasmid named N_HistEGFP_40 also carried a phleomycin cassette2. The transformation of protoplasts from S. commune haploid strain ura-1-9, carrying mating type genes A43B41 and being auxotrophic for ura-1 gene, led to the isolation of several transformants expressing histone H2B with green fluorescence. The fluorescence of EGFP labeled H2B was concentrated in the nuclei, with very little background in the cytoplasm. Combined confocal and phase contrast microscopy revealed one nucleus in each hyphal compartment of the haploid hyphae. Comparable cloning of mRFPruby labeled H2B histone is currently under way. This latter construct will be transformed in a haploid S. commune strain either fully or semi compatible with strain ura-1-9. Light microscopic investigation of DAPI stained nuclei in compatible hyphae have shown regularly dividing nuclei at hyphal fusions3, although a cessation in cell cycle is thought to be associated with reciprocal exchange and migration of nuclei at mating. This discrepancy could be resolved by observing nuclear behavior in living hyphae, with the approach also providing valuable insights as to differences in nuclear behavior in vegetative and mating hyphae. 1Ohm et al. (2010). Nature Biotechnology 28, 957-63, 2Schuren, F.H.J., Wessels, J.G.H., 1994. Curr. Genet. 26, 179–183. 3 Raudaskoski, M. (1998) Fungal Genetics and Biology 24, 207-227.

123 LASER MICRODISSECTION AND TRANSCRIPTOMICS OF INFECTION CUSHION DEVELOPMENT OF 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). 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. 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 separately epiphytically growing runner hyphae and infection cushions. Several hundred runner hyphae and infection cushions grown on wheat glumes were isolated and RNAseq perfomed. Quantitative expression analysis show marked differences in gene expression patterns between runner hyphae and infection cushions. We will discuss specifically differences in carbohydrate active enzymes including cell wall degrading enzymes, putative effector proteins, and secondary metabolites. 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.

124 LIGHT SENSING IN FUSARIUM FUJIKUROI: INTERDISCIPLINARY ANALYSIS OF THE FUNGAL RHODOPSIN CARO

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Many substantial processes in filamentous fungi are controlled by light. Fungi usually possess light sensors reacting to different wavelength in the VIS spectrum. Green light is sensed by fungal rhodopsins, a family of photoreceptors with a highly conserved structure consisting of seven transmembrane helices. The structure includes an internal pocket that harbours the chromophore all-trans-retinal, which is covalently bound to the protein via a protonated Schiff-base. Upon light activation, retinal changes its conformation from all-trans to 13-cis, leading to either proton-pumping or sensory function depending on the protein. However, detailed knowledge of the physiological functions of fungal rhodopsins is still missing. The phytopathogenic fungus Fusarium fujikuroi contains two rhodopsin encoding genes, carO and opsA. The carO gene is linked to and co-regulated with genes coding for enzymes for retinal synthesis, whose expression is strongly induced by light. In order to figure out its possible biological role, we fused CarO to a yellow fluorescent protein (YFP) and expressed the fusion protein in F. fujikuroi mycelia (carO promoter), yeasts (AOX1-promoter), and mammalian cells (CMV-promoter). Combined biophysical analysis (confocal laser scanning microscopy (cLSM), super-resolution fluorescence imaging (dSTORM), and patch-clamp techniques of CarO::YFP revealed that CarO is an efficient proton pump which is expressed in spores and hyphae, both in the cytoplasm membrane and in inner organelles. Unexpectedly, CarO pumping activity is enhanced in the presence of gluconate, a salt or ester of gluconic acid. This compound may be used as a carbon source, and its addition to the medium strongly influences F. fujikuroi growth and secondary metabolism in wild type and carO mutants. Interestingly, though carO mutants exhibit no apparent phenotypic alterations under standard culture conditions compared to control strains, they showed growth alterations when exposed to light under certain pH in media based on ammonium chloride and various carbon sources.

125 MAS1, A 91AA PROTEIN CONFERS NEUROSPORA CRASSA SENSITIVITY TO A NOVEL METABOLITE PRODUCED BY A SPONGE-ASSOCIATED STRAIN OF ASPERGILLUS TUBINGENSIS

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Sponge-associated fungi are a promising source of natural products, due to the unique ecological niche in which they reside. We have isolated several Aspergillus spp. from the Mediterranean marine sponge Ircinia variabilis. One of them, an A. tubingensis strain, was found to secrete metabolites that inhibit the growth of several fungi (Alternaria alternata, Rhizoctonia solani and Neurospora crassa). At least two novel metabolites with antifungal activity were purified and their structures elucidated. The compounds inhibited N. crassa growth (MIC=210μM) and affected hyphal morphology. Using random tagged mutagenesis, we have identified N. crassa mutants exhibiting resistance to the compounds. Plasmid rescue analysis indicated that a defect in a yet-uncharacterized gene (NCU03140.4), designated mas-1, whose product is a cytosolic protein (as determined by localization of a GFP:: MAS1 fusion protein), confers resistance to the compound. This was confirmed by analysis of the appropriate N. crassa knock-out strain. Furthermore, complementation of the knockout strain restored sensitivity to the compound.We analyzed the sensitivity of a Δmas-1 strain to several fungicides whose function results in impaired hyphal integrity. When grown in the presence of either tebuconazole (an ergosterol biosynthesis inhibitor) or fludioxonil (an activator of the hyperosotic stress response pathway), no significant difference between the Δmas-1 strain and the wild type were observed. Unexpectedly, the Δmas-1 strain was only about half as sensitive to sublethal concentrations of the chitin synthase inhibitor polyoxin D. The expression of chitin synthase genes was highly elevated in the Δmas-1 strain when compare to the wt, suggesting alterations in the cell wall of Δmas-1, which may involve changes in chitin deposition.

126 MATURATION OF EXTREME STRESS-RESISTANT ASCOSPORES OF NEOSARTORYA FISCHERI INVOLVES REDUCTION OF BULK WATER AND ACCUMULATION OF TREHALOSE AND TREHALOSE-BASED OLIGOSACCHARIDES

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Neosartorya fischeri ascospores survive stresses such as high temperature (85 °C) and drought (<0.5 % RH). In this study, acquisition of stress resistance during maturation of N. fischeri ascospores was related to accumulation of compatible solutes, the presence of bulk water, and redox stability. Ascospores of 11-day-old cultures were killed by a 2 min treatment at 85 °C, while spores of 15-50 day-old cultures survived this treatment. Spores of 50-day-old cultures even resisted a 50 min treatment at 85 °C. Individual ascospores isolated from 11- and 15-day-old cultures contained 3.9 pg (454 mM) and 12.1 pg (1027 mM) compatible solutes, respectively. This amount increased to 15.4 pg (1051 mM) in ascospores of 50-day-old cultures. The composition of the compatible solutes in the ascospores changed during growth of the culture. Glycerol levels had disappeared in ascospores of 15-day-old-cultures, while mannitol levels decreased after day 20. In contrast, the relative amount of trehalose and trehalose-based oligosaccharides increased until 50 days of culturing. Bulk water, as measured by electron spin resonance (ESR) spectroscopy, was much higher in spores of 11-day-old cultures when compared to spores of 15- to 50-day-old cultures. Ascospore maturation also coincided with increased redox stability. This stability gradually increased during maturation. Dry heat storage of 3 days at 60 °C didn't affect the spin probe immobility or the redox stability of the polar cytoplasmic environment of dried ascospores. However, the redox stability of the more hydrophobic cytoplasmic environment (possibly in the proximity of lipid membranes) did decrease due to dry heat storage. Taken together, this study distinguishes two maturation stages of ascospores. The first stage is accompanied by a reduction of bulk water in the spores, the second stage is characterized by an increase of trehalose and TOS. Redox stability build up was observed during both stages.

127 MUTATIONAL ANALYSIS OF THE PH-SENSING RECEPTOR PALH

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There is a well-conserved signalling pathway among ascomycete fungi to mediate environmental pH sensing. This pathway, involving six proteins (PalA, PalB, PalC, PalF, PalH and PalI) in Aspergillus nidulans, mediates the activation of the transcription factor PacC under alkaline conditions. PalH is a seven-transmembrane domain protein reminiscent of G Protein Coupled Receptors (GPCR) but, instead of signalling through a heterotrimeric G protein, it is coupled to the positive-acting, arrestin-like protein PalF through the two PalF Binding Domains located in its cytosolic tail. PalH is phosphorylated exclusively under alkaline pH. However, this phosphorylation is not essential for pH signalling, and hence its physiological role remains unclear.
In addition, site-directed mutagenesis analyses of PalH interhelical loops and transmembrane helices have been carried out. This screening has led to the identification of several loss-of-function mutations and, importantly, of weak gain-of-function mutations, a class of mutations in the pH signalling receptor that had not been previously described. In this mutant background, a certain degree of PacC proteolytic processing activation is detectable under acidic conditions. With previously reported data indicating that PalH acts upstream of all other Pal proteins with the exception of PalI (a traffic 'policeman'), these results strongly indicate that PalH is the pH sensing receptor in the Pal/pH signalling pathway.

128 MYCELIAL GROWTH UNDER HYPOXIA AND ANOXIA PRODUCE CONIDIA WITH ALTERED STRESS TOLERANCE IN ENTOMOPATHOGENIC FUNGI

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Hypoxia (low oxygen concentrations) and anoxia (lack of oxygen) in filamentous fungi causes significant changes in their metabolism, germination, mycelial growth, and conidial production. The conidial thermotolerance and conidial tolerance to menadione-induced oxidative stress were studied when conidia were produced under hypoxia (Petri dishes sealed tree times with Parafilm), anoxia (the cultures were grown for 24 h under normoxia and transferred to anaerobiosis jars for five days, then transferred back to normoxia for eight days), and compared with conidia produced under normoxia (normal oxygen concentrations), and minimal medium. Ten insect-pathogenic fungal species were used in this study, including Aschersonia aleyrodis, Beauveria bassiana, Isaria fumosorosea, Lecanicillium aphanocladii, Metarhizium. anisopliae s.l. M. brunneum, M. robertsii, Tolypocladium cylindrosporum, T. inflatum, and Simplicillium lanosoniveum. Conidia of three fungal species T. cylindrosporum, M. anisopliae, and L. aphanocladii produced under hypoxia have increased thermotolerance. Only A. aleyrodis conidia produced after the anaerobiosis condition were more resistant to heat. The nutritive stress induced higher conidial thermotolerance on M. brunneum, M. robertsii, T. inflatum, and M. anisopliae. Mycelial growth under hypoxia conditions also had induced increased tolerance to menadione in four fungal species, B. bassiana, M. brunneum, M. robertsii, and M. anisopliae. Again, only conidia of the fungus A. aleyrodis produced after the anaerobiosis condition were more resistant to oxidative stress. MM induced high menadione tolerance on six fungal species B. bassiana, M. brunneum, M. robertsii, T. cylindrosporum, M. anisopliae, and S, lanosoniveum. In conclusion, conidia produced under hypoxia increased tolerance of three fungal species to heat and four species to menadione in relation to conidia produced in normoxic conditions. The anoxic stress only induced higher tolerance to heat and menadione in Aschersonia. The nutritive stress, however, induced higher thermotolerance in four species and higher menadione tolerance in six species. The hypoxic condition did not decrease conidial production in the majority of species; the only reduction was for S. lanosoniveum and A. aleyrodis. The anoxic condition caused mycelial death of four isolates, but on the other isolates, this condition generally did not harm conidial production. The conidial production on MM was negligible.

129 NGS ANALYSIS REVEALED NEW MUTATIONS IN NSDA STERILE MUTANT OF ASPERGILLUS NIDULANS

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Sexual development and fruiting body production of fungi play pivotal roles in production of ascospores by meiosis as well as adaptation of various environmental changes. In a homothallic fungus Aspergillus nidulans, many environmental factors and genes affecting sexual development have been elucidated. One of the first and important attempts for understanding the sexual development of A. nidulans was isolation of NSD mutants, which are defective in the process. NSD mutants are divided into four different complementation groups, NSDA-D, and the two genes responsible for the nsdC and nsdD mutation have already been isolated and characterized. However, nsdA4 and nsdB5 mutations from NSDA and NSDB mutants, respectively, are remained to be unveiled. Since classical complementation experiments by transforming genomic DNA library to the mutants were not successful, we analyzed the whole genome sequence of NSDA mutant obtained from Next Generation Sequencing (NGS) to identify the nsdA4 mutation. As a result of analysis of mutation sites, we previously found several NSDA mutant-specific mutations and confirmed the mutations by PCR followed by sequencing analysis. Three mutations including AN3939 locus, which encodes SCF ubiquitin ligase subunit CulC, were investigated but none of the knock-out of the candidate genes showed nsd- phenotype. However, recent intensive mutation analysis revealed that the NSDA mutant strain carries missense mutations both in the nsdD and nsdC ORF region, suggesting that phenotype of NSDA mutant might be derived from the two simultaneous mutations of the previously identified important genes for sexual development. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (NRF-2012R1A1A4A01012864).

130 NON-MATING G-PROTEIN COUPLED RECEPTORS IN THE MUSHROOM SCHIZOPHYLLUM COMMUNE

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The fungus S. commune is known for its complex tetrapolar mating type system with the mating type loci A and B and their subloci Aα, Aβ, Bα and Bβ. Different specificities of the encoded genes, either for homeodomain transcription factors in case of the A loci, or pheromones and the recognizing receptors with the B loci, lead to an estimated number of over 23.000 possible mating types for a wild-type individual. Most interesting for our study are the B-receptors (Ste3-like, seven transmembrane domains, G-protein coupled), recognizing pheromones of non-self specificity and inducing signal transduction pathways and specific gene regulation. Besides these genes, homologs to the known B-specific receptors were found after sequencing of strain H4-8: four new Ste3-like GPCRs with three of these so called B-receptor like genes (brl's) being localized adjacent to the B locus. Their function is unknown, because a B-locus defective strain without any interactions seen in B-dependent development still contains these four brl's, which obviously do not respond to any wild-type pheromone. However, our results indicate conservation by sequence identity. Two new genomes of the strains TatD and LoeD showed an identical localization in close proximity to the true B receptor genes, but less sequence conservation. Gene expression was observed e.g. by microarray analyses, disproving a potential pseudo gene nature. Expression was further investigated by qRT-PCR during early and late mating interactions and in monokaryotic strains, showing comparable regulation only between gene brl4 and the true mating receptor bar2, with the others being highest expressed under monokaryotic conditions. To gain insights into the function over-expressions are tested. Tagging the receptors for visualization is performed to localize the Brl's and to examine interacting proteins by co-immunoprecipitation.

131 NON-SELF RECOGNITION INDUCES COMMUNICATION INTERFERENCE IN NEUROSPORA CRASSA

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Genetically identical germlings undergo chemotropic interactions and cell fusion in a highly regulated process which is associated with the early colony establishment in Neurospora crassa. However, when cell fusion between genetically different individuals occurs, non-self recognition and a type of programmed cell death occur, called heterokaryon incompatibility (HI). HI prevents transmission of potentially infectious cytoplasmic elements, such as mycoviruses and senescence plasmids. To assess whether chemotropic interactions prior to cell fusion are affected when germlings are genetically different, we compared the frequency of communication between the N. crassa lab strain FGSC2489 and 112 wild type isolates of one population group (Louisiana). Communication frequency between genetically identical germlings was typically between ~75-95%, while communication between genetically different individuals could be drastically reduced to less than 10%. From these analyses, we defined three communication groups (CG) within the 112 N. crassa isolates. Germlings within a CG communicated and fused frequently, while germlings of different CG avoided each other. These data indicate that N. crassa germlings can distinguish individuals of different CGs at a distance. The CG phenotype segregated 1:1 in a cross between a CG1 and CG2 strain, indicating that a single locus determines the affiliation of a strain to a CG. A bulked segregant analysis identified a 100 kbp region of the right arm of linkage group V that showed segregation of SNPs at 100% frequency in CG1 vs. CG2 progeny. Analysis of 24 sequenced isolates of different CGs revealed two divergent genes (communication group determinant; cgd-1 and cgd-2) that were highly polymorphic between individuals from different CGs. The genes cgd-1 and cgd-2 encode hypothetical proteins with two predicted transmembrane domains. A CG1 strain deleted for cgd-1 showed a reduction in communication frequency, but fused at this same frequency with germlings from all three CGs. These data indicate that cgd-1 is involved in both self and non-self communication that affects chemotropic interactions of germlings. GFP-fusions and allele swapping will give us further insight into the function of these proteins during chemotropic interactions between genetically identical versus genetically different germlings.

132 NOX-DEPENDENT GENETIC PATHWAYS GOVERN SEXUAL DEVELOPMENT AND ASCOSPORE GERMINATION IN THE FUNGUS SORDARIA MACROSPORA

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NADPH oxidase (NOX)-derived reactive oxygen species (ROS) act as signaling determinants that induce different cellular processes. To characterize NOX function during fungal development, we utilized the genetically tractable ascomycete Sordaria macrospora. Genome sequencing of a sterile mutant led us to identify the NADPH oxidase encoding nox1 as a gene required for fruiting body formation, regular hyphal growth and hyphal fusion. These phenotypes are shared by the nor1 mutant, lacking the NOX regulator NOR1. Further phenotypic analyses revealed a high correlation between increased ROS production and hyphal fusion deficiencies in the nox1 mutant and other sterile mutants. A genome-wide transcriptional profiling analysis of mycelia and isolated protoperithecia from wild type and the nox1 mutant revealed that nox1 inactivation affects the expression of genes related to cytoskeleton remodeling, hyphal fusion, metabolism, and mitochondrial respiration. Genetic analysis of the nox2 mutant, lacking the NADPH oxidase 2 gene, the nor1 mutant, and transcription factor deletion mutant ste12, revealed a strict melanin-dependent ascospore germination defect, indicating a common genetic pathway for these three genes. We report that gsa3, encoding a G-protein α subunit, and sac1, encoding cAMP-generating adenylate cyclase, act in a separate pathway during the germination process. The finding that cAMP inhibits ascospore germination in a melanin-dependent manner supports a model in which cAMP inhibits NOX2 activity, thus suggesting a link between both pathways. Our results expand the current knowledge on the role of NOX enzymes in fungal development and provide a frame to define upstream and downstream components of the NOX signaling pathways in fungi.

133 NUCLEAR DYNAMICS AND MITOSIS IN FUSARIUM OXYSPORUM IS MORE COMPLEX THAN PREVIOUSLY DESCRIBED

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The plant pathogen Fusarium oxysporum f. sp lycopersici (Fol) has previously been described as a filamentous fungus with uni-nucleated compartments, where the apical compartment undergoes mitosis. Our observations suggest that nuclear behavior in Fol can be more complex. For example, we frequently encountered multi-nucleated compartments and intercalary mitosis during side-to-side fusion of vegetative hyphae. Additionally, we observed that in fast growing hyphal tips at the colony edge several nuclei undergo mitosis simultaneously. Similar to Colletotrichum lindemuthianum, Fol appears to exhibit a diversity of mitotic patterns in different cells and/or developmental stages.

134 NUCLEAR TRANSPORT AND CELLULASE PRODUCTION IN TRICHODERMA REESEI

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The filamentous fungus Trichoderma reesei is an important industrial producer of cellulolytic and hemicellulolytic enzymes, which are used for biofuel production from lignocellulosic biomass. Induction of cellulases involves nuclear import of the main transcriptional activator XYR1 from the cytoplasm. The mechanisms of nuclear import of XYR1, however, remained so far unknown in T. reesei. Nuclear import (and export) generally occurs through nuclear pore complexes and the action of nuclear carriers of the karyopherin-ß superfamily. In order to characterize the nuclear import dynamics of transcriptional regulators relevant for cellulase gene expression, we have screened the T. reesei genome for genes encoding nuclear transport protein - importins and exportins - and examined the phenotype of deletion mutants. Functional characterization of the karyopherins shows that three karyopherin genes are essential in T. reesei, including the alpha importin encoding kap 1, the beta importin encoding kap2 and kap5. Knock-out of the non-essential beta importin kap8, strongly decreased cellulase gene expression and nuclear localization of a GFP-tagged XYR1.

135 OXYGEN AND AN EXTRACELLULAR PHASE TRANSITION INDEPENDENTLY CONTROL CENTRAL REGULATORY GENES AND CONIDIOGENESIS IN ASPERGILLUS FUMIGATUS

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Conidiogenesis is the primary process for asexual reproduction in filamentous fungi. As the conidia resulting from the conidiogenesis process are primarily disseminated via air currents and/or water, an outstanding question has been how fungi recognize aerial environments suitable for conidial development. In this study, we documented the somewhat complex development of the conidia-bearing structures, termed conidiophores, from several Aspergillus species in a subsurface (gel-phase) layer of solid media. A subset of the isolates studied was able to develop conidiophores in a gel-phase environment, but exposure to the aeriform environment was required for the terminal developmental transition from phialide cells to conidia. The remaining Aspergilli could not initiate the conidiogenesis process until they were exposed to the aeriform environment. Our observations of conidiophore development in high or low oxygen conditions in both aeriform and gel-phase environments revealed that oxygen and the aeriform state are positive environmental factors for inducing conidiogenesis in most of the aspergilli tested in this study. Transcriptional analysis using A. fumigatus strain AF293 confined to either the aeriform or gel-phase environments revealed that expression of a key regulatory gene for conidiophore development (AfubrlA) is facilitated by oxygen while expression of another regulatory gene controlling conidia formation from phialides (AfuabaA) was repressed regardless of oxygen levels in the gel-embedded environment. Furthermore, by comparing the developmental behavior of conidiation-defective mutants lacking genes controlling various regulatory checkpoints throughout the conidiogenesis pathway, we propose that this aerial response by the fungus requires both oxygen and the phase transition (solid to aeriform), with these environmental signals integrating into the upstream regulatory pathway and central regulatory pathway of conidiogenesis, respectively. Our findings provide not only novel insight into how fungi respond to an aerial environment to trigger development for airborne conidia production but also the relationship between environmental factors and conidiogenesis regulation in aspergilli.

136 PERCEIVING THE HOST: DIFFERENTIAL GENE EXPRESSION BETWEEN HEMILEIA VASTATRIX APPRESSORIA PRODUCED IN PLANTA AND IN VITRO

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The fungus Hemileia vastatrix is the causal agent of coffee leaf rust one of the most devastating diseases affecting coffee production worldwide. Like other rust fungi, this obligate biotroph develops specialized structures to proceed with the host entry through stomata and cell invasion for nutrient uptake. The plant-fungus dialogue starts as early as the appressorial stage. Appressoria are the first infection structures developed by the fungus and require specific topographic signals from the host for their differentiation from the germ tubes. Like in several other rusts, certain rough artificial surfaces are also capable of inducing appressoria differentiation in H. vastatrix, such as polyethylene or oil-collodion membranes. This easy way to obtain appressoria has allowed the development of several studies concerning gene expression and gene discovery at appressorial level. However, in other pathosystems, it was found that gene expression in appressoria seems to be influenced by host-derived signals, suggesting that, while still inducing appressoria differentiation, artificial rough surfaces may not be useful for appressorium-based gene expression studies. To elucidate the differences between gene expression in appressoria obtained in vitro and in planta, we have constructed a subtractive suppressive hybridization library enriched in genes expressed during in planta appressoria differentiation. Besides revealing genes that are expressed specifically in response to the presence of the host plant, and we expect that our findings could give some insights about factors governing the pathogen-host interaction and mutual recognition.

137 PHOTODYNAMIC INACTIVATION OF THE PLANT-PATHOGENIC FUNGI COLLETOTRICHUM ACUTATUM AND COLLETOTRICHUM GLOEOSPORIOIDES WITH NOVEL PHENOTHIAZINIUM PHOTOSENSITIZERS

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Colletotrichum is a large genus of ascomycete fungi containing several species that are common pathogens to a wide array of crops and non-cultivated plant species. The control of plant pathogenic fungi faces some of the problems that have been observed in the related clinical area, including the selection of antifungal tolerant strains and the relatively few classes of currently available and effective fungicides. Antimicrobial photodynamic treatment (APDT) is an alternative and promising antifungal discovery platform that can be used to control localized mycoses or to kill fungi in the environment. The approach is based on the use of a photosensitizer (PS) that accumulates in the target fungal cell. Subsequent exposure of the PS to light of an appropriate wavelength starts a photochemical process that produces several reactive oxygen species, such as peroxides and singlet oxygen, leading to non-specific oxidative damage and causing the subsequent death of the fungal cells without significant harm to the host. We evaluated the effect of APDT with four phenothiazinium derivatives [methylene blue (MB), new methylene blue N (NMBN), toluidine blue O (TBO) and the novel pentacyclic phenothiazinium photosensitizer S137] on conidia of three fungal species (Colletotrichum acutatum, C. gloeosporioides and Aspergillus nidulans). The efficacy of APDT with each PS was determined, initially, based on photosensitizer minimal inhibitory concentration (MIC). Additionally, the effects of APDT with two selected PS (NMBN and S137) were evaluated on survival of the conidia. Subcellular localization of the PS was determined in C. acutatum conidia. The effects of photodynamic treatments on leaves of the plant pathogens host Citrus sinensis were also investigated. APDT with S137 showed the lowest MIC. MICs for S137 were 5 μM for the three fungal species when a fluence of 25 J cm-2 was used. APDT with NMBN (50 μM) and S137 (10 μM) resulted in a reduction of approximately 5 logs in the survival of the conidia of all species to fluences ≥ 15 J cm-2. Washing the conidia before light exposure did not prevent the photodynamic inactivation. Both NMBN and S137 accumulated in cytoplasmic structures such as lipid bodies of C. acutatum conidia. No damage to orange tree leaves was observed after APDT. Our results open the interesting perspective of using APDT to control plant-pathogenic fungi.

138 PHYSIOLOGICAL PROCESSES REGULATED BY NITRIC OXIDE BOTRYTIS CINEREA

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Botrytis cinerea is a wide host range pathogen. It is a necrotroph that grows preferentially on senescent or dead tissues, but it can also infect healthy plant tissues. It has been observed that the fungus benefits from the host cellular dead program activated in the plant in response to pathogens' attack. The ROS and RNS produced during this process create a hostile environment for microorganisms. B. cinerea, however, survives in these conditions. Functional analysis of fungal components involved in the response to high levels of ROS and of NO, and considered to be important for pathogenicity, suggest the fungus does not experience a strong oxidative nor nitrosative stress in planta. Remarkably, it has been demonstrated that the fungus itself produces ROS that might contribute to the oxidative status in planta. In addition to their participation in pathogenicity processes, a more basic role related to the regulation of differentiation and virulence processes has been proposed for fungal produced ROS. Similarly, the production of NO by the fungus has also been demonstrated, being this production regulated by the development stage of the fungus, the exposure to exogenous NO and the host. On a first attempt to identify the physiological processes in which NO participates in B. cinerea, we have carried out pharmacological studies in which germinating spores and mature mycelium were exposed either to NO donors or to NO scavengers. The results obtained point out that NO affects germination. Transcriptomic analyses of germinating spores exposed to DETA-nonate and to cPTIO showed that NO modulates the expression of numerous genes. GO terms highly represented among the up- and down-regulated genes in response to NO are oxidation-reduction processes, nitrogen metabolic processes and gene expression and regulation of transcription. Interestingly, the GO terms DNA repair, DNA replication, chromatin remodeling, chromosome segregation and cell cycle progression were also identified.In order to obtain information about the genetic factors mediating the responses to NO in B. cinerea, several genes identified in our transcriptomic analysis have been selected for functional characterization. In this work we present and discuss the results obtained in the course of the analysis of Bcnor1 and Bcnor2, two B. cinerea nitric oxide responding genes encoding each a C6 transcription factor.

139 PKCA LOCALIZATION DEPENDS ON TWO HIGHLY CONSERVED REGIONS

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The Aspergillus nidulans orthologue of Protein Kinase C (PkcA) is involved in the organism's putative cell wall integrity (CWI) pathway. The pkcA gene is essential and reduced expression of PkcA under control of an inducible promoter (alcA) causes rupture of hyphae. A strain bearing the G564R substitution adjacent to the PkcA C1B domain is hypersensitive to cell wall perturbing agents including Calcofluor White and Caspofungin. We have demonstrated PkcA localization to sites of cell wall synthesis, septa and growing hyphal tips. Localization to septa is transient; initially accumulating as a ring at the septation site, contracting as septum construction progresses, and dissipating once a septum is complete. In the current work we identify the regions within PkcA that are responsible for its localization to hyphal tips and septation sites. To this end, we designed serially truncated PkcAs and expressed them as green fluorescent protein (GFP) chimeras. We deleted sequence at both the N- and C-termini. Those that were C-terminally truncated were GFP labeled at the C-termini and expression was driven by 600 base pairs of 5'-untranslated sequence. The N-terminally truncated chimeras were labeled at the N-termini and driven by the alcA promoter. We identified two regions that direct PkcA localization. The first, a 10 amino acid sequence near the carboxyl end of the C2 domain, is required for localization to hyphal tips. Constructs containing this sequence also localize to septation sites. However, the isolated C2 domain localizes to the cytosol. We propose that PkcA localization and subsequent participation in a putative CWI pathway is facilitated by motif-requiring protein-protein interactions with other tip growth and septation related proteins. A second region between C2 and C1B (encompassing C1A) is sufficient for localization to septation sites, but not hyphal tips. Both sequences we identified are highly conserved among various fungi. Taken together, these results suggest a bipartite model for PkcA interaction with other septation proteins that may work as a team or independently to escort or tether PkcA to septation sites.

140 PROPER ACTIN RING FORMATION AND SEPTUM CONSTRICTION REQUIRES COORDINATED REGULATION OF SIN AND MOR PATHWAYS THROUGH THE GERMINAL CENTRE KINASE MST-1

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Regulation of cell polarity and cytokinesis is highly complex and involves a large number of components that form elaborate interactive networks. Fungal nuclear Dbf2p-related (NDR) kinases function as effector kinases of the morphogenesis (MOR) and septation initiation (SIN) networks, which have opposite functions during septum formation, and are activated by the pathway-specific germinal centre (GC) kinases POD6 and SID1, respectively. We characterized a third GC kinase, MST-1, that connects both kinase cascades. Genetic and biochemical interactions with SIN components and life imaging identify MST-1 as SIN-associated kinase that functions in parallel with the GC kinase SID-1 to activate the SIN-effector kinase DBF-2. Aberrant cortical actomyosin rings are formed in Δmst-1, which result in miss-positioned septa and irregular spirals, indicating that MST-1-dependent fine-tuning of the SIN is required for proper formation and constriction of the septal actomyosin ring. However, MST-1 also interacts with several components of the MOR network and modulates MOR activity at multiple levels. These data specify an antagonistic relationship between the SIN and MOR during septum formation that is, at least in part, coordinated through the GC kinase MST-1.

141 RAPID DISORGANIZATION OF THE GOLGI APPARATUS BY BLOCKING THE EXIT OF COPII TRAFFIC FROM THE ENDOPLASMIC RETICULUM

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The mechanistic bases of the biogenesis and maintenance of the cisternae of the Golgi apparatus are still subject to debate. Proteins and lipids synthesized at the endoplasmic reticulum (ER) are sent to the Golgi apparatus into vesicles. The formation of these vesicles is a crucial step of the secretory pathway involved in Golgi function and maintenance. Coat protein complex II (COPII) is a set of highly conserved proteins that mediates the biogenesis of those membrane vesicles. Sar1 is a p21 GTPase that triggers and regulates the assembly of COPII. sarA, the Aspergillus nidulans sar1 orthologue, is essential. We generated a saturated library of mutant alleles by random PCR combined with gene replacement and selected a collection of temperature-sensitive alleles that are useful to specifically block traffic from the ER to the Golgi apparatus. Following a temperature shift-up, we found that Sec23, a subunit of COPII, shifts its localization from the ER to the cytosol, suggesting that COPII vesicles do not form. In addition, an acute and rapid disorganization of the Golgi apparatus occurs: RerA (Rer1), a Golgi resident protein that cycles between Golgi and ER to retrieve proteins to the ER, abnormally labels ER membranes, indicating that its steady-state equilibrium has changed, and the late Golgi marker PHosbp becomes cytosolic. Our data seem incompatible with the stable cisternae model, supporting instead cisternal maturation.

142 REGULATION OF SNARES IN THE ENDOVACUOLAR SYSTEM OF ASPERGILLUS NIDULANS

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Aspergillus nidulans presents many advantages for the study of the traffic within the endocytic pathway. Probably, the main one is the ease to differentiate by fluorescent microscopy highly motile early endosomes (EEs) from late endosomes (LEs), larger and relatively static, and from vacuoles, spherical, static and with an optically visible lumenal space. Previous work in our lab defined Rab5 and Rab7 domains in EEs and vacuoles, respectively, and also provided evidence of maturation of the former into the latter [1,2,3]. Maturation of EEs into LEs is essential but, once EEs has matured, homotypic fusion of LEs/vacuoles is not vital. In the present work we address the study of endosomal maturation focusing in the regulation of syntaxins, one kind of t-SNAREs, in the endovacuolar system. Pep12 is the sole syntaxin of A. nidulans in this context, which lacks a Vam3 homolog. Vps45 and Vps33 are SM proteins that positively regulate syntaxins. Vps45 binds tightly to the late-Golgi/endosomal syntaxin Tlg2 but surprisingly, inactivation of Tlg2 gave rise to no growth alterations while a vps45 null mutant is markedly affected. These data suggest that Vps45 must be regulating an additional syntaxin and we hypothesize that Pep12 is this second target. In fact, there is evidence of functional [4] and, debatably, physical [5,6] interaction between both proteins in yeast and deletion of A. nidulans pep12 recapitulates the vps45Δ phenotype. We have proved experimentally that rabbit polyclonal antiserum against Pep12 is able to specifically immunoprecipitate Vps33 and Vps45 but not Sly1, the SM of the early-Golgi syntaxin Sed5, while Tlg2 antiserum exclusively and strongly immunoprecipitate Vps45. We believe that Pep12 must be regulated at three different levels by two SM proteins: Vps33, in the EEs CORVET and LEs/vacuole HOPS contexts, and Vps45, in the Golgi-to-endosomes traffic. Additionally, we are identifying all the SNARE complexes formed in the endovacuolar system to establish a physical map of interactions. Even though much more effort is required to understand the source of all interactions our results indicate that Pep12 is regulated by two different SM proteins along the endovacuolar system.

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143 RELATIONSHIPS BETWEEN GROWTH AND CELL CYCLE IN USTILAGO MAYDIS

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During proliferation, the progression through cell cycle has to be coordinated with the cell growth. In fungal cells, during vegetative growth, there is a clear correlation between active cell cycle and the ability to growth. For instance, in filamentous fungi, basal compartments that are cell cycle arrested also diminished its ability to growth, keeping a fixed size. Only cell compartments that are active at cell cycle also are active at growth. This fact implies that most likely there are connection between the elements involved in cell cycle regulation and the elements involved in growth regulation, to promote both processes at the same time. The corn smut fungus, Ustilago maydis, has to produce a specific structure called infective filament consisting of a dikaryotic hyphae, which is required to penetrate the plant tissue. However, a peculiar characteristic of the U. maydis dikaryotic filament is that in spite of an active growth, this infective filament has its cell cycle arrested. This combination of growth without cell cycle progression produces a filament of around 100µm in length (vegetative U. maydis cells are around 17 µm in length). How cell growth seems to be disconnected from cell cycle progression in this structure is unknown. In order to address this issue, we should know in first place how cell growth and cell cycle progression are coordinated in U. maydis. Here we will introduce our initial attempts to uncover these connections.

144 ROLE OF CELL WALL ON SENSITIVITY OF FUNGI TO CHITOSAN

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Chitosan is a natural polymer of β-1,4-glucosamine subunits derived from chitin. This biopolymer displays antifungal activity against yeast and filamentous fungi. Permeabilization plasma membrane has also been demonstrated to explain the fungicidal effects of chitosan. We have studied the role of the fungal cell wall on the sensitivity of fungi to chitosan. Neurospora crassa recently prepared protoplasts are more sensitive to chitosan than conidia or protoplasts regenerating the cell wall. This indicates the protective role of the fungal cell wall to chitosan stress. Chitosan is synergic with fungal cell wall stressing compounds. This is specially evident on chitosan sensitive fungi such as Neurospora crassa. We are also investigating the role of the cell wall on putative chitosan gene targets identified in a previous chemogenomic study in Saccharomyces cerevisae. This study opens new ground in the knowledge of the mode of action of chitosan, specially regarding cell wall and plasma membrane liaisons.

145 ROLE OF OLIGOMERIZATION IN THE TRAFFICKING AND VACUOLAR TURNOVER OF A PURINE TRANSPORTER IN A MODEL FUNGAL SYSTEM

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The uric acid/xanthine transporter UapA of the model fungus Aspergillus nidulans has been used as a prototype cargo for studying membrane trafficking and endocytosis. In the presence of ammonium ions or substrates, UapA is ubiquitinated, internalized from the plasma membrane and sorted to the MVB/vacuolar pathway. Interestingly, substrate-elicited endocytosis operates only for functional UapA molecules. However, inactive UapA versions are endocytosed if co-expressed with active UapA molecules. The latter phenomenon, designated in trans endocytosis, prompted us to investigate whether UapA homo-oligomerizes. Here, we confirm the oligomerization of UapA using two different approaches; in vivo bimolecular fluorescence complementation (BiFC) and direct pull-down assays of differentially tagged UapA molecules. We show that functional UapA oligomers are initially formed in the ER membrane and remain stable in the plasma membrane. Using UapA mutants showing ER-retention, we subsequently identify an N-terminal motif and other elements affecting oligomerization. Finally, we show that substrate-elicited endocytosis, unlike ammonium-induced, coincides with the dissociation of transporter oligomers, prior to their internalization. Our findings suggest that UapA oligomerization, analogously to some plant and mammalian transporters, is critical for ER-exit, sorting to the plasma membrane and endocytosis. At present, we examine the possible role of the Sec23/24 ER-exit molecular machinery on UapA oligomerization and vice versa.

146 ROLES OF ALTERNATIVE G1-LIKE CYCLINS IN POLAR GROWTH IN USTILAGO MAYDIS

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The basidiomycete fungus Ustilago maydis causes smut disease in maize. The activation of the virulence program requires the mating of a pair of compatible haploid yeast–like cells after pheromone signaling to produce an infectious dikaryotic hyphae. The production of all these structures relies on the activation of a sustained and strong polar growth. Because this, it is not surprising that cytoskeleton regulators, like Rac1, or molecular motors, such as myosin V, are required for pathogenic development in U. maydis. However, how these housekeeping elements are differentially regulated during the pathogenic development is not currently understood. Our laboratory described the requirement of a Cyclin-dependent kinase, Cdk5, as well as virulence-specific cylin Pcl12 for the proper formation of conjugative hyphae as well as infective filaments.

G1-like CDK activity seems to promote polar growth in well-known systems such as S. cerevisiae and the molecular details are starting to be understood. In other fungal system able to produce dimorphic growth, such as Candida albicans, it has been reported that alternative CDK complexes with G1-like activity are responsible of the induction of the polar growth required for the dimorphic switch. Our hypothesis is that the Pcl12-Cdk5 complex is acting as a G1-like CDK complex and thereby promoting polar growth in U. maydis during the pathogenic development. In this communication we will introduce our research efforts to elucidate which elements are located downstream of this CDK complex in order to activate the polar growth in U. maydis.

147 STEROL STRUCTURE SPECIFICALLY IMPACTS INTERCELLULAR COMMUNICATION AND CELL FUSION IN NEUROSPORA CRASSA

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Cell fusion is a fundamental process among all eukaryotic organisms. We use Neurospora crassa as a model system to explore the genetic bases of intercellular communication and fusion. During early colony formation, germinating vegetative spores of N. crassa mutually attract and grow towards each other, establish physical contact, and undergo cell wall breakdown and plasma membrane fusion. Cell-cell signaling in N. crassa involves an unusual mode of interaction. During directed growth, the MAP kinase MAK-2 and the cytoplasmic protein SO are recruited to germling tips in a highly dynamic, oscillatory manner. Upon cell-cell contact, both proteins concentrate at the site of fusion. To understand the formation of these membrane-associated protein complexes containing MAK-2 and SO, we studied the role of the plasma membrane during signaling and fusion. Since sterols are crucial constituents of eukaryotic cell membranes with many biological functions, we analyzed the behavior of mutants affected in the biosynthesis of ergosterol. Deletion of the erg-2 gene, which encodes an enzyme that mediates the last step in this biosynthesis pathway, strongly reduces interactions between germlings. Interestingly, those few cells of the mutant that still attract each other are unable to arrest growth after physical contact and rarely undergo fusion. A detailed comparison of different sterol biosynthesis mutants of N. crassa revealed that not the absence of ergosterol but the accumulation of a sterol intermediate in Δerg-2 impacts germling fusion. Strikingly, only those sterol precursors with two double bonds in the side chain of the molecule specifically provoke Δerg-2-like defects. While the recruitment of MAK-2 in Δerg-2 is comparable to wild type, the SO protein is only poorly recruited and strongly mislocalizes all over the plasma membrane, even after cell-cell contact. Right after wild-type cells touch, the MAP kinase MAK-1 accumulates at the contact site, presumably regulating cell wall breakdown during fusion. In contrast, we never observed recruitment of MAK-1 in Δerg-2 pairs, suggesting a failure to initiate cell wall remodeling. Interestingly, by manipulating the stability of SO and the activity of MAK-1, we were able to provoke defects similar to those of Δerg-2. These data suggest a functional link between the pathways that mediate intercellular signaling and cell wall integrity during germling fusion, both depending on specific structural features of sterols.

148 STRUCTURE AND FUNCTIONS OF THE NADPH OXIDASE COMPLEXES IN BOTRYTIS CINEREA

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Reactive oxygen species (ROS) are widely generated and act as messenger molecules for intercellular signaling or play a role during defense mechanisms against pathogens (Heller and Tudzynski, 2011). Their most common enzymatic producers are NADPH oxidases (Nox). In fungi they have been shown to be involved in various differentiation processes like fruiting body development or sclerotia production, in addition in pathogenic fungi they can be involved in virulence as we have shown e.g. for the gray mold fungus Botrytis cinerea. Botrytis cinerea is an airborne, necrotrophic plant pathogen with more than 200 hosts world wide. Among those are economically important crops such as strawberries, grapes or tomatoes.In this fungus two NADPH oxidase isoforms (BcNoxA and BcNoxB) as well as their putative regulator (BcNoxR) were previously identified (Segmueller et al., 2008). Besides their involvement in pathogenicity and sclerotia production, deletion studies have revealed that BcNoxA and BcNoxR are also involved in hyphal germling fusions (Roca and Weichert et al., 2012). Fusion of the respective proteins with fluorescence markers showed a localization of the catalytical subunits BcNoxA and BcNoxB primarily to the ER, while the regulator BcNoxR is localized in vesicles throughout the hyphae and at the hyphal tips (Siegmund et al., 2013). In contrast to the mammalian system, not all components of the fungal Nox components have been identified, yet. For B. cinerea interaction studies with potential candidates confirmed an interaction of the small GTPase BcRac with the regulator BcNoxR. Furthermore, analyses of the tetraspanin BcPls1 reveal a similar deletion phenotype as the BcNoxB isoform (Siegmund et al., 2013), while the putative ER protein Pro41 seems to have similar functions as BcNoxA. An involvement of these two proteins with BcNoxA and BcNoxB might be a first step to understand how the two isoforms are differentially regulated in B. cinerea.

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149 T. REESEI MEIOSIS GENERATES SEGMENTAL ANEUPLOID TO ENHANCE PRODUCTION OF HEMICELLULASES

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Hypocrea jecorina is the sexual form of industrial workhorse fungus Trichoderma reesei QM6a. QM6a and its derivates are the main industrial sources of cellulases and hemicellulases used to degrade bimass to simple sugars. H. jecorina sexual reproduction yields hexadecad asci with 16 linearly arranged ascospores. Our results reveal that these ascospores are generated via two rounds of postmeiotic mitosis following the two meiotic divisions. The QM6a genome sequencing project revealed 89 assembled scaffolds (sets of ordered and oriented contigs) totaling ~ 34 Mb. Here, we also report that the genome of H. jecorina CBS999.97 wild isolate comprises large DNA rearrangements in scaffold 33 and scaffold 36. Remarkably, due to chromosome heterzygosity, the hexadecad asci of CBS999.97 wild isolate frequently (>90%) contain four or eight inviable ascospores with an equal number of viable segmentally aneuploid ascospores. Array-based comparative genomic hybridization (aCGH) experiments revealed that all the viable segmentally aneuploid progenies have a large chromosomal duplication (~500 kb) and a short chromosome deletion (~33 kb). Intriguingly, segmental duplication contains genes involved in xylan degradation and enhances expression of several carbohydrate-active enzymes, particularly cell wall degrading hemicellulases. These results suggests additional impacts of meiosis in promoting genetic heterogeneity and genome plasticity.

150 THE CATALYTIC SUBUNIT 1 OF SERINE/THREONINE PROTEIN PHOSPHATASE 2A (PP2AC1) CONNECTS HIGHLY CONSERVED SIGNALLING PATHWAYS

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Using protein-protein interaction studies, we present an approach to investigate a putative protein supracomplex controlling the sexual development in Sordaria macrospora. This ascomycete serves as an excellent model organism for development of fruiting bodies [1, 2]. To determine their role for sexual differentiation we deleted the genes encoding the catalytic subunits of the major serine/threonine protein phosphatase 2A family PP2A, PP2Ac1 and PP2Ac2. PP2A balances various signal transduction pathways by negatively regulating kinases or other signaling modules. PP2A is highly conserved throughout eukaryotes and dephosphorylates a multitude of cellular proteins involved in e.g. cell cycle control and gene regulation. PP2A typically assembles as a heterotrimer including a structural A-, a regulatory B- and a catalytic C- subunit [3, 4]. A pp2Ac1 deletion strain shows a sterile phenotype. Data from tandem affinity purifications with PP2Ac1 as bait followed by MudPIT (multidimensional protein identification technology) mass spectrometry revealed a strong interaction of PP2Ac1 with PRO22 and two A phosphatase associated protein (TAP42). The putative alternative phosphatase subunit TAP42 suggests a link to the target of rapamycin (TOR) pathway [5]. Moreover, PP2Ac1 may connect different signalling pathways leading to a controlled reaction on a multitude of signals. Using yeast two-hybrid analysis we found that both catalytic subunits of PP2A interact not only with subunits from the striatin interacting phosphatase and kinase (STRIPAK) complex but also with a PRO40-MAPK interaction network. The STRIPAK complex is highly conserved within eukaryotes and the first description in filamentous fungi was done recently in S. macrospora [6]. The STRIPAK complex consists of at least six subunits, among them PP2AA and PRO22 and PRO45, while the PRO40-MAPK interaction network comprises PRO40 and the three kinases of the cell wall integrity pathway. Both complexes play a crucial role in signalling during the transition from vegetative growth to sexual development.

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[5]Van Hoof et al. (2005) Biochem. J. 386, 93–102 // [6]Bloemendal et al. (2012) Mol. Microbiol. 84: 310-323.

151 THE CELL WALL STRESS RESPONSE OF ASPERGILLUS NIGER INVOLVES THE ACTIVITY OF AT LEAST TWO TRANSCRIPTION FACTORS: RLMA AND MSNA

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Comprehensive understanding on how fungi adapt and survive cell wall stress conditions is still missing. Recently, we have shown the importance of the cell wall integrity pathway and its downstream targets RlmA (transcription factor) and AgsA (alpha-1,3 glucan synthase) for Aspergillus niger to survive sublethal concentrations of caspofungin, fenpropimorph and the antifungal protein AFP (Meyer et al 2007, Hagen et al. 2007) . In this study, we extended these transcriptomic and physiologic analyses to study the response of A. niger towards aureobasidin A (AbaA), an inhibitor of the sphingolipid biosynthesis and FK506, an inhibitor of the calcium-calcineurin signaling pathway. Upon AbaA treatment, 237 responsive genes were found which were mainly assigned to function in (i) lipid metabolism, (ii) cell wall remodelling, (iii) vesicle transport, (iv) nutrient transport and (vi) proteasomal degradation. Expression of 96 genes changed upon FK506 treatment, which are predicted to function in (i) ion homeostasis, (ii) calcium signaling, (iii) protein folding and maturation and (iv) vesicle trafficking. In silico analysis of all responsive genes and their promoter regions predicted that beside RlmA, another transcription factor, MsnA, might guard A. niger against these cell wall stressors. Analysis of the phenotype of A. niger when depleted for MsnA indeed confirmed that MsnA is important for A. niger to withstand cell wall stress.

152 THE CELL-END MARKER TEAA AND THE MICROTUBULE POLYMERASE ALPA CONTRIBUTE TO MICROTUBULE GUIDANCE AT THE HYPHAL TIP CORTEX OF ASPERGILLUS NIDULANS TO PROVIDE POLARITY MAINTENANCE

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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 at the cortex. Further analysis suggested a functional connection between TeaA and AlpA (an ortholog of the MT polymerase Dis1/CKAP5/XMAP215) for proper regulation of MT growth at hyphal tips. AlpA localized at MT plus-ends, and bimolecular fluorescence complementation assays suggested that it interacted with TeaA after MT plusends reached the tip cortex. In vitro MT polymerization assays showed that AlpA promoted MT growth up to sevenfold. Addition of the C-terminal region of TeaA increased the catastrophe frequency of the MTs. Thus, the control of the AlpA activity through TeaA might be a novel principle for MT growth regulation after reaching the cortex.
Reference: Takeshita et al., (2013) J. Cell Sci. 126, 5400-5411.

153 THE FUNCTIONAL ORTHOLOGUE OF THE HUMAN TUMOR SUPPRESSOR APC PROTEIN MIGA PLAYS A ROLE IN POLARITY DETERMINATION IN THE FILAMENTOUS FUNGUS ASPERGILLUS NIDULANS

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Polarity establishment and maintenance is an essential process conserved in all kingdoms and very obvious in filamentous fungi like A. nidulans. The cell needs a orchestrated 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 (MAP's) such as microtubule plus-end tracking proteins (+TIP's) play key roles in establishing and maintaining an internal polarity axis. In addition, microtubules define the site of actin polymerization through the delivery of cell end marker proteins (1). Here we describe MigA (Microtubule guiding protein A) 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. MigA interacts with the membrane associated ApsA protein and is involved in spindle positioning during mitosis. Since MigA is related to the yeast Kar9, this function is conserved in comparison to Saccharomyces cerevisiae. Moreover, MigA is also associated with septal and nuclear microtubule organizing centers (MTOC's) and localizes in an EbA-dependent manner to assembling and retracting microtubule plus-ends. This characteristic classifies MigA as a +TIP. A. nidulans MigA forms a homodimer and is able to bind filamentous α-tubulin autonomously. In contrast to Kar9, MigA 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. MigA also interacts with the class V myosin MyoV (3). Taken together we propose an active Actin-MyoV-MigA-dependent guidance mechanism of microtubules in the hyphal tip, which we named MigA-pathway. Hence, actin and microtubule organization depend on each other.

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154 THE NICOTINATE UTILIZATION PATHWAY OF ASPERGILLUS NIDULANS

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Many microorganisms can utilise nicotinic acid as a Nitrogen source, however the degradation pathway of nicotinic acid was studied only in a few prokaryotes. In eukaryotes the only known enzyme of this pathway is HxnS of Aspergillus nidulans (purine hydroxylase II, encoded by hxnS) that hydroxylates nicotinic acid to 6-hydroxy-nicotinic acid as a first step of the degradation. We have identified three genomic clusters, NDC1 (Nicotinic acid Degradation Cluster 1 - hxnS, hxnR, hxnP, hxnT, hxnY, hxnZ) and NDC2 (hxnX, hxnV) separated by 40 kb from each other on chromosome IV and NDC3 (hxnN, hxnM) is on chromosome I. Some genes in the chromosome VI clusters correspond exactly to mutations identified in the 70s by Kelly and Scazzocchio in a classical genetic screen (unpublished). Genes in the three clusters are under the control of the HxnR transcription factor, included in the NDC1 cluster, and are induced by a metabolite of nicotinate degradation. Deletion mutants for each NDC cluster genes (hxnR, -P, -T, -Y, -Z of NDC-1, hxnX, -V of NDC2 and hxnN, -M of NDC 3) led to the identification of several steps in the nicotinate degradation pathway. Besides HxnS, three gene products, HxnX and V from NDC2 and HxnM from NDC3 are involved in the degradation pathway. We also determined that the true inducer of the pathway is the intermediate metabolite of the nicotinic acid degradation that is produced in the third step of degradation by HxnV or HxnX enzymes. This research was supported by the Hungarian Scientific Research Fund (OTKA-K101218) and previously to EU grants to C. Scazzocchio.

155 THE PHOTORECEPTOR WCOB ACCUMULATES IN THE CYTOPLASM AND INTERACTS WITH THE CAROTENE OXYGENASE CARS IN THE FUNGUS PHYCOMYCES BLAKESLEEANUS

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Phycomyces blakesleeanus is sensitive to several environmental signals such as light, wind, gravity and pressure. Light modifies the direction of growth of the fruiting body, sporangiophore, (phototropism), stimulates the production of beta-carotene in the mycelium and regulates the development of the sporangiophores. Blue light is sensed through the Mad complex, a blue-light regulated transcription factor complex composed of MadA and MadB. MadA and MadB are homologs of WC-1 and WC-2 from Neurospora crassa. MadA contains a LOV domain for chromophore binding, and both MadA and MadB contain PAS domains for protein-protein interactions and a Zn finger domain for DNA binding. The Mad complex is required for sensing blue light, and for the regulation by light of transcription. Phycomyces genome has three genes homologs to wc-1: madA, wcoA and wcoB; and four genes homologs to wc-2: madB, wctB, wctC and wctD. WcoB contains a LOV domain and two PAS domains, but lacks the Zn finger domain. WcoB is homologous to MCWC-1B of Mucor circinelloides, a protein that regulates the biosynthesis of beta-carotene in coordination with CrgA. We have characterized the localization of WcoB in the mycelium of Phycomyces using an antibody raised against a peptide of WcoB. The gene wcoB is induced by light in vegetative mycelia, but WcoB was present in mycelia kept in the dark or exposed to light. The induction by light of transcription did not result in a major change in the amount of WcoB. In order to identify the cellular localization of WcoB we performed cellular fractionations using cultures grow in the dark or exposed to 30 min of light. We detected WcoB in the cytoplasmic fraction of cellular extracts, while the nuclear fraction was devoid of WcoB. Immunofluorescence assays with spores or germinating mycelia showed that WcoB was detected as localized patches in the cell membrane. Our results suggest that WcoB does not act as a transcription factor. In order to identify proteins that interact with WcoB we performed immunoprecipitation assays. We detected several proteins that immunoprecipitated with WcoB. One of the putative WcoB-interacting proteins was identifed as CarS, a carotenoid oxygenase that breaks beta-carotene in the first step of the biosynthesis of the sexual hormones. It is possible that WcoB provides light-sensing capabilities to CarS through this interaction, allowing the postranslational regulation by light of beta-carotene and sexual hormone biosynthesis.

156 THE RELEVANCE OF MATING TYPE AND SEXUAL COMPETENCE FOR ENZYME PRODUCTION IN TRICHODERMA REESEI

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Trichoderma reesei QM6a is the parental strain of all T. reesei strains currently used in research and industry. Sexual development of this strain can be used to combine characteristics of different production strains or to introduce properties of nature isolates into production strains. However, although this strain is able to undergo sexual development, it was also shown to be female sterile. We were therefore interested whether full sexual competence or mating type is relevant for biotechnological production of enzymes. We analyzed sexually competent strains with the genetic background of QM6a along with the fully sexually competent nature isolate CBS999.97. We found that the growth characteristics of strains with the two different genetic backgrounds vary considerably on diverse carbon sources. However, no significant difference in carbon utilization was observed in strains of different mating types. Transcriptome analysis of these strains upon growth on cellulose accordingly only showed few differences in gene regulation between mating types except for the characteristic mating type genes. In contrast, gene regulation in strains of different genetic background was considerably altered, which is also in agreement with growth data. Investigation of female fertile strains compared to female sterile QM6a revealed more than 90 genes with different transcript levels including glycoside hydrolases, transporters and a strongly regulated dehydrogenase. Therefore we developed a PCR based screening method for identification of female fertility or sterility in sexual crosses of QM6a derivatives. In conclusion, progeny of both mating types can be used for biotechnological fermentations, while female fertility might cause altered gene regulation.

157 THE ROLE OF ENDO-BETA-N-ACETYLGLUCOSAMIDASES (ENGASES) IN THE ENDOPLASMIC RETICULUM ASSOCIATED DEGRADATION PROCESS (ERAD) OF MISFOLDED GLYCOPROTEINS IN THE MYCOPARASITIC ASCOMYCETE TRICHODERMA ATROVIRIDE

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N-glycosylation is an important post-translational modification of proteins, which mainly occurs in the endoplasmic reticulum (ER). Glycoproteins that are unable to fold properly are exported to the cytosol for degradation by a cellular system called ER-associated degradation process (ERAD). Once misfolded glycoproteins are exported to the cytosol, they are subjected to deglycosylation by peptide:N-glycanase (PNGase), generating free oligosaccharides with a chitobiose residue (fOs-GN2). Then fOSs-GN2 are trimmed by endo-beta-N-acetylglucosamidase (ENGase) responsible for cleaving the N,N'-diacetylchiotobiose moiety. The enzymatic function of ENGases seems to be crucial for free oligosaccharides further trimming by aplha-mannosidases. Finally,free N-glycans are transferred to the lysosome for final degradation. ENGases belong to two different glycoside hydrolase (GH) families, 18 and 85.In this study we investigated the role of ENGases in the mycoparasite Trichoderma atroviride. This species contains two GH18 ENGases, one is predicted to be cytosolic (Eng18B) while the other is putatively secreted (Eng18A). We expected an important role of ENGases in this species, since genome analysis revealed that the only PNGase-encoding gene carries mutations in the catalytic domain indicating an inactive enzyme. Deletion of the Eng18B gene had a severe impact in phenotype. Furthermore, the antagonistic ability against Botrytis cinerea was highly reduced in the Eng18B deletion strain. By heterologous expression in Saccharomyces cerevisiae, both Eng18A and Eng18B were shown to degrade the highly glycosylated RNAse B protein, indicating active deglycosylation enzymes. However, we showed that only Eng18B was able to degrade the RTL protein complex (consists of ricin A-chain, a transmembrane domain and a cytoplasmic leucine protein), which is known to be a substrate for PNGases. This result is possibly attributed to the different localization of ENGases in yeast cells. Finally, analysis of free N-glycans with HPLC and MS-MS spectrometry revealed that no fOSs were generated in the Eng18B deletion strain, implying that this enzyme is the only factor for free N-glycans formation in T. atroviride. To the best of our knowledge, this is the first enzymatic characterization of the cytosolic ENGase in filamentous fungi, and furthermore we showed that free N-glycans are formed by a different route in T. atroviride compared to yeast and mammalian cells.

158 THE ROLE OF FLD IN THE REGULATION OF CONIDIATION IN NEUROSPORA CRASSA

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Conidiophore development in Neurospora crassa requires several genes and their genetic interactions have been established. Strains with mutations in aconidiate-2 (acon-2) or fluffyoid (fld) are blocked in the transition from filamentous to budding growth. Mutations in aconidiate-3 (acon 3) or fluffy (fl) allow the production of minor, but not the major constriction chains that are produced prior to the formation of conidia. Mutations in two conidial separation genes (csp-1 and csp 2) prevent the separation of cross walls to release free conidia. A strain with a mutation in gene fld is blocked at the formation of minor constrictions like acon-2 mutants but can produce some flecks of conidia under certain conditions of temperature or carbon starvation. The fld mutation was isolated spontaneously and was mapped by classical methods on chromosome IV between arg 14 and his-5. We looked for genes in this genomic region that could be responsible for the fld phenotype. One of them, locus NCU09739, had a point mutation in the fld strain: a deletion of a G nucleotide in position 1686 that lead to a change in the reading frame and a premature STOP codon. The resultant protein lacks 112 residues at C-terminus in fld mutant strain. In addition, we have found that the mutation in NCU09739 is inherited by fld strains after genetic crosses further supporting the proposal that fld is NCU09739. To further confirm that fld is NCU09739 we created a strain with a deletion of G1686 that showed the fld phenotype. The FLD protein is a putative zinc finger protein of 676 amino acids that contains a binuclear zinc finger cluster (Zn2Cys6) at N-terminus. The gene fld is not induced by light, but it is missregulated in the absence of fl suggesting an interaction between FLD and FL. FLD localizes in the nucleus and in the cytoplasm during vegetative growth in the wild type strain, and accumulates in aerial hyphae during conidiation. The localization of FLD during vegetative growth and during conidiation in mutants altered in conidiation will help us to understand the role of this transcription factor during conidial development.

159 THE ROLE OF SUPEROXIDE DISMUTASES IN PODOSPORA ANSERINA LIFESPAN CONTROL

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Within biological systems reactive oxygen species (ROS) are formed as by-products of the respiratory chain, during photosynthesis or are actively generated by enzymes like NADPH oxidases. Besides their role as signaling molecules, ROS are involved in damaging cellular components including DNA, lipids and proteins. Accumulation of molecular damage leads to impairments of cellular functions, degeneration, and aging. Fortunately, all biological systems have evolved pathways to control the levels of ROS 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), the only enzymes capable of degrading superoxide. The product of this reaction, hydrogen peroxide, can subsequently be degraded into water by catalases and peroxidases. 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, PaSOD3 and PaSOD4 are proteins with a manganese binding domain. PaSOD2 localizes to the perinuclear ER while PaSOD3 and PaSOD4 are found in mitochondria where ROS arises at the electron transport chain (Zintel et al. 2010). Here we report data of investigations to elucidate the role of PaSOD3 on growth and aging. We present results demonstrating an effect of manganese sulfate in restoring the wild-type phenotype of PaSod3 over-expression strains. In addition, we identified differential protein patterns of the wild type and the PaSod3 over-expressors in two-dimensional protein gels.

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

160 THE SPF27-HOMOLOGUE NUM1 CONNECTS SPLICING AND CYTOPLASMIC TRAFFICKING PROCESSES IN USTILAGO MAYDIS

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The conserved NineTeen protein complex (NTC) is an integral subunit of the spliceosome and required for intron removal. The NTC mediates conformational changes of core spliceosomal components, stabilizing RNA-RNA- as well as RNA-protein interactions. In addition, the NTC is involved in cell cycle checkpoint control, response to DNA damage, as well as formation and export of mRNP-particles. In the basideomycetous fungus Ustilago maydis, we have identified the Num1 protein as the homologue of SPF27, one of NTC core components. Num1 is required for polarized growth of the fungal hyphae, and, in line with the described NTC functions, affects the cell cycle and cell division. RNA-Seq analysis revealed that in num1 deletion strains splicing is affected on a global scale. In a screen for Num1 interacting proteins, not only NTC core components as Prp19 and Cef1 were identified, but several proteins with putative functions during vesicle-mediated transport processes. Among others, Num1 interacts with the motor protein Kin1 in the cytoplasm. Similar phenotypes with respect to filamentous and polar growth, vacuolar morphology, as well as the motility of early endosomes corroborate the genetic interaction between Num1 and Kin1. Our data implicate a previously unidentified connection between a component of the splicing machinery and cytoplasmic transport 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 proteins functions in the coordination of pre-mRNA splicing with nuclear-pore complex dependent export of mRNP-particles and microtubule-based mRNA-transport.

161 THE TRANSCRIPTOMIC AND PHYSIOLOGIC CONSEQUENCES OF RACA ACTIVATION AND INACTIVATION FOR POLAR GROWTH OF ASPERGILLUS NIGER

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RacA is the main Rho GTPase in Aspergillus niger regulating polarity maintenance via controlling actin dynamics. Both deletion and dominant activation of RacA (RacG18V) provoke an actin localization defect and thereby loss of polarized tip extension, resulting in frequent dichotomous branching in the racA deletion strain and an apolar growing phenotype for RacG18V. In the current study the transcriptomics and physiological consequences of these morphological changes were investigated and compared with the data the morphogenetic network model for the dichotomous branching mutant ramosa-1. This integrated approach revealed that polar tip growth is most likely orchestrated by the concerted activities of phospholipid signaling, sphingolipid signaling, TORC2 signaling, calcium signaling and CWI signaling pathways. The transcriptomic signatures and the reconstructed network model for all three morphology mutants (racA deletion, RacG18V, ramosa-1) imply that these pathways become integrated to bring about different physiological adaptations including changes in sterol, zinc and amino acid metabolism and changes in ion transport and protein trafficking. Finally, the fate of exocytotic (SncA) and endocytotic (AbpA, SlaB) markers in the racA deletion mutant was followed, demonstrating that hyperbranching does not per se result in increased protein secretion.

162 TRACING CELLULASE SECRETION IN NEUROSPORA CRASSA

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Fungal based bioprocessing is of increasing importance for the production of renewable fuels and chemicals using lignocellulosic feedstocks. In nature, highly diverse filamentous fungi secrete large amounts of hydrolytic enzymes like cellulases in order to feed on their extracellular lignocellulosic substrate. Creating an understanding of the underlying genetic and physiological mechanisms of cellulase secretion down to the molecular level combined with rational genetic engineering aims to make the production of second generation biofuels economically viable by reducing enzyme production costs. Eukaryotic protein secretion is yet characterized to a significant extend in yeast and mammals. Although the basic components are also conserved in filamentous fungi, little is known about crucial differences accounting for their unique life-style based on secretion for highly polarized growth and nutrition. The model fungus Neurospora crassa secretes naturally a host of cellulases to allow it to grow on burnt vegetation. The tractability of N. crassa makes it an excellent model to study secretion of industrially relevant cellulases. To achieve this goal we are characterizing the cellulase secretion pathway in N. crassa by following the trafficking of fluorescently tagged Endoglucanase 2 (EG-2), a major secreted endocellulase. To determine the compartments through which cellulases traffic we are co-localizing EG-2-GFP with fluorescently-tagged markers of the ER, Golgi, endosomes, and the Spitzenkorper and are assaying the consequences to EG-2-GFP trafficking of blocks to secretion imposed by pharmacological or mutational insults. Our initial results indicate that EG-2-GFP shows localization to the ER and is mostly absent from the Spitzenkorper, suggesting trafficking through a classical ER to Golgi secretory pathway and terminal secretion along lateral hyphal walls. Additionally, targeted blocks to the secretory pathway indicate a potential role of endosomes in EG-2-GFP trafficking.

163 TRANSCRIPTION FACTOR PRO1 REGULATES MULTICELLULAR DEVELOPMENT IN SORDARIA MACROSPORA

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The homothallic ascomycet Sordaria macrospora was used as a model organism for fungal sexual development since the late 50s. In a forward genetic approach a set of mutants was generated showing a sterile phenotype. Within this collection of mutants we recently characterized mutant pro1, which generates only immature fruiting bodies (protoperithecia) but never mature fruiting bodies (perithecia). The mutant carries a gene deletion of transcription factor PRO1, which is characterized by a Zn(II)2Cys6 binuclear cluster. Furthermore, PRO1 contains a DNA binding motif at the N-terminus, and a putative nuclear localization signal (NLS)1. Thus, pro1 is related to the well-characterized transcription factor GAL4 from yeast. Using a GFP-tagged version of PRO1, we were able to demonstrate a nuclear localization. The construct will be used to identify transcription factor binding sites. For this purpose, we use chromatin immunoprecipitation coupled with high throughput sequencing (ChIP-seq). We also aim to identify interaction partners of PRO1 using tandem affinity purification (TAP) together with mass spectrometry2. The combined application of both techniques will improve our understanding of a regulatory network controlling cellular differentiation.

1 Masloff et al. Genetics 152: 191-199 (1999); 2 Bloemendal et al. Mol Microbiol 84: 310-323 (2012)

164 TREHALOSE IS A SIGNALING MOLECULE THAT REGULATES GROWTH OF NEUROSPORA CRASSA

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Trehalose is a disaccharide consisting of two α,α-1,1-linked glucose moieties. Recently, trehalose has been implicated in coordination of transcription and metabolism. Knockouts of trehalose-synthases show altered transcriptional profiles and display severe developmental phenotypes in several model organisms including A. thaliana, S.cerevisiae and filamentous fungi. In Neurospora crassa trehalose-synthase is encoded by the clock controlled gene 9 (ccg-9) and the absence of functional trehalose synthase (ccg-9RIP) affects vegetative growth and clock-controlled rhythmic conidiation via unknown mechanisms (Shinohara ML et al., 2002). We show that ccg-9RIP produces less conidia than WT. Yet, the glucose-dependence of conidiation is similar in WT and ccg-9RIP. Analysis of the Neurospora transcriptome during vegetative growth and asexual development indicates that metabolic pathways are not significantly affected in the ccg-9RIP strain. However, key transcription factors required for asexual development are severely misregulated in ccg-9RIP. Our data indicate that trehalose regulates growth of Neurospora via transcription factor signaling.

165 VEL1 OF TRICHODERMA REESEI HAS AN AUXILIARY ROLE IN IN CELLULASE GENE EXPRESSION AND IS REQUIRED FOR SEXUAL AND ASEXUAL DEVELOPMENT

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The heterotrimic velvet complex comprised VelB/VeA/LaeA proteins, was introduced as coordinator of photo signal to fungal development and secondary metabolites production. We have recently undertaken a study towards elucidating a possible role of VEL1, the T. reesei orthologue of A. nidulans VeA, in T. reesei development and cellulase gene expression. Deletion of this gene causes a complete loss of conidiation in light and darkness and loss of formation of perithecia during mating in the presence of light. Overexpression of vel1 under the constitutive expression signals of tef1 does not significantly increase conidiation in light or darkness, and leads to infrequent formation of infertile perithecia in the dark. Moreover, expression of T. reesei vel1 gene occurs at a low level, which is regulated by the carbon source and in most cases higher during cultivation in the dark. We will also show here that a loss of vel1 function does not affect cellulase gene expression, but vel1 overexpression strongly can enhance it. Consistent findings were also obtained for the formation of xylanases and ß-xylosidases. Furthermore, stimulation of cellulase gene expression by overexpressing vel1 is dependent on a functional lae1 allele – the putative protein methyltransferase which controls the expression of cellulase and hemicellulase encoding genes in T. reesei (Seiboth, Karimi et al. 2013). In order to correctly assess the impact of VEL1 on the physiology of T. reesei in general and on the (hemi)cellulase formation in particular under conditions reminiscent of an industrial enzyme fermentations process, we employed semi-pilot scale bioreactors to analyze the performance of all the mutant strains mentioned above.

166 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. Like many other secondary metabolism genes, the genes for penicillin biosynthesis were transferred from pro- to eukaryotes by horizontal gene transfer and are thus clustered. The regulation of these primarily bacterial genes in eukaryotes occurs mainly through global regulators. It is supposed that subunits of the velvet complex, which were recently detected for P. chrysogenum, function as such global regulators, although the exact regulatory mechanisms still have to be elucidated. Core components of the velvet complex are VelA and LaeA, which regulate secondary metabolite production, hyphal morphology, conidiation, and pellet formation [1]. As novel subunits, we recently identified VelB, VelC, and VosA [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 clearly indicate that velvet subunits like VelB and VelC have opposing roles in the regulation of penicillin biosynthesis and light-dependent conidiation [2]. 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 unexpected 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 IPNS 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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[2] Kopke K, Hoff B, Bloemendal S, Katschorowski A, Kamerewerd J, Kück U (2013) Eukaryot Cell 12: 299-310

167 VIPA – A NOVEL PART OF THE LIGHT REGULATOR COMPLEX IN ASPERGILLUS NIDULANS?

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In the filamentous ascomycete A. nidulans development and metabolism are strongly regulated by light. In light A. nidulans undergoes an asexual life cycle with formation of conidiophores and conidiospores whereas in the dark sexual development with ascospore formation and production of secondary metabolites takes place [1]. For light detection of several wavelengths A. nidulans harbors different photosensors like the phytochrome FphA for red light sensing and the White Collar homologue LreA for blue light detection. A central regulator is the Velvet protein, a FphA interaction partner [2]. Here, we report about a novel Velvet interaction partner, VipA (velvet interacting protein A). VipA is a 334aa protein including a FAR1 domain standing for putative DNA binding capacity. FAR1 proteins are well known from plants like Arabidopsis thaliana where members of this protein family are involved in phytochrome controlled far-red light responses [3,4]. In A. nidulans a vipA deletion in different strain backrounds leads to reduction of conidiospore amounts compared to wildtype and complemented strains up to 64%. First countings also point to lower cleistothecia amount and therefore also an influence on sexual development. These findings suggest an activating role of VipA in asexual and sexual development. VeA – VipA interaction was shown by yeast-two hybrid analysis and bimolecular fluorescence complementation. The two proteins interact in the nuclei. Furthermore BIFC analysis of VipA points to positive interaction with LreA/B in the nucleus. This would suppose VipA to be part of the light regulator complex. VipA represents a new element in the regulatory network of spore formation in A. nidulans. Detailed analyses on gene regulation through VipA and its relation to other light regulators are under way.

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168 YEAST TO HYPHAL SWITCHING IN TAPHRINA DEFORMANS, THE CAUSATIVE AGENT OF PEACH LEAF CURL DISEASE

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Taphrina is a fungal genus in the phylum Ascomycota comprising 31 currently accepted species, which are all plant parasites. Taphrina deformans is the causative agent of peach leaf curl disease causing disease mainly in peaches and nectarines. The life cycle of T. deformans is characterized as a dimorphic organism switching between yeast and hyphal stages. The yeast is saprobic and the hyphal stage pathogenic. Thus, like other dimorphic pathogens, T. deformans requires yeast-to-hyphal switching as a mechanism to promote virulence. There are few examples of experimental systems for the study of plant-associated and plant-pathogenic yeasts. We use T. deformans as a model pathogen to study plant-yeast interactions, which requires the ability to manipulate the organism in culture. The aim of this study is to understand factors controlling the induction of the pathogenic hyphal form. The hyphal stage of T. deformans has been assumed to be an obligate parasite whereas the yeast stage can be grown in vitro. Culture conditions under which predominately supports yeast or hyphal cell types have been established. We have been able to grow and induce switching between the different cell morphotypes in a culture media including the pathogenic hyphal form. Additionally, a new intermediate cell morphotype has been observed that can be maintained indefinitely on plates. We studied the physiological function of this new cell type, which we call prehyphal cells. As compared to the yeast cells, pre-hyphal cells profusely grow into the hyphal form. We have also performed morphological analysis and results showed that the prehyphal cells are morphologically distinct from the yeast and the hyphal cell types. We concluded that the prehyphal cells might be a transitionary cell morphotype between the yeast and the hyphal cell stages. To study the possibility that sexual reproduction is linked to T. deformans morphotype switching, we have manually curated the MAT loci in T. deformans recently published genome. As a part of curation process, additional components of the MAT loci were identified. The possible role of MAT loci in the regulation of yeast to hyphal switching is currently being investigated and results will be presented.

169 A HIGHLY CONSERVED GENE CLUSTER FOR THE BIOSYNTHESIS OF PIGMENTS IN THE FUNGAL KINGDOM

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The basidiomycete subphylum Pucciniomycotina is composed of two major groups, the rusts and the red yeasts. These generic names are due to the biosynthesis of certain carotenoids. To identify the genes responsible for this pigmentation, we performed an insertional mutagenesis in the red yeasts Sporobolomyces sp. and Rhodotorula graminis. The analyses of insertion sites in the white mutants, together with the genome sequences, revealed a cluster of three genes that encode a bifunctional lycopene cyclase/phytoene synthase, a phytoene dehydrogenase and a predicted carotene oxygenase. The third oxygenase-encoding gene was disrupted in Sporobolomyces sp., and the carotenoid content analyzed in the mutants by TLC and HPLC. The car0 deletion strains do not synthesize retinal, indicating a role of the protein in cleaving the middle of β-carotene. The organization of this CAR gene cluster is similar to that characterized in an ascomycete, Fusarium fujikuroi. Phylogenetic analysis shows that these homologs from the red yeasts are consistent with a basidiomycete origin, rather than a horizontal transfer. Rather, this gene cluster is evident throughout the Ascomycota and in some Basidiomycota, representing the most conserved metabolic gene cluster known to date in the fungi. Inclusion of genes for carotene oxygenases indicates that the coordinated synthesis of metabolites derived from cleaving β-carotene into two molecules of retinal, rather than carotenes, is likely responsible for the advantages of clustering these genes. The presence of opsin homologs in a subset of the ascomycete gene clusters supports a hypothesis that the pathway developed to produce retinal for detecting or using light.

170 A HYBRID-ASSEMBLY YIELDS A NEAR GAPLESS DRAFT GENOME OF THE MILD PLANT PATHOGEN VERTICILLIUM TRICORPUS

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The relatively small fungal genus Verticillium encompasses only ten species, of which few are causal agents of vascular wilt diseases. Vascular wilts belong to the most destructive plant diseases, affecting hundreds of economically and ecologically important crops. V. dahliae is considered as a notorious plant pathogen, although strains may differ significantly in host range and aggressiveness. In contrast, V. tricorpus is less commonly observed as causal agent of vascular wilt and only weakly pathogenic on a narrow host range. Thus, inter-species comparisons may reveal components that determine host range and pathogenicity within the Verticillium genus.
We developed a novel hybrid sequencing and assembly approach that combines second and third generation sequencing techniques to facilitate affordable high-quality genome assembly. We applied our approach to the genome of V. tricorpus, yielding a 36Mb near gapless draft genome of which the quality is superior to previously generated and more expensive genome assemblies within this genus. With comparative genomics we identified genomic features that are shared between members of this genus, such as a genomic organization that involves islands of genes enriched for those encoding secreted proteins that may have a role in virulence. We hypothesize that this genomic organization facilitates rapid adaptation, possibly utilizing genomic rearrangements that have recently been observed in V. dahliae. Moreover, we identified orthologs for several classes of known fungal effector proteins, thereby providing a first glimpse of the effector catalog of V. tricorpus. Consequently, our study highlights the technical advances of a hybrid sequencing and assembly approach and provides a novel resource to study the genome organization and virulence of this intriguing genus that contains major plant pathogens.

171 AN IN SILICO SEARCH FOR GLUCOSE-METHANOL-CHOLINE OXIDOREDUCTASES WITH INTERESTING FEATURES IN TEN POLYPORALES GENOMES

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The aim of this work was to find new glucose-methanol-choline (GMC) oxidoreductases with potential biotechnological applications taking advantage of the great deal of fungal genomes currently available. In order to do so, ten fungal species were selected (Bjerkandera adusta, Phlebia brevispora, Ganoderma sp., Fomitopsis pinicola, Phanerochaete chrysosporium, Dichomitus squalens, Ceriporiopsis subvermispora, Trametes versicolor, Rhodonia placenta and Wolfiporia cocos) from the order Polyporales, which possess the ability of degrading wood and, hence, have the degradative machinery encoded in their genomes. We performed an in silico search through protein sequence homology using cloned enzymes (aryl-alcohol oxidases, glucose oxidases, methanol oxidases, pyranose oxidases, cellobiose dehydrogenases and pyranose dehydrogenases) from related fungi. Once the putative enzymes of each class chosen, their sequences manually curated and annotated, we: i) analyzed their evolutionary relationships by constructing gene phylograms based on their predicted protein sequences; and ii) established their duplication/reduction history during fungal evolution by investigating the number of genes of each enzyme type most probably present at every node in the species evolutionary tree (by reconciliation between our constructed gene tree and the species tree available). Moreover, we modelled almost all the GMC sequences out of the 195 found in the ten genomes using the crystallographic structures of related enzymes as templates to gain insight into their structural variation and hypothesize their probable catalytic properties.

172 AN UPDATE OF ON-GOING WORK WITH CADRE AND ASPERCYC

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The Central Aspergillus Data Resource (CADRE; www.cadre-genomes.org.uk) gathers automated and manual annotation efforts for this genus, providing enriched data for each genome. This information flows into AsperCyc (www.aspercyc.org.uk), an online resource of predicted metabolic pathways for the Aspergillus genus. Recently, much work has been going on that will filter into these online resources. We have recently been involved in an EC-FP7 funded systems biology study of fungal pathogens (Sybaris) during which we sequenced and annotated nine Aspergillus fumigatus strains, including CEA10 and AF300, and one A. nidulans strain (F8226) using data from CADRE. Currently, we are involved in another EC-FP7 funded project (NOFUN; www.nofunproject.org) that builds on our earlier work and aims to identify novel drug targets and to develop novel classes of antifungal drugs. Both projects have and will involve RNAseq analyses, data that we can make available, along with the strains, within CADRE.

173 ANALYSIS OF A KINOME-WIDE KNOCKOUT LIBRARY REVEALS THE ASPERNIDINE A BIOSYNTHESIS GENE CLUSTER IN ASPERGILLUS NIDULANS

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The sequenced genome of Aspergillus nidulans shows that this organism harbors the genetic potential to produce more secondary metabolites than have been discovered. However, many of these genes are silent, and therefore the inability to activate and gain access them stands as a major obstacle. Here, we report that by using a genome-wide kinase knockout library we were able to find a kinase deletion strain with an activated silent secondary metabolism pathway. Protein kinases play key roles in regulation and signal transduction. To explore the possibility of activating silent secondary metabolite gene clusters in A. nidulans by manipulating kinase expression, we examined the secondary metabolite profiles of a genome-wide kinase knockout library. We found that a mitogen-activated protein kinase, mpkA, deletion strain produces aspernidine A. By creating a series of targeted deletions in this mpkA deletion strain we identified a cluster of six genes—one polyketide synthase, one cytochrome P450 monooxygenase, one prenyltransferase, and three additional genes— that are required for aspernidine A biosynthesis. Two of the gene deletions gave novel intermediates that provided information about the biosynthesis of this metabolite. We believe the approach using a genome-wide kinase knock out library we have presented is general and could be applied for the genome mining of secondary metabolites in other organisms.

174 ANALYSIS OF GLOBAL REGULATION OF GENE EXPRESSION OF CARBON CATABOLITE REPRESSION DURING THE FORMATION OF CELLULASES BY TRICHODERMA REESEI (HYPOCREA JECORINA)

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The ascomycete Hypocrea jecorina (anamorph of Trichoderma reesei) is one of the most well studied cellulolytic fungi and widely used in the biotechnology industry. The carbon catabolite repression mechanism adopted by T. reesei is mediated by the transcription factor CRE1 and consists in the repression of genes related to the production of cellulase when a readily available carbon source is present in the medium. This study aims to contribute to understanding the mechanism of carbon catabolite repression during the formation of cellulases, by comparing the mutant strain of T. reesei Δcre1 with its parental, QM9414. For this, the cDNA libraries of strains QM9414 and Δcre1 grown in 1% cellulose, 1 mM sophorose and 2% glucose were sequenced by RNA-seq by LGC Genomics GmbH in Berlin/Germany, using the equipment Illumina/HiSeq2000. The results of the sequencing were analyzed by the alignment software Bowtie and DEseq package, which makes the analysis of differentially expressed genes. We obtained a total of 264 million of reads that, when analyzed, showed 815 genes differentially expressed in Δcre1 in relation to the parental QM9414 on cellulose, 2368 on sophorose and 697 on glucose, for a total of 9129 genes in the genome of T. reesei. Most of genes that were up- or down-regulated in the mutant belonged to Gene Ontology categories described as metabolic processes, membrane, oxidoreductase activity, carbohydrate metabolism and transport. Cellulolytic enzymes, genes related to the transport of substances and other transcription factors were targeted for carbon catabolite repression mediated by CRE1, in a carbon source-dependent manner. Validation of differentially expressed genes by qRT-PCR showed a high correlation between the two techniques, which can be demonstrated by a high linear coefficient of Pearson ($r2 = 0.94$). It is hoped that these results contribute to a better understanding of the mechanism of carbon catabolite repression in T. reesei, enhancing the application of this fungus in several biotechnology sectors in which is used.

175 APPLICATIONS AND LIMITS OF RNASEQ IN EXPLORING A FUNGAL-ALGAL SYMBIOSIS IN NATURE

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Fungal-algal symbioses, collectively called lichens, were among the first recognized symbioses and are one of the only terrestrial symbioses to form complex architectures that resemble neither symbiont in isolation. However, the molecular mechanisms that enable this relationship remain elusive as the whole symbiosis is not practical to synthesize in culture. Transcriptome-level work on wild samples is complicated by the presence of contaminating endolichenic fungi and the lack of reference genomes. We examined transcriptomes of two putative species of Bryoria (Parmeliaceae; Lecanoromycetes; Ascomycota). One of the species (B. fremontii) was used as food by Native Americans while the other (B. tortuosa) was recognized by them as toxic. Despite being phenotypically distinct, recent rDNA evidence suggests they are identical. We data-mine the transcriptomes of both species using both differential expression and nucleotide divergence estimates and examine the role of environmental contaminants in constraining the data set.

176 BIODIVERSITY OF CHAETOMIACEAE FOR EFFICIENT PLANT BIOMASS DEGRADATION

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A major challenge in the sustainable production of biofuels and biochemicals is efficient enzymatic conversion of plant biomass into monomeric sugars. Currently, most enzyme mixtures are produced by a small selection of fungal species (e.g. Trichoderma reesei, Aspergillus niger). However, the fungal kingdom holds many more fungal species which produce enzyme mixtures with beneficial characteristics such as high hemicellulase activity and high thermostability. Strains within fungal family Chaetomiaceae can grow efficiently on plant substrates and they have a large variability in optimal growth temperatures. This family already harbours industrially-relevant fungi like Thielavia terrestris and Myceliophthora thermophila. This study will investigate the potential of 32 Chaetomiaceae species to degrade plant biomass.The 10 Myceliophthora and 22 Thielavia species of Chaetomiaceae fungi differed in their optimal growth temperature from 25°C (T. antarctica) to 45°C (e.g. M. thermophila and M. heterothallica). There was a large difference in preference of growth for the tested plant substrates: wheat straw, spruce, and sugarbeet pulp. For instance, spruce was for most fungi a difficult substrate to degrade, while most fungi grew well on sugarbeet pulp. The thermophilic fungi within Chaetomiaceae showed to produce enzymes with a good stability at 70°C. M. heterothallica was one of the thermophiles with good growth on a large range of substrates. This species was also able to produce offspring with a large physiological and genetic variety. Mating and evolutionary engineering strategies were used to further improve M. heterothallica capability to degrade sugarbeet pulp. The physiology of M. heterothallica strains altered strongly after a few rounds of sexual crosses and selection for sugarbeet pulp saccharification. Together with the extracellular pH of the culture, the produced enzyme set changed in pectin hydrolase and lyase activities. The change in enzyme composition was mimicked by evolving M. heterothallica strains on sugarbeet pulp media with different pH. This study showed the strategic strength of combining biodiversity with specific selection strategies to find enzyme mixtures with interesting industrial properties.

177 CHROMOSOME NUMBER REDUCTION BY TWO TELOMERE-TO-TELOMER FUSIONS IN THE EREMOTHECIUM CORYLI GENOME

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Fungi of the genus Eremothecium belong to the Saccharomyces complex of pre-Whole Genome Duplication yeasts. We established the 9.1. Mb draft genome of Eremothecium coryli, which encodes 4682 genes, 186 tRNA genes, and harbors several Ty3 transposons as well as at least 65 remnants of transposition events (LTRs). Conserved syntenic gene arrangements led to the identification of eight E. coryli loci that bear centromeres in the closely related species E. cymbalariae. Two of these E. coryli loci, however, lacked conserved centromere DNA elements. Using a plasmid stability assay we could show that CEN1 and CEN8 have been decommissioned in E. coryli, which thus has evolved to harbor six chromosomes. A comparative genomics approach was employed to determine the mechanism of this chromosome number reduction in E. coryli. The identification of scaffold positions of genes located to telomeres in E. cymbalariae and the reconstructed yeast ancestor revealed two telomere-to-telomere fusion events In E. coryli. These involved in one case the right ends of the yeast ancestral telomeres of chromosomes 3 and 8 (Anc3R + Anc8R), and in the other a fusion of the telomeres of Anc6R and Anc7L. To further explore the genome evolution in the Eremothecium genus we used the genome sequences of three sequenced species plus the yeast ancestral gene order and based on conserved block synteny we could reconstruct the gene order of three complete chromosomes of the Eremothecium ancestor. This opens a view on the series of inversions and reciprocal translocations in the yeast ancestor and in the three Eremothecium species and identified E. coryli with the lowest amount of rearrangements to the Eremothecium ancestor.

178 CLASS-II PEROXIDASE GENES IN THE OYSTER MUSHROOM GENOME: HETEROLOGOUS EXPRESSION, MOLECULAR STRUCTURE, CATALYTIC AND STABILITY PROPERTIES AND LIGNIN-DEGRADING ABILITY

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The genome of Pleurotus ostreatus has been completed, as an important edible mushroom and as a model lignin-degrading organism. Heterologous expression of the nine class-II peroxidase genes, followed by kinetic studies, confirmed the preliminary structural-functional classification for all but one of them. The resulting inventory revealed the presence of three versatile peroxidases (VPs) and six manganese peroxidases (MnPs), two of which were solved at 1.0-1.1 Å, and the absence of lignin peroxidases (LiPs). Gene number expansion supports the importance of both peroxidase types in the white-rot life-style of this fungus. Using 14C-labeled model dimer and synthetic lignin we showed that P. ostreatus VP is able to degrade lignin. Moreover, the dual Mn-mediated and Mn-independent activity of P. ostreatus MnPs justifies their inclusion in a new peroxidase subfamily, with a role oxidizing phenolic degradation products. The availability of the whole POD repertoire enabled to investigate the existence of duplicated genes at a biochemical level. Interestingly, the isoenzymes not only differ in kinetic constants. Comparison of temperature and pH stabilities revealed surprising differences in activity T50 (43-63 ºC, after 10 min at pH 5), and residual activity at both acidic (0-96% after 4 h at pH 3) and alkaline pH (0-57% after 4 h at pH 9). Crystal structures and homology models, together with CD and UV-visible spectroscopy results, explained some of the stability differences, including the highest β-turn proline numbers in thermostable VP1, and the tightly-coordinated structural Ca2+ ions and number of exposed lysines in MnP4 being stable at both acidic and alkaline pH. The analysis of P. ostreatus genome reveals a lignin-degrading system where the role generally played by LiP has been assumed by VP. Moreover, it enabled the first comprehensive characterization of peroxidase isoenzymes in a basidiomycete, providing some clues on the high stability properties of some of them.

179 CLUSTER HYPOTHESIS GUIDED CHARACTERIZATION OF FUNGAL SECONDARY METABOLITE GENE CLUSTERS

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In the last decade, a large variety of genes that are involved in the biosynthesis of fungal secondary metabolites have been identified and characterized. It has long been known that these genes are clustered, often being apart from each other by less than 2kb on the chromosome. Several hypotheses have been put forth to explain the physical clustering or fungal secondary metabolite genes. One is that clustering may allow the constituent genes to be coregulated by transcription factors in a similar chromatin environment. Another reason that has been proposed is that the "selfish" clusters confer selective advantage to themselves and clustering optimizes the horizontal gene transfer (HGT) of intact gene clusters between species.
Here we present the work of characterization of the two separate biosynthetic gene clusters involved in meroterpenoids austinol biosynthesis, one compact cluster for terretonin biosynthesis, one compact cluster for epipolythiodioxopiperazine biosynthesis and one intertwined super cluster for the biosynthesis of both fumagillin and pseurotin. Combination of the above results allows us to reexamine the cluster hypothesis and how the clustering of these secondary metabolite genes might be changed through the long course of evolution.

180 COLLETOTRICHUM ACUTATUM SENSU LATO GENOMES

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Colletotrichum acutatum sensu lato includes a number of important pathogens that cause economically significant losses of various crops. C. acutatum has a wide host range in both domesticated and wild plant species, and its capability to infect different types of hosts such as insects has also been described. Members belonging to this complex are able to develop three different types of interaction with plant hosts including biotrophic, necrotrophic and hemibiotrophic infections and are also capable of surviving on weeds and non-hosts without causing visible symptoms. They are mainly asexual, but some have a teleomorphic state called Glomerella and can be either homothallic or heterothallic. The sexual behaviour in Glomerella is more complicated than in most ascomycetes, and strains within the same species do not show a typical MAT1-1/2 system. Previous results suggest the existence of C. acutatum populations, related to their reproductive behaviour and host association patterns, now described as new species belonging to "C. acutatum species complex". The wide host range and the different lifestyles (necrotrophic, biotrophic, hemibiotrophic and quiescent) suggest C. acutatum is a suitable system for studying evolution, speciation process and host association through whole genome comparisons. One representative isolate has been sequenced, assembled and annotated. The isolate was chosen based on its wide host range including strawberry and the phylogeographic position. Resulting data have been used for a wider Colletotrichum comparative genomics with the aim to investigate gene family expansions in non-specificity host. The data suggest an interesting expansion of several gene families, such as those encoding carbohydrate-active enzymes, secondary metabolites pathways and effectors that could be associated with the host range. The new knowledge and resources developed with the genome analyses along with the results of the population level diversity studies provide a platform for future comparative and functional genomics investigations to advance this research.

181 COMBINING POPULATION GENOMICS, RNA-SEQ AND MINIATURE TRANSPOSABLE ELEMENT (MITE) PRESENCE TO IDENTIFY THE AVR2 GENE OF THE MELON PATHOGENIC FUNGUS FUSARIUM OXYSPORUM F. SP. MELONIS

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Fusarium oxysporum (Fo) is a soilborne fungus that causes Fusarium wilt disease in many plant species by colonizing and eventually blocking the host xylem vessels. The F. oxysporum species complex is a collection of apparently asexual non-pathogenic and pathogenic clonal lineages. Many lineages harbour unique genomic sequences residing mostly on extra chromosomes. One class of lineage-specific genes encodes effector proteins that are crucial determinants of virulence on a given host species. Some of these effectors, called avirulence proteins are recognized by plant resistance proteins. Effector recognition makes the plants resistant to the pathogen expressing the so-called avirulence (AVR) gene. To identify the Fom effector gene AVR2, we have sequenced several Fom isolates that differ in their recognition by the melon Fom-2 resistance protein. All effector proteins in the tomato wilt strain Fo f. sp. lycopersici harbor a miniature transposable elements (MITE) in their promoter. We have exploited the presence of the MITE to predict the effector complement of each sequenced Fom isolate. We combined this analysis with RNA sequencing of Fom-infected melon roots to pinpoint those effector candidates that are expressed during melon infection. Currently, we are testing the recognition by the melon Fom-2 gene of an AVR2 candidate from our combined analyses.

182 COMPARATIVE ANALYSIS AND EVOLUTION OF ABC PROTEINS IN BASIDIOMYCETES

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ABC (ATP-binding cassette) proteins constitute one of the largest families of proteins whose representatives are found in virtually every living cell. A large fraction of ABC proteins known as ABC transporters is involved in transport of various substrates across biological membranes. However, ABC proteins are also implicated in many other cellular processes, including ribosome biogenesis, assembly of translation pre-initiation complex, and regulation of transcription and translation. Despite their great functional diversity, all ABC proteins share a common structural motif, namely a nucleotide-binding domain, which is capable of binding and hydrolysing ATP. The energy released upon ATP hydrolysis is used by the protein to perform its biological function. With the advance of fungal genomics, the number of known fungal ABC proteins increases rapidly, but the information on their biological functions remains scarce. Here, we report the results of an extensive survey of ABC proteins from the genomes of a representative set of basidiomycetes. We have analysed genomes of species with different ecological adaptive strategies (biotrophs, necrotrophs, saprotrophs; white rot and brown rot wood-degrading fungi, mycorrhiza-formers, plant and animal parasites) representing various phylogenetic lineages within the phylum Basidiomycota. Our survey included the identification of genes encoding ABC proteins, their manual annotation, cataloguing and phylogenetic analysis. Obtained results show that the number of ABC proteins may vary significantly between the species. This is particularly true for ABC transporters from the subfamilies ABC-B, ABC-C and ABC-G, whereas ABC proteins without transmembrane domains (subfamilies ABC-E and ABC-F) show little variation in their copy number. We have observed patterns of extensive gene amplification and gene loss in certain groups of ABC proteins. Interestingly, some of the identified ABC transporters have probably acquired additional domains resulting in a novel domain structure that has not been previously reported for eukaryotic ABC proteins. The comparison of sets of ABC proteins present in the genomes of basidiomycetes and ascomycetes revealed the existence of two groups of ABC proteins specific for basidiomycetes. Results of our survey should contribute to the better understanding of evolution of ABC proteins in fungi and support further experimental work on their characterization.

183 COMPARATIVE GENOME ANALYSIS OF A WORLD-WIDE POPULATION OF MAGNAPORTHE ORYZAE ISOLATES

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The causal agent of the rice blast, Magnaporthe oryzae, has been intensively studied to unveil the molecular genetic mechanisms underlying the host and pathogen interaction for several decades as a model organism. In order to understand the fungus at the population level, a total of 37 isolates, which are representative isolates of each lineage collected from six countries, were sequenced. The average size and the number of predicted genes for the 37 genomes were 36.41 Mb and 13,403, respectively. They showed similar distribution of attributes, including genome size, average gene length, GC ratio and protein domain profile. However, the number of structural variations and gene family annotation exhibited differential distribution. Genome and proteome conservation analysis revealed that all the genomes share the majority of genetic materials in common, when compared in a pairwise manner. In addition, the size of core and pan-genome turned out to be 8,262 and 20,655, respectively, reflecting genome conservation and variations among the field isolates. Sequence analysis of an avirulence gene AVR-Pita1 revealed that the insertion of DNA transposon Pot3 was occurred in eight out of eleven Chinese isolates, causing gain of virulence in seven isolates. To assist comparative and evolutionary genomics of M. oryzae, all the available resources including genome sequences, gene family annotation and bioinformatics tools were integrated to develop a web-based genomics portal, Magnaporthe Atlas (http://www.magnaporthe.org/).

184 COMPARATIVE GENOME-SCALE RECONSTRUCTION OF GAPLESS METABOLIC NETWORKS FOR PRESENT AND ANCESTRAL SPECIES

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We introduce a novel computational approach CoReCo for comparative metabolic re-construction and provide genome-scale metabolic network models for 49 important fungal species. Leveraging on the exponential growth in sequenced genome availability, our method reconstructs genome-scale gapless metabolic networks simultaneously for a large number of species by integrating sequence data in a probabilistic framework. High reconstruction accuracy is demonstrated by comparisons to the well-curated Saccharomyces cerevisiae consensus model and large-scale knock-out experiments. Our comparative approach is particularly useful in scenarios where the quality of available sequence data is lacking, and when reconstructing evolutionary distant species. Moreover, the reconstructed networks are fully carbon mapped, allowing their use in 13C flux analysis. We demonstrate the functionality and usability of the reconstructed fungal models with computational steady-state biomass production experiment, as these fungi include some of the most important production organisms in industrial biotechnology. In contrast to many existing reconstruction techniques, only minimal manual effort is required before the reconstructed models are usable in flux balance experiments. CoReCo is available at http://esaskar.github.io/CoReCo/. Originally we used KEGG as the compound and reaction database in our reconstruction pipeline. To further improve our reconstructions a new improved database of compounds and electron and atom balanced reactions was created to replace KEGG. Compounds and reactions from YMDB, HMDB, ChEBI, KEGG, MetaCyc and Rhea databases and from metabolic models of several microorganisms were used. Results of fungal metabolic model reconstruction using the combined database will be presented.

185 COMPARATIVE GENOMICS OF COLLETOTRICHUM GRAMINICOLA ISOLATES SHEDS LIGHT ON THE MECHANISMS OF PATHOGENICITY

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Understanding genetic variation, the processes that create it, and its consequences is important for understanding the genetic basis of the huge diversity of life. In the case of pathogens, these studies may additionally help us to learn more about genomic traits shaping their ability to attack other organisms. To better understand patterns of genetic variation in the maize pathogen Colletotrichum graminicola, we sequenced the genomes of seven field isolates showing a variable range of virulence to maize and collected from different regions of the world. We analyzed genomic structural variations and patterns of gene gain and loss using genomic sequences obtained from various assembly and gene annotation strategies. We found that the amount of genomic variation in each chromosome is correlated with the amount of repetitive DNA. We identified sets of unique genes in each isolate, and discovered that they are significantly enriched with genes coding for small secreted proteins (putative effectors), which could represent evolutionary innovations directly involved in host specificity or environment adaptation. When comparing the genomes of pathogenic and non-pathogenic isolates, we identified genes partially disrupted or completely lost in the genome of the non-pathogenic isolate, suggesting their involvement in the pathogenic lifestyle of C. graminicola. Overall, genomic variation and patterns of gene gain/loss provide a valuable resource for selecting targets for further functional and population genetic analyses aimed at identifying genes involved in the development of maize anthracnose.

186 COMPARATIVE GENOMICS OF DRY ROT FUNGUS SERPULA LACRYMANS AND ITS ECOLOGICAL TRANSITION FROM LIVING IN NATURE TO RESIDING IN BUILDINGS

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The genus Serpula (Basidiomycota, Boletales, Serpulaceae) consists mainly of brown rot fungi that efficiently decompose wood in temperate parts of the world. The species Serpula lacrymans is known to cause dry rot in buildings and is an economic disaster to homeowners with infected houses. There are two main lineages of the species, Serpula lacrymans var. shastensis residing in natural habitat in the Cascade Mountain Range in North America and Serpula lacrymans var. lacrymans that colonize buildings in most continents. Serpula var. lacrymans is further subdivided into two invasive groups, one from Japan and another present in Europe, North America and Australia/New Zealand. Our aim in this project is to compare their genomes to reveal how the genetic variation is distributed within the lineages and to identify important genetic factors in the ecological transition where var. lacrymans adapted to human made environments. The Joint Genome Institute (JGI) has sequenced two monokaryotic var. lacrymans genomes from Europe, and one dikaryotic Serpula himantioides that serves as a outgroup in the analyses. Seven more strains of Serpula from Europe, Asia and North America have been sequenced by Illumina GAII, assembled by Velvet, and genes are predicted using MAKER. In addition, all MAKER predicted transcripts have been clustered by orthoMCL, and the clusters are screened for genes under strong selection pressure (Dn/Ds). The genome wide comparison may provide information about which genes/ gene families that have been important for the transition. We expect that the genomes of var. lacrymans are more homogeneous compared to var. shastensis, except for some genomic regions that are under strong selection.

187 COMPARATIVE GENOMICS OF MULTIPLE COLLETOTRICHUM ISOLATES FROM THE GLOEOSPORIOIDES SPECIES COMPLEX

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Members of the gloeosporioides clade of Colletotrichum represent a diverse group of pathogens whose members infect a wide range of commercially important plants. We have sequenced the genomes of 11 strains of gloeosporioides across the clade isolated from different hosts including six closely related strains isolated from strawberry with different levels of virulence. This allowed us to take a comparative genomics approach in analysing the gloeosporioides clade, with particular focus on features potentially involved in virulence. This revealed that all members of the genus, including those with lower levels of virulence, have expanded numbers of proteases, carbohydrate degrading proteins and secondary metabolite synthesis genes relative to other fungal genomes including other members of the genus. Further results of analyses to identify key factors differentiating virulent and less virulent strains will be presented. This work was supported by the Programme for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry to K.S., Y.T. and Y.N.

188 COMPARATIVE GENOMICS REVEALS REPEATED SEQUENCES AS MAIN DIVERSITY SOURCE FOR BLACK TRUFFLE

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The Périgord Black truffle (Tuber melanosporum Vittad.) is an ectomycorrhizal filamentous fungus endemic of south European forests and considered as a gastronomy delicacy. The genetic diversity of this fungus is questioned since 15 years. The aim of this study was to assess the overall genetic diversity and the factors driving genome plasticity of this species by resequencing six T. melanosporum isolates. These strains were chosen in different populations covering the whole natural distribution of this species. These isolates were sequenced using Illumina technology single end reads of 76 bp for a coverage of ~ 20 X. Considering the mapping of the reads against the reference genome previously sequenced, the T. melanosporum core genome was estimated to ~110 Mbp. Most of the regions without mapped reads corresponded to repeated sequences. A total of 442,326 SNPs corresponding to 3,540 SNPs/Mbp were identified among the seven genomes. T. melanosporum presented a genetic diversity similar to other filamentous fungi. The SNPs were more frequent in repeated sequences (85 %) although 4,716 SNPs were also identified in the coding region of 2,655 genes. Differences in insertion point for gypsy retrotransposons as well as high frequencies of SNPs suggested that transposable elements are among the main plasticity factor for the black truffle. In conclusion, this study proposed a set of SNPs that could be used in the future for analyzing black truffle population genetic. To our knowledge this study presents the first large-scale identification of polymorphisms for a mycorrhizal species by genome resequencing.

189 COMPARATIVE PHENOTYPIC ANALYSIS OF CANDIDA PARAPSILOSIS AND CANDIDA ALBICANS

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Candida albicans and Candida parapsilosis are both members of the CUG clade, and it is generally assumed that they share common biological properties. However, the two organisms have variable virulence characteristics, and different phenotypes. To enable a detailed comparison of the two species, we generated homozygous (double allele) deletions of >100 genes in C. parapsilosis including transcription factors and protein kinases, and compared the phenotypes to the corresponding deletions of C. albicans. Two independent C. parapsilosis deletions were constructed for each target gene. Growth in >40 conditions was tested, including carbon source, temperature, and the presence of antifungal drugs. We found that many phenotypes are shared in the two species, such as the role of Upc2 as a regulator of azole resistance. However, other characteristics are unique to one species. For example, Cph2 plays a role in the hypoxic response in C. parapsilosis and not in C. albicans, and SEF1, a regulator of iron uptake in C. albicans, is required for utilization of different carbon sources only in C. parapsilosis. Some of the biggest differences relate to the regulation of biofilm development. We identified 7 transcription factors that are required for biofilm development in C. parapsilosis. Only three (Efg1, Bcr1 and Ace2) are shared with C. albicans. In contrast, some transcription factors required for biofilm formation in C. albicans do not have the same role in C. parapsilosis. We also compared the transcription profile of C. albicans and C. parapsilosis biofilms. Our analysis suggests the processes shared between the two species are predominantly metabolic, whereas C. albicans-specific genes are associated with hyphal growth. Overall, whereas there is a significant overlap in gene function between C. albicans and C. parapsilosis, there are also considerable differences. We are currently testing the effect of the gene knockouts on virulence, using the model organism Galleria mellonella.

190 COMPARATIVE PROTEOMIC ANALYSIS OF BOTRYTIS CINEREA MEMBRANOME

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The high versatility exhibit by B. cinerea as a phytopathogenic fungus, is due to its ability to handle a large number of different mechanisms and infection strategies, based on a large number of pathogenicity/virulence factors. Some of these fungal tools as well as its role in the infection cycle has been study one by one through molecular approaches based on site specific mutations procedures describing the mutant phenotype. Most of these factors, are related to signal transduction cascades, where membrane proteins, must play key role as a bridge between environmental conditions and intracellular molecular processes. Nowadays, proteomics approaches has showed its potency to unravel complex biological processes, proving its advantages as an alternative against other "-omics" methods. Moreover, data from obtained peptides may help in the functional annotation of sequenced genome. This work describes the first proteomic approach to study the membrane proteins in order to determine its role in the infection process, by analyzing those membrane proteins specifically expressed by B. cinerea under different pathogenicity conditions. Membrane protein extraction was optimized under two different pathogenicity states by using different plant-like elicitors; a) Glucose as a constitutive response and b) desproteinized Tomato Cell Wall as a virulence state inductor. 2-DE protein profiles were obtained, showing, in both stages, a subproteome located between 4 and 8 of pI with a molecular weight from 116 to 14 kDa. Analysis of these profiles showed a differential expression of membrane proteins. We detect common and specific spots in each assayed carbon source, which has been analysed by MALDI TOF/TOF. Many of these protein spots, particulary those expresed exclusively under pathogenicity induction could have a role in the infection process. To avoid the problems associated with the high hydrophobicity showed by this protein we run in parallel an study by LC-MS/MS resulted in a large number of detected peptides (7507) and identified proteins (2203). The analysis of these membrane protein will provide more information about its nature and function, allowing us to determine which of them are involved in the infection cycle of the fungus and helping us to understand its possible role as a pathogenicity/virulence factor revealing new components of the signalling network.

191 COMPUTATIONAL EXAMINATION OF SECONDARY METABOLISM POTENTIAL OF THE FUSARIUM GRAMINEARUM GENOME REVEALS NEW PUTATIVE GENE CLUSTERS AND INDICATES HORIZONTAL GENE TRANSFER EVENTS

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The genome sequence of the plant pathogen Fusarium graminearum exhibits a diverse enzymatic potential for secondary metabolism synthesis. Previously described gene clusters that synthesize mycotoxin compounds like Trichothecene or Zearalenone play an important role in plant pathogenicity. However, the chemical nature of most of the products of predicted secondary metabolism enzymes and their ecological role, in particular in virulence, is largely unknown. In this work we demonstrate in a computational approach that a great portion of the predicted signature enzymes show evidence to be part of a secondary metabolism pathway. In total we identified 19 novel gene clusters that are significantly enriched with secondary metabolism connected functions. We further show co-regulation during host infection and condition specific expression patterns of these clusters in analyzing 12 publicly available microarray experiments. Additionally we determined promoter motifs that correlate with gene expression and are highly overrepresented in gene clusters compared to the genome wide distribution. A search for orthologous copies using a protein similarity database reveals conserved clusters outside the Fusarium phylum, some of them show evidence of horizontal gene transfer as orthologs can be found exclusively in representatives of the Botrytis or Cochliobolus lineage. The presented 19 candidates are strongly suggested as novel gene clusters and will provide valuable targets for further experimental examination.

192 CONSERVATION AND DIVERSITY OF SUGAR-RELATED CATABOLIC PATHWAYS IN FUNGI

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Plant polysaccharides are among the major substrates for many fungi. After extracellular degradation, the monomeric components (mainly monosaccharides) are taken up by the cell and used as carbon sources to enable the fungus to grow. This would also imply that the range of catabolic pathways of a fungus may be correlated to the composition of the polysaccharides it can degrade. In this study we have tested that hypothesis by analyzing the presence, absence and redundancy of genes of a number of catabolic pathways in selected fungi from the Ascomycota, the Basidiomycota and the Zygomycota. This involved first the identification of the catabolic pathway genes which was performed by automated ortholog and paralog searches. The expression of the genes was evaluated for those species for which transcriptome data was available. The results were then compared to growth profiling data of the species on a set of plant-related poly- and monosaccharides to determine to which extent the genome content fits the physiological ability of the species.

193 CRYPTIC FUNCTIONS AND EVOLUTION OF SUBSTRATE SPECIFICITIES IN THE FUR-LIKE TRANSPORTER FAMILY IN ASPERGILLUS NIDULANS

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The sequenced genomes of bacteria, archaea, fungi and plants comprise thousands of genes encoding transporters belonging to the Nucleobase Cation Symporter 1 (NCS1) family. This family comprises transporters specific for purines, pyrimidines, allantoin, thiamine and pyridoxine. NCS1 can be subdivided into the Fcy- and Fur-like subfamilies, characterized by specific amino acid sequences. The crystallographic structure of a bacterial NCS1 transporter, Mhp1, showed the existence of 12 transmembrane segments (TMS), of which TMS1-10 are arranged in two inverted intertwined repeats of five α-helices. In addition, structures of the outward-facing open and substrate-bound occluded conformations of Mhp1 were solved, showing how the outward-facing cavity closes upon binding of the substrate. All Aspergilli possess several NCS1-like proteins, most of which are of unknown function and specificity. We have characterized three of the A. nidulans proteins. FcyB is a purine-cytosine/H+ symporter, whereas FurD and FurA are uracil and allantoin H+ symporters, respectively. A structural model of FcyB was also reported, which together with systematic mutational analysis identified amino acid residues essential for substrate binding. Here we investigate the function of all functionally uncharacterized Fur-like transporters in A. nidulans, namely FurB, C, E, F and G. As relevant Fur null mutants do not lead to detectable phenotypes, we proceeded to over-express these functionally uncharacterized Fur transporters in a mutant strain that genetically lacks all seven known transporters involved in nucleobase-related transport (uapAΔ uapCΔ azgAΔ furDΔ furAΔ fcyBΔ cntAΔ). FurE can function as a low capacity uracil, allantoin and uric acid transporter, while FurC, F and G can mediate low capacity uracil transport. Fur-GFP chimeric proteins show variable sensitivity to ammonium or substrate-elicited endocytosis, which may reflect differences in the N- or C-terminal regions of Furs. No function could be detected for FurB, which shows increased instability and problematic targeting in the plasma membrane. Targeted mutational analysis facilitated by the solved structure of Mhp1, direct genetic screens designed to obtain FurA and FurD versions with altered specificities and phylogenic analyses, are leading to an understanding of the molecular basis and the evolution of substrate specificity in the Fur family.

194 DBHIMO: A WEB-BASED GENOMICS PLATFORM FOR HISTONE-MODIFYING ENZYMES

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Over the past two decades, epigenetics has evolved into a key concept for understanding regulation of gene expression. Among many epigenetic mechanisms, covalent modifications such as acetylation and methylation of lysine residues on core histones emerged as a major mechanism in epigenetic regulation. Here we present the database for Histone Modifying enzymes (dbHiMo) aimed at facilitating functional and comparative analysis of histone modifying enzymes. Histone modifying enzymes were identified applying a search pipeline built upon profile hidden Markov model (HMM) to proteomes. The database houses 22,169 histone modifying enzymes identified from 342 species including 214 fungal, 33 plants, and 77 metazoan species. The dbHiMo provides users with web-based personalized data browsing and analysis tools, supporting comparative and evolutionary genomics. With comprehensive data entries and associated web-based tools, our database will be a valuable resource for future epigenetics/epigenomics studies.

195 DE NOVOASSEMBLY OF PYRENOCHAETA LYCOPERSICI GENOME, A WELL KNOWN FUNGAL PATHOGEN OF TOMATO

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Pyrenochaeta lycopersici is the soil-transmitted ascomycete responsible for corky root rot (CRR) disease in tomato. The pathogen causes significant yield losses, both in the greenhouse and in the field, in many tomato-growing areas of the world, reducing fruit yields up to 75%. De novo assembly led to a 54.9 Mb P. lycopersici draft genome and we annotated approximately 17,000 genes. The assembly was based on Illumina sequencing and was carried out integrating the RNA-Seq data from the transcriptome of the P. lycopersici mycelium grown on artificial culture medium. From the 31,746,550 reads, we obtained 27,982 putative transcripts, 27,574 (98.5%) of which could be mapped onto the assembled genome, further validating the comprehensiveness of the gene space represented. The hemibiotrophic lifestyle of P. lycopersici was confirmed by the comparison of P. lycopersici genome sequence with those of other fungi, this showed a clear phylogenetic relationship with hemibiotrophic and necrotrophic plant pathogens. Moreover, the analysis of gene functions and comparison of gene sequences showed that a large fraction of genome is devoted to pathogenetic activity and showed a large overlap of the gene inventory to that of other already sequenced fungal plant pathogens. In detail, P. lycopersici genome reveals a significative expansion of gene families related to plant cell wall degradation, nutrient absorption and the detoxification of fungicides. Within the carbohydrate degradation arsenal (CAZymes), a significative expansion of the GH61 family has emerged. One of the enzymes belonging to GH61 class, expressed during the tomato root infection, was identified and characterized. We also observed a significant expansion of the gene families associated with heterokaryon incompatibility (HI), which represents an important source for this imperfect fungus for increasing genetic variability. The obtained P. lycopersici assembly constitutes an invaluable support to understand the phenotypical and physiological features of this pathogen; it will allows to investigate the molecular basis of the reproductive behavior and of the mechanisms involved in plant-pathogen interaction. The research was supported by the Project RESPAT funded by MiPAAF and by Fondazione Cariverona (Completamento e attività del Centro di Genomica Funzionale Vegetale).

196 DEGRADATION OF DIFFERENT PECTINS BY FUNGI: CORRELATIONS AND CONTRASTS BETWEEN THE PECTINOLYTIC ENZYME SETS IDENTIFIED IN GENOMES AND THE GROWTH ON PECTINS OF DIFFERENT ORIGIN

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Pectins are diverse and very complex biomolecules and their structure depends on the plant species and tissue. It was previously shown that derivatives of pectic polymers and oligosaccharides from pectins have positive effects on human health. To obtain specific pectic oligosaccharides, highly defined enzymatic mixes are required. Filamentous fungi are specialized in plant cell wall degradation and some produce a broad range of pectinases. They may therefore shed light on the enzyme mixes needed for partial hydrolysis. The growth profiles of 12 fungi on four pectins and four structural elements of pectins show that the presence/absence of pectinolytic genes in the fungal genome clearly correlates with their ability to degrade pectins. However, this correlation is less clear when we zoom in to the pectic structural elements. This study highlights the complexity of the mechanisms involved in fungal degradation of complex carbon sources such as pectins. Mining genomes and comparative genomics are promising first steps towards the production of specific pectinolytic fractions.

197 DEVELOPMENT OF SYSTEM-WIDE FUNCTIONAL ANALYSIS PLATFORM FOR PATHOGENICITY GENES IN THE RICE BLAST FUNGUS

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Null mutants generated by targeted gene replacement are frequently used to reveal function of the genes in fungi. However, targeted gene deletions may be difficult to obtain or it may not be applicable, such as in the case of redundant or lethal genes. Constitutive expression system could be an alternative to avoid these difficulties and to provide new platform in fungal functional genomics research. Here we developed a novel platform for functional analysis genes in Magnaporthe oryzae by constitutive expression under a strong promoter. Employing a binary vector (pGOF1), carrying EF1� promoter, we generated a total of 4,432 transformants by Agrobacterium tumafaciens-mediated transformation. We have analyzed a subset of 54 transformants that have the vector inserted in the promoter region of individual genes, at distances ranging from 44 to 1,479 bp. These transformants showed increased transcript levels of the genes that are found immediately adjacent to the vector, compared to those of wild type. Ten transformants showed higher levels of expression relative to the wild type not only in mycelial stage but also during infection-related development. Two transformants that T-DNA was inserted in the promotor regions of putative lethal genes, MoRPT4 and MoDBP5, showed decreased conidiation and pathogenicity, respectively. We also characterized two transformants that T-DNA was inserted in functionally redundant genes encoding alpha-glucosidase and alpha-mannosidase. These transformants also showed decreased mycelial growth and pathogenicity, implying successful application of this platform in functional analysis of the genes. Our data also demonstrated that comparative phenotypic analysis under over-expression and suppression of gene expression could prove a highly efficient system for functional analysis of the genes. Our over-expressed transformant library would be a valuable resource for functional characterization of the redundant or lethal genes in M. oryzae and this system may be applicable in other fungi.

198 DIDYMELLACEAE GENOMICS – THE CASE OF SPECIES CAUSING ASCOCHYTA BLIGHT IN FIELD PEA

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Ascochyta blight (AB) is a major yield constraint in field pea (Pisum sativum) worldwide. It is caused by Peyronellaea pinodes, Peyronellaea pinodella, Ascochyta pisi and Phoma koolunga of the Didymellaceae family, in the order of Pleosporales. In the field, the species can co- or independently infect pea; they induce similar symptoms and disease development. While P. pinodes is homothallic, the others are heterothallic, a major determinant of disease epidemiology. As a first step in elucidating the molecular basis of population structure, host-pathogen or pathogen-pathogen interaction, the genomes of a couple of isolates per species were sequenced. Peyronellaea pinodes, the main AB pathogen, was subjected to in-depth sequencing with multiple libraries of different insert size sequenced using Illumina and 454 technologies. Genomes were assembled using SOAPdenovo2. RNASeq data derived from in vitro cultures were used to correct ab-initio annotation. The genome assembled into 33.2Mb with L50 of 817,551bp and N50 of 15 scaffolds, close to estimated total number of chromosomes. The assembly contains 4.6% repetitive DNA and most of the genes as estimated by CEGMA, 96.4% complete. Similarly, the other genomes (Illumina libraries) span 30.9-31.9Mb with 93.5-96.8% complete gene models. No major genome rearrangements were observed between the species; the Peyronellaea genomes showed high levels of synteny between each other, while some translocations and inversions were observed between P. pinodes and Ph. koolunga. As expected, a high level of mesosyntheny was observed between these species and other pathogens within Pleosporales. According to reciprocal best BLASTp hit; 67% of P. pinodes genes had orthologs in Ph. koolunga, A. pisis, or P. pinodella, and 57% genes had orthologs in at least one other Pleosporales. In order to identify pathogenicity related genes, identification of secreted proteins and those involved in secondary metabolism were undertaken. In P. pinodes, 795 genes were unique; 91 of which were small secreted proteins, likely to be involved in host-pathogen interaction. Peyronelleae species contain at least 2 fold higher numbers of terpene synthase genes (58 - 68) and half of the genes encode non-ribosomal peptide synthase (21-31) compared to A. pisi and Ph. koolunga, with 33-37 and 60-78, respectively. Further comparative analyses, functional characterization and in planta expression studies will serve to elucidate disease determinants.

199 DIVERSITY OF FERULOYL ESTERASE ACTIVITY IN 14 ASPERGILLI SPECIES

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Feruloyl esterases (ferulic acid esterases, EC 3.1.1.73) are a subclass of carboxylic acid esterases, which catalyze the hydrolysis of ester linkage of ferulic acid and other cinnamic acids from plant cell wall polymers. Feruloyl esterases cleave phenolic acids (ferulic acid and p-coumaric acid) and their dimers from naturally occurring hemicelluloses and pectins. Hemicelluloses represent about 20–35% of the lignocellulosic biomass, and therefore there is a large potential for industrial applications of feruloyl esterases.
The presence of feruloyl esterase encoding genes varies significantly in fungi, even between species of the same genus. For instance, orthologs for Aspergillus niger faeA were only found in some Aspergilli, while the number of candidate feruloyl esterases of carbohydrate esterase family 1 varies between one and four in these species.
Plant cell wall derived substrates have been reported to induce feruloyl esterase activity in fungi. We studied the ability of 16 Aspergilli to release ferulic acid from sugar beet pulp and wheat bran. Total feruloyl esterase activity was detected by using different methyl esters as substrates. Feruloyl esterase activity profile of Aspergilli strains on different growth media and substrate specificity will be discussed.

200 ESSENTIAL GENE DISCOVERY IN THE BASIDIOMYCETE CRYPTOCOCCUS NEOFORMANS FOR ANTIFUNGAL DRUG TARGET PRIORITIZATION

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Fungal diseases represent a major health-care burden globally. Like other pathogenic microbes, there are a limited number of agents suitable for use in treating fungal diseases and resistance to these agents can develop rapidly. Cryptococcus neoformans is a basidiomycete fungus that causes cryptococcosis worldwide in both immunocompromised and healthy individuals. As a basidiomycete, it diverged from other common pathogenic or model ascomycete fungi more than 500 million years ago. Here we report the identification of C. neoformans genes essential for viability through forward and reverse genetics approaches in an engineered diploid strain. The forward genetic screen relies on the ability to generate T-DNA insertional mutants in a diploid strain, meiosis and sporulation are induced, and selection for the insertion event in haploid cells on selective media. More than 2,500 mutants were analyzed, and T-DNA insertions in more than 15 genes required for viability were identified. These genes includes the thioredoxin-reductase TRR1, the Lysyl (K) tRNA Synthetase KRS1, the ribosome assembly RSA4, the mRNA-capping CET1, and others. However, a considerable number of insertional mutants that were impaired or unable to produce haploid basidiospores were also initially selected as potentially inviable strains, representing a caveat of the forward genetic approach. For targeted gene replacement, the C. neoformans homologs of 34 genes required for viability in Ascomycete fungi were disrupted, meiosis and sporulation induced, and haploid progenies evaluated for their ability to grow on selective media. 20 (59 %) were found to be required for viability in C. neoformans. These gene are involved in mitochondrial translation, ergosterol biosynthesis, RNA-related functions and others. Generated diploid mutants were also evaluated by haploinsufficiency tests on a number of perturbing agents and drugs, revealing interesting phenotypic traits due the loss of one copy of an essential gene in C. neoformans. In conclusion, this study expands the knowledge of the essential genes in fungi, and sought to define their function in the cell by haploinsufficiency tests. Genes that have no mammalian homologs and are essential in both Cryptococcus and ascomycete human pathogens would be ideal for the focused development of antifungal drugs with broad spectrum activity.

201 EVOLUTION AND ADAPTATION OF DETOXIFICATION SYSTEM OF TRAMETES VERSICOLOR, A WOOD DEGRADER FUNGUS

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Wood decaying fungi and in particular saprophytic Agaricomycotina play a major role in the carbon cycle and are of great interest for various potential applications in green chemistry for instance. These fungi are able to degrade all wood components thanks to the secretion of a complex extracellular enzymatic system. Besides this system, wood decayers have also developed complex intracellular detoxification networks, called xenome, able to deal with the myriad of potential toxic molecules generated during wood degradation. The main hypothesis supporting this work is that wood chemical composition is likely a major factor for (i) driving the adaptation of wood-decaying fungi to the diversity of their substrate and (ii) explaining the rapid evolution of their detoxification system. To test this hypothesis, we have selected the white-rot Trametes versicolor as a model, a fungus easily found in temperate forest ecosystems on various tree species. Ten strains from different origins (type of wood and geographical area) were selected and analyzed using several phenotypic tests: growth rate, ability to degrade wood (beech, oak and spruce), extracellular enzymatic activities during oak degradation and secretome composition… Besides this phenotypical study, the different strains have been re-sequenced and the first genomic analysis tends to corroborate the observed phenotypic polymorphism suggesting that tree origin could be an essential factor governing the wood-decaying fungi adaptation.

202 EVOLUTIONARY SCENARIO FOR MADS-BOX GENES IN FUNGI DIFFERS FROM THAT FOR ANIMALS AND PLANTS AND PUTS FUNGI IN THE INTERMEDIATE POSITION

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MADS-box genes, encoding MADS-domain transcription factors (MADS TFs), are known in almost all eukaryotes and well investigated in animals and plants. The studies of the evolutionary history of these genes suggest that two types of MADS-box genes (Type I (SRF-like) and Type II (MEF2-like)) likely existed already in the most recent common ancestor of plants and animals. However, little is known about the evolutionary dynamics of these genes in fungi. Fungal MADS-box genes are involved in a variety of important functions, including cell cycle control, cell and mating-type differentiation, cell wall integrity, hyphal growth and pheromone response. To better understand the evolution of fungal MADS-box genes, we analyzed the sequences of these genes, including all functionally characterized ones, in 87 fungal species. The analysis of the conservation of structure and function of the two MADS types revealed an interesting feature of these genes in fungi: in Type I, stronger conservation of structure is accompanied by the more variable functions, whereas the Type II genes are less conserved structurally, but are also less variable in their functions. In other words, the genes with the more conserved domain demonstrate more functional diversity than the less conserved genes. In plants, however, Type I genes have much higher birth-and-death rates and sequence variability than Type II genes, so the conservation of structure and function correlate. In contrast to both plants and fungi, animals do not demonstrate variability in the MADS-box genes, keeping them ultra-conserved and in very low copy-number through all the kingdom. In fungi, MADS TFs gene number considerably depends on the lineage, being less variable in the derived fungi but much more multiple in the basal fungi. The highest number of the MADS is observed for Mucorales (up to eleven genes), whereas in asco- and basidiomycetes we can find 2-4 genes per genome. Interestingly, in some species the Type II genes are much less conserved possibly due to adaptations to individual (e.g. pathogenic) lifestyle. In conclusion, the evolutionary scenario for MADS-box genes in fungi seems to lie in the middle between those of animals (ultra-conservation) and plants (much higher variability, with lineage-specific clades generated by high birth-and-death of the Type I genes and ancient, highly conserved clades of the Type II genes).

203 EXPANSION OF THE ENZYMATIC REPERTOIRE OF THE CAZY DATABASE TO INTEGRATE AUXILIARY REDOX ENZYMES

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Since its inception, the carbohydrate-active enzymes database (CAZy; www.cazy.org) has described the families of enzymes that cleave or build complex carbohydrates, namely the glycoside hydrolases (GH), the polysaccharide lyases (PL), the carbohydrate esterases (CE), the glycosyltransferases (GT) and their appended noncatalytic carbohydrate-binding modules (CBM). The recent discovery that members of families CBM33 and family GH61 are in fact lytic polysaccharide monooxygenases (LPMO), demands a reclassification of these families into a suitable category. Because lignin is invariably found together with polysaccharides in the plant cell wall and because lignin fragments are likely to act in concert with (LPMO), we have decided to join the families of lignin degradation enzymes to the LPMO families and launch a new CAZy class that we name “Auxiliary Activities” in order to accommodate a range of enzyme mechanisms and substrates related to lignocellulose conversion. Comparative analyses of these auxiliary activities in hundreds of fungal genomes reveal a pertinent division of several fungal groups and subgroups combining their phylogenetic origin and their nutritional mode (white vs. brown rot). The new class introduced in the CAZy database extends the traditional the traditional CAZy families, and provides a better coverage of the full extent of the lignocellulose breakdown machinery.

204 FUG1, A NOVEL FUNGAL-SPECIFIC GENE, IS A KEY REGULATOR OF MORPHOGENESIS, CONIDIATION, AND PATHOGENESIS IN MULTIPLE FUSARIUM SPP.

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The number of sequenced fungal genomes is rapidly increasing. However, the functional characterization of novel genes has not progressed at a comparable rate. In pathogenic fungi, some uncharacterized genes likely represent novel virulence factors or anti-fungal targets. To fully understand the mechanisms underlying fungal pathogenesis, characterizing genes with no known or predicted function is of significant importance in the post-genomic era. Fusarium verticillioides is a ubiquitous pathogen of maize, causing reductions in grain quality due to contamination with fumonisin mycotoxins. Recently, we identified the novel gene FUG1 (Fungal Unknown Gene 1) in F. verticillioides via a reverse genetics approach designed to identify uncharacterized pathogenicity genes. Despite broad conservation among phylogenetically diverse groups of fungi, putative FUG1 orthologs have not been functionally characterized and have no predicted biological function. Targeted deletion of FUG1 in F. verticillioides resulted in a pleiotropic phenotype, including reduced radial growth and conidiation, altered colony morphology, and increased pigmentation. Deletion of FUG1 impaired colonization of maize kernels and stalks. Additionally in the kernel environment, fumonisin B1 biosynthesis per unit growth was significantly reduced in the FUG1 deletion strain. The deletion strain was more sensitive to oxidative stress, which implicates FUG1 in responses to host defense mechanisms. Deletion of FUG1 in the related pathogen Fusarium graminearum resulted in altered colony morphology and reduced conidiation. Moreover, the F. graminearum FUG1 deletion strains were significantly reduced in virulence in maize silks and wheat heads, suggesting the involvement of FUG1 in plant pathogenesis may be broadly conserved. This study directly implicates FUG1 in development and pathogenesis in two Fusarium spp. and represents the first step towards explaining how FUG1 functions at the molecular level to regulate multiple biological processes.

205 FUNCTIONAL ANALYSIS OF A YIPPEE-LIKE (YPEL) GENE FAMILY IN DEVELOPMENT AND PATHOGENICITY IN THE RICE BLAST PATHOGEN MAGNAPORTHE ORYZAE

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Magnaporthe oryzae is an ascomycete fungus and the causal agent of rice blast, one of the most destructive diseases of rice. This pathogen is considered as a good model organism to study phytopathogenic fungal development. Like most fungal pathogens, conidia (asexual spores) of M. oryzae play a key in the disease cycle. Transcriptional regulation is an important process for development and pathogenicity in response to environmental factors. Therefore, transcription factors (TFs) is critical for understanding regulation of fungal development and pathogenicity. Yippee-like (YPEL) proteins exist in eukaryotes including fungi, plants and animals, and are highly homologous across different taxa. YPEL contains a putative zinc-finger binding domain, which has diverse functions such as binding DNA and RNA, and mediating protein folding and protein-protein interaction. For humans, five YPEL proteins were found. Analysis of human YPELs suggests their distinct functions in regulation of cell division and development. However, functions of YPEL proteins are still unknown in most organisms including fungi. Recently, we identified two YPEL genes in the genome of Magnaporthe oryzae. The two M. oryzae loci MGG_06263 and MGG_00255 share a significant sequence homology with human's YPEL proteins, and therefore named as MoYPEL1 and MoYPEL2, respectively. To elucidate function of MoYPEL1 and MoYPEL2 genes, we generated deletion mutants for each gene via homology-dependent gene replacement. In brief, the deletion mutant △Moypel1 showed a remarkable reduction in conidiation, reduced mycelial growth and no appressorial formation on hydrophobic slide glass, compared to the wild-type. In addition △Moypel1 showed a little appressorial formation and penetration in planta. On the other hand, the deletion mutant △Moypel2 showed no phenotypes in germination and appressoria formation, except for a reduced pathogenicity, compared to the wild-type. Also, △Moypel2 appears to increase conidiation and produce multiple appressorium. These data indicate that two YPEL proteins are important in development and pathogenicity.

206 FUNCTIONAL ANALYSIS OF THE ZTSTUA TRANSCRIPTION FACTOR IN THE FUNGAL WHEAT PATHOGEN ZYMOSEPTORIA TRITICI

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Septoria tritici blotch (STB) caused by Zymoseptoria tritici (teleomorph: Mycosphaerella graminicola) is one of the most damaging fungal wheat diseases especially in regions with high rainfall. Despite its tremendous economic importance, and the progress being made over the last decade, the molecular mechanisms controlling the pathogenicity of this fungus are still poorly understood. We identified and characterized the transcription factor ZtStuA in Z. tritici using Agrobacterium tumefaciens-mediated transformation to improve the understanding of the molecular mechanisms that underlie pathogenicity. ZtStuA is the orthologue of the Aspergillus nidulans StuAp transcription factor belonging to the APSES family that is involved in key developmental processes such as dimorphism, mating and sporulation. Deletion of ZtStuA in Z. tritici resulted in severely defected yeast-like spore production, induced autolysis, hampered melanisation and absence of the dimorphic switch. In addition, we showed that ZtStuA is involved in virulence, as the deleted mutants were non-pathogenic on susceptible wheat cultivars. Ongoing characterization and the results of complementation will be presented.

207 FUNGAL CU-METALLOTHIONEINS: NEW FUNCTIONS AND GENOME MISANNOTATION

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Copper is an essential metal that is toxic at high concentrations. Fungi, as other organisms, have developed homeostatic mechanisms to avoid high Cu concentrations, among which metallothioneins (MTs) are prevalent. The fungal pathogen Cryptococcus neoformans causes opportunistic respiratory infections that end up in lethal meningitis if disseminated to the brain. In response to C. neoformans colonization, host macrophage mobilize Cu as an antifungal defense, this resulting in the induction of a Cu-operon, which includes two MT genes (CnMT1 and CnMT2) [1]. CnMT1 and CnMT2 are essential for fungal virulence and pathogenicity. The analysis of the recombinantly expressed Cu-CnMT1 and Cu-CnMT2 complexes showed that these MTs are extraordinary long (122 and 183 aa's) and present repeated Cu-coordinating domains, which confer them a large capacity for Cu+ binding in relation to other fungal MTs [2]. In pathogenic fungi, selection pressure to detoxify copper MT may have caused the tandem amplification of a basic Cu-binding block, which is surprisingly very similar to the well-known Neurospora crassa or Agaricus bisporus Cu-thioneins (25 aa's). Surprisingly, genes and cDNAs encoding for CnMT1 and CnMT2 were wrongly annotated in the corresponding genome database [2]. With the aim of studying different features of diverse fungal MTs (gene/cDNA/protein sequence and copper coordination abilities), and in order to correlate them with functional (virulence/detoxifying capacity) and evolutionary trends, we undertook a survey of the presence of MT-like peptides in different fungi. Our approach combines in silico manual search of putative MT ORFs, and in vivo experimentation by growing different species in Cu-enriched cultures and isolating mRNA/cDNAs of putative MTs for sequencing analysis. Results on the screening of Aspergillus fumigatus, Fusarium verticilloides, Scedosporium prolificans and Sporothrix brasiliensis, (all from the Ascomycota phylum) genomes show that MT are likely to be encoded by most of them, but they are not listed as annotated genes or even as predicted ORF, probably due to errors in genome annotation and to the fact that small ORF are often disregarded by automatic annotation algorithms.

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208 GENOME-WIDE EXPRESSION RESPONSES CAUSED BY THE LACK OF ATFA IN STRESS-EXPOSED AND UNSTRESSED CULTURES OF ASPERGILLUS NIDULANS

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The role of the bZIP-type transcription factor AtfA in controlling the global transcriptional stress response in Aspergillus nidulans was studied. Transcript changes elicited by menadione sodium bisulfite (MSB), H2O2, t-butylhydroperoxide (tBOOH), diamide and NaCl exposures were determined in the atfA deletion mutant and appropriate control strain. Genes showing at least two-fold induction or repression were grouped according to their annotations. In total, 1,577 genes were up-regulated and 1,380 genes were down-regulated at least under one of the stress conditions tested in a wild type strain. The majority (approximately 67%) of the induced or repressed genes was affected by only one stress treatment and only five genes responded to all types of stress. Stress responsive genes have their versatile physiological functions in DNA replication, transcription, translation, cell wall homeostasis and cell division as well as in carbohydrate, lipid, nitrogen and secondary metabolisms under all stress conditions studied. Stress-specific responses included for example down-regulation of endoplasmic reticulum functions and induction of the FeS protein assembly genes under MSB exposures or repression of cell wall homeostasis genes and induction of lipid and monosaccharide metabolic genes under NaCl elicited stress. The deletion of atfA affected transcription of 199 genes (97 genes up- and 102 genes down-regulated) under unstressed conditions. Among them, induction of genes related to secondary metabolism (17), repression of stress response genes (7) and transcriptional changes in cell wall homeostasis genes (9) were notable. On the other hand, transcription of approximately 50 % of the stress responsive genes was influenced by the deletion of atfA under stress conditions. AtfA contributed remarkably to the induction of secondary metabolite and DNA repair genes, and it was also important in the regulation of trehalose metabolic and iron-sulfur cluster assembly genes. Furthermore, the deletion of atfA also affected induction or repression of genes, which were not stress-responsive in wild type strains. For example, 72 % of the genes induced by H2O2 in the atfA deletion mutant were not up-regulated and 82 % of the genes repressed by MSB in the mutant were not affected negatively by MSB stress in a wild type strain. This project was supported by OTKA (K100464).

209 GENOME-WIDE TRANSCRIPTIONAL RESPONSE TO AMBIENT PH CHANGES IN FUSARIUM GRAMINEARUM: A LARGE METABOLIC REORGANIZATION CONTROLLED BY PAC1

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The pathogenic fungus Fusarium graminearum produces type B trichothecene mycotoxins during wheat infection that are now recognized as virulence factor. These toxins accumulate in cereal grains and represent a threat for health of animal and human consumers. Early steps of infection and induction of toxin biosynthesis can respond to various ambient signals, which can be determinant for the establishment of infection and the levels of contamination with toxins. During the initial stages of infection, the fungus can be subjected to drastic pH changes. Expression studies of the Tri genes implicated in trichothecene biosynthesis have demonstrated that acidic extracellular pH is a determinant inducer. It was shown that the pH regulatory factor FgPac1 negatively regulates the expression of Tri genes under neutral or basic pH. Beside their role in regulating secondary metabolites in fungi, Pac transcription factors are also known to regulate various classes of genes, especially the genes whose roles involve adaptation to the environment. In this study, a genome-wide transcriptional analysis conducted in two different pH conditions using a strain deleted for FgPac1 and a strain expressing a constitutively active form of FgPac1 was carried out to investigate global regulation by the pH in F. graminearum. Our data identifies a set of genes related to various functions which expression levels are affected by pH change. As expected, our results enlighten a general switch from basic to acidic metabolic activities upon acidification. In addition, our results further point towards a potential role of a calcium-mediated regulation in response to ambient pH, and identify the specific activation of the GABA shunt by acidic pH. Finally, a clustering approach followed by cis-regulatory motifs search highlight the presence of complex stress-response regulatory circuits impacted by the change in external pH.

210 GENOMIC AND KARYOLOGIC CHARACTERISATION OF COLLETOTRICHUM KAHAWAE

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The genus Colletotrichum was recently elected one of the most important group of plant pathogenic fungi in the world, based essentially on their economic importance and scientific relevance. The thorough understanding of Colletotrichum biology greatly depends on a consensus classification and on robust identification tools. The species complex C. gloeosporioides includes a number of plant pathogens, causing diseases within a wide variety of host plants. According to evolutionary studies, these fungi have recently speciated via a host-jump process, giving rise to the species C. kahawae. This later species is defined by its unique capacity of infecting unripe coffee berries in Coffea arabica, causing Coffee Berry Disease (CBD). Although CBD is at the present restricted to Africa, the risk of its introduction to America and Asia is currently a major concern. In an effort to identify pathogenicity factors that enabled the acquisition of this unique capacity, differentiating these fungi from closely related, non CBD-causing, we have compared CBD-causing and non CBD-causing fungi for their genome size and chromosome number. Furthermore, the identification of supranumerary chromosomes can be of great relevance as these may harbour key pathogenicity factors. For this we used different approaches that include chromosome separation and characterization by pulse field electrophoresis, cytogenetic analysis by microscopy, chromosome number estimation by telomeric fingerprinting and genome size determination by flow cytometry. Putative differences in genomic composition (genome size and chromosome number) may prompt the analysis of specific pathogenicity factors in extra chromosomes, as well as aid the taxonomy of C. kahawae.
Acknowledgments: This work is being funded by Portuguese national funds through Fundação para a Ciência e a Tecnologia (project PTDC/AGR-GPL/114949/2009 and grants SFRH/BPD/47008/2008, SFRH/BPD/65686/2009 and SFRH/BPD/88994/2012 attributed to AL, ASP and PT respectively).

211 GENOMIC AND TRANSCRIPTOMIC ANALYSES UNRAVEL EFFECTOR REPERTOIRE OF COLLETOTRICHUM ACUTATUM DURING THE INFECTION ON CAPSICUM ANNUUM

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Colletotrichum acutatum is one of the most notorious fungal pathogens and has a broad host-range associated with significantly economic fruits. In Taiwan, the anthracnose disease of chili pepper (Capsicum spp.) is mainly caused by C. acutatum via a hemibiotrophic lifestyle that contains an initial biotrophic stage in living host cells and a subsequent necrotrophic stage in dead host cells. Here, we have assembled a high-quality draft genome of hypervirulent strain of C. acutatum (Coll-524). After gene model prediction from 245 supercontigs and singletons, draft genome of C. acutatum Coll-524 revealed 339 putative secreted small proteins that might function as effector proteins. Through the time-course transcriptomes of C. acutatum infecting on Ca. annuum cultivar Group Zest, candidate effector proteins (CEPs) were revealed. Their expression profiles could be clustered into at least five differential expression patterns. They were significantly expressed at different stages of the infection on Ca. annuum fruit including appressoria formation, biotrophic stage, the switch from biotrophy to necrotrophy, and necrotrophic stage, but some have no expression during the infection. Among these CEPs, CEPs003 was in planta-induced and highly expressed during the switch of biotrophic and necrotrophic stage. The function of CEPs003 during the infection will be presented and discussed.

212 GENOMIC CpG DEFICIENCIES IN BASIDIOMYCETES LOOK SIMILAR TO THE PRODUCTS OF RIP IN ASCOMYCETES

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Many Basidiomycetes display fractions of their genomes that are markedly deficient in specific cytosine dinucleotides. This feature is similar to that found in Ascomycete genomes, but with the difference that only CpG dinucleotides are affected. Also, as in Ascomycetes, members of Basidiomycete transposable element families within each genome differ by an excess of transition mutations, predominantly in CpG context. However, while RIP in Ascomycetes can lead to transition/tranversion ratios of over 1000, the highest figure found among 85 Basidiomycetes is 99. Similar findings were seen in some lower fungi and two simple plants, all of which have been shown to share with Basidiomycetes the property of having heavy cytosine-5 methylation in CpG contexts of repeat sequences, but much lower methylation of genes. It is apparent that mutational destruction of repeats in these organisms is less thorough than in Ascomycetes, which has allowed the well-documented expansion of gene families. CpG loss can, nevertheless, be seen in a few fragmentary gene family members. There are two plausible explanations that could link CpG methylation and mutation: (1) although Basidiomycetes lack good homologues of the Neurospora crassa rid gene that is essential for RIP, some other RIP-like mechanism may be responsible for C to T transitions, with subsequent DNA methylation as in N. crassa; (2) alternatively, cytosine methylation may precede, and lead to, C to T mutation by spontaneous or enzymatic deamination of 5-methylcytosines.

213 HORIZONTAL GENE TRANSFER OF SECONDARY METABOLITE CLUSTERS BETWEEN FUNGI

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In recent years the number of horizontal gene transfer cases detected between fungi has increased notably. They not only encompass one single gene, but transfer events have been described that involved complete pathways and even whole chromosomes. The great impact that many of the secondary metabolites have in our health and economy makes them an important field of study. Transfers of complete clusters with the ability to synthesize these compounds can have a profound efect in our way of life, therefore it is important to have a clear view of the propensity fungal species may have to transfer these groups of genes. Here we present a study in which whe have analysed the presence of 88 known secondary metabolism clusters in 80 fully sequenced fungal species. In several instances, the evolutionary distance found between fungal species containing a given metabolite cluster clearly points to a transfer event between the fungal species. Such is the case of the cluster that encodes the loline metabolite. This compound, which acts as an insecticide, is usually synthesized by endophytic fungi, yet we found a close homolog of the cluster encoded in the apple pathogen Penicillium expansum. A more in depth analysis showed clear signs that the cluster had indeed been transferred and likely underwent a change of function. Another aspect that can be exploited when studying transferences of secondary metabolism clusters between fungal species is the possibility of identifying unknown clusters. Conserved groups of genes across two different species that are distantly related but known to synthesize the same or similar compounds can be the clue needed to associate a metabolic cluster and compound it synthesizes. So we used comparative genomic tools to identify the putative gene cluste that is responsible for the synthesis of Chaetoglobosin and which is found in the distantly related species Chaetomium globosum and Penicillium expansum. The joint study of secondary metabolism clusters and horizontal gene transfer allows us the oportunity to further study transference of groups of genes between fungal species. In additional it can provide us with an important tool for the identification of secondary metabolism clusters considering many of the metabolites still remain unmatched to their genetic component.

214 IDENTIFICATION OF TWO FUSARIUM CULMORUM GENES INVOLVED IN FOOT AND ROOT ROT ON DURUM WHEAT BY TRANSPOSON-MEDIATED MUTAGENESIS WITH THE MIMP1/IMPALA DOUBLE-COMPONENT SYSTEM

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Fusarium culmorum is an ubiquitous soil-borne plant pathogenic fungus that produces type B trichothecene mycotoxins. On wheat and other small grain cereals, it induces both a direct damage following production loss and an indirect damage due to mycotoxins contaminating grains. We adopted a transposon tagging approach with the mimp1/impala double component system to select mutants altered in their metabolic or morphological processes and/or impaired in their aggressiveness during the first step of interaction between this fungus with the host plant. In vitro bioassays were carried out to identify altered phenotypic characters in putative mutants growing on potato dextrose agar (PDA) amended with 2 M sorbitol, 1 M NaCl (osmotic stress), 30 mM potassium persulphate (oxidative stress), 0.02% sodium dodecylsulphate (SDS) and 0.5 ppm tebuconazole. To test thermal stress resistance of putative mutants, radial colony growth was tested on PDA at 37°C and 8°C. An in vitro pathogenicity assay was performed by placing one durum wheat seed onto each one of ten mycelium plugs in a Petri dish and incubating 3-6 days in the dark at 25°C. To confirm the result obtained in vitro, in planta assays were performed in greenhouse conditions. Two F. culmorum mutants (coded R38 and R386) were selected with altered phenotypic characters, including complete loss of pathogenicity towards wheat stem base/root tissue, altered pigmentation and stunted vegetative growth. Cloning of sequences flanking the mimp1 reinsertion by splinkerette PCR allowed us to identify an hypothetical gene with orthologs only in the fungal domain (R38) and a conserved hypothetical protein localized in the cytoplasm and endoplasmic reticulum (R386). These are the first two mimp1-tagged gene involved in FRR pathogenicity, adding to the few other genes known to play a role in this disease. Research funded by Regione Autonoma della Sardegna (Legge Regionale 7 agosto 2007, n. 7 “Promozione della ricerca scientifica e dell’innovazione tecnologica in Sardegna”) and by the Ministry of University and Research (PRIN 2010: Cell wall determinants to improve durum wheat resistance to Fusarium diseases). Barbara Scherm acknowledges support by P.O.R. SARDEGNA F.S.E. 2007-2013 - Obiettivo competitività regionale e occupazione, Asse IV Capitale umano, Linea di Attività l.3.1 (research project Identification of natural and natural-like molecules inhibiting mycotoxin biosynthesis by Fusaria pathogenic on cereals).

215 IDENTIFICATION OF VIRULENCE GENES IN DIFFERENT HOST-PATHOGENIC FORMS OF FUSARIUM OXYSPORUM

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The species complex Fusarium oxysporum (Fo) represents one of the most abundant and widespread microbes of the soil microflora, including plant-pathogenic strains that, together, are able to infect a broad host range. Essential for avoiding PTI responses and manipulating host defences are effector proteins. Fo genomes can be separated into two parts: core chromosomes that contain housekeeping genes and are syntenic to closely related Fusarium species; and lineage-specific (parts of) chromosomes that are enriched for transposable elements and genes related to pathogenicity or other niche-specific traits. A transposable element, "miniature Impala" (mimp), is always present in the promoter of effector genes, which provides a way to identify new putative effectors from Fo genome sequences. By screening a region downstream of this mimp for ORFs that contain a signal peptide (SP), we generated lists of candidate effector genes in strains of, amongst others, Fo f. sp. melonis and Fo f. sp. cubense. We also started investigation of cucumber-infecting strains. A diverse set of eight Fo f. sp. cucumerinum (Foc) and three Fo f. sp. radicis-cucumerinum (Forc) isolates was selected for genome sequencing. Additionally, for two strains from each forma specialis the transcriptome was sequenced. From these sequences three datasets were generated: i) 2kb regions downstream of a mimp, ii) ORFs containing a SP and iii) active in planta transcripts. By comparing these datasets putative effectors were identified. This set of effectors forms the basis of our investigation of the molecular basis of host-specificity and host immunity in Fo-cucurbit interactions.

216 INFERRING THE ECOLOGICAL ROLE OF THE ROOT-ASSOCIATED FUNGUS ARCHAEORHIZOMYCES FINLAYI USING GENOME AND TRANSCRIPTOME ANALYSES AND CLASSICAL CHARACTERIZATION

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Archaeorhizomyces finlayi is the only species formally described in the class Archaeorhizomycetes (Taphrinomycotina, Ascomycota). This fungus is characterized by the absence of distinctive reproductive structures, slow growth in culture, and an apparent association with roots of coniferous plants. Archaeorhizomycetes have recently been identified as one of the most abundant groups of fungi in soil samples from boreal ecosystems, but the lack of isolates and diagnostic species descriptions have hindered investigations of the ecological role of these fungi. The aim of this work is to uncover possible ecological roles of Archaeorhizomycetes using whole genome and transcriptome comparisons of A. finlayi against known fungal and bacterial genomes, together with classical lab-based physiological characterization. Total DNA and RNA from dikaryotic mycelia were purified and sequenced using Illumina and Ion Torrent, with both the genome and transcriptome assembled using a variety of bioinformatic tools. Protein-coding genes were predicted using AUGUSTUS and MAKER, and automatic and manual gene annotations were carried out using NCBI and KGCC databases along with microsynteny-driven ortholog detection. The genome assembly of A. finlayi is about 22.5 Mbp in size, with more than 12000 predicted genes. The majority of the genes associated with the following pathways have been annotated: production of ergosterol, pheromone recognition, hypotonic shock, high osmolality, starvation, and the mitotic and meiotic cell cycles. Genes associated with the metabolism of essential amino acids, lipopolysaccharide, vitamins, and nitrogen, among others, and catabolic pathways of plant polymers such as starch, glycans, styrene, butanoate, and lipoic acids have also been found. At the same time, genes that may be involved in plant-pathogen interactions where also noted. Overall, the analysis of the genome of A. finlayi suggested that this species might be a root-associated fungus capable of decaying several sugars, absorbing, and transforming nitrite to nitrate or ammonia in agreement with preliminary lab-based characterization. Furthermore, one of the most expressed proteins was a putative heterokaryon incompatibility protein (HET) suggesting that A. finlayi may have retained the capacity to undergo sexual reproduction; this capacity is currently being explored in vitro.

217 INSIGHTS ON THE EVOLUTION OF MYCOPARASITISM FROM THE GENOME OF CLONOSTACHYS ROSEA

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The mycoparasitic fungus Clonostachys rosea is an efficient biological control agent (BCA) under field conditions for a variety of plant diseases on agricultural crops. C. rosea belongs to the same order (Hypocreales), but to a different family (Bionectriaceae), than the more studied Trichoderma spp. BCAs. Comparative studies between C. rosea and Trichoderma spp. BCAs may thus improve our understanding of critical components of the mycoparasitic lifestyle. We sequenced the genome of C. rosea strain IK726 using Illumina/SOLiD technology, and transcriptomes from C. rosea interacting with Botrytis cinerea and Fusarium graminearum. Comparative genomics revealed a significant (Pd0.05) increase in the number of ABC-transporters, polyketide synthases, cytochrome P450 monooxygenases, pectin lyases and GMC oxidoreductases compared with other filamentous ascomycetes, including T. atroviride and T. virens. Interestingly, the increase of ABC-transporter gene number in C. rosea was associated with phylogenetic subgroup G (pleiotropic drug resistance transporters), while ABC-transporter gene number changes in Trichoderma spp. involved subgroup C that is putatively involved in secondary metabolite export. Gene expression data indicated that certain C. rosea subgroup G ABC-transporter genes were induced by exposure to the Fusarium mycotoxin zearalenone (ZEA), and deletion of a single ABC-transporter gene (abcG5) resulted in reduced growth rate (P=0.001) on ZEA-containing media and on fungicide-containing media. Deletion of the abcG5 ABC-transporter gene or the zearalenone lactonohydrolase gene zhd101, previously shown to encode an enzyme that detoxifies ZEA, resulted in mutants that failed to protect wheat and barley seedlings against F. graminearum foot rot disease in growth chamber tests. The comparative genomics study between C. rosea and Trichoderma spp. suggests that these BCAs rely on different mechanisms for attack. T. atroviride and T. virens contain many genes encoding hydrolytic enzymes that degrade fungal cell walls, and proteins involved in secondary metabolite biosynthesis, but few membrane transporters. This is in sharp contrast to the situation in C. rosea that contains only few genes encoding fungal cell wall degrading enzymes, but high numbers of proteins involved in secondary metabolite biosynthesis and membrane transport. In summary, our data suggest that mycotoxin tolerance/detoxification is an important component of the biocontrol ability of C. rosea.

218 INVESTIGATION OF PROTEIN PHOSPHATASES IN TRICHODERMA REESEI

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Perception of external environment changes and detection of intracellular energetic status allows the balance of requirements for growth and cell survival. Cellular responses are regulated by different processes as post-translational modifications by phosphorylation and dephosphorylation. In the biotechnological workhorse Trichoderma reesei, signaling pathways transmitting light signals and nutrient signals were shown to influence expression of plant cell wall degrading enzymes. Therefore we aimed to characterize protein phosphatases of T. reesei in order to assess their potential for improvement of enzyme expression. We performed hierarchical cluster analysis of transcript patterns of phosphatase genes in different mutants upon growth on cellulose and on inducing or repressing carbon sources. The profiles in photoreceptor mutants still showed light specific clustering, suggesting that the photoreceptors influence light dependent transcription of phosphatase genes. In agreement with literature, also the G-protein beta and gamma subunits and the phosducin like protein PhLP1 have a light dependent influence. Additionally, the transcript profiles of the phosphatases largely clustered according to the inducing or repressing effect of the carbon source used. Functional category analysis of coregulated genes enable us to assign putative functions to groups of phosphatases. Thereby we could distinguish between phosphatases predominantly involved in metabolic functions or in energy supply and protein synthesis. Analysis of deletion strains revealed functions in development (conidiation, fruiting body formation or ascosporogenesis), growth on different carbon sources and formation of cellulases in light and darkness. In summary we provide first insights into the functions of protein phosphatases in T. reesei.

219 LOOKING INTO THE FUNGAL GENOMES FOR NOVEL LIPASES/STEROL-ESTERASES WITH BIOTECHNOLOGICAL INTEREST

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Lipases are the enzymes more used in industrial applications, are able to catalyze synthesis and hydrolysis reactions of triglycerides [1]. Sterol-esterases share the same α/β-fold structure, and are able to catalyze synthesis and hydrolysis reactions of sterol-esters that may be useful in certain biotechnological applications [2]. In some cases, such as the Candida rugosa-like lipase family, the same enzyme presents wide substrate versatility being active against triglycerides and sterol-esters [2, 3, 4]. Nowadays, with the development of massive DNA sequencing, the genomes of an enormous number of organisms can be studied in a short time. The Joint Genome Institute (JGI) (http://www.jgi.doe.gov/) was pioneer in this kind of projects and has available in its website hundreds of fungal genomes. In an attempt to identify and characterize novel enzymes with enhanced properties and potential application in the industry, we carried out an in silico search of new sterol-esterases and lipases sequences from 128 genomes of environmental fungi with potential biotechnological interest. The candidate genes were selected analyzing the conserved motifs detected in the Candida rugosa-like lipase family and performing phylogeny studies. The kinetic properties of the new putative enzymes are predicted on the basis of their sequence and three-dimensional model structure. Selected candidates have been expressed in Pichia pastoris and their physic-chemical characterization is being carried out.

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220 MEIOTIC RECOMBINATION IN BISPORIC SUB-SPECIES AND INTERVARIETAL HYBRID OF AGARICUS BISPORUS

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A potentially informative model organism to study meiotic recombination is the button mushroom Agaricus bisporus. This species is represented mainly by two sub-species differing in type of life cycle. In A. biporus sub sp. bisporus (bisporic sub-species), most of the basidia produce two spores, each of which receives predominantly two non-sister nuclei with opposite mating types which germinate into fertile mycelia (heterokaryons). A minority of basidia producing four spores, each of which receives only one nucleus and germinate into homokaryons. This type of life cycle is designated as secondary homothallic. A. bisporus sub sp. burnettii (tetrasporic sub-species), has a predominantly heterothallic life cycle, i.e. most of the basidia produce four spores, each of which germinate into homokaryons. These sub- species differ not only in life cycle, but also in level of meiotic recombination. Studies on bisporic sub-species showed that recombination per chromosome per individual averaged 0.10, resulting in short genetic linkage maps with a total length of 164 cM. Despite the low meiotic recombination frequency (MRF), homologous chromosomes appear to segregate normally. Previous study indicated that MRF in the tetrasporic sub-species is normal. To identify genes involved in differences in MRF, we generated a set of 138 haploid homokaryotic offspring isolated from an intervarietal hybrid bisporic × tetrasporic. SNP markers derived from whole genome sequence were mapped onto 13 linkage groups. Average recombination frequency per chromosome per individual in tetrasporic sub-species was substantially greater (0.8) than the average in bisporic sub-species leading to a longer genetic map length (1020 cM) compared with the bisporic sub-species. Observed differences between MRF in bisporic sub-species and intervarietal hybrid of bisporic × tetrasporic sub-species indicate that MRF in tetrasporic sub-species is normal and at least partially dominant. That allows the mapping of genes involved in differences in recombination frequency, knowledge that can be useful for mushroom breeding.

221 MOLECULAR DISSECTION OF AMYLOLYSIS IN FUSARIUM VERTICILLIOIDES

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Maize is a globally important crop that serves as a food staple throughout the world. Fusarium ear rot, caused by Fusarium verticillioides, is a devastating maize disease due to the accumulation of fumonisins within infected kernels. Fumonisins are polyketide-derived mycotoxins associated with various mycotoxicoses of humans and livestock. Although previous work has established a link between starch metabolism and fumonisin biosynthesis in F. verticillioides, little is known about the mechanisms underlying starch sensing or the genetic regulation of amylolysis within infected maize kernels. A combination of forward and reverse genetic approaches is being applied to dissect the molecular mechanisms of starch sensing and metabolism in F. verticillioides. The forward genetic component utilizes random insertional mutagenesis to identify novel genes involved in amylolysis. Two separate forward genetic screening methods have been utilized to assess virulence in insertional mutants: a live ear assay has identified mutants impaired in kernel colonization, while a cracked kernel assay is being utilized to quantify amylolysis and fumonisin biosynthesis. A preliminary live ear screen of 490 insertional mutants identified 36 strains displaying reduced kernel colonization. The reverse genetic component consists of targeted deletion of six candidate regulatory genes to dissect signal transduction pathways involved in amylolysis. Targeted deletion mutants are being evaluated for impaired starch hydrolysis, abnormalities in growth and development, and impaired fumonisin biosynthesis. The combination of forward and reverse genetic approaches will provide new insight into amylolysis and kernel colonization in F. verticillioides. An improved understanding of these processes will lead to a new working model explaining kernel colonization and the genetic linkage between starch metabolism and fumonisin biosynthesis.

222 MOLECULAR MECHANISMS IN HYPERPARASITISM OF T. GUIZHOUENSE NJAU 4742 ON FUSARIUM OXYSPORUM F. SP. CUBENSE 4 REVEALED THROUGH THE WHOLE GENOME-WIDE TRANSCRIPTIONAL RESPONSE

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Trichoderma guizhouense NJAU 4742 is the newly recognized member of the Harzianum clade. This strain is capable to suppress various disease caused by fungal pathogens including soil-born pathogen Fusarium oxysporum f. sp. cubense 4 (Foc4), the causative agent of banana wild disease. NJAU 4742 has superior mycoparasitic abilities in darkness that in case of some selected preys are essentially weakened in light. in this study the molecular mechanisms in hyperparasitism NJAU 4742 and mechanisms employed by FOC4 to protect itself against Trichoderma parasitism were explored from based on the genome-wide transcriptomic analysis in comparison to the type strain of T. harzianum CBS 226.95 that is a relatively weak mycoparasite. As both members of the Harzianum clade, NJAU 4742 and CBS 226.95 are powerful environmental opportunist and both posses mat1-2. The size of genome of NJAU 4742, is 38.29 Mbp, compared with 40.98 Mbp, CBS 226.95. The method newly reported[1] was used to freshly annotate and analyze two strains, to detect genomic deficiencies making CBS 226.95 less efficient in antagonism of FOC4. Mycelia of NJAU 4742 were harvested for transcriptomic analysis of crosstalk when it encounter it-self, similar species (CBS 226.95), and prey (Foc4) by de novo sequencing. The de novo sequencing was also used to help understand colony morphology, conidiation profile of NJAU 4742, and hyperparasitism to Foc4, which essentially altered by light, while CBS 226.95 is insensitive to illumination. Considering the different interaction of Foc4, transcriptomic analysis contributed to comprehend the mechanisms employed by FOC4 in resistance against Trichoderma parasitism.

223 MUSHROOMICS: COMPARATIVE MUSHROOM GENOMICS AND TRANSCRIPTOMICS

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The era of mushroom omics has come amongst the mushrooming of omics in all life science fields. I propose to name “omics” of mushrooms Mushroomics. My laboratory has been working on the genomics and transcriptomics of Shiitake mushroom Lentinula edodes and inky cap mushroom Coprinopsis cinerea. We sequenced the genome of the L. edodes monokaryon L54A using the Roche 454 and ABI SOLiD sequencing platforms. Over 13,000 protein-coding genes were predicted from the 40.2 Mb draft genome. We constructed a high-density genetic linkage map that was useful to link scaffolds into super-scaffolds. We conducted comparative analyses on public genome sequences of basidiomycetes and ascomycetes, and revealed genes expanded in genomes of mushroom-forming fungi. The expanded genes included specific types of regulators, ubiquitin ligases, protein-binding proteins, protein kinases, and transcription factors. In particular, F-box and paracaspase domain proteins were significantly expanded. We performed RNA-Seq of multiple stages of L. edodes. We also analyzed the transcription profiles of different stages of C. cinerea using NimbleGen microarrays. Genes differentially expressed during fruiting body initiation and development in these mushrooms were identified. Transcriptome age index (TAI) profile and transcriptome divergence index (TDI) profile showed a molecular hourglass pattern over the developmental lifecycle of L. edodes and C. cinerea. Young fruiting bodies is the bottleneck with high conservation, expressing the evolutionarily oldest and most conserved transcriptome. We compiled the genome sequences of L. edodes and other fungi into an Ensembl-based platform, equipped with a battery of genomic analysis tools, for comparative mushroom genomic analysis. Our works have generated rich resources for the analysis of genomics and transcriptomics of mushroom-forming fungi. Our analyses also provided insights into the molecular mechanisms of fruiting body development in fungi and the evolution of fungal complex multicellularity. Indeed, our works showed that the era of Mushroomics has arrived.

224 NON-CONSERVED CHROMOSOMAL SEGMENTS IN THE FUSARIUM GRAMINEARUM GENOME: ORIGIN AND BIOLOGICAL RELEVANCE

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Non-conserved Chromosomal Segments in the Fusarium graminearum genome: Origin and biological relevance Whole genome comparisons between closely related species, often show uneven evolution across chromosomes. In many cases non-conserved regions were identified in specific regions of chromosomes or even as entire chromosomes[(1)]. The origin and biological relevance of these non-conserved chromosomal regions are still largely unknown. Here we studied the role of non-conserved regions in adaptation and evolution in Fusarium graminearum. In contrast to some other plant-pathogenic fungi like Fusarium oxysporum and Zymoseptoria tritici [(2)], in which non-conserved regions are largely confined to lineage specific chromosomes, the genome of F. graminearum harbours thirteen non-conserved regions dispersed across all four[(3)]. Our studies, using RNA-Seq data from the F. graminearum isolate PH-1 [(4)] show that the expression of genes in conserved regions is stable, while gene expression in non-conserved regions is strongly influenced by the developmental stage. Genes involved in the production of secondary metabolites or secreted proteins, are enriched in these non-conserved regions, suggesting that these regions are important for adaptations to new environments, including adaptation to new hosts. Expression analysis of PH-1 and a knock-out of a master regulator of transcription (PH-1 delta ebr1; 5) allowed the identification of several new gene clusters that are co-regulated and potentially involved in the production of secondary metabolites. Clustering of genes in non-conserved genomic regions may facilitate co-regulated activation or repression of specific sets of genes.

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225 ORCINOL IS PRODUCED BY POLYKETIDE SYNTHASE 14 IN FUSARIUM GRAMINEARUM

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Filamentous fungi are producing a wide array of secondary metabolites such as polyketides, which are produced by polyketide synthases (PKS). The bioactivity of the secondary metabolites can vary. Some of the secondary metabolites have benne linked to the infection of a host or increasing its competing abilities for nutrients amongst other microorganisms. Furthermore has the pharmaceutical industry gotten an interest in these compounds, as they have the potential of becoming new kinds of drugs. In search for new compounds we have focused on the global plant pathogenic fungus Fusarium graminearum. Genome annotations indicate that it contains 15 PKS genes, of which only 6 has been linked to a product. To remedy this, we focused on PKS14, which has only been shown to be expressed during plant infections or when cultivated on rice or corn meal (RM) based media. To enhance the production of the resulting product we introduced a constitutive promoter in front of PKS14 and cultivated two of the resulting mutants on RM medium. This led to the production of a compound, which was only detected in the PKS14 overexpressing mutants and not in the wild type or PKS14 deletion mutants. The compound was isolated by preparative HPLC and identified as orcinol by UHPLC-MS/HRMS and NMR by comparing spectra to commercial available standards. Orcinol has previously been found in lichen fungi, but it has not previously been detected in Fusarium sp. This polyketide has been shown to be synthesized through decarboxylation of orsellinic acid (OA) in other fungal genera and phylogenetic analyses of PKSs placed PKS14 in a subcluster of known OA synthases. Based on expression analyses the PKS14 gene cluster is predicted to include six additional genes, suggesting that orcinol may not be the end product of the biosynthetic pathway.

226 PATTERNS OF NATURAL SELECTION IN CODING AND NONCODING DNA SEQUENCES IS ASSOCIATED WITH VIRULENCE IN THE PLANT PATHOGEN COLLETOTRICHUM GRAMINICOLA

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Pathogen and host populations are constantly imposing on each other selection pressures. For plant pathogens, sequences coding for proteins implicated in the evasion or suppression of host defenses or in the generation of new mechanisms of infection are expected to be the most affected by the action of selection. Furthermore, many phenotypic adaptations are likely to be consequence of changes in the regulatory regions, but little evidence exists about selection acting at this level in plant pathogenic fungi. In this work, we used whole genome sequences of eight strains of the maize pathogen Colletotrichum graminicola to investigate patterns of selection in different regions of the genome. Our study shows an excess of low and high frequency polymorphisms, as well as positive selection, affecting both, coding and non-coding DNA sequences close to protein-coding genes involved in pathogenicity. Interestingly, we found that genes coding for effector proteins and secondary metabolites show evidence of positive selection in the coding sequence, whereas genes upregulated during infection in maize are enriched with 3'UTRs under positive selection and 5'UTRs sequences with evidence of balancing selection. Based on our results, we suggest that even though adaptive substitutions on coding sequences are important for proteins that interact directly with the host, polymorphisms in the regulatory sequences may confer flexibility in the virulence processes of this important pathogenic fungus . This analysis enabled us to develop a list of candidate list of genes encoding putative effectors that have evidence of positive selection. One gene has been functionally characterized and has been shown to be an effector protein that is translocated to the plant nucleus.

227 PHENOTYPE-BASED SCREENING METHOD OF SECRETED PROTEINS FOR FILAMENTOUS FUNGI

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Identifying a repertoire of secreted proteins is crucial for understanding fundamental biological processes underlying lifestyle of fungi. However, unlike secreted proteins of oomycetes possessing a distinctive translocation signal, it appears that fungal counterparts do not have such pattern. As a result, in-silico analyses depending primarily on the presence of signal peptide predicted overwhelming number of candidate secreted proteins. Current approaches to test secretion of such proteins are dependent upon the use of fluorescence marker or heterologous expression (for example, yeast secretion trap) and have limitations in either time required and/or confidence of interpretation. Here we describe the method relying on complementation of water-soaking phenotypes in a mutant strain lacking hydrophobin gene of the rice blast fungus, Magnaporthe oryzae. Deletion of MPG1, a gene encoding hydrophobin results in easily wettable phenotype (Talbot et al. 1996). When a query protein is fused to a truncated hydrophobin lacking signal peptide in Δmpg1 mutant, secreted proteins can be identified simply by visual examination of wettability of fungal colonies. To evaluate our method, we tested two known secreted proteins, AvrPita and MSP1 and two known non-secreted proteins, THL1 and ACT1. Constructs containing AvrPita or MSP1 were able to complement wettability phenotype of the mutant in most of the transformants, whereas complementation efficiency was low in transformants carrying constructs containing THL1 or ACT1. Spray-inoculation of the transformants expressing AvrPita-MPG1 fusion protein onto rice plants carrying Pita, a cognitive R gene led to development of hypersensitive responses, confirming that the fusion protein is indeed secreted. Our data indicates that our methods can effectively and quickly screen for secreted proteins having different properties and secretion efficiency. Considering that hydrophobin genes are ubiquitous among fungal species, we believe that our method would be applicable to any fungal species for which genetic manipulation is available and therefore help us to understand the molecular basis of fungal lifestyles ranging from saprobic to pathogenic ones.

228 PHYLOGENOMICS OF BASIDIOMYCOTA IDENTIFIES TARGETS FOR EVOLUTIONARY STUDIES OF ADAPTATION AND PATHOGENICITY IN RUST FUNGI

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Understanding the origin and evolution of pathogenicity of rust fungi (Pucciniales) has remained a conundrum for decades. Extensive work has been carried out but most studies have focused on a limited number of species and genes. Recently, the publication of two rust fungi genomes, Puccinia graminis f. sp tritici and Melampsora larici-populina presented an important step forward towards the understanding of rust pathogenicity, revealing expansions of gene families and large repertoires of small secreted proteins, i.e. candidate pathogenicity effectors. However, the role of natural selection on genes shared between rust fungi and other Basidiomycota remains little explored. In this work, we employed a phylogenomic approach focused on single-copy genes shared by several Basidiomycota species to understand how they were shaped by natural selection. We have identified 614 shared orthologs across 38 Basidiomycota species, which were used to reconstruct their evolutionary relationships. Large scale screening of positive selection acting on a subset of amino acids on the basal branch of the rusts uncovered 832 selected sites across 142 genes, which is surprisingly high given the fact that these genes are common across the Basidiomycota. Gene ontology analyses reveal that the selected genes are enriched for metabolic process pathways, lyase activity and small molecular processing. Of the 832 selected sites, 353 (42%) represent amino acid sites with a variant exclusive to all rust fungi and 111 (13%) represent amino acid sites mostly conserved across all targeted Basidiomycota species. This suggests that while the largest share of selected sites are de novo mutations unique to the Pucciniales and prime candidates for genes underlying the common adaptive path of rust fungi, there is a non-negligible signal for sites with strong codon usage bias (i.e., genes with the same translated amino acid but using a different codon) and a moderate signal for selected sites whose variants are shared between rust fungi and other Basidiomycota. This work aims to be a future hypothesis-generator framework of loci responsible for the adaptation and pathogenicity of rust fungi.

229 PHYLOGENOMICS OF BEETLE-VECTORED SYMBIOTIC FUNGI

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Recent eruptive bark beetle outbreaks have surpassed all previously documented outbreaks in frequency, range, and scale, with over 36 million hectares of forests effected in the Western U.S. and Canada. These outbreaks impart large-scale landscape transformation via changes in tree species composition, herbaceous plant cover, and carbon cycling/sequestration. Much attention has been paid to the eruptive dynamics of beetle populations from an entomological perspective, but less work has been done on the beetle's symbiotic fungi. This is surprising, given the fungi are required for beetle survival. Whole genome sequencing of Grosmannia clavigera, a primary Mountain Pine Beetle (MPB) symbiont, has provided some insight into fungal pathogenicity. However, to most effectively leverage genomic data, it is useful to compare several genomes derived from species at multiple evolutionary distances. This allows insight into the co-evolutionary changes that have occurred between the beetle and their fungal symbionts as well as adaptations occurring in the fungi that allow expansion to new environments. We recently sequenced the genomes and transcriptomes of seven different ambrosial beetle symbionts within Ophiostomatales and Microascales, all of which exhibit varying degrees of host-beetle association. These data, combined with other recently published fungal symbiont genomes (Ophiostoma ulmi, Ophiostoma novo-ulmi, Ophiostoma piceae, Leptographium longiclavatum and Grosmannia clavigera), can be compared to gain insight into beetle-symbiont interactions including the role symbionts play in beetle defense, nutrition, thermal tolerance, and potential geographic range. While these genomic data provide a unique opportunity to study the evolution of fungal pathogenicity and life history across an array of ecologically and economically relevant species, characterizing transitions in these traits requires a robust phylogeny. The explosion of genomic data sets over the last decade has resulted in a corresponding increase of phylogenomic analyses. This influx of data has helped resolve many difficulties in phylogenetic reconstruction associated with sampling too few loci. However, it has also introduced additional complexities to phylogenomic inference that can mislead analyses. Here we present a Maximum Likelihood phylogeny for Ophiostomatales estimated from phylogenomic data using methods that account for biases inherent in genome-scale datasets.

230 PHYLOGENOMICS OF TRICHODERMA

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According to the current hypothesis Trichoderma and some other Hypocreales evolved as biotrophic associates of wood rotting fungi (mycoparasites of Basidiomycetes) and later on followed their preys/hosts into their habitats. Thus, an outstanding opportunistic potential of several Trichoderma species allowed them to establish as efficient saprotrophs in such ecological niches as soil, rhizosphere, and dead wood (Druzhinina et al., 2011). Many of the Trichoderma species are able to establish also biotrophic associations with plants (endophytism) and animals (opportunistic pathogens of humans). However, a profound affinity to mycotrophy remains to be the major ecological descriptor of the genus. The pilot phylogenomic analysis of the first three genomes of Trichodema, T. atroviride, T. reesei and T. virens, showed that T. atroviride most closely resembles the ancestral state of the genus, while T. reesei is the most derived taxon from these three (Kubicek et al., 2011). In this report we present the refined phylogeny of the genus Trichoderma combining the previously available materials with the data from the five newly sequenced genomes from T. harzianum, T. guizhouense NJAU 4742, T. asperellum, T. longibrachiatum and T. citrinoviride,. We have retrieved, manually curated and aligned 105 proteins from 26 Hypocreales genomes that are currently available. The resulting length of our alignemnet arrived at 54 000 aa residues. Our analysis has revealed that Trichoderma shares the last common ancestor with those Hypocreales families that include insect pathogens (Clavicipitaceae, Cordycipitaceae and Ophiocordycipitaceae) but not with families enriched with plant-pathogens (Fusarium). The simultaneous analysis of several Trichoderma genomes allowed us to detect the relatively ancient origin of the genus that essentially predates the divergence time for numerous families within the order. Interestingly, all Trichoderma species with sequenced genomes represent different infragenetic clades but are ecologically resembling each other: they all are powerful environmental opportunists with strong mycoparasititc potential. It forces us to focus on the similarity found between Trichoderma species and their cumulative differences to the next neighbours. For this purpose we have selected several other mycoparasitic fungi from Hypocreaceae for which no genomes are available for publics. Results of the phylogenetic analysis will be presented.

231 POLYKETIDE SYNTHASES FROM FUSARIUM: CLONING, EXPRESSION AND PURIFICATION FOR STRUCTURAL ANALYSIS

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Fungi produce a wide array of secondary metabolites with interesting bioactivities by help of a number of enzyme complexes. Polyketide synthases (PKS's) are a class of multidomain enzymes producing a class of secondary metabolites called polyketides. Only few structures of PKS's have been described, even fewer from fungi and none from Fusarium species. Multidomain proteins can be quite challenging to work with, which is why the project intends to solve the 3D-structures of single domains of PKS's one by one. In this project, the plan is to work with the cloning and expression of different domains from a PKS from Fusarium.
The genes of different Acyl-carrier protein (ACP) domains of the PKS' from Fusarium graminearum were expressed in E. coli thorugh a vector containing tags for purification and a TEV protease cleaving site. The expression was induced using Isopropyl β-D-1-thiogalactopyranoside (IPTG) and the ACP protein purified through steps of tag purification, TEV protease cleavage, spin column and gel filtration. Results were analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) which showed that the ACP domain was successfully expressed, cleaved and purified. Since the purification was a success, further structure analysis through X-ray chromatography and Nuclear magnetic resonance (NMR) will be performed. We also tried to purify β-ketosynthase (KS) domains from F. graminearum.

232 PROTEOMICS APPROACHES TO WINE YEAST S. BAYANUS VAR UVARUM

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Saccharomyces cerevisiae is the typical wine fermentations yeast. It has been widely used to develop different improvement during must fermentation. To acquire wines specificity and distinction is essential to have a broad knowledge of microbial fermentation processes, as the diversity of yeasts involved in this process that provides much of complexity to the wines.
After several years of analysis, our group found different population of wine yeast during different vintages of red grapes, showing that, in spite of the relevance of S. cerevisiae, other specie has a relevant role during this process. The specie Saccharomyces bayanus var uvarum was detected in a high percentage, producing a specific profile of organoleptic properties of special relevance. Moreover, this strain has the ability to work nicely at low temperature, increasing the formation of volatile compounds responsible for the different wine organoleptic characteristics.
We had developed a differential proteomics analysis to elucidate the proteins involved in the winemaking process at low temperature by comparing the profiles obtained at 13ºC (low temperature) and 25ºC (high temperature) by two-dimensional electrophoresis (2DE) and mass spectrometry (MALDI TOF/TOF). The proteome of S. bayanus var. uvarum was located between pI 3/10 and Pm 116/14 kDa. Comparing the obtained 2DE profiles, we detect differentials specific proteins from each physiological stage. This proteins were selected for identification by MALDI TOF / TOF in collaboration with The Cambridge Centre For Proteomics (University of Cambridge). Through a detailed study of these differences we will be able to elucidate the role that each of them during fermentation, which could be the basis for new strains selection criteria and/or the quest of biomarkers of wine quality.

233 QTL MAPPING OF MELANIZATION IN THE WHEAT PATHOGEN ZYMOSEPTORIA TRITICI

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Zymoseptoria tritici is found worldwide and is considered one of the most important wheat pathogens in Europe. Little is known about the genetic architecture of important life history traits that could affect pathogenicity, including the production of melanin pigments. Melanin plays an important role in virulence and antimicrobial resistance in several pathogenic fungi. To identify genes involved in melanin production, we mapped quantitative trait loci (QTLs) in progeny of two mapping populations. We genotyped 263 (cross#1) and 261 (cross#2) progeny at 9745 and 7333 single nucleotide polymorphisms (SNP) respectively, using restriction site associated DNA sequencing (RADseq), enabling construction of two highly dense linkage maps. The estimated total genome size was 4255 cM in cross#1 and 4979 cM in cross#2, with an average spacing between SNP markers of 0.44 cM and 0.68 cM. To map QTLs affecting melanization, we measured grey values of single spore colonies growing on Petri dishes containing potato dextrose agar (PDA). As melanin production is stress-related, each progeny was submitted to 2 stress treatments and one control treatment. Grey values were measured using digital images taken at 8, 11 and 14 days post inoculation (dpi) with a novel image processing approach that allowed high-throughput phenotyping. In cross#1 we detected 6 unique QTLs over all three treatments. Cross#2 revealed 9 QTLs, of which 3 overlapped with cross#1. QTLs varied by treatment and over scoring days. By investigating non-synonymous SNPs within QTL confidence intervals, we were able to identify several candidate genes, including PKS1, which encodes a polyketide synthase. PKS1 was shown to be involved in melanin production and virulence in other plant pathogenic fungi, but has not previously been associated with these traits in Z. tritici. This study is the first to map QTLs affecting melanin production in a fungal plant pathogen and the first to apply RAD-seq as a next generation sequencing tool to identify SNPs used for QTL mapping in Z. tritici. This study illustrates the power of this approach and may help identifying QTLs for several important quantitative traits.

234 QUANTITATIVE PROTEOMIC AND TARGETED METABOLOMIC ANALYSIS REVEAL UNEXPECTED EFFECTS OF INTERFERENCE WITH GLIOTOXIN BIOSYNTHESIS IN ASPERGILLUS FUMIGATUS

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Previous work has shown that deletion of the gliK gene from the gli cluster in A. fumigatus resulted in the abrogation of gliotoxin biosynthesis and led to a significant increase in sensitivity to exogenous gliotoxin, relative to the parent strain (≥ 1 μg/ml gliotoxin; $p < 0.001$). Comparative proteomic profiling of the gliotoxin-sensitive mutant, ΔgliK, revealed perturbation of translation in response to gliotoxin and also indicated dysregulation of the unfolded protein response (UPR) to endoplasmic reticulum (ER)-associated stress. This informs on the mechanisms involved in gliotoxin-mediated toxicity and may extend to other gliotoxin-sensitive species. Additionally, enzymes involved in the methyl/methionine cycle, including methylenetetrahydrofolate reductase (MTHFR) and methionine synthase, were significantly up-regulated in ΔgliK upon exposure to gliotoxin ($p < 0.05$). Up-regulation of the methionine cycle in response to gliotoxin, as revealed by comparative proteomics, may therefore indicate an attempt to compensate for disruption in mechanisms required for gliotoxin methylation in ΔgliK. Metabolomic investigation of ΔgliK revealed a reduced capacity to produce bis-methyl gliotoxin (BmGT), or S-methylate exogenous gliotoxin, relative to the parent strain. However, this inefficient conversion of gliotoxin to BmGT does not appear to specifically contribute to the increased sensitivity of ΔgliK to gliotoxin. LC-MS analysis, using 13C-labelled-methionine (via S-adenosylmethionine; SAM) revealed Met as the source of the methyl groups on BmGT. Additionally, deletion of gliK, but not gliT, from A. fumigatus also resulted in over-production of two diketopiperazines (6-methoxyspirotryprostatin B (m/z 394) and 18-oxotryprostatin A (m/z 396)) which are unrelated to gliotoxin and previously only detected in a marine species, Aspergillus sydowi. This suggests that disruption of gliotoxin biosynthesis, and unexpected trans effects, specifically alters the biosynthesis of additional secondary metabolites and may represent a novel strategy for activating silent gene clusters.

235 REGULATORY NETWORKS AND REGULATORS OF CHROMATIN STRUCTURE GOVERNING GLOBAL RESPONSES TO CHANGES IN LIGHT AND TIME

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Most fungi are highly responsive to their immediate environment, having developed sophisticated means to respond acutely to changes in their photic environment as well as circadian clocks to anticipate repeating environmental changes. Neurospora has proven to be a tractable model for understanding the proteins and networks underlying these responses. In this organism, blue light is detected by FAD stably bound by the transcription factor WC-1, eliciting photochemistry that drives a conformational change in the complex of WC-1 and WC-2 (WCC) resulting in activation of gene expression from promoters bound by the WCC. The circadian system allows anticipation of recurring environmental changes, and comprises a negative feedback loop wherein the WCC, in the dark, drives expression of frq. FRQ, an intrinsically disordered protein, stably interacts with casein kinase 1 and with FRH (a putative RNA helicase that does not function enzymatically in the clock; Hurley et al., Molecular Cell, 2013), and after phosphorylation-mediated delays, the complex downregulates the WCC (Baker, Loros, & Dunlap, FEMS Microbiol. Rev.36: 95-106, 2012). Using the tools of next generation sequencing, recombineering, and luciferase reporters, the molecular details of the clock itself as well as network ramifying from the clock out to primary, secondary, and tertiary targets of light and clock control can now be described. In a case study of regulation, structure/function analysis of WC-1 identified a region of 100 amino acids essential for frq circadian expression. A proteomics-based search for coactivators interacting with the WCC via this region of WC-1 uncovered the SWI/SNF (SWItch/Sucrose NonFermentable) complex: SWI/SNF interacts with WCC in vivo and in vitro, binds to the Clock box in the frq promoter, and is required both for circadian remodeling of nucleosomes at frq and for rhythmic frq expression. These data suggest a model in which WC-1 recruits SWI/SNF to remodel and loop chromatin at frq thereby activating frq expression to initiate the circadian cycle. These data, considered in the context of the genome, allow us to compare and contrast light-regulation of gene expression and clock-regulation of gene expression.

236 REVERSE GENETICS IN FUSARIUM SOLANI: LINKING COMPOUNDS TO NRPSS GENES

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Filamentous fungi produce a broad range of small molecules known as secondary metabolites that can have different biological activities. One of the classes of secondary metabolites is nonribosomal peptides, which are produced by huge multi-modular sythetases (NRPSs). NRPSs can contain several modules with different catalytic domains such as adenylation (A), peptide acyl carrier (T) and condensation (C) domains. The A domains recognize specific amino acids and modifies it as an aminoacyl-AMP. The modified amino acid is transferred by a carrier domain (T) through a tioester bond to the C domain where the peptide bond is formed. Besides the common domains, others domains can generate different modification on the amino acids. There are only 5 known compounds produced by NRPSs describes in Fusarium. These are malonichrome, ferricrocin, fusarinine, enniatin and beauvericin. Fusarium species are important pathogens of a lot of our crops that we consume as tomatoes, potatoes, cereals, maize and some others. Fusarium has been related to the production of N-sansalvamide isolated from green algae. The compound belongs to a type of NRPs known as cyclic depsipeptides. The compounds in this group can have different biological activities as cytotoxic, antibiotic and antifungal properties. N-sansalvamide has cytotoxic effects and they have been proved in cancer cells. Recently a related compound, neo-N-methylsansalvamide, has been discovered from Fusarium solani isolated from potatoes. Its structure has been characterized, consisting in 5 amino acids (Phe, Leu, Val, N-MeLeu and O-leu): (S)-2-hydroxy-4-methylpentanoic acid, N-methyl-L-leu-L-val-L-leu-L-phe. The characteristics of the new peptide as well as being found in Fusarium make us think about the option that we are in front of a secondary metabolite produced by any NRPSs. In the present study we try to find the gene responsible of the production of the compound by using different reverse genetics technics in F. solani. The use of molecular biology technics allow us to overexpress or knockout different parts of the F. solani genome and analyse the metabolite profile of the fungus comparing it to the F. solani wild type.

237 RNA-SEQ ANALYSIS OF THE EFFECTS OF GLIOTOXIN ON A. FUMIGATUS WILD-TYPE AND A. FUMIGATUS ΔGLIT REVEAL A DYSREGULATION OF CYSTEINE AND METHIONINE METABOLISM

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Aspergillus fumigatus produces a number of secondary metabolites, one of which, gliotoxin, has been shown to exhibit anti-fungal activity. Thus, A. fumigatus must be able to protect itself against gliotoxin. Indeed, one of the genes in the gliotoxin biosynthetic gene cluster in A. fumigatus, gliT, is required for self-protection against the toxin- however the global self-protection mechanism deployed is unclear. RNA-Seq was employed to identify genes differentially regulated upon exposure to gliotoxin in A. fumigatus wild-type and A. fumigatus ΔgliT (hypersensitive to gliotoxin). A total of 164 genes were differentially regulated (log2 fold change of 1.5) in A. fumigatus wild-type when exposed to gliotoxin, consisting of 101 genes with up-regulated expression and 63 genes with down-regulated expression. Interestingly, a larger number of genes, 1700, were found to be differentially regulated (log2 fold change of 1.5) in A. fumigatus ΔgliT challenged with gliotoxin. These consisted of 508 genes with up-regulated expression, and 1700 genes with down-regulated expression. In both strains primary metabolic functions, including amino acid metabolism and carbohydrate metabolism were affected. Both strains exhibited differential regulation of genes involved in secondary metabolism, in particular gliotoxin and helvolic acid biosynthesis, cellular transport, and cell rescue and defence. Interestingly, genes involved in cysteine and methionine metabolism along with sulphur metabolism were differentially expressed in A. fumigatus ΔgliT upon addition of exogenous gliotoxin. Quantitative determination of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) levels revealed a significant depletion of SAM ($p < 0.05$) in A. fumigatus ΔgliT upon exposure to gliotoxin and a significant increase ($p < 0.05$) in SAH levels, not observed in A. fumigatus wild-type. Comparative proteomics identified the presence of two isoforms of S-adenosylhomocysteine hydrolase differentially expressed in A. fumigatus ΔgliT following exogenous gliotoxin addition. One isoform is increased in intensity while the other decreases compared to the control, indicating a possible switch in activity due to post-translational modification. In the absence of GliT, addition of exogenous gliotoxin results in a dysregulation of cysteine and methionine metabolism, as evidenced particularly by the increased SAH levels in A. fumigatus ΔgliT, consequently resulting in a hypersensitive phenotype to gliotoxin.

238 ROLLING-CIRCLE TRANSPOSONS IN FUNGI: DIVERSITY, STRUCTURE AND TRANSCRIPTIONAL ACTIVITY

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Eukaryotic rolling-circle transposons, also known as helitrons, are a type of class-II transposons recently discovered by computational analyses in plant and animal genomes [1]. They bear conserved structural hallmarks such as a 5' - TC terminus, a subterminal palindromic hairpin followed by a 3'- CTRR terminal end, and display an insertion preference at AT dinucleotides. Putative autonomous elements previously described in plants, animals and fungi encode a protein (RepHel) containing a motif conserved in the replication initiator (Rep) of plasmid rolling-circle replicons, as well as a DNA helicase (Hel) domain. The presence of these domains as well as the absence of target site duplications flanking helitron boundaries suggest that these elements could transpose by a rolling circle mechanism [1]. Previous studies have demonstrated their capacity to capture and mobilize gene fragments and complete genes in plant and animal genomes [1]. For this reason they are thought to play an important role in the evolution of their host genomes. We have used a bioinformatic approach for the identification of fungal helitrons based on de novo detection of their structural features [2] and homology-based searches of helitron-specific helicases. We have observed that among the fungal kingdom, basidiomycota division has experienced a wide colonization of helitrons. The homology based searches of RepHel proteins yielded significant hits (e < 10-5) to 78 % of the basidiomycetes analyzed (81 out of 104 genomes). Surprisingly, helitron-specific helicases were found only in 11% of the ascomycetes analyzed (23 out of 204 genomes). The vast majority of basidiomycete helitrons were truncated non-autonomous copies, and in most cases 5' and 3' boundaries of the putative autonomous elements weren't found. A deeper analysis performed on the two haploid protoclones of the basidiomycete Pleurotus ostreatus revealed the presence of 4 helitron families dispersed along ten out of its eleven chromosomes. The two homologous genomes displayed differential helitron content (0.12% and 0.31% of their genome size, respectively). This analysis revealed several breaks in gene colinearity as a result of recent mobilizations of autonomous elements. Moreover, some of these elements were transcriptionally active in several media.

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239 SEVERAL RUST FUNGI (PUCCINIALES) POSSESS SOME OF THE LARGEST FUNGAL GENOME SIZES KNOWN

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Rust fungi (Pucciniales) represent one of the most important groups of plant pathogens. Pucciniales have developed specific characteristics, among them the obligate biotrophic feeding strategy, which they share with the mildew fungi. Biotrophic specialisation among fungi in general has led - in evolutionary terms - to expansions or reductions in genome size. Several rust genomes have been sequenced recently, with sizes ranging from 68 Mb for Puccinia striiformis to 101 Mb for Melampsora larici-populina. The genome size expansions due to the abundance of transposable elements recorded for rust genome sequences has been suggested as a strategy to create diversity, alternative to the rare sexual stages. In this work we have used flow cytometry to estimate the genome size of several rust fungi. Besides corroborating a recent report for a genome size of ca. 730 Mb for Hemileia vastatrix, which fit among the largest detected so far in fungi, large values of genome size were also recorded for P. oxalidis, Phakopsora pachyrhizi and several Uromyces spp. These results clearly reinforce the identification of a trend for genome size expansion in rusts. Acknowledgments: This work is being funded by Portuguese National Funds through Fundação para a Ciência e a Tecnologia (project PTDC/AGR-GPL/114949/2009 and grants SFRH/BPD/65686/2009, SFRH/BPD/65965/2009 and SFRH/BPD/88994/2012 attributed to ASP, ST and PT respectively), and by the Deutscher Akademischer Austauschdienst (DAAD, Germany) in the scope of the Portuguese-German bilateral collaboration project Functional and cytological characterisation of Hemileia vastatrix genes expressed during the infection process of coffee leaves.

240 SUPERFAMILY OF ACTINOPORIN-LIKE PROTEINS IN FUNGI

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Superfamily of actinoporin-like proteins (ALPs) comprises diverse protein families that show structural similarity to actinoporins. Actinoporins (20 kDa) are potent cytolytic toxins isolated from sea anemones, structurally defined by a rigid beta sandwich flanked by two alpha helices (PF06369). We determined the distribution of ALP sequences in fungi for some ALPs families like fungal fruit body lectins, necrosis inducing proteins and aegerolysins. Fungal fruit body lectins are mostly fungal proteins. Their domain fold is named FB lectin (PF07367) and it is not related to any other of several lectin folds, however, it shows significant structural similarity to actinoporins. The lectin XCL from Boletus chrysenteron induces drastic changes in the actin cytoskeleton after sugar binding at the cell surface and internalization and has potent insecticidal activity (Birck et al., 2004). Necrosis inducing proteins belonging to NPP1 domain (PF05630) are present in oomycetes, fungi and bacteria. Infiltration of NPP1 into leaves of Arabidopsis thaliana plants result in transcript accumulation of pathogenesis-related genes, production of ROS and ethylene, callose apposition and cell death (Fellbrich et al., 2002). Aegerolysins were discovered in fungi, oomycetes and bacteria. They share common aegerolysin fold (PF06355). The bacterial members of this family are expressed during sporulation. Aegerolysins have been described to exhibit pleiotropic functions; some of them are haemolytic in the presence of another MACPF-domain containing protein (Tomita et al., 2004; Ota et al., Shibata et al., 2010). Aegerolysin and ostreolysin are expressed during formation of fungal primordia and fruiting bodies, and may play an important role in the initial phase of fungal fruiting (Berne et al., 2002). The occurrence of ALPs in fungi was highly heterogeneous, with aegerolysins and NPP1 overrepresented, while the other ALPs were more rarely identified and we observed no obvious correlation to taxonomy or pathogenous lifestyle. At least part of ALPs may be considered as small secreted proteins, even though sometimes no recognizable signal peptide was observed. ALPs from Aspergillus niger were chosen for cloning and further characterisation studies, NPP1 protein represented here and aegerolysins elsewhere (poster M. Novak).

241 SURVEY OF THE LIGNOCELLULOLYTIC CAPABILITIES OVER THE ORDER POLYPORALES (FUNGI, BASIDIOMYCETES)

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Saprotrophic Basidiomycetes from the order Polyporales are efficient decayers of a broad range of woody substrates. Polyporales include brown-rot and white-rot fungi that show high diversity in enzymatic properties and can be exploited for their selective ability to breakdown the lignocellulose polymers i.e. cellulose, hemicellulose and/or lignin. A collaborative project has been initiated with the Joint Genome Institute (DOE, U.S.) for sequencing the genomes of 40 Polyporales strains from the CIRM-CF collection maintained in Marseille, France (http://www6.inra.fr/cirm_eng/). The suite of Polyporales strains has been selected based on
- abilities to degrade/deconstruct plant biomass
- phylogenetic position in the taxon
- geo-climatic origin with emphasis on tropical regions where biodiversity is higher and underexplored.
In order to facilitate genome assembly, monokaryotic strains have been obtained from dikaryotic strains by in vitro fructification or protoplastization. A consortium of 12 laboratories will analyze and compare the gene repertoires in these Polyporales with other sequenced genomes and explore the diversity of lignocellulose-acting enzymes fungi use to retrieve carbon from plant biomass. Expert annotation of carbohydrate-active enzymes and redox auxiliary activities that target lignin will be run in parallel with comparative transcriptomics to identify the genes induced on various ligno- and cellulosic substrates. In addition, comparative proteomics will identify the secreted enzymes active on these substrates. This trans-disciplinary analysis will provide a better understanding of the enzyme machineries involved in lignocellulose deconstruction by wood decayers and allow identification of novel enzymes for efficient plant biomass transformation in a wide panel of green chemistry and white biotechnology applications. Consortium Partners: Bernard Henrissat (AFMB, Marseille), Dan Cullen (Forest Products Laboratory Madison, WI), Régis Courtecuisse (Université de Lille), David Hibbett (Clark University, Worcester, MA), Ursula Kües (University of Göttingen, Germany), Francis Martin (INRA-Nancy), Angel Martinez (Biological Research Centre, Madrid, Spain), Antonio Pisabarro (Universidad Publica de Navarra, Spain), Ronald de Vries CBS, Utrecht, The Netherlands), Han Wösten (Utrecht University, The Netherlands).

242 THE COMPACT MITOCHONDRIAL GENOMES OF THE ETHANOL-METABOLIZING FUNGI BAUDOINIA COMPNIACENSIS AND ZASMIDIUM CELLARE

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Fungi in the class Dothideomycetes often live in extreme environmental niches or have unusual physiology. Two of these, the warehouse-staining fungus Baudoinia compniacensis and the wine cellar fungus Zasmidium cellare, are unusual because they can metabolize ethanol vapor. Exposure to ethanol vapor also allows B. compniacensis to become thermal tolerant, but its effect on the physiology of Z. cellare is not known. The nuclear genome of B. compniacensis is the smallest published in the Dothideomycetes at only 21.9 Mb with a reduced gene set compared to most other species. However, nothing is known about the mitochondrial genomes of either ethanol-metabolizing fungus. To fill this gap, the mitochondrial genomes of both species were assembled from genome-sequencing projects at the Joint Genome Institute of the U.S. Department of Energy. The mitochondrial genome of Z. cellare, at only 22,976 bp, is the smallest reported for a filamentous fungus, with that of B. compniacensis only slightly larger at 25,970 bp. Both mitochondrial genomes contained the complete set of 14 protein-coding genes seen typically in other filamentous fungi, including the three atp synthase subunits atp6, atp8 and atp9, cytochrome b, cytochrome oxidase subunits cox1-cox3, and the NADH dehydrogenase subunits nad1-nad4, nad4L, nad5-nad6, none of which contained any introns. The mitochondrial genome of B. compniacensis contained 26 predicted tRNA genes decoding all 20 amino acids, while that for Z. cellare contained only 19 tRNA genes with the ability to decode only 15 amino acids. Each mitochondrial genome had genes encoded on both strands with a single change of direction, different from most other fungi but consistent with the two Dothideomycetes mitochondrial genomes published previously. There was very little synteny between the two mitochondrial genomes, except for several groups of conserved genes that usually were in pairs. The five missing tRNA genes in the Z. cellare mitochondrial genome were clustered in that of B. compniacencis and could have been lost though a single deletion. Each mitochondrial genome contained a unique open reading frame potentially coding for a protein of unknown function. The small sizes of the mitochondrial genomes of these two ethanol-metabolizing fungi were due to lack of introns and very few ORFs for genes of unknown function relative to other fungi and not a loss of genes, except for the missing tRNA genes of Z. cellare.

243 THE F. OXYSPORUM PHYLOME REVEALS DIFFERENT EVOLUTIONARY TRAJECTORIES OF GENES RESIDING ON LINEAGE SPECIFIC CHROMOSOMES

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Four out of fifteen chromosomes of the reference strain of the tomato pathogen Fusarium oxysporum f. sp. lycopersici (Fol) are lineage specific (LS) and enriched for transposable elements and pathogenicity-related genes. The smallest of these LS chromosomes can transfer between Fol and a harmless F. oxysporum strain, causing the latter to become pathogenic on tomato. This provides an explanation for the polyphyly of host specificities within the F. oxysporum species complex. Preliminary analyses suggest that genes on LS chromosomes were acquired via horizontal transfer from a fungal donor, further extending the potential role of horizontal gene/chromosome transfer in the evolution of pathogenicity. In other pathogens, lineage specific duplications and losses have been designated as the main drivers behind evolution of pathogenicity. Here we investigate whether proteins that reside on LS chromosomes share a common history and which evolutionary processes had the most profound influence. We inferred trees for 15276 Fol proteins, 2254 of which are encoded on LS chromosomes. We clustered these trees to identify different evolutionary trends and found that two large clusters each account for ~20% of proteins encoded on the LS chromosomes. Most genes encoded on LS chromosomes result from expansion of multiple distinct protein families. One large cluster consists of trees in which a clade of Fol specific duplications, neighbors a protein from Fusarium verticillioides, in accordance with the species tree. This pattern suggests that these LS-encoded proteins originate from a translocation of a gene from the core to an LS chromosome, which was followed by duplication events. The other large cluster consists of trees in which a clade of Fol specific duplications, neighbors a protein from Fusarium solani. This suggests either massive independent loss of potentially entire chromosomes in Fusarium verticillioides and Fusarium graminearum, or horizontal transfer of potentially entire chromosomes, from a donor related to Fusarium solani.

244 THE GENOME OF THE EARLY DIVERGING ASCOMYCETE, TAPHRINA BETULINA, CAUSATIVE AGENT OF THE BIRCH WITCH'S BROOM DISEASE

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Taphrina are pathogenic yeasts frequently causing tumor-like symptoms on their hosts, which are mostly woody plant species. These early diverging Ascomycetes are related to the model organism Schizosaccharomyces pombe and the human pathogen Pneumocystis jirovecii, all belonging to Taphrinomycotina subphylum. There are a variety of Taphrina species reflecting the wide host range of this genus. T. deformans is the causative agent of peach leaf curl disease. T. betulina is a pathogen of birch trees causing witch's broom disease, which remodels the birch into tumorous brooms. The production of auxin has been documented in several Taphrina species and is widely believed to be involved in host symptom formation, although this has not been rigorously proven. We are interested in the biology of these Taphrina species as well as developing systems to study the interaction with their respective hosts. The draft genome of T. deformans has been published by our collaborators. The de novo genome sequence of T. betulina has been obtained in our lab using first the 454- and then the Pacbio-platforms. Four full length cDNA libraries from T. betulina cultures were prepared, with in house technology, for transcriptome sequencing with Illumina miseq platform (300 bp paired-end reads). Separate assemblies have been created using both of the T. betulina genome sequencing datasets and current work focuses on combining these data into a single final assembly. The recently obtained Pacbio assembly gave a genome size estimate of 12.7 Mbp with 46x genome coverage and only 60 contigs. The maximum contig was over 1 Mbp and the contig N50 value was 320 899 bp. The 454 assembly is also of good quality (estimated 13.7 Mbp genome size, 22x coverage, 329 contings representing 12.5 Mbp, N50=82,086 bp) and has been used to produce a preliminary gene annotation, which predicted 7133 genes with an average gene size 1356 bp and a low repetitive sequence content. Transcript sequencing produced over 1 million reads that will be mapped to the final T. betulina genome to aid accurate gene prediction especially producing insight to gene start sites, untranslated regions and intron exon structures. Using the two sequenced Taphrina genomes, we have searched for and manually curated potential auxin biosynthesis genes for further study. Our preliminary characterization of Taphrina species, ongoing work with putative auxin biosynthesis genes and analysis of the T. betulina genome will be presented.

245 THE GENOMIC ARCHITECTURE OF ECTOMYCORRHIZAL SYMBIOSIS IN THE GENUS AMANITA

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The ectomycorrhizal (ECM) symbiosis of fungi and trees is a successful evolutionary strategy, and has evolved repeatedly and independently, spawning many species-rich lineages among the fungi. Comparative genomics of two unrelated ECM fungi, the black truffle Tuber melanosporum, an ascomycete, and Laccaria bicolor, a basidiomycete, reveal commonalities in the gene repertoires of the two fungi, for example loss of plant cell wall degrading enzymes (PCWDEs). Nevertheless, the genomic architectures of the two species are dramatically different. The truffle has a very large, yet gene poor genome, densely populated by transposable elements (TEs) and with few gene families. In contrast, L. bicolor contains a large repertoire of genes, the majority of which are part of abundant gene families. Obviously, different gene sets and different genomic architectures can lead to the same kind of symbiosis, raising interesting questions about its evolvability. The genus Amanita encompasses approximately 500 species, the majority of which are ectomycorrhizal. However, a basal clade within the genus is asymbiotic and the single origin of symbiosis within the Amanita allows for a close investigation of the differences between free-living and ECM niches. We have sequenced the genomes of five species of Amanita, three ECM species and two asymbiotic species; as well as the asymbiotic outgroup Volvariella volvacea. We will discuss differences in gene content among the species, the origin of different gene families on the phylogeny, changes in overall genome architecture and possible evolutionary mechanisms. ECM Amanita are characterized by increased gene content compared to asymbiotic species. Patterns are similar to those discovered in L. bicolor: expansions in gene families involved in signaling, protein-protein interactions and small secreted proteins, but losses of PCWDEs and other secreted enzymes. It appears that loss of PCWDEs was an important early step towards the ECM symbiosis in this clade, but the asymbiotic species A. inopinata also shows significant reductions in PCWDEs. We find abundant numbers of TEs in two of the three ECM species and will highlight their potential influence on genome architectures.

246 THE RESPONSES OF ASPERGILLUS NIGER TO DIFFERENT LIGNOCELLULOSIC SUBSTRATES HIGHLIGHT BOTH SIMILARITIES AND SUBSTRATE-SPECIFIC DIFFERENCES

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Fungi are major degraders of lignocellulose in nature and are the main sources of enzymes used to saccharify lignocellulose in the production of second generation biofuels. The cost of producing these enzyme cocktails is a major barrier to cheaper biofuels. One path to reducing costs is through a better understanding of the response of fungi to lignocellulose. Aspergillus niger has an extensive repertoire of genes encoding enzymes that hydrolyse the components of lignocellulose (cellulose, hemicelluloses and pectin) as well as various accessory proteins. This repertoire of genes makes A. niger an excellent model to investigate fungal response to lignocellulosic substrates over time. Previously we investigated the global transcriptional response of A. niger to wheat straw (Delmas et al., 2012) and more recently have compared this response with the response to stems from a willow tree at a single time point. At the transcriptional level, there are many similarities in the responses to the two substrates such as the large increase in carbohydrate active enzyme (CAZy) transcripts from glycosyl hydrolase (GH) families. Some of the differences in the responses can in part be explained by the differences in composition of the substrates. At the biochemical level, enzymes prepared from A. niger exposed to wheat straw were used in a saccharification assay and were more effective at saccharification of wheat straw than willow. In further work at the transcriptional level, we are investigating with the Joint Bioenergy Institute (JBEI) and the Joint Genome Institute (JGI) the responses of A. niger to untreated and pre-treated (ionic liquid or hydrothermal) Miscanthus stems and wheat straw at nine time points from three hours until five days after transfer from a simple carbon source. Here we aim to elucidate the transcriptional response of A. niger as a function of time and lignocellulosic substrate and we will present some preliminary results from our study. In terms of fungal biology, our work shows that A. niger has some ability to distinguish between different lignocellulosic substrates and that is likely to be related to the differences in inducing molecules derived from these different substrates and the evolutionary selective advantage to the fungus of preferentially synthesising appropriate enzymes for available carbon sources. In terms of applications,our work could improve functionality of enzyme cocktails and reduce the costs of their production.

247 THIAMIN BIOSYNTHESIS IN FUNGI

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Thiamin is produced by bacteria, plants and a range of fungi but not by animals. Its major biologically active derivative is thiamin diphosphate (TPP) which serves as a cofactor for several enzymes involved in carbohydrate and amino acid metabolism. In the fungal pathway of thiamin de novo synthesis, two precursors are produced, the thiazole heterocycle 5-(2-hydoxyethyl)-4-methylthiazole phosphate (HET-P) by THI4 and a yet unknown NUDIX hydrolase and the pyrimidine heterocycle 4-amino-5-hydroxymethyl-2-methylpyrimidine (HMP) diphosphate (HMP-PP) by THI5 and THI20. HET-P and HMP-PP are then fused by THI6 to form thiamin phosphate (TP). A phosphatase converts TP into thiamin changed by THI80 into the active TPP. We took the key proteins of Saccharomyces cerevisiae for thiamin biosynthesis in tblastn searches of fungal genomes on the JGI website and in the NCBI database. Microsporidia seem to have none of the genes. Mucor circinelloides and Rhizopus oryzae from the Mucoromycotina have all genes but Phycomyces blakesleeanus lost thi4, thi5 and thi80 and Umbelopsis ramanniana thi4. Many of the ascomycetes have all of the key enzymes, but others have lost all genes, amongst are many yeasts (e.g. Candida maltosa, Saccharomyces bayanus) and several pathogenic filamentous species (e.g. Metarhizium acridum, Claviceps purpurea). In some species, one or more genes are lost with other genes retained: Aspergillus oryzae and nidulans (thi80 lost), Aspergillus niger (thi20, thi80 lost), Ascobolus immersus (thi4, thi5, thi80 lost) and Epichloë festucae (all lost but thi4) to name some examples. Few Basidiomycotes have the complete sets of genes: the rusts Cronartium quercuum fusiforme, Melampsora laricis-populina and Puccinia graminis, the smuts Sporisorium reilianum and Ustilago maydis and the dandruff fungus Malassezia globosa, the yeast Cryptococcus vishniacii and the Agaricomycetes Gloeophyllum trabeum, Neolentinus lepideus and Wallemia sebi. Ustilago hordei has only thi5 whereas thi5 is gone lost in most other Basidiomycetes, sometimes together with one or more further genes of the pathway. Resulting thiamin auxotrophy might be overcome by addition of thiamin or the thiazole and pyrimidine precursors, depending on which of the genes are left. Uptake of thiamin and its precursors by thiamin-dependent organisms implies presence of suitable transporters. Thiamin transporters have evolved several times in fungi and an account of their distribution will also be presented.

248 THIRD GENERATION SEQUENCING PAVES THE WAY FOR RAPID AND COMPLETE FUNGAL GENOME SEQUENCING

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Verticillium dahliae, causal agent of vascular wilt disease, is one of the most notorious plant pathogens on tomato. By using population genome sequencing with the Illumina platform, we have recently shown that frequent genomic rearrangements drive the evolution of lineage-specific regions that establish virulence and niche adaptation in this species. Due to limitations associated with second generation sequencing, in particular the short read length and lack of coverage of repetitive sequence stretches, the exact genomic signatures at the site of the chromosomal rearrangements, and thus the molecular mechanism that establishes these modifications, remain largely unknown.

In order to obtain detailed genomic information on the recombination sites, we re-sequenced the ~37 Mb genome of V. dahliae strain JR2 using single-molecule real time (SMRT) sequencing with PacBio technology. To this end, we generated ~8 Gb of sequencing data. De novo assembly was performed resulting in 66 contigs of which 16 covered 99% of the complete genome as inferred from an optical map. Subsequent manual and software-guided scaffolding resulted in a gapless assembly of all eight complete chromosomes. Thus, the re-assembled V. dahliae strain JR2 genome represents the first finished, gapless fungal genome. We subsequently applied comparative genomics and inferred the exact positions of the previously identified genomic rearrangements. Our approach to identify genomic signatures at the recombination sites will be discussed. This study highlights the superior genome assembly qualities of third generation sequencing technologies and exemplifies the pivotal role of a finished genome in understanding the evolution and biology of fungal pathogens. We anticipate that third generation sequencing will pave the way for rapid and affordable genome sequencing approaches aiming for finished assemblies in fungal and also other eukaryotic species.

249 TRANSCRIPTOMIC PROFILING OF THE HYPOXIC RESPONSE OF THE HUMAN-PATHOGENIC FUNGUS ASPERGILLUS FUMIGATUS CULTIVATED IN AN OXYGEN-CONTROLLED CHEMOSTAT

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Aspergillus fumigatus is a ubiquitous, filamentous fungus, which may cause a broad spectrum of disease in the human host, ranging from allergic or locally restricted infections to invasive mycoses. During the course of the infection A. fumigatus has to cope with different kinds of stress conditions including low oxygen levels (hypoxia). Just recently it was shown that hypoxia adaptation is an important virulence attribute of A. fumigatus. To identify novel hypoxia-sensing and adapting pathways we have characterized the changes of the A. fumigatus transcriptome in response to long periods (7-10 days) of hypoxia (1% O2) in an oxygen-controlled chemostat. Under these conditions, genes involved in glycolysis, energy conversion, amino acid metabolism/transport, NO-detoxification, cell wall metabolism and nuclear transport showed an increased level. In contrast, genes involved in phospholipid metabolism, cell growth and morphogenesis and the cytoskeletal organization were down-regulated. To get a deeper knowledge about the specific role of metabolic pathways in adaptation to hypoxia, we have started to characterize candidate genes, which may contribute to the survival and the maintenance of redox balance during hypoxia. These included enzymes of the fumarate metabolism, the NO-detoxifiying system, nitrate assimilation and respiration. First data will be presented and discussed.

250 USE OF CHIP-SEQ TECHNOLOGY FOR THE FUNCTIONAL CHARACTERIZATION OF THE MATING TYPE PROTEIN MAT1-1-1 FROM THE INDUSTRIAL PENICILLIN PRODUCER PENICILLIUM CHRYSOGENUM

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Penicillium chrysogenum is the major industrial producer of the ß-lactam antibiotic penicillin which is widely used in the treatment of infections caused by gram positive bacteria. We have studied the regulation of secondary metabolism and morphology of this filamentous fungus to broaden our current understanding of these fundamental processes. Recently we were able to show that the MAT1-1-1 mating-type protein, playing a crucial role in governing sexual identity, also is involved in the regulation of a wide range of genes with biotechnological relevance ranging from regulators of penicillin production, hyphal morphology and conidial formation [1]. In order to understand the regulatory functions of MAT1-1-1, chromatin immunoprecipitation combined with next generation sequencing (ChIP-seq) was performed. Bioinformatic analysis was used for the identification of putative DNA-binding regions of MAT1-1-1 as well as for de novo motif analysis. qPCR confirmed the enrichment of MAT1-1-1 specific DNA-regions in ChIP-DNA compared to input-DNA and was used for the verification of peak quality scores generated during bioinformatic analysis. Furthermore, we performed qRT-PCR analyses to distinguish genes that are specifically regulated by MAT1-1-1 from those that are unaffected by overexpression and deletion of the MAT1-1 gene. In order to conduct electrophoretic mobility shift assays (EMSA), we fused the MAT1-1 gene to the glutathione S-transferase (GST) gene for overexpression in E. coli. The purified fusion protein was used to verify the binding of MAT1-1-1 to putative DNA binding sites. Taken together, our data from ChIP-seq analysis extends the current knowledge of the regulatory network controlling both penicillin biosynthesis and morphogenesis in P. chrysogenum. Hence, our experiments will identify new starting points for targeted genetic engineering of P. chrysogenum, which is crucial for further optimization of industrial production strains.

[1] Böhm et al. (2013) Sexual reproduction and mating-type-mediated strain development in the penicillin-producing fungus Penicillium chrysogenum. PNAS 22;110:1476-81

251 WHOLE GENOME ANALYSIS OF BEAUVERIA BASSIANA ISOLATES DIFFERING IN VIRULENCE TOWARDS MALARIA MOSQUITOES

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Malaria, kills 660,000 persons each year. A demonstrated effective way to alleviate the burden of the disease is to control its vector (anopheline mosquitoes) using insecticides either through impregnated bed nets (ITNs) or indoor residual spraying (IRS). These strategies have limitations because of the rapid spread of insecticide resistance in mosquitoes. Entomopathogenic fungi have been proposed as a novel biopesticide to kill malaria mosquitoes and have been shown to successfully reduce the lifespan of mosquitoes under laboratory and field conditions. To make this approach more effective, it is crucial to study the components and mechanisms of fungal virulence by exploring the potential development of fungal resistance by the mosquito. Previously, we characterized the natural variation in virulence of 29 isolates of Beauveria bassiana and showed that there were pronounced differences between isolates. In this study, we selected 5 isolates representing the extremes of low/high virulence for further genomic analysis. We performed de novo genome assembly using sequencing data obtained by Illumina HiSeq2000 (short and long insert libraries). The comparative analysis of the genomes of isolates with contrasting virulence levels will be presented. The implications of these findings for malaria vector control will be discussed.

252 WHOLE-GENUS SEQUENCING OF THE ASPERGILLUS GENUS

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The genus Aspergillus is a highly important genus of organic matter-degrading filamentous fungi. This genus is species-rich and is currently composed of more than 300 species. There are few groups of organisms with a higher number of potential products per species than the fungi; for Aspergilli, above 250 carbohydrate-active enzymes and 30-100 secondary metabolites have been identified in each species examined so far. The group is ubiquitous and diverse, and includes key cell factories for enzyme production, model organisms, fermenters of foodstuffs, plant pathogens, producers of animal and human mycotoxins, and degraders of organic matter ranging from dung over plant matter to dried and semidried fruits. In collaboration with a consortium including the US Joint Genome Institute and the Joint Bioenergy Institute, we are at DTU Systems Biology currently advancing research in the Aspergillus species and in fungi in general by de novo genome sequencing of all unsequenced species (>280) in the genus. This is estimated to identify >75,000 carbohydrate-active enzymes and > 1000 new biosynthetic gene clusters for secondary metabolism and aims to answer key questions about the linkage between physiology and phylogeny of this important genus. Here we present the preliminary results for the first app. 50 species of the genus focusing on biological insights and genome dynamics. We will also present the current status of this ongoing work, and the planned order of the next genomes to be sequenced.

253 A LIGHT INDUCIBLE SYSTEM IN NEUROSPORA CRASSA TO UNCOVER THE MOLECULAR MECHANISTIC ORIGIN UNDERLYING THE REFRACTORY PERIOD FOLLOWING A BURST OF TRANSCRIPTION

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Single-cell studies of gene expression have revealed that transcription is discontinuous and occurs in bursts of transcription. Recent analyses have indicated the existence of refractory periods during which genes stay inactive for a certain time before switching on again. However to explore this observed mechanism, it would be necessary to analyze biochemically transcription at single active genes in vivo. Such techniques are not available. To tackle this issue, we setup a cellular system based on a light-activated circadian transcription factor in Neurospora crassa. The fast activation and self-inactivation features of the transcription factor enable time resolution of single cell transcription dynamic signatures on a cell population level. Here we show that during the refractory time following a burst of transcription, phosphorylation of the RNA polymerase II CTD is impaired. These results provide mechanistic insights into the refractoriness and a new powerful cellular assay to analyze transcription discontinuity.

254 A NOVEL PENICILLIN REGULATOR (PCFKH1) IN PENICILLIUM CHRYSOGENUM

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In the filamentous fungus Penicillium chrysogenum, a complex regulatory network of different transcriptional factors controls the expression of the penicillin biosynthetic gene cluster. In this work, we have characterized a novel transcriptional factor PcFKH1 (Pc18g00430) in P. chrysogenum. This gene, hereafter referred to as Pcfkh1 (2302 bp, 2 introns), codes a protein (PcFKH1) of 718 amino acids with strong similarity to forkhead transcription factor Fkh1/2 from different filamentous fungi (i.e. 82% similarity and 76% identity to forkhead transcription factor Fkh1/2 from Aspergillus fumigatus and 49% similarity and 34% identity to the Acremonium chrysogenum cephalosporin regulator FKH1). Analysis of the DNA-binding domain of the P. chrysogenum and A. chrysogenum FKH1 orthologs revealed 75% similarity and 68% identity among these two protein domains. The promoter regions of the penicillin biosynthetic genes were analysed in search for putative FKH1 binding sites. No PcFKH1-binding sites were found in the promoter region of the pcbAB gene. However, two binding sites were identified in each of the pcbC and penDE gene promoters. Expression analyses carried out inPcfkh1 knock-down mutants confirmed that PcFKH1 has a positive effect on the expression of the penDE gene, confirming the involvement of this transcription factor in the control of penicillin biosynthesis.

255 AN ENDOGENOUS PROMOTER FOR CONDITIONAL GENE EXPRESSION IN ACREMONIUM CHRYSOGENUM: THE XYLAN AND XYLOSE INDUCIBLE PROMOTER XYL1P

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Acremonium chrysogenum is the natural producer of the beta-lactam antibiotic cephalosporin C and therefore of significant biotechnological importance. Here we identified and characterized the xylanase-encoding xyl1 gene and demonstrate that its promoter, xyl1P, is suitable for conditional expression of heterologous genes in A. chrysogenum. This was shown by xylose and xylan-inducible xyl1P-driven expression of genes encoding green fluorescence protein and phleomycin resistance. Moreover, we demonstrate the potential of the xyl1P promoter for selection marker re-use. Taken together, these findings will help to overcome the limitation in genetic tools in this important filamentous fungus.

256 ASSEMBLY AND CHARACTERIZATION OF BACTERIAL ARTIFICIAL CHROMOSOME (BAC) CLONES OF THE CELLULOLYTIC FUNGUS TRICHODERMA HARZIANUM REVEALS NEW INSIGHTS INTO BIOMASS-DEGRADING GENES AND ADJACENT REGULATORY REGIONS

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Cellulases are glycosyl-hydrolases that catalyse the hydrolysis of carbohydrate polymers in cellulose and hemicellulose to fermentable monomers. This class of enzymes has many applications in industry, including the bioconversion of cellulose into ethanol. However, the large-scale production of these enzymes represents a challenge for the biofuel industry. Several microorganisms have been studied with respect to cellulase production, especially fungi from Trichoderma genera. Trichoderma reesei was the first observed fungus with high capacity to degrade cellulose, and most of the studies about fungal cellulases focus in this species. Trichoderma harzianum is a well-known fungus used as biocontrol agent, and lately some strains have been studied due to their high production of cellulases, sometimes at more significant levels than those from T. reesei. Since there is limited genomic data available for T. harzianum and most of the studies focus only on the isolated cellulolytic enzymes, it is of great importance the genomic analysis of these genes and their regulatory regions. Thus, the aim of the present study was the construction of a bacterial artificial chromosome (BAC) library of T. harzianum IOC-3844 and the sequencing of selected BAC clones in order to analyze the cellulase genes as well as the genomic regions in which they are inserted. The genomic BAC library was constructed and consisted of 5,760 clones. Analysis by PFGE indicated the average size of insert DNA to be 85 kbp, and it was estimated the library covers about 10x the T. harzianum genome. Initial screening of the library was performed by the method of plate pool, based on the combination of 384 clones (1 plate). The plate pools were screened using primers for endoglucanase, cellobiohydrolase, b-glucosidase, xylanase and swollenina genes, and for each positive pool further PCR-amplifications were performed for each clone individually. Following the fingerprinting profile by PFGE and BAC-end sequencing, the target BAC inserts were sequenced through 454 GS-FLX Plus pyrosequencing (Roche) and cellulase-related genes and genomic regions were assembled. The annotations and comparative analysis through public databases are in progress. Results are providing new insights into regulation and activity of the cellulase complex genes in the promising fungus T. harzianum. Furthermore, our findings may contribute to a better understanding of evolution of fungal biomass-degrading genes.

257 CELL WALL STRESS INDUCED GENES USE AS NEW TARGETS FOR ANTIFUNGALS IN ASPERGILLUS FUMIGATUS

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A. fumigatus infections are a demanding problem for immunocompromised patients. Invasive aspergillosis has a poor prospect with mortality up to 90%. Upcoming problem is resistance to antibiotics of pathogenic fungi. It is important to identify new targets for antifungals. The cell wall is an essential organelle for fungi and not present in mammalian cells. Similar to S. cereviseae, cell wall stress induces cell wall related genes via the cell wall integrity (CWI) pathway in A. fumigatus.
The role of PkcA and RlmA in the cell wall integrity pathway was studied in a loss-of-function pkcA mutant (Campos Rocha et. al. 2012) and a rlmA deletion mutant (Malavazi, unpublished data). The mutants are used to study expression of cell wall stress related genes upon calcofluor white stress by Northern blot analysis, RNAseq and introduction of cell wall stress reporter constructs.
This study presents the involvement of PkcA and transcription factor RlmA in the CWI pathway in A. fumigatus. The results show also new RlmA induced gene(s), thereby presenting the first indication of RlmA regulated gene(s) in A. fumigatus. The RlmA induced genes are useful tools for development of an A. fumigatus cell wall stress reporter strains.

258 CHARACTERIZATION OF A NUCLEAR RECEPTOR-LIKE DOMAIN OF THE XYLANASE REGULATOR 1

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In case of industrially used organisms such as Trichoderma reesei the engineering of transcription factors is an interesting research target that gains increasing attention. T. reesei is of interest because of its high secretory capacity of native and heterologously expressed enzymes and compounds. Recently, a single point mutation in the main transactivator of xylanase and cellulase expression in T. reesei, Xyr1, was found to be responsible for a strongly deregulated and enhanced xylanase expression. By circular dichroism spectroscopy we found that this mutation caused a change in secondary structure of the protein. According to electrophoretic mobility shift assays and the determination of the equilibrium binding constants, the DNA-binding affinity of the mutated Xyr1 was considerably reduced compared to the wild-type Xyr1. Both mentioned techniques were also used to investigate the allosteric response of the two different proteins to carbohydrates (D-glucose-6-phosphate and D-xylose), which are known to signal repressors or inducers of Xyr1 target genes. The mutated Xyr1 no longer exhibited a conformational change in response to these carbohydrates, indicating that the observed deregulation is not a simple matter of a change in DNA-binding of the transactivator. Altogether, we postulate that the part of Xyr1, where the mutation is located, functions as a nuclear receptor-like domain that mediates carbohydrate signals and modulates the Xyr1 transactivating activity.

259 CHARACTERIZATION OF THE N-ACETYLGLUCOSAMINE CATABOLISM GENE CLUSTER IN FILAMENTOUS FUNGI

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The aminosugar N-acetylglucosamine (GlcNAc) is the monomeric subunit of chitin, which is an important structural component of the fungal cell wall and the exoskeletons of protists and arthropods. Fungi, together with bacteria, are the primary degraders of chitin in nature. They usually have a wide range of different chitinases belonging to glycoside hydrolase (GH) family 18. The catabolism of GlcNAc, however, and regulatory aspects connected to that topic, have not been investigated in filamentous fungi so far. In Candida albicans GlcNAc is a potent inducer of hyphal growth and is related to its virulence, but also induces the expression of the genes needed to catabolize GlcNAc. We analyzed the genomic organization of GlcNAc catabolism genes in filamentous fungi and found that in most fungi the three genes responsible for the conversion of N-acetyl-glucosamine to fructose-6-phosphate are clustered. In addition, this cluster contains a gene encoding an NDT80-like transcription factor. Moreover, a gene encoding a GH family 3 enzyme, that exhibits similarities to beta-N-acetylhexosaminidases of bacteria, belongs to the cluster. Neurospora crassa and two Trichoderma spp. - the mycoparasite Trichoderma atroviride and the saprotroph Trichoderma reesei - were chosen for further studies. Although a comparison of the GlcNAc gene cluster among these fungi showed high conservation, growth behavior on GlcNAc was strongly different. T. reesei and T. atroviride showed fast growth on medium containing GlcNAc as sole carbon source, whereas N. crassa exhibited almost no growth. Interestingly, despite this finding, all three tested fungi are able to grow equally well on chitin. Analysis of N. crassa gene knockout strains related to regulation of catabolism will lead to further insights into this aspect. Transcriptional profiling of the GlcNAc catabolism cluster genes in Trichoderma spp. showed that, when grown on GlcNAc or chitin, the expression of the three genes encoding catabolizing enzymes is strongly upregulated, while the expression of GH3 and TF was not altered under inducing and not-inducing conditions in both fungi. In order to further investigate the functions and regulatory aspects of the GlcNAc catabolism genes, knockout strains of the respective genes will be studied.

260 CO-TRANSFORMATION WITH A SECOND MARKER-LESS PLASMID DOUBLES NUMBERS OF TRP1+ TRANSFORMANTS OF COPRINOPSIS CINEREA COMPARED TO SINGLE TRP1+ VECTOR TRANSFORMATION

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For transformation of <I>Coprinopsis cinerea</I>, Binninger et al. (1987) cloned a 6.5 kb PstI genomic fragment with the tryptophan synthetase gene (trp1+) into <I>Escherichia coli</I> vector pUC9 to give pCc1001 that allows complementation of trp1 auxotrophies. During fungal transformation, vector DNA integrates at multiple ectopic sites into a host nucleus, singly or in tandem copies. Integrations at multiple ectopic sites offer the chance of co-transformation of two or more different vector constructs at the same time. With equal amounts of two vectors, equal numbers of nuclear places should become occupied by the two distinct DNAs in co-transformation. Using vector pCc1001 in several transformation experiments however revealed a surprising phenomenon. pCc1001 alone gives only a low number of transformants whereas co-transformation with a marker-less plasmid always yields about 2x more trp1+ transformants. Truncations of the <I>C. cinerea</I> DNA in pCc1001 to just the trp1+ gene sequence and changing the vector backbone did not alter the effect. Different relative trp1+ vector to second vector concentrations in transformations were tested. Keeping the trp1+ vector high caused a rapid decrease in numbers of trp1+ transformants and keeping the second vector high a slow decrease. The results suggest that initial trp1+ transformants are lost due to uptake of trp1+ at multiple ectopic nuclear sites. Accordingly, single and co-transformation rates of trp1+ vectors were equal in a deltaku70 strain that is defective in the pathway of ectopic DNA integration. The phenomenon of loss of transformants by multiple ectopic integrations is specific to the trp1+ gene. Single and co-transformation using a vector with pab1+ (encoding the p-aminobenzoic acid synthetase) for selection always resulted in the same numbers of transformants. Tryptophan biosynthesis in <I>C. cinerea</I> is under feedback inhibition by tryptophan on the first enzyme of the pathway, anthranilate synthase. Integration of more ectopic trp1+ copies into a nucleus might cause an overexpression with a strong feedback effect up to an irreversible shut-down of the pathway. In line with this, addition of the tryptophan precursors anthranilate or indole strongly reduced the numbers of trp1+ transformants in both single and co-transformations. Addition of phenylalanine or tyrosine had similar effects since aromatic amino acid productions start all at chorismate and are under cross-pathway control.

261 CONNECTION BETWEEN CARBON AND NITROGEN METABOLISM REGULATION IN N. CRASSA. ROLE OF THE PROTEINS NIT2 AND NMR1

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In N. crassa, the regulatory protein NMR1 controls the use of alternative nitrogen sources guarantying the assimilation of the preferred ones such as ammonium and glutamine. The GATA factor NIT2 is responsible for the global regulation of structural genes involved in the catabolism of secondary nitrogen sources. NIT2 is regulated by NMR1, which blocks the NIT2-binding site to its DNA motif. In a genome-wide screen of transcription factor mutant strains set we identified the NIT2 protein as a potential transcription factor regulating glycogen metabolism. This data led us to start investigating the connection between nitrogen and carbon metabolism regulation by analyzing the role of NIT2 and NMR1 proteins in glycogen metabolism and in carbon catabolite repression. The glycogen content was analyzed by growing the wild-type, nit-2KO and nmr-1KO strains in VM medium containing 2% sucrose (control) and then transferring the mycelia to media containing different nitrogen sources (either N-free or N-free plus glutamine or N-free plus KNO3). We have not observed differences in the glycogen accumulated by all strains in media containing different nitrogen sources, however the mutant strains accumulated higher glycogen levels than the wild-type strain in VM medium. The NIT2 motif (5-GATA-3) was identified in the promoters of all genes encoding enzymes of the glycogen metabolism. Gene expression analyzed by qPCR showed that the nitrogen source strongly influenced gene expression and that the genes were overexpressed in the nmr-1KO strain in a nitrogen source-independent way. ChIP-PCR analyzes confirmed binding of His::NIT2 recombinant protein to the NIT2 motifs in the gsn and gpn promoters. The carbon repression was evaluated by growing the strains in media containing different nitrogen sources in the presence or not of 2-deoxyglucose (2-DG). The nitrogen source did not influenced in carbon repression. We also evaluated the NIT2 protein location in the nit-2KO complemented (his-3::Pccg-1-nit-2-sfgfp) strain in the same growth conditions. The NIT2::sfGFP protein was concentrated at the nuclei when mycelium was transferred to N-free and N-free plus KNO3 (derepressing conditions). All results together suggest that the regulatory proteins NIT2 and NMR1 connect the regulation between nitrogen and carbon metabolism in N. crassa, however NMR1 seems to play a more global control.
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262 DEPICTING THE MECHANISMS REGULATING THE EXPRESSION OF THE GLYCOSIDE HYDROLASES-ENCODING GENES IN PENICILLIUM FUNICULOSUM

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Penicillium funiculosum is a filamentous fungus that produces and secretes a mixture of enzymes that is commercialized under the name of Rovabio TM. This enzymatic cocktail is used as animal feed additive to enhance the hydrolysis of plant polymers present in the seed coat. After a proteomics approaches for cocktail characterization, we are now carrying out transcriptomics analyses to unravel the mechanisms regulating the expression of the genes coding for these hydrolytic enzymes. On the one hand, we undertook a genome-wide analysis, using RNA-seq, of P. funiculosum exposed to glucose or milled wheat straw, a complex lignocellulosic material. When the mycelium was grown on glucose and then transferred to wheat straw, 926 genes were differentially expressed between the two conditions. Amongst the genes that were induced in response to lignocellulose, we found genes coding for swollenins, hydrophobic surface binding proteins and most of the hydrolytic enzymes. On the other hand, we explored the expression of a subset of genes encoding glycoside hydrolases and other auxiliary proteins by qPCR under a large set of 25 different culture conditions covering stress and different carbon sources including monosaccharides, disaccharides or plant polymers. The data revealed that, in some cases, the expression patterns diverged from one gene to the other even within a same CAZy family, with different responses to the sugars tested. We also showed that the optimal response of genes coding for hydrolytic enzymes was induced by the most complex plant polymers, which might be due to the synergistic effect of several inducers. The role of key transcriptional factors, such as xlnR, creA, araR and clrA in this complex response is currently under study using deletion mutants that we recently constructed using the popIN/popOUT system. Our approach may help providing a global view of the network that regulates the expression of genes involved in the degradation of plant polymers by P. funiculosum.

263 DEVELOPMENT OF A HIGH THROUGHPUT GENE EXPRESSION PLATFORM FOR EXPRESSION OF TRANSCRIPTION FACTORS IN ASPERGILLUS NIDULANS

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Aspergillus nidulans is a well characterized model organism for which many genetic engineering tools have been developed. This makes it an ideal system to study and uncover the mechanisms that control fundamental physiological aspects of fungal life forms i.e. cell differentiation, signaling and metabolism. In order to address fungal physiology in a systems perspective we are in the process of making an overexpression library of all 490 putative and annotated transcription factors (TF) contained in the Aspergillus genome. To facilitate the construction of this overexpression library we have developed a high throughput (HTP) gene expression platform with background free cloning vectors and background free integration systems. Furthermore, to limit the manual work most of the construction of gene targeting substrates has been automated, including the validation of PCR fragments. Here we present the results of the first generation of the library, which is composed by all 52 TF on chromosome I expressed under the control of the inducible Tet-on promoter. The initial characterization identified several strains with an altered metabolite profile. Hence, some showed up-regulation, or down-regulation, of a few secondary metabolites indicating that the library contains both activators and repressors. Among the up-regulated compounds some are potentially novel. Interestingly, overexpression of some of the TFs had significant impact on the morphology, conidiation and growth rate. Together our results show that the high throughput gene expression platform is an efficient and suitable way to construct a TF library, which can be used to study the regulation of the secondary metabolism as well as various other aspects of fungal physiology like cell cycle regulation, cell differentiation and signaling.

264 DIFFERENTIAL EXPRESSION OF LIGNINOLYTIC PEROXIDASE GENES BY PLEUROTUS OSTREATUS GROWING ON LIGNOCELLULOSE MEDIUM UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

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Pleurotus ostreatus is an important edible mushroom and a model lignin degrading organism, whose genome contains nine genes of ligninolytic peroxidases, characteristic of white-rot fungi. These genes encode six manganese peroxidase (MnP) and three versatile peroxidase (VP) isoenzymes that differ in their catalytic and stability properties. Using liquid chromatography coupled to tandem mass spectrometry, secretion of four of these peroxidase isoenzymes (VP1, VP2, MnP2 and MnP6) was confirmed when P. ostreatus grows in a lignocellulose medium (pH 5.5) at 25 ºC (three more were identified by only one unique peptide). Then, the effect of environmental parameters on the expression of the above nine genes was studied by reverse transcription-quantitative PCR (RT-qPCR) by changing the incubation temperature and medium pH of the P. ostreatus cultures pre-grown under the above conditions (using two reference genes for normalization of the RT-qPCR results). The cultures maintained at 25 ºC provided the highest levels of peroxidase transcripts and the highest total activity on Mn^{2+} (a substrate of both MnP and VP) and Reactive Black 5 (a VP specific substrate). After global analysis of the expression patterns, peroxidase genes were divided into three main groups according to the level of expression at optimal conditions (vp1/mnp3 > vp2/vp3/mnp1/mnp2/mnp6 > mnp4/mnp5). Adjusting the culture pH to acidic or alkaline conditions (pH 3 and 8) or decreasing/increasing the incubation temperature (to 10 ºC/37 ºC) led to downregulation of most of the peroxidase genes (and decrease of the enzymatic activity) in most of the cases. The analysis also reveals differences in the transcription levels of the peroxidase genes when the culture temperature and pH parameters were changed, suggesting a possible adaptive expression according to environmental conditions. pH modification produced more dramatic effects than temperature modification, with vp expression resulting more affected than mnp expression. While mnp3 was the less affected gene under temperature modified conditions, mnp4 and mnp5 were the only peroxidase genes being slightly upregulated under alkaline pH conditions.

265 DST2 OF SCHIZOPHYLLUM COMMUNE IS INVOLVED IN THE TRANSITION OF PRIMORDIA INTO MATURE FRUITING BODIES

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The basidiomycete Schizophyllum commune is a model organism for mushroom development. Blue light plays a key role in the switch from dikaryotic vegetative growth to initiation of mushroom formation. The role of blue light sensing has been appointed to White Collar 1 (WC-1) and WC-2, which form a complex of photoreceptor and transcription factor. Deletion of either gene in S. commune results in a blind phenotype (Ohm et al. 2012). An additional protein, Dst2, was found to play a role in light perception in Coprinopsis cinerea. Deletion of this gene resulted in a "dark stipe" phenotype (Kuratani et al. 2010). Inactivation of dst2 in S. commune did not result in a blind phenotype in S. commune. The transformants grew slower compared to the parental strain but formation of aggregates and primordia was not affected. However, formation of mature mushrooms was highly reduced and only cone-shaped fruiting bodies were found. Together these data indicate that Dst2 does not play a role in sensing blue light. Analysis of the Dst2 sequence shows a FAD-binding domain, also present in WC-1. This, however, is not a conventional FAD-binding domain found in photoreceptors and is more likely involved in oxidation of substrates. Currently we attempt to purify the Dst2 protein using a linked His-tag in S. commune. This will enable further analysis of the protein and identification of its substrate.

266 DUAL DNA BINDING AND COACTIVATOR FUNCTIONS OF ASPERGILLUS NIDULANS TAMA, A ZN(II)2CYS6 TRANSCRIPTION FACTOR

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Transcription factors that contain DNA binding domains generally regulate transcription by direct interaction with DNA. Zn(II)2Cys6 zinc binuclear cluster proteins – the largest family of transcription factors in fungi – regulate genes of diverse pathways, including primary and secondary metabolism and development. For most transcription factors, including Zn(II)2Cys6 proteins, the DNA binding motif is essential for function. However, Aspergillus nidulans TamA and the related Saccharomyces cerevisiae Dal81p protein contain Zn(II)2Cys6 motifs that previous studies have shown are dispensable for function. TamA acts at several promoters as a coactivator of the global nitrogen GATA transcription factor AreA. We now show that the TamA DNA binding motif mediates regulation of the key nitrogen metabolism gene gdhA, which encodes NADP-glutamate dehydrogenase. Therefore TamA has dual DNA binding and non-DNA binding coactivator functions. Using electrophoretic mobility shift assay (EMSA) and chromatin-immunoprecipitation (ChIP) we detect binding of FLAG-epitope-tagged TamA to the promoter region of gdhA and show that the TamA DNA binding motif is required for DNA binding. Additionally, we show that TamA and AreA are reciprocally required for full binding at the gdhA promoter under conditions where AreA activates gdhA but is inactive at most promoters. Finally, using ChIP-seq we map binding of TamA throughout the genome and identify novel TamA targets. The dual functions of TamA provide an additional level of combinatorial control for transcription factors to mediate gene-specific expression.

267 EPIGENETIC CONTROL OF EFFECTOR GENE EXPRESSION IN THE PLANT PATHOGENIC FUNGUS LEPTOSPHAERIA MACULANS

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Plant pathogens secrete an arsenal of small secreted proteins (SSPs) acting as effectors that modulate host immunity to facilitate infection. In Eukaryotic phytopathogens, SSP-encoding genes are often located in particular genomic environments and show waves of concerted expression at diverse stages of plant infection. To date, little is known about the regulation of their expression. Leptosphaeria maculans is an ascomycete fungus responsible for the most devastating disease of oilseed rape (Brassica napus). The sequencing of its genome revealed a bipartite structure only described so far in higher Eukaryotes, alternating gene rich GC-equilibrated isochores and gene poor AT-isochores made up of mosaics of transposable elements. The AT-isochores encompass one third of the genome and are enriched in putative effector genes that present the same expression pattern (no or a low expression level during in vitro growth and a strong over-expression during primary infection). Here, we investigated the involvement of one histone modification, histone H3 lysine 9 methylation (H3K9me3), in epigenetic regulation of concerted effector gene expression in L. maculans. For this purpose, we silenced expression of two key players in heterochromatin assembly and maintenance, HP1 and DIM5, by RNAi. By using HP1-GFP as a heterochromatin marker, we observed that almost no chromatin condensation is visible in a silenced-dim5 background. Whole genome oligoarrays performed on silenced-hp1 and silenced-dim5 transformants background revealed an over-expression of pathogenicity-related genes during in vitro growth, with a favored influence on SSP-encoding genes in AT-isochores. That increase of expression during in vitro growth was associated with a reduction of H3K9 trimethylation at two SSP-encoding gene loci. The ectopic integration of four effector genes in GC-isochores led to their overexpression during growth in axenic culture. These data strongly suggest that an epigenetic control, mediated by HP1 and DIM5, represses the expression of at least part of the effector genes located in AT-isochores during growth in axenic culture. Our hypothesis is that changes of lifestyle and a switch toward pathogenesis lift chromatin-mediated repression, allowing a rapid response to new environmental conditions.

268 EVIDENCE OF MICROBIAL EPIGENETICS; LOSS-OF-FUNCTION MUTANT OF THE BCK1 HOMOLOG, CPBCK1, FROM THE CHESTNUT BLIGHT FUNGUS CRYPHONECTRIA PARASITICA RESULTED IN THE SECTORING ACCOMPANIED WITH THE CHANGES IN DNA METHYLATION

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The Cpbck1 gene of Cryphonectria parasitica, an ortholog of Saccharomyces cerevisiae Bck1 that encodes a mitogen-activated protein (MAP) kinase kinase kinase and functions downstream of PKC in the cell wall integrity pathway, was isolated and characterized. Colony morphology of the Cpbck1-null mutants differed dramatically from the wild type that mutants showed the invasive growth pattern characterized by slower growth rate, absence of distinctive aerial hyphae resulting in almost absence of conidia-bearing structure and conidia, sparse mycelial growth on the surface of agar plate with abnormal pigmentation, and irregular mycelial mat within the restricted area. Feeding hyphae growing under the plate showed less branched and relatively slower growth pattern. Interestingly, the Cpbck1-null mutant produced sectors appeared as thick rubbery patches of matted growth without pigmentation and sporulation. In addition, these characteristics of sectoring were maintained when they were transferred into a new plate. Complementation of the Cpbck1-null mutant with a wild-type allele rescued mutant phenotypes indicating that the mutant phenotypes were due to the absence of the Cpbck1 gene. However, complementation of the sector was not successful indicating that the characteristics of sectored cell were irreversible and inheritable. Intracellular structure observed by electron microscope revealed both invasive growth-type and sectored-type showed the occurrence of hypertrophy of cell wall, multiple nuclei within swollen cells and intrahyphal hyphae. Compared to the hyphae of the original Cpbck1-null mutant showing hyphal tip swelling as well as atypical branches emerging below the swellings, the hyphae of the sector showed more compact mycelial distribution with a lot of aggregation of individual hypha. DNA methylation, an indicative of epigenetic marker, examined by Southern blot analysis and bisulfite DNA modification of putative target genes revealed that there was difference in the DNA methylation pattern between original Cpbck1-null mutant and sectored isolate. This study suggests that epigenetic changes are predisposed by the loss of function mutation of a specific gene Cpbck1 and how the fungal signaling pathway implicates in the control of epigenetic processes, without which resulted in abnormal degeneration such as sectoring.

269 EXPRESSION OF CARS, A NEGATIVE REGULATOR OF CAROTENOGENESIS IN FUSARIUM

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Fusarium fujikuroi is a rice pathogen that produces a large array of secondary metabolites, which includes bikaverin, gibberellins, fusarubins, fusaric acid, fusarins and carotenoids among others. We formerly described the genes that participate in carotenoid metabolism in this fungus: carRA, carB, carT, carD, involved in the synthesis neurosporaxanthin, and carX, responsible for the production of retinal. The same set of genes is found in the genome of the pathogenesis model Fusarium oxysporum. A major regulatory factor controlling the expression of the car genes in both species is light. Additionally, the pathway is downregulated by the product of gene carS, a RING finger protein with a LON domain, orthologous to the ubiquitin ligase CrgA of Mucor circillenoides. The mutants of this gene, both in F. fujikuroi or F. oxysporum, are deeply pigmented and accumulate large amounts of neurosporaxanthin independently of illumination. Two T-DNA insertional mutants of F. oxysporum, currently under investigation, exhibit a similar phenotype, and contain sequence alterations in the intergenic region close to carS gene. We are interested in the mechanism of action of the CarS protein in Fusarium. As a first approach, we have used antibodies raised against a CarS epitope to investigate its expression at protein level. Supporting the validity of the CarS antibody, no signal of the expected size was found in extracts of mutant SG1, holding a premature stop mutation in its carS allele. Subcellular fractionation experiments showed that CarS protein is mainly found in the cytoplasm, and purified GST-CarS fusion protein did not bind to a bidirectional promoter of the structural genes carRA and carX. These results lead us to hypothesize that CarS may control carotenogenesis by interacting with other regulatory proteins, probably responsible for the transcriptional activation of the carotenoid genes. Consistent with former data on carS transcriptional regulation, the CarS protein was detected in cell-free extracts from wild type strain of F. fujikuroi grown in darkness, but the amounts were significantly higher in the light. Unexpectedly, the CarS protein was detected in higher amounts in carS mutants than in the wild type, suggesting a feed-back control mechanism by the functional CarS protein. Data of carotenoid content, CarS protein levels and transcripts of carS and structural car genes in light induction experiments will be presented.

270 EXPRESSION OF NYV1 IS REPRESSED BY TWO TRANSCRIPTIONAL REPRESSORS NRG1 AND MIG1 IN DELETION MUTANTS FOR SEVEN ESCRT COMPONENTS

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Conserved in eukaryotes, endosomal sorting complex required for transport (ESCRT) components function to form multivesicular bodies for sorting of proteins destined for the yeast vacuole or the mammalian lysosome, and their mutations cause neurodegenerative diseases and other cellular pathologies in humans (1-3). Deletion mutants for seven ESCRT components, Snf7, Snf8, Stp22, Vps20, Vps25, Vps28 or Vps36, are calcium-sensitive and show a defect in Rim101 activation (4-8). Our previous study shows that the calcium sensitivity of these ESCRT mutants is mainly due to repressed expression of the ER/Golgi calcium pump gene PMR1 through the Rim101/Nrg1 pathway in budding yeast [(9)]. Here, we show that this calcium sensitivity could be partially suppressed by the release of the calcineurin-dependent inhibition of Vcx1. Overexpression of PMC1, encoding the vacuolar calcium pump, or NYV1 encoding a negative regulator of Pmc1 activity [(10)], suppresses or increases the calcium hypersensitivity of these ESCRT mutants, respectively. In addition, deletion of NYV1 suppresses the calcium hypersensitivity of these ESCRT mutants. The lacZ reporter assay, promoter mutagenesis and gel electrophoretic mobility shift assay demonstrate that expression of NYV1 in these ESCRT mutants is repressed by two transcriptional repressors Nrg1 and Mig1 through their binding motifs in the NYV1 promoter.

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271 FUNCTIONAL ANALYSIS OF CLBR AND CLBR2 CONTROLLING THE CELLULOSIC BIOMASS DEGRADING ENZYME GENE EXPRESSION IN ASPERGILLUS ACULEATUS

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Aspergillus aculeatus possesses at least two signaling pathways controlling gene expression in response to cellulosic biomass. The cellobiose- and cellulose-responsive induction of the FIII-avicelase (cbhI), FII-carboxymethyl cellulase (cmc2), hydrocellulase (cel7b), and FIa-xylanase (xynIa) genes is under the control of the XlnR-independent signaling pathway, while the cellulose-, D-xylose-, and arabinose-responsive induction of the FI-carboxymethyl cellulase (cmc1) and FIb-xylanase (xynIb) genes is under the control of the XlnR-dependent-signaling pathway. By screening the T-DNA insertion mutant library in A. aculeatus, we have identified a cellobiose response regulator (ClbR), a DNA-binding protein possessing the Zn(II)2Cys6 binuclear cluster domain. Although ClbR participates in the cellulose-inductive expression regardless of XlnR dependency, the effect of the clbR overexpression was limited. It increased the amount of the xynIa transcripts, while it decreased the cel7b transcripts and had no effect on the others. On the presumption that the multiple effects of the clbR overexpression were due to transcription factor titration, we screened ClbR-interactor(s) by Yeast-two hybrid system. ClbR paralog, namely ClbR2 showing 42% identity with ClbR, was isolated. Genetic analysis of clbR and clbR2 revealed that both factors control the expression of cbhI, cmc2, and manR, a transcription factor controlling the expression of not only mannanolytic enzyme genes but also cellulolytic enzyme genes in A. oryzae. Although we confirmed that ManR also controlled the cellulose-induced expression of cbhI and cmc2 in A. aculeatus, neither ManR nor ClbR2 regulated the xynIa expression. We next investigated the binding properties of recombinant MalE-ClbR(1-250) and ManE-ClbR2(1-295) proteins to the xynIa promoter by electrophoresis mobility shift assay (EMSA). EMSA demonstrated that MalE-ClbR(1-250) and MalE-ClbR2(1-295) independently bound to the xynIa promoter, while mixture of both proteins did not yield an additional shift band, suggesting that MalE-ClbR(1-250) and MalE-ClbR2(1-295) competitively bind to the same region. Now, we are analyzing the binding properties of both recombinant proteins to the manR promoter.

272 FUNCTIONAL ANALYSIS OF GENES ENCODING ABC TRANSPORTER AND CYTOCHROME P450 IN COLLETOTRICHUM LINDEMUTHIANUM

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The abcCl1 and cypCl1 genes encoding ABC transporter and cytochrome P450, respectively, were isolated from Colletotrichum lindemuthianum, the etiological agent of the anthracnose in the common bean plant (Phaseolus vulgaris) and functionally analyzed. Each gene is found in one copy and clustered in the genome of the fungus. The genes showed an increased expression in response to the following different toxic compounds: eugenol, hygromycin and pisatin phytoalexin. We isolated abcCl1 mutant with loss of function and it was evaluated for their morphological characteristics and pathogenicity. The abcCl1 mutant showed a decrease in colony size, and less pigment in relation to wild type, in addition to a significant decrease in the production of conidia. Appressoria showed changes and no acervula production at seven days after infection. This mutant showed less aggressiveness on detached bean leaves. The assessment of relative gene expression during infection abcCl1 revealed a higher expression from the fifth day of inoculation, confirming its role in initiating the necrotrophic phase. The AbcCl1 and CypCl1 proteins can be involved in the same functional process, that is, the fungus resistance to the toxic compounds produced by plants or antagonistic microorganisms. The cytochrome P450 codified by the cypCl1 gene can represent a complementary detoxification mechanism used by this fungus during the establishment of the disease.

273 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.

274 FUNCTIONAL COMPLEMENTATION AND ETHYLENE RESPONSE OF PLANT-FUNGAL FUSION HISTIDINE KINASE IN YEAST AND FILAMENTOUS FUNGI

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The two-component signal transduction system (TCS) has been conserved widely in bacteria and eukaryotes, including plants and fungi. TCS typically consists of two types of common signal transducers: histidine kinase (HK), a response regulator (RR). In plant Arabidopsis thaliana, the ethylene receptor AtETR1 acts as HK and their HK activities are regulated by ethylene. AtETR1 contains (i) an ethylene-binding domain (EBD) consisting of three transmembrane helices (TM) in the N-terminal half, (ii) an HK domain (HKD) containing HK, and (iii) the receiver domains of RR in the C-terminal half. As well as AtETR1, fungal HKs also consist of an N-terminal sensor detecting environmental stimuli, an HKD, and a RR in the same order of functional domains in AtETR1. Signal transduction pathways caused by these fungal sensors control cellular responses to extrinsic and intrinsic signals. If these fungal sensor domains are replaced by EBD of AtETR1 and fungal pathways can be controlled by ethylene, the hybrid HKs would be useful as a new gene regulation system in fungal industry. To create a novel system of gene regulation by ethylene, we constructed and examined expression systems of plant-fungal fusion HKs in yeast and filamentous fungi. Here, we report functional complementation and ethylene response of plant-fungal fusion HKs in a temperature-sensitive sln1 yeast mutant and Aspergillus nidulans.

275 FUSARIUM VERTICILLIOIDES SGE1 IS REQUIRED FOR FULL VIRULENCE AND REGULATES EXPRESSION OF PROTEIN EFFECTOR AND SECONDARY METABOLITE BIOSYNTHETIC GENES

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The transition from one lifestyle to another in some fungi is initiated by a single orthologous gene, SGE1, that regulates markedly different genes in different fungi. Despite these differences, many of the regulated genes encode effector proteins or proteins involved in the synthesis of secondary metabolites (SMs), both of which can contribute to pathogenicity. Fusarium verticillioides is both an endophyte and a pathogen of maize and can grow as a saprophyte on dead plant material. During growth on live maize plants, the fungus can synthesize a number of toxic SMs, including fumonisins, fusarins, and fusaric acid, that can contaminate kernels and kernel-based food and feed. In this study, the role of F. verticillioides SGE1 in pathogenicity and secondary metabolism was examined by gene deletion analysis and transcriptomics. SGE1 is not required for vegetative growth or conidiation but is required for wild-type pathogenicity and affects synthesis of multiple SMs including fumonisins and fusarins. Induced expression of SGE1 enhanced or reduced expression of hundreds of genes, including numerous putative effector genes that could contribute to growth in planta, genes encoding cell surface proteins, gene clusters required for synthesis of fusarins, bikaverin and an unknown metabolite, as well as the gene encoding the fumonisin cluster transcriptional activator. Together, our results indicate that SGE1 has a role in global regulation of transcription in F. verticillioides, but that its role in regulation of pathogenicity and secondary metabolism is limited as is evident by partial reduction rather than elimination of virulence on maize and production of fumonisins.

276 GENE SILENCING ON DEMAND: ESTABLISHMENT OF THE TET-OFF SYSTEM FOR ASPERGILLUS NIGER

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The tetracycline-expression system is a versatile tool to control and fine-tune gene expression in eukaryotic cells in a metabolism-independent manner. By the addition of doxycycline, genes can either be switched on (Tet-on system) or switched off (Tet-off system). Recently, the Tet-on system has successfully been established for Aspergillus niger (Meyer et al., 2011). In the current study, we tested and evaluated different variations of the Tet-off system for use in A. niger by using luciferase as a reporter gene. Transformants with single or multiple copies of the Tet-Off system were generated and their expression levels determined and compared with a respective control strain. By adding various concentrations of doxycycline to the cultivation medium, it could be shown that the expression of the reporter gene could indeed be down-regulated in a concentration-dependent manner. Most importantly, it was possible to completely shut down luciferase expression. Furthermore, the system turned out to be not only tuneable dependent on the concentrations of doxycycline. Also multiple copies of the Tet-off cassette changed the intensity of expression and its respective down-regulation kinetics.

277 HETEROCHROMATIN PROTEIN 1 OF TRICHODERMA REESEI IS REQUIRED FOR TRANSCRIPTION OF CAZYME GENES AND CONIDIATION

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HP1 is a fundamental protein of constitutive heterochromatin, specifically enriched at centromeres and telomeres of most eukaryotic chromosomes. These conserved chromo domain proteins have important functions in the cell nucleus including gene repression by heterochromatin formation and transcriptional activation. In fungi, histone H3 at lysine 9 (H3K9) is subject to methylation by the methyltransferase KMT1 (aka Clr4, DIM-5, ClrD) and H3K9me3 is recognized by HP1 (Swi6, HepA, MOD1) [Smith et al. 2012]. In some fungi, LaeA acts as positive global regulator of secondary metabolite cluster genes, potentially counteracting H3K9me3 and HP1 binding by unknown mechanisms [Strauss and Reyes-Dominguez 2011]. Recently, we reported that the LaeA ortholog of Trichoderma LAE1 is essential for cellulase gene expression in T. reesei but does not directly influence the methylation of H3K4 or H3K9 at the CAZymes loci [Seiboth and Karimi et al. 2012, Karimi-Aghcheh et al. 2013]. Here we show that loss of T. reesei hep1 also results in decreased cellulase activity. In the presence of light, deletion of hep1 decreased conidiation to 18% of normal whereas in darkness only half as many conidia were produced when compared to the parent strain (QM9414). Chromatin immunoprecipitation (ChIP) followed by high-throughput sequencing with antibodies against H3K9me3 (typical for heterochromatin regions) and H3K9/14ac (indicative of active transcription) showed enrichment with H3K9me3 in 201 out of 228 CAZyme genes in Δhep1 strain as compared to wild type. Our data show that HP1 affects asexual development in a light-dependent manner. In addition, they suggest that this protein is required for cellulase genes transcription in T. reesei and may exert its function indirectly through as-yet-unknown elements.

278 HIGH-AFFINITY GLUCOSE TRANSPORT IN ASPERGILLUS NIDULANS IS MEDIATED BY THE PRODUCTS OF TWO RELATED BUT DIFFERENTIALLY EXPRESSED GENES

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Independent systems of high and low affinity effect glucose uptake in the filamentous fungus Aspergillus nidulans. Low-affinity uptake is known to be mediated by the product of the mstE gene. In the current work two genes, mstA and mstC, have been identified that encode high-affinity glucose transporter proteins. These proteins' primary structures share over 90% similarity, indicating that the corresponding genes share a common origin. Whilst the function of the paralogous proteins is little changed, they differ notably in their patterns of expression. The mstC gene is expressed during the early phases of germination and is subject to CreA-mediated carbon catabolite repression whereas mstA is expressed as a culture tends toward carbon starvation. In addition, various pieces of genetic evidence strongly support allelism of mstC and the previously described locus sorA. Overall, our data define MstC/SorA as a high-affinity glucose transporter expressed in germinating conidia, and MstA as a high-affinity glucose transporter that operates in vegetative hyphae under conditions of carbon limitation.

279 IDENTIFICATION AND CHARACTERIZATION OF THE ASPERGILLUS NIDULANS ZN(II)2CYS6 TRANSCRIPTION FACTOR RHAR THAT MEDIATES L-RHAMNOSE UTILIZATION AND THE PRODUCTION OF ALPHA-L-RHAMNOSIDASES

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L-Rhamnose is a naturally occurring deoxyhexose sugar that can be assimilated by numerous yeasts and filamentous fungi when preferred carbon sources such as glucose are limited or absent. L-Rhamnose is widely distributed in plants where it is commonly found glycosydically bound to other sugars and organic moieties including the primary cell wall pectic polysaccharides rhamnogalacturonan I and II, hemicellulose, glycoproteins and diverse secondary metabolites, some of the latter being important bioactive compounds. L-rhamnose released from the degradation of these plant materials induces the production of diverse enzymes appropriate for the continued depolymerisation/modification and utilization of these substrates. L-rhamnose and rhamnosides are promising candidates for use in the fields of food, cosmetics, agriculture and health. Thus, alpha-L-rhamnosidases - catalyse the hydrolysis of terminal non-reducing L-rhamnose residues in oligosaccharides and alpha-L-rhamnosides - find a variety of uses in industry that include the reduction of citrus juice bitterness, improvement of the release of aromas in musts and wines, increase the bioavailability of food ingredients, drug development, etc. In addition, these enzymes also are involved in the detoxification of plant secondary metabolites and hence they could play a role in evading plant defences against fungal attacks. In this study we have indentified in Aspergillus nidulans and Neurospora crassa the rhaR gene that encodes a putative Zn(II)2Cys6 DNA-binding protein. Genetic evidences indicate that the product of rhaR acts in a positive manner to induce transcription of the A. nidulans L-rhamnose regulon. rhaR-deleted mutants showed a reduced ability to induce the expression of the alpha-L-rhamnosidase genes rhaA and rhaE and concomitant reduction in alpha-L-rhamnosidase production. The rhaR deletion phenotype also results in a significant reduction in growth on L-rhamnose that correlates with reduced expression of the L-rhamnonate dehydratase gene lraC, evidencing that RhaR also controls the expression of the L-rhamnose catabolic pathway. Expression of rhaR alone is not sufficient for induction since its mRNA accumulates even in the absence of L-rhamnose, therefore the presence of both functional RhaR and L-rhamnose are absolutely required. In N. crassa, deletion of rhaR also impairs growth on L-rhamnose. This work is founded by the Spanish Ministerio de Economía y Competitividad grant number AGL2011-29925

280 IDENTIFICATION OF A NOVEL MASTER REGULATOR OF CELLULASE AND HEMICELLULASE PRODUCTION IN TRICHODERMA REESEI USING GENOME-WIDE APPROACH

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Trichoderma reesei (anamorph Hypocrea jecorina) is an efficient producer of enzymes degrading lignocellulosic biomass. The cellulases and hemicellulases produced by the fungus are widely employed in industry, and this production system has a central role in biorefinery applications. Various environmental and metabolic factors together with the physiological state of the cell affect the enzyme production in T. reesei. Thus, a complex signalling cascade and regulatory network is needed to control the pattern of enzyme activities produced. In previous studies, both positively and negatively acting regulatory factors for cellulase and hemicellulase genes have been characterised in T.reesei. In this study, an expression microarray data on T. reesei cultivated in the presence of different carbon sources was analysed in order to identify additional regulatory genes for cellulase and hemicellulase production. In total, 28 putative regulatory factors were chosen to be over-expressed in T. reesei based on the fact that they were induced by lignocellulosic substrates. A part of these genes are also in genomic regions together with glycoside hydrolase genes co-regulated in the inducing conditions. In the primary screening, over-expression of seven of these factors led to increased production of cellulases and/or xylanases. Among these factors is a novel master regulator of the cellulose and hemicellulose genes designated ace3. Its over-expression increased cellulase production 2-4 –fold and also enhanced hemicellulase production. Its deletion abolished cellulase production totally, decreased hemicellulase production, and in the ace3 deletion strains the major cellulase gene transcripts were at extremely low levels. Interestingly, the modifications of ace3 also affected the mRNA levels of the previously identified hemicellulase and cellulase master regulator xyr1, suggesting interplay between these factors.

281 IDENTIFICATION OF CRUCIAL MOTIFS IN THE TRANSCRIPTION FACTORS ACE1 AND XYR1 IN TRICHODERMA REESEI

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The filamentous ascomycete Trichoderma reesei is industrially deployed for the production of cellulases. These cellulases find various applications (paper and pulp, food and feed, textile industry) and represent a bottle-neck for the cost-effective production of second generation biofuels. Gene expression of the two major cellobiohydrolases CBHI and CBHII as well as the endoglucanase EGLI is mainly regulated by the central transactivator Xyr1. Additional transcription factors were found to be involved in their regulation. Cre1 mediates carbon catabolite repression downregulating the expression of the latter genes directly or indirectly by repressing gene expression of their activator Xyr1. However, all industrially used strains are Cre1 deficient. The transcription factor Ace1 is involved in the regulation of the major cellulases and is still expressed in industrial strains. Interestingly, T. reesei uses the very same transcription factors for the regulation of its main hemicellulases, e.g. the two endoxylanases XYNI and XYNII. They show a different pattern of inducibility than the cellulases even if they share the same main transcription factors. Ace1 and Xyr1 have been suggested to bind to the same sites in the xylanase activating element of the xyn1 promoter. It was postulated that different protein-protein-DNA complexes are built under inducing, non-inducing and repressing conditions. We investigated the underlying mode of action of the two transcription factors Ace1 and Xyr1 in regard of their (hetero)dimerization and subsequent impact on DNA binding. We could identify several potentially crucial motifs within the primary structure of Ace1 and Xyr1 via an in silico analysis (e.g. coiled coils and phosphorylation sites). Different variants of Ace1 and Xyr1 with mimicked (de-)phosphorlyation or bearing modified crucial motifs were heterologously expressed in E. coli and subjected to electrophoretic mobility shift assays using probes from several target upstream regulatory regions.

282 IDENTIFYING THE RNASE III INVOLVED IN A DICER-INDEPENDENT NON-CANONICAL RNA SILENCING MECHANISM IN MUCOR CIRCINELLOIDES

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RNA silencing is a conserved gene regulatory mechanism in eukaryotes that represses gene expression in a homology-dependent manner. The canonical silencing mechanism requires the presence of the RNase III Dicer to process double-stranded RNA (dsRNA) precursors into small interfering RNAs (siRNAs), which guide the RNA-induced silencing complex (RISC) to degrade target mRNAs. In the basal fungus Mucor circinelloides, the silencing pathway also requires RNA-dependent RNA polymerase (RdRP) enzymes to produce dsRNAs using single-stranded transcripts as template. Analyses in the wild-type strain and mutants of M. circinelloides affected in silencing genes, indicated that its canonical dicer-dependent silencing mechanism is involved in the production of endogenous small RNA (esRNA) that regulate the expression of protein-coding genes. These analyses also revealed that, besides the canonical silencing pathway, M. circinelloides presents a non-canonical RNA silencing mechanism. This mechanism is RdRP-dependent but Dicer-independent and regulates the expression of more than 500 protein-coding genes through the production of esRNAs. To identify the gene encoding the RNase III that participates in the non-canonical silencing pathway, four candidate genes were selected by screening the M. circinelloides genome for RNase III-like domains, and null mutants of each of those genes were obtained by using gene replacement techniques. Northern blot analysis of reporter genes regulated by the non-canonical mechanism identified one of those RNase III-encoding genes, named r2d2, as the putative RNase III enzyme involved in this mechanism, since the reporter genes presented increased expression in the null r2d2 and rdrp mutants, relative to wild type and dicer mutant strains. esRNA analysis in the r2d2 mutant and wild type strain confirmed the involvement of r2d2 in the non-canonical RNA silencing mechanism, since accumulation of esRNAs derived from loci regulated by this mechanism is significantly reduced in the r2d2 mutant. To investigate the requirement of the RNase III domain for the r2d2 function, point mutations at the catalytic region of the RNase III domain were generated. The analysis of strains carrying this mutant allele agree with the role of r2d2 as the RNase III enzyme involved in the non-canonical silencing pathway in M. circinelloides. This work was funded by the Spanish MICINN (BFU2009-07220) and MINECO (BFU2012-32246) co-financed by FEDER.

283 IMPROVEMENT OF CELLULASE AND HEMICELLULASE EXPRESSION USING CHIMERIC TRANSCRIPTION FACTORS IN TRICHODERMA REESEI

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Trichoderma reesei is widely used by the enzyme industry as a producer of cellulolytic and hemicellulolytic enzymes and also increasingly used as a production host for heterologous proteins. Despite the high protein secretion capacity, the enzyme price is still a major concern for the cost effectiveness. Numerous approaches have been used in attempts to improve the activity of cellulase and hemicellulase promoters to increase the production yields. This work aimed at improvement of cellulase and hemicellulase expression by using chimeric transcription factors in regulation of cellulase and hemicellulase promoters. Chimeric transcription factors studied in this work consist of an endogenous DNA binding domain fused to an activation domain from heterologous origin.
Proteins selected for the chimeric constructs studied in this work were XYRI (transcriptional activator of xylanase and cellulase genes in filamentous fungi), CREI (carbon catabolite repressor), ACEI (repressor for cellullase genes) and two acidic activation domains, GAL4 (from Saccharomyces cerevisiae) and VP16 (from human herpes virus, HPV). The effect of transcription factor chimera expression was determined by analysis of cellulase and hemicellulase enzyme activity measurements from cultivations induced by different carbon sources.
Results obtained in this study show potential of using chimeric transcription factors to increase the transcription of genes encoding cellulase and hemicellulases. The information gathered in this study can provide further insights to the molecular mechanism of cellulase and hemicellulase gene regulation.

284 INDIVIDUAL TRANSCRIPTION FACTOR REPERTOIRES OF FUNGAL GENOMES REVEAL REGULATORY BASIS OF LINEAGE- AND SPECIES-SPECIFIC TRAITS

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Families of transcription factors (TFs) are not distributed evenly across the fungal kingdom. Combining the information about the expansions and losses of the TF families in different genomes with the specific biological traits of the species, we can obtain a valuable source of insights into the evolutionary history and functional importance of particular features, in which the TFs are involved. This gets a special value when we discuss such features as virulence of pathogenic fungi, or specific traits of symbionts, adaptation to a particular niche, etc. Having developed a tool for the genome-wide prediction of TFs, we collect and compare the whole TF repertoires of the available fungal genomes. We consider different fungal lineages, paying special attention to the basal fungi, as they are probably the least investigated in this respect. The comparison reveals interesting deviations, some of which can be associated with virulence (e.g., expanded heat shock factors in Lichtheimia corymbifera), the other reflect re-direction of functions from one family to another (e.g., in asco-and basidiomycetes Cys6 Zn cluster TFs play superior role, whereas in the basal fungi they lose it to Cys2His2 Zn fingers). Another exciting expansion, which is evidently characteristic for all Mucorales, is a duplication of the general transcription factor TBP (TATA binding protein). Normally eukaryotic genomes have just one copy of this gene and the duplication, if it happens, is associated with gaining a new function. In higher eukaryotes, the duplications of TBP can be involved in modulating gene- and cell-type-specific programs of transcription, such as tissue differentiation, development, etc. In fungi, the event of TBP duplication is exceptionally rare. Our survey of all so far sequenced genomes revealed only 4 examples of such duplication besides Mucorales (3 in Ascomycetes/Sordariomycetes (Chaetomium globosum, Grosmannia clavigera and Podospora anserina) and 1 in a basidiomycete Laccaria bicolor). We can suppose that the duplicated TBP-like factors can take over an additional role in some condition-specific responses, which makes them likely candidates as virulence factors in pathogenic species. The presented work is based on the TF annotations made by our new tool for the genome-wide prediction of TFs. The tool scans the genomes for hidden Markov models for known DNA-binding domains, is implemented in R and will be soon publicly available on-line.

285 INSIGHTS INTO THE ROLE OF THE CRE-1 TRANSCRIPTION FACTOR IN THE CROSSTALK BETWEEN THE CIRCADIAN CLOCK AND CARBON CATABOLIC REPRESSION IN NEUROSPORA CRASSA

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Circadian clocks are autonomous timers composed of interconnected transcriptional/transcriptional feedback loops. They are thought to confer a selective advantage by enabling processes to occur at appropriate times of the day. In the model organism Neurospora crassa, ~20% of its genes are under circadian control and interestingly; many of them are related to metabolism. The different pathways involved in relaying the time-of-day information to the expression of these genes, however, remain unclear

We are interested in elucidating the transcriptional mechanisms interconnecting circadian and metabolic processes, particularly regarding organismal fitness. Thus, we are analyzing carbon catabolite repression (CCR) and cellulolytic capabilities in a circadian context, using N. crassa as a model.

Evaluating the role of CRE-1, a crucial transcription factor involved in several important cellular processes such as cellulose degradation and carbon catabolic repression, we found evidence that suggests that this transcription factor acts as a link between both pathways. These data provide insights on how the circadian clock is influencing Neurospora physiology, potentially impacting a biotechnological relevant process like biomass conversion.

286 INTERACTION OF ASPERGILLUS NIDULANS GALR, XLNR AND ARAR IN D-GALACTOSE AND L-ARABINOSE CATABOLISM

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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 make 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, and a role for GalR in L-arabinose catabolism could explain the observed difference 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. Interactions between all three regulators with respect to pentose catabolic and D-galactose catabolic gene expression were observed.

287 INVOLVEMENT OF AN APSES REGULATOR IN THE MORPHOGENESIS OF TRICHOPHYTON RUBRUM

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The dermatophyte Trichophyton rubrum is a human fungal pathogen and the most prevalent causative agent of clinical cases of skin and nail mycoses. Specific adaptations during host-pathogen interaction allow this keratinolytic pathogen to adhere, invade and colonize the keratinized host tissues, causing dermatophytosis. In order to survive, the pathogens have to obtain nutrients and adjust their metabolism, overcoming the host defense mechanisms and modulating their genetic responses to the environment. Transcription factors play crucial roles in the cell, controlling key events in several processes and the APSES regulators have been reported to play important roles in morphogenesis, metabolism and stress responses in several fungi species. Five genes encoding distinct proteins containing the APSES domain were found in the genome of T. rubrum, suggesting that these regulators may be implicated in different processes in the cell. In this work, the expression profile of these genes was evaluated during keratin and nail growth, suggesting that these transcription factors are important for growth in keratinized tissues. One of these genes was inactivated by gene targeting and the mutant strain was subjected to functional characterization regarding to growth rate in several nutritional sources and morphological aspects. Colony morphology was distinct and growth rate was slightly lower in the mutant strain in the conditions tested. Moreover, hyphae and conidia are abnormal in shape and size in the mutant strain, compared to the wild type, providing evidences that this APSES transcriptional factor plays a role in conidial and hyphal morphogenesis in T. rubrum.
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288 KDMB, A HISTONE H3 LYSINE K9/K36 DEMETHYLASE MODULATES GENE EXPRESSION OF SECONDARY METABOLITE GENE CLUSTERS

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Chromatin remodelling at secondary metabolite (SM) gene clusters is an important mechanism regulating the biosynthesis of natural products. Mechanisms such as histone acetylation/deacetylation, methylation/demethylation and the associated binding of non-histone proteins, such as heterochromatin protein-1 (HepA) profoundly alter the chromatin landscape and associated gene expression profiles in toxigenic Aspergilli or pathogenic Fusarium species. These processes proofed to be important for controlling the ON/ OFF state of SM biosynthetic genes as well as the fine tuning of their expression. Here, we present the dynamic process of histone H3 demethylation by a jumonji-type demethylase termed KdmB. The purified enzyme removes the methyl mark from tri-methylated H3K9me3 and H3K36me3, but not from H3K4me2 in vitro. Chromatin immunoprecipitation (ChIP) revealed that KdmB is involved in counteracting the heterochromatic state mediated by H3K9 methylation at several SM gene clusters, e.g. penicillin (PEN) or sterigmatocystin (ST) during transition from silent to actively transcribed loci. Transcriptome analysis of a kdmB null mutant exhibits repression of several SM gene clusters with concomitant loss of corresponding SM production. Our data revealed surprising differences in the chromatin-mediated mechanisms regulating ST and PEN cluster gene expression in respect to the function of HepA and the global regulator of SM gene expression, LaeA. Moreover, kdmB deletion is associated with the loss of H3 serine 10 phosphorylation, a histone mark usually associated with actively transcribed genes. Strikingly, ΔlaeA strains showed the same effect at ST gene cluster indicating a genetic interaction between KdmB and the complex networks regulating SM cluster gene expression.

289 LIGHT-DEPENDENT AND CIRCADIAN TRANSCRIPTION DYNAMICS IN VIVO RECORDED WITH A DESTABILIZED LUCIFERASE REPORTER IN NEUROSPORA

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We show that firefly luciferase is a stable protein when expressed at 25°C in Neurospora, which limits its use as transcription reporter. We created a short-lived luciferase by fusing a PEST signal to its C-terminus (LUC-PEST) and applied the LUC-PEST reporter system to record in vivo transcription dynamics associated with the Neurospora circadian clock and its blue-light photosensory system over the course of several days. We show that the tool is suitable to faithfully monitor rapid, but also subtle changes in transcription in a medium to high throughput format.

290 LIGHT-RESPONSIVE TRANSCRIPTION FACTORS (LTFS) REGULATE DIFFERENTIATION AND VIRULENCE IN THE GRAY MOLD FUNGUS BOTRYTIS CINEREA

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Botrytis cinerea 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. 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; 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 TF in virulence, and discovered furthermore its 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 in B. cinerea B05.10 including five further TF-encoding genes (BcLTF2-6), and that the expression levels of the majority of these genes (66%) are modulated by BcLTF1. Bcltf2 encodes a C2H2-TF which is the homologue of N. crassa SAH-1 (short-aerial-hyphae-1). Expression levels are increased in mutants exhibiting a hyper-conidiation phenotype such as deletion mutants of BcLTF1, the VELVET protein BcVEL1 and the WHITE COLLAR-like TF BcWCL1, suggesting a role of BcLTF2 in regulation of conidiation. Indeed, Δbcltf2 mutants do not produce conidia in axenic culture and in planta. Notably, mutants form sclerotia under all illumination conditions indicating that the suppression of sclerotial development by light is likely due to the induction of conidiation rather than due to a direct suppression of the sclerotium differentiation program.

291 MAGNAPORTHE ORYZAE RBP35/CFI25 COMPLEX PROMOTES DISTAL POLYADENYLATION SITES IN SEVERAL INFECTION-RELATED GENES

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The 3’ end polyadenylation of pre-mRNAs is a two-step process. First, pre-mRNAs are cleaved at their 3' end. The second step involves the addition of the poly(A) tail by RNA polymerases. Presence of multiple potential 3’ end cleavage sites is common in eukaryotic genes, and the selection of a proper 3´ end cleavage site represents an important step of regulation of gene expression during development and in response to cellular cues. Several proteins of the polyadenylation machinery have been shown to regulate alternative polyadenylation (APA), including Rbp35/CfI25 complex in Magnaporthe oryzae and Hrp1 in yeast. The M. oryzae Rbp35/Cfi25 complex is not essential for viability but regulates the length of 3’UTRs of transcripts with developmental and virulence-associated functions. The response of cells to changes in their environment often requires co-regulation of gene networks, but very little is known how this occurs at post-transcriptional level in fungi. M. oryzae has to adapt to the plant nutritional environment during host invasion. M. oryzae is an hemibiotrophic fungus and the molecular mechanisms that regulate the switch from biotrophic to necrotrophic stage are unknown. Possibly, one of the cues that trigger this switch is the lack of carbon sources within the host cell. Combining RNA Sequencing with a T-fill method, we have performed a comprehensive map of polyadenylation sites and quantified their usage under metabolic changes (cultured in rich medium or exposed to 12 hours of carbon depletion). We have observed that APA is involved in regulating M. oryzae gene expression in response to nutritional fluctuations. Interestingly, under carbon starvation, genes that are alternatively polyadenylated show a preference for distal poly(A) sites, i.e. they possess long 3’UTRs. By contrast, the Δrbp35 mutant lacks precision in the cleavage and shows an increase of proximal cut sites in pre-mRNAs. Results of these polyadenylation maps will be presented, including APA gene targets, sequence motifs present in long 3' UTRs and RBP35-dependent mRNA isoforms. In summary, our results suggest that Rbp35/CFI25 complex regulates distal poly(A) site selection in approx. 20% of total pre-mRNAs, including those involved in the response to carbon depletion. The identification of RBP35/CF25 as a component of the alternative polyadenylation machinery is an important step to unravel post-transcriptional networks that regulate M. oryzae plant colonization.

292 MOLECULAR DETERMINANTS OF SPORULATION IN ASHBYA GOSSYPII

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Sporulation is a key mechanism used by fungi to be able to survive in harsh conditions. This process is tightly regulated to ensure that sporulation is only initiated when required. We have identified a set of genes that regulate sporulation in the homothallic ascomycete Ashbya gossypii. Three genes, STE11, STE7 and STE12 result in a hypersporulation phenotype when deleted. STE11 and STE7 are both components of the conserved pheromone MAPK cascade and STE12 is the main transcription factor of the cascade. This is in sharp contrast to Saccharomyces cerevisiae where deletion of any of these genes leads to sterility. Further on we show that two of the conserved homologs to S. cerevisiae involved in karyogamy (nuclear fusion) affect sporulation upon deletion. Deletion of KAR3 causes severely reduced sporulation while a KAR4 deletion strain is non-sporulating. Similarly to kar4, deleting the homologs of the main regulators of sporulation in S. cerevisiae, IME1, IME2, IME4 and NDT80 also results in sporulation deficient strains. By using RNAseq transcript profiles of ime1, ime2, kar4 and ndt80 we could identify a set of 67 down-regulated genes, most of which were upregulated in the oversporulating ste12 strain. One of these genes was the endoglucanase encoded by ENG2 that is involved in hyphal fragmentation to generate single cell sporangia at the end of the sporulation. Interestingly, in sporulation conditions sporangia without spores are produced in the ime1, ime2, kar3, kar4 and ndt80 mutants as well as in the wildtype strain. Supply of new nutrients to these sporangia enables return to vegetative growth indicating that these cells are not locked in meiosis. Furthermore we show that double-strand breaks (DSBs) by Spo11 are not required for sporulation; however, deletion of DMC1 which repairs DSBs in S. cerevisiae reduced sporulation in A. gossypii. Finally, to get deeper insight into the regulation of sporulation in A. gossypii we have characterized the promoter region of the homolog to the main regulator of sporulation in S. cerevisiae, IME1. Our RNAseq transcript profile data indicate that IME1 has a central role in sporulation also in A. gossypii. However, the regulation might differ since the intragenic region is significantly shorter compared to the 2.1 kb IME1 promoter of S. cerevisiae. To this date we show that a considerable shorter IME1 promoter of only 544 bp is sufficient to induce sporulation in A. gossypii.

293 MONITORING EXPRESSION OF PKS14 FROM FUSARIUM GRAMINEARUM DURING INFECTION

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Fusarium graminearum is a global plant pathogen, which infects numerous important crops including small grain cereals and maize. During infection F. graminearum is able to produce several known secondary metabolites including deoxynivalenol, zearalenone and aurofusarin. We have recently discovered that the polyketide synthase (PKS) 14 gene cluster in F. graminearum is responsible for production of orsellinic acid and orcinol. Transcriptome analyses showed that the gene cluster is expressed during plant infection, but the levels and the actual role of these compounds are not known. To monitor when PKS14 is expressed during infection we will clone the promoter region of PKS14 and insert it into a transformation vector where it controls the yellow fluorescent protein (YFP). The vector will be introduced into F. graminearum by agrobacterium mediated transformation followed by homologue recombination. Conidia of the resulting mutants will be used to inoculate spikes of Brachypodium distachyon. The infection and fluorescence will then be monitored by confocal microscopy which can be used to determined when and where PKS14 is expressed. To further investigate the role of orsellinic acid and orcinol in plant pathogenicity we will use deletion and overexpression mutants of PKS14 in infection experiments and examine whether they have altered aggressiveness towards B. distachyon.

294 NEW INSIGHTS INTO REGULATION OF BIOMASS DEGRADING ENZYMES BY XYR1 IN TRICHODERMA REESEI

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Trichoderma reesei is a filamentous ascomycete fungi of great industrial importance since it produces enzymes needed to biomass degradation into soluble monosaccharides. Xyr1 is a transcription factor recognized as an essential activator for most hydrolytic-enzyme encoding genes in T. reesei. The aim of this study was to analyze the transcriptional profile of T. reesei mutant strain Δxyr1 comparing to T. reesei parent strain (QM9414) in the presence of cellulose, sophorose and glucose as carbon sources using RNA-seq approach. The strains were grown in Mandels-Andreotti medium supplemented with 1% cellulose, 2% glucose or 1 mM of sophorose. After growth, the total RNA was isolated using TRIzol reagent®. RNA-seq experiments were performed by LGC Genomics GmbH (Berlin/Germany) using the Illunina/HiSeq2000 platform. It was obtained approximately 117 million 100 bp paired-end reads for the QM9414 strain and 144 million 100 bp paired-end reads for the mutant strain. Bioconductor DESeq package was employed to differential expression analysis, using two-fold change cuttoff, i.e., log2 fold change ≥ 1 or ≤ -1 and adjusted p-value ≤ 0.05 as thresholds. Gene expression analysis revealed that of the 9129 genes that compose T. reesei genome, 2185 genes were differentially expressed in cellulose, 2124 genes in sophorose and only 46 genes in glucose. The differentially expressed genes were also identified as up-regulated or down-regulated (749 and 700, respectively). The 1379 differentially expressed genes were used to build a heatmap to verify the clustering of genes and conditions. The regulatory network of these 1379 genes differentially expressed was also generated to verify the interrelationship of the genes between the conditions, highlighting a greater number of exclusive genes in inducing conditions (cellulose and sophorose) and few genes in repressing conditions (glucose). These results will contribute to the elucidation of the complexity of the formation of biomass-degrading enzymes with applications in biotechnology, involving the XYR1 transcription factor. However, more studies are still needed to elucidate the entire regulatory network involved in the formation of enzymes of biotechnological importance.

295 PARTICIPATION OF THE PHOTORECEPTORS WCOA, CRYD AND VVDA IN PHOTOINDUCTION OF CAROTENOGENESIS IN F. FUJIKUROI

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Fusarium fujikuroi is a model system in the investigation of secondary metabolism, which includes the production of gibberellins, bikaverin, fusarins and carotenoids. The synthesis of the major carotenoid product in this fungus, the acidic xanthophyll neurosporaxanthin, is stimulated by light through the transcriptional activation of three of the structural genes of the pathway, carRA, carB, and carT. We are investigating the molecular basis of this photoresponse. In other fungi, such as Neurospora crassa or Mucor circinelloides, carotenoid photoinduction is mediated by a White Collar protein. We formerly found that the mutants of the white collar gene of F. fujikuroi, wcoA, contain the same amount of carotenoids under continuous illumination than the control strain, but they exhibit a reduced induction of the car genes. The genome of F. fujikuroi includes genes for other photoreceptors, such as the DASH cryptochrome CryD and the small flavoprotein VvdA, orthologous of VIVID of N. crassa. As the wcoA mutants, the cryD mutants exhibit the same carotenoid content than the wild type under continuous illumination, but the vvdA mutants accumulate about 40% less carotenoids. Here we report on the effect of the mutations of these photoreceptor genes on carotenoid content and car mRNA levels in light induction kinetics experiments. The wild type exhibit a biphasic response, with a rapid increase in carotenoid content in the first 6 hours followed by a slow increase in the following 42 hours. The mutants of the three photoreceptors exhibit different kinetic responses. The wcoA mutants are defective in the rapid initial response, whereas the cryD mutants are affected in the second slower response. Unexpectedly, in the vvdA mutants, the fast response was enhanced while the second response was attenuated. The data are consistent with the transcriptional analyses, which showed a strong reduction of photoinduction of mRNA levels in the wcoA mutants, and a minor reduction in the CryD mutants. However, the vvdA mutation had no effect on carRA and carB photoinduction. Taken together, our data point to a cooperative participation of WcoA and CryD in photoinduction of carotenoid biosynthesis in F. fujikuroi. The function of VvdA is uncertain, but it could affect in different ways the activity of WcoA and CryD.

296 PCRFX1 CONTROLS THE EXPRESSION OF THE PENICILLIN BIOSYNTHETIC GENE CLUSTER: VALIDATION OF BINDING SITES

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Penicillium chrysogenum is the filamentous fungus used for the industrial production of penicillins. No penicillin pathway-specific regulators have been found in the amplified region containing the penicillin gene cluster so far and therefore, penicillin biosynthesis seems to be controlled directly by global regulatory factors. In P. chrysogenum, the global transcriptional factor PcRFX1 has been identified and it has been proven to control the biosynthesis of penicillin. Based on DNA binding sequences reported for PcRFX1 orthologs, we have established a new consensus sequence for the binding of PcRFX1 to the penicillin biosynthetic gene promoters (NNRCCNNRSHWAY).Using this consensus sequence, two putative binding sites upstream of pcbAB, one upstream of pcbC and another one in the promoter region of penDE, were found. The functionality of those putative PcRFX1 DNA binding sequences was verified using gene reporter assays. Furthermore, by means of EMSA assays we have proven that PcRFX1 interacts specifically with the putative DNA binding sequences located in the penicillin biosynthetic gene promoters.

297 POTENTIAL PROTEIN PARTNERS OF RRMA, THE REGULATOR OF TRANSCRIPT STABILITY IN ASPERGILLUS NIDULANS

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Regulation of transcript stability is an effective way to modulate gene expression at posttranscriptional level in response to environmental stimuli. RrmA, the RNA-binding protein from Aspergillus nidulans, was shown to regulate the stability of several mRNAs in response to nitrogen source and oxidative stress (Krol et al, 2013). We looked for potential protein partners of RrmA using an in vitro and in vivo approach. In the first approach, RrmA was expressed and purified from bacteria and, subsequently, used for standard pull down in vitro assays with protein extracts from A. nidulans mycelium grown under nitrogen repression or oxidative stress conditions. Proteins interacting with RrmA in vivo were identified using the strain expressing RrmA::GFP fusion and immunoprecipitation with GFP-Trap®_A (Chromotek). Proteins interacting with RrmA were identified using Mass Spectrometry (MS) analysis. Several proteins participating in RNA metabolism were identified, like subunit of Lsm complex and translation factors.
Ref: Krol, Morozov, Jones, Wyszomirski, Weglenski, Dzikowska, Caddick (2013) RrmA regulates the stability of specific transcripts in response to both nitrogen source and oxidative stress Mol. Microbiol. 89:975-988

298 REGULATION OF CELLULASE GENES THROUGH PH SIGNALING PATHWAY IN ASPERGILLUS NIDULANS

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In Aspergillus nidulans, expression of cellulase genes is cooperatively regulated by the pathway specific transcription factor ManR/ClrB and the wide-domain transcription factor McmA. The expression appeared to be also under control of the pal-pacC pH signaling, as the palC mutant displayed decreased cellulase productivity. Higher productivity of endoglucanases A and B (EglA and EglB) at neutral pH compared to acidic pH and impaired production of the enzymes in the pacC deletion strain confirmed the involvement of the pal-pacC signaling. qRT-PCR analysis indicated that expression of not only eglA and eglB but also the other cellulase genes such as cbhA and cbhD, which are under control of ManR/ClrB and McmA were rapidly induced by cellobiose at alkaline pH in the wild type strain, while their expression was delayed and decreased by the pacC deletion. PacC appeared to regulate indirectly transcription of eglA based on mutational analysis of its promoter, but expression of the transcription factors known to date to be involved in cellulase regulation, including manR/clrB and mcmA, was not regulated by PacC. Nevertheless, genome-wide identification of PacC-dependent genes under cellobiose-induced conditions at alkaline pH demonstrated that the genes highly-expressed in a PacC dependent manner were significantly overlapped with those regulated by ManR/ClrB. PacC might control uptake of cellobiose, the most possible inducer of ManR/ClrB-mediated regulation, because cellobiose consumption was delayed in the pacC deletion strain.
This work was supported by the Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry.

299 REGULATION OF CELLULOLYTIC AND HEMICELLULOLYTIC GENES BY MCMA AND MANR/CLRB IN ASPERGILLUS NIDULANS

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Fungal cellulolytic and hemicellulolytic enzymes are promising tools for industrial hydrolysis of cellulosic biomass. The regulatory system involved in their production had not been well understood, but recent discovery of new transcriptional activators are now providing the keys to solve the complexity of the regulatory system. In Aspergillus, XlnR regulates xylanase and cellulase genes, and ManR/ClrB does mannanase and cellulase genes. McmA also regulates cellulase genes in A. nidulans. We report here that cellulase and mannanase genes are regulated by ManR/ClrB, McmA, and the ManR paralog ManS and that the factor(s) responsible for the regulation differs genes to genes. Transcription of highly expressed cellulolytic genes including endoglucanse genes eglA and eglB, cellobiohydrolase genes cbhA and cbhD, were regulated by both McmA and ManR/ClrB in response to cellobiose. Cellobiose also induced some mannanase genes (manB, manC, mndB) in a ManR/ClrB dependent manner, while galactomannan induced a different set of mannanase genes (manC, manE, manF), which were under control of ManS. McmA was not required at least for expression of the beta-mannosidase gene mndB. CeRE (Cellulose Responsive Element, CCGN2CCN6GG) is the sole cis-element responsible for the induction of eglA. We have shown that McmA binds to CeRE on the eglA promoter by EMSA (electrophoretic mobility shift assay). In the assays to detect DNA binding of ManR/ClrB, DNA binding domain of ManR fused to Flag-tag (ManR-DBD) was used. ManR-DBD displayed very weak binding to the eglA CeRE by itself, but significantly increased binding was detected in the presence of McmA. Examination of binding to the eglB promoter exhibited the similar results, that is, ManR could not bind to the eglB CeRE without assistance of McmA. These observations indicate that cellulase genes are regulated by cooperative binding of ManR/ClrB and McmA to CeRE. The expression of mndB was not McmA dependent and its promoter lacked CeRE. This suggested that ManR/ClrB can bind to the promoter without assistance of McmA. EMSA using various probes with intact and mutated sequences revealed that ManR/ClrB should bind to CGGN8CCG. This discovery provided a reference to locate the binding sites on other ManR/ClrB-dependent genes. The arising question is why cellulase genes require McmA for its expression.
This work was supported by the Programme for Promotion of Basic and Applied Research for Innovation in Bio-oriented Industry.

300 REGULATION OF FUSARIN PRODUCTION IN FUSARIUM FUJIKUROI

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Fusarin is a polyketide synthesized by enzymes encoded by fus genes that are clustered in the Fusarium fujikuroi genome. The main enzyme, FusA, is a polyketide synthase with a nonribosomal peptide synthase. Transcript amounts of gene fusA are higher in high-nitrogen (high N) than in low-nitrogen (low N) media. To determine if this regulation is mediated by AreA, we have investigated the effect of its deletion on fusarin production. ΔareA mutants secreted lower amounts of fusarin than the wild type in media with high concentration of organic nitrogen sources and produced only trace amounts in media with $NaNO_3$, implicating AreA in fusarin regulation. Fusarin production is reduced by light, partly due to lower levels of fusA mRNA and partly due to light degradation. Former studies on F. fujikuroi photoreceptors showed that the mutants of the genes for the White Collar protein WcoA and the DASH cryptochrome CryD are affected in the production of other nitrogen-regulated secondary metabolites. In order to establish a possible role of these photoproteins in the regulation of fusarin production, we have studied the regulation of this pathway in null wcoA and cryD mutants. The ΔwcoA mutants decreased their fusarin production in high N medium, both in dark and in light, indicating that WcoA protein has an important role in fusarin production. However, ΔcryD mutants, formerly found to be upregulated for bikaverin production in low N medium under light, were not affected significantly in the production of fusarin, either in dark or in light and irrespective of the nitrogen concentration. These results indicate that CryD is not involved in the control of fusarin production. Other regulatory genes involved in the production of gibberellins, bikaverin and carotenoids, such as the adenylyl cyclase gene acyA or the carotenogenesis-related gene carS were also implicated in fusarin production. Fusarin levels dropped in ΔacyA mutants, especially in high N medium. However, the pattern observed in the carS mutants depended both on nitrogen and light. carS mutants grown in high N medium under illumination showed a reduction in fusarin content. However, in low N medium we observed an increase both in light and dark. RT-PCR analyses on fusA mRNA levels will be reported for all the mutants under investigation to assess the possible roles of AreA, CryD, AcyA and CarS on fusA transcription.

301 REGULATORY NETWORKS DRIVING OXIDATIVE STRESS RESPONSE IN FUSARIUM GRAMINEARUM

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The filamentous fungus Fusarium graminearum infects cereals and corn. It is one of the main causal agent of “Fusarium Head Blight” and “Maize Ear Rot”. During infection, it produces the mycotoxins type B trichothecenes, including deoxynivalenol, secondary metabolites that accumulate in the grains. Although the biosynthetic pathway involving specific Tri genes has been elucidated, the global regulation of toxin production remains enigmatic. In filamentous fungi, a large variety of transcription factors form a regulatory network activated by several oxidative stimuli, linked with secondary metabolism. We have recently identified a transcription factor, Fgap1, involved in response to oxidative stress coupled with trichothecene production in F. graminearum. Here, an integrated transcriptomic approach is used to identify candidate Fgap1-mediated regulatory networks involved in responding to redox variations in a deoxynivalenol-producing F. graminearum strain, wild type as well as genetically modified to either lack the gene Fgap1 or constitutively over-express it. Our findings include the identification of specific functional genes sets regulated by redox variations of the environment, in an Fgap1-dependant or not manner. For example, genes involved in the mitochondrial function or in the ribosomal function are differentially regulated by Fgap1. As a whole, our results indicate that Fgap1 plays a central role in response to oxidative stress in F. graminearum. Functional re-wiring of regulatory networks in the Fgap1-deleted strain is currently being investigated.

302 REVERSIBLE OXIDATION OF A CONSERVED METHIONINE IN THE NUCLEAR EXPORT SEQUENCE DETERMINES SUBCELLULAR DISTRIBUTION AND ACTIVITY OF THE FUNGAL NITRATE REGULATOR NIRA

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Nitrate is an important source of nitrogen for plants and microbes and nitrogen availability also regulates production of secondary metabolites. Dynamics of nitrate induced gene expression in fungi is tightly regulated and mediated by the synergistic action of a nitrogen status-sensitive chromatin regulator/transcriptional co-activator, together with a nitrate-responsive activator (AreA and NirA, respectively, in the Aspergillus nidulans model system). We have previously shown that exposure of A. nidulans cells to nitrate disrupts the interaction between the nuclear export signal (NES) in NirA and the exportin KapK/Crm1 leading to fast NirA nuclear accumulation and activity. Here we present that inactive NirA in nitrate deprived cells carries methionine sulfoxide (MetOx) in the NES whereas reduced MetR is found in nuclear and active NirA. Our results show that Met-oxidation is dependent on the function of FmoB, a flavin-containing monooxygenase whereas the MetOx reduction pathway remains obscure, yet, it is independent from classical methionine sulfoxide reductases (MsrA, MsrB). In vitro binding and in vivo co-immunoprecipitation assays revealed that KapK binds the NES with high affinity despite the presence of MetOx in the target sequence because under nitrate-depleted conditions, the NES is exposed. In contrast, under nitrate-inducing conditions, where the NES carries MetR, KapK is unable to bind presumably due to a NirA conformation which blocks NES accessibility. A genetic screen and mutagenesis experiments identified a small region in NirA which could serve as nitrate-responsive regulatory domain (NiRD) and this region may be responsible for shielding the NES under inducing conditions. In addition, the C-terminal activation domain (AD) appears to regulate the NES-NiRD interaction. Collectively, our data suggest a model of a nitrate-triggered redox-sensitive conformational change which brings either the NES or the AD into a functional state.

303 RNAi-DEPENDENT EPIMUTATIONS EVOKE ANTIFUNGAL DRUG RESISTANCE IN THE ZYGOMYCETE FUNGAL PATHOGEN MUCOR

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Microorganisms evolve via a panoply of mechanisms spanning aneuploidy, sexual/parasexual reproduction, mutators, Hsp90, and even prions. The pathogenic fungus Mucor circinelloides grows as a hyphae aerobically, but as a yeast in anaerobic conditions or in the presence of the immunosuppressive drug FK506. The antifungal activity of FK506 is exerted in complex with FKBP12, a protein folding enzyme conserved throughout eukaryotes. The FKBP12- FK506 complex inhibits the protein phosphatase calcineurin and thereby blocks hyphal growth of M. circinelloides. Continued exposure to FK506 yields resistant isolates, which exhibit hyphal growth emerging from the yeast colony. Some isolates harbor a variety of Mendelian mutations in the fkbA gene that encodes FKBP12 or the calcineurin genes, conferring stable drug resistance. However, other isolates harbor no mutations in the target genes. These unusual epimutant isolates also revert frequently within several generations of vegetative growth in drug-free media and are restored to wild-type (yeast growth in the presence of FK506). Northern and Western analyses revealed a loss of fkbA mRNA and FKBP12 protein in the epimutants. High-throughput sequencing and Northern blot also detected sRNA generated from fkbA in the epimutant strains, revealing a new role for RNAi in the development of transient, reversible resistance to an antifungal drug treatment. RNAi activation involves generation of a double-stranded RNA trigger intermediate using the fkbA mature mRNA as template. Our results reveal a novel epigenetic RNAi-based epimutation mechanism controlling phenotypic plasticity in fungi.

304 SHEDDING LIGHT ON PYRONEMA CONFLUENS: A MODEL ORGANISM FOR SEXUAL DEVELOPMENT, LIGHT SIGNALING AND CIRCADIAN CLOCK?

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In recent years, 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 transcriptomes of the Pezizomycete Pyronema confluens. Now the 50 Mb sequenced genome, which encodes 13369 predicted protein-coding genes, is available (Traeger et al., 2013). 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. qRT-PCR 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. 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. Moreover we analysed if transcription of the major clock gene Pcfrq is regulated in a circadian manner. The results indicate circadian rhythmicity of Pcfrq under standard conditions. Continuing experiments will investigate if this rhythmicity is stable at different temperatures. Taken together the data suggest that P. confluens could be not only used as a model organism for fruiting body development, like it was used in the late 19th and early 20th century, but also as model for light signaling and circadian clock studies in fungi.

305 SIGNALLING PROCESS AND ACTIVATION OF SLTA, A TRANSCRIPTION FACTOR INVOLVED IN CATION/ALKALINITY STRESS RESPONSE

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Many microorganisms, including fungi, have developed genetic strategies to survive to environment stresses, such as variations in pH, temperature, nutrient availability, reactive oxygen or diverse saline concentrations. In the filamentous fungus and model organism Aspergillus nidulans, tolerance to an alkaline ambient pH requires the activities of three high hierarchy 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. Here we present our latest results in the signalling process and the activation of SltA, in addition to its transcriptional regulatory activity. Signalling of SltA requires its proteolytic processing, an extreme post-translational modification mechanism that shares with PacC. To understand how SltA is signalised and 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 lethal 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. sltB gene encodes for a protein of 1272 amino acids, also specific to filamentous fungi, with two putative functional domains. The N-terminal pseudokinase domain is involved in the proteolysis of native SltA 78 kDa to a 32 kDa form. A second domain is similar to a trypsin-like protease, and our data suggest that SltB is auto-proteolysed through this protease activity. Finally, we have determined that SltB is expressed in a SltA dependent manner. A model of regulation of SltA through SltB activity is presented for this novel cation/alkaline pH regulatory pathway in filamentous fungi.

306 STUDYING THE EXPRESSION OF TERPENE CYCLASE GENES, KEY ENZYMES IN THE SECONDARY METABOLISM OF THE PHYTOPATHOGENIC FUNGUS BOTRYTIS CINEREA

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Botrytis cinerea is a phytopathogenic fungus that shows a wide variety of mechanism for infecting plant material. B. cinerea has a complex secondary metabolism, allowing infecting more than two hundreds plant species. Until date, several families of cell wall degrading enzymes and toxins have been described for this fungus1, including two important families of toxins: the sesquiterpene botrydial (and related compounds), and botcinic acid and its derivated2,3. In the genome of this fungus, six putative sesquiterpene cyclases and three diterpene cyclases genes have been annotated1, but not all of them have been totally characterized yet. It is known that STC1 encodes for a sesquiterpene synthase (BcBOT2) which plays a crucial role in the biosynthesis pathway of toxin botrydial3. The biological role of the other STC and DTC genes is currently being elucidated.
This study presents the analysis of expression profiles shown by quantitative reverse transcription-PCR of the terpene gene family (STC and DTC) encoding sesquiterpene cyclase and diterpene enzymes in B. cinerea. We used an OSMAC approach (one strain many compounds) trying to stimulate the secondary metabolisms of this fungus, and we described the evolution of the gene expression profiles during several days of fermentation.

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307 THE BINDINGS SITE OF A TRANSCRIPTION FACTOR ENCODED ON BOTH CORE AND ACCESSORY GENOMES OF FUSARIUM OXYSPORUM F.SP. LYCOPERSICI IS ENRICHED IN THE PROMOTERS OF EFFECTOR GENES

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In the tomato pathogen Fusarium oxysporum f. sp. lycopersici, most known effector genes reside on an accessory chromosome that can be exchanged between strains through horizontal transfer. Expression of these effector genes is massively upregulated upon infection, but the mechanism by which this is regulated is unknown. In addition to effector genes, the accessory chromosome also encodes 10 predicted transcription factor. Among these are three homologs of FTF1 (Fusarium transcription factor 1, Reyes-Dominguez ea 2012) and one copy of EBR2 (Enhanced Branching 2, Jonkers ea 2013). Of all transcription factor genes on the accessory chromosome, except one, there is a homolog in the core genome. We wanted to know i) whether the transcription factors on the accessory chromosome affect effector gene expression, ii) what could be their (other) targets, and iii) whether they have a different function compared to their core genome homologs. Of five of the transcription factor genes on the accessory chromosome, overexpression transformants were generated, and for one of those, FOXG14275, overexpression enhanced expression of the effector gene SIX1. To identify the transcription factor binding sites, the same five transcription factors, an FTF1 homolog from another accessory region, and all core genome homologs were fused to an N-terminal GST-tag, expressed in E.coli and hybridized on oligo-DNA arrays. From this, binding sites could be reliably inferred for four of the transcription factors on the accessory chromosome and three of the homologs on the core genome. The binding site for core and accessory homologs is in all cases highly similar or identical. Remarkably, the DNA bindingsite for FTF1 (accessory) and FTF2 (core) corresponds to a motif found earlier to be enriched in effector (SIX) gene promoters (Schmidt ea 2013).

Reyes-Dominguez ea, FGB, 2012
Jonkers et al, Environ. Microbiol. 2013
Schmidt et al, BMC Genomics, 2013

308 THE CCAAT-BINDING-COMPLEX MEDIATES IRON REGULATION IN ASPERGILLUS FUMIGATUS

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Iron is essential for a wide range of cellular processes but its excess is toxic. Therefore, microorganisms evolved fine-tuned mechanisms for uptake and storage of iron, to sustain iron homeostasis. In the opportunistic fungal pathogen Aspergillus fumigatus, the bZIP-type transcription factor HapX mediates adaption to iron starvation by activating siderophore biosynthesis and repressing iron-dependent pathways. HapX-deficiency attenuates the virulence of A. fumigatus underlining the importance of adaptation to iron starvation in pathogenicity. The HapX N-terminal amino acid sequence predicts interaction with the DNA-binding, heterotrimeric CCAAT-binding complex (CBC), which is conserved in all eukaryotes and believed to co-regulate up to 30% of all genes. Here, we characterized the role of the CBC in iron regulation of A. fumigatus by analysis of the phenotypic consequences of genetic inactivation of the CBC subunit HapC. HapC-deficiency was deleterious during both iron starvation as well as iron sufficiency, demonstrating iron-independent regulatory functions of the CBC. In contrast, HapX is important during iron starvation only. Whole transcriptome sequencing during iron starvation conditions revealed that HapC-deficiency upregulates 562 genes, of which 214 (38 %) are also upregulated by HapX-deficiency, and down-regulates 635 genes, of which 397 (60 %) are also downregulated by HapX-deficiency. Deficiency in both HapX and CBC derepressed genes involved in iron-consuming pathways, but decreased production of siderophores as well as certain secondary metabolites. Inhibition of reductive iron assimilation by ferrous iron chelation blocked colony formation of both HapC-deficient and HapX-deficient conidia. Moreover, inactivation of HapC was epistatic to HapX-deficiency. Taken together, these data indicate that the CBC mediates both the activating and repressing functions of the iron-regulatory transcription factor HapX. The central role of the CBC in environmental adaptation is underlined by HapC-deficiency rendering A. fumigatus avirulent in a murine model of aspergillosis.

309 THE CONSERVED MAP KINASE MPKB AFFECTS DEVELOPMENTAL PROCESSES BUT NOT SECONDARY METABOLISM IN ASPERGILLUS FLAVUS

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In eukaryotes, MAP kinase pathways play important roles in regulation of growth, development, and various stress responses. To characterize the function of MAP kinase in an important pathogenic and toxigenic fungus Aspergillus flavus, the AflmpkB gene (AFL2G_02589), an orthologue of the yeast fus3 gene, was deleted. In Aspergillus nidulans, previous studies revealed that MpkB positively regulates the sexual and asexual differentiation as well as secondary metabolite production. In this study, deletion of AflmpkB resulted in no mycelial growth change, while the conidial production was reduced about 60% comparing to the wild-type. Moreover, the mutant produced immature and abnormal conidiophores such as vesicular dome-immaturity in the conidiophore head, decreased number of the phialides and very short stalks, although expression of the brlA gene, a key regulator of conidiation, was up-regulated in the mutant. Also, AflmpkB couldnt produce any sclerotia, suggesting that the AflmpkB gene is important to conidiophore and sclerotia development. However, AflmpkB mutants produced normal level of aflatoxin B1. Taking together, A. flavus MpkB plays a positive regulatory role in the production of the conidiation and the sclerotia but not in the production of the secondary metabolite such as aflatoxin B1.
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310 THE FUNCTIONAL CHARACTERIZATION OF THE NEUROSPORA CRASSA HAC-1 TRANSCRIPTION FACTOR REVEALS A CRUCIAL ROLE FOR THE UNFOLDED PROTEIN RESPONSE IN PLANT CELL WALL DECONSTRUCTION

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High secretion capacity in filamentous fungi requires an extremely efficient system for protein synthesis, folding and transport. When the folding capacity of the endoplasmic reticulum (ER) is exceeded, a pathway known as the unfolded protein response (UPR) is triggered allowing cells to mitigate and cope with this stress. In yeast, this pathway relies on the transcription factor HAC1, which mediates the up-regulation of several genes required under these stressful conditions. In this work, we identify and characterize the HAC1 orthologue in the filamentous fungus Neurospora crassa. We show that hac-1 mRNA undergoes an ER stress-dependent unconventional splicing reaction, which in Neurospora removes a 23 nt intron, leading to a change in the open reading frame and the production of a functional transcription factor. By disrupting hac-1, we determined this gene to be crucial for activating UPR and for proper growth in the presence of ER stress-inducing chemical agents. Neurospora is naturally found growing on dead plant material and it has become a model organism for plant cell wall deconstruction studies. Notably, we found that Hac-1 is necessary for growth on cellulose or Avicel (crystalline cellulose). Further characterization of this phenomenon revealed that it is due, in part, to a drastic reduction in the levels of secreted proteins. Unexpectedly, however, we also observed that the expression of cellulolytic genes is partly impaired in the hac-1 KO strain, which correlates with poor induction of key transcription factors (CLR) required for deconstruction of cellulose. Nevertheless, growth of hac-1 deficient strains on xylan, cellobiose or glucose is not impaired, which can be partially explained by less challenging protein secretion demands. The characterization of this signaling pathway in Neurospora will help in the study of fungal plant cell wall deconstruction, highlighting UPR as relevant process that can be further manipulated with important biotechnological applications. FONDECYT1131030, MN-FISB NC120043.

311 THE GATA-FACTOR AREB IN FUSARIUM FUJIKUROI ACTS BOTH AS ACTIVATOR AND REPRESSOR OF NITROGEN-REGULATED GENE SETS

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The phytopathogenic ascomycete Fusarium fujikuroi produces a broad spectrum of interesting secondary metabolites, including the agriculturally applied phytohormones gibberellins (GAs), which stimulate growth and developmental processes in plants. Biosynthesis of GAs and other secondary metabolites is repressed by high concentrations of nitrogen. This Nitrogen Metabolite Repression (NMR) of GAs is mediated by the global GATA transcription factors AreA and AreB. While the role of AreA as transcriptional activator of many NMR-controlled genes has been shown in several fungi, the role of AreB is not well understood. Here, we report the functional characterization of AreB and its interplay with AreA in F. fujikuroi. The areB locus produces three different transcripts that each code for functional proteins fully complementing the areB deletion mutant regarding growth and GA production. However, under nitrogen repression, the AreB isoforms differ in subcellular localization indicating distinct functions under these conditions. In addition, AreA and two isoforms of AreB co-localize in the nucleus under nitrogen starvation, but their nuclear localization disappears under nitrogen sufficient conditions. Using a bimolecular fluorescence complementation (BiFC) approach we showed for the first time that one of the AreB isoforms interacts with AreA under nitrogen starvation conditions. Furthermore, AreA activates the expression of areB while AreB does not affect the expression of areA. Cross-species complementation revealed that some AreB functions are retained between F. fujikuroi and Aspergillus nidulans while others have diverged. In contrast to A. nidulans, where AreB was postulated to function as a negative counterpart of AreA, AreB can act both as repressor (e.g. for amino acid permeases) and activator (e.g. for GA biosynthetic genes) of transcription in F. fujikuroi.

312 THE GENE CARC ENCODES A GERANYLGERANYL PYROPHOSPHATE SYNTHASE IN PHYCOMYCES BLAKESLEEANUS

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Carotene biosynthesis in the fungus Phycomyces blakesleeanus is regulated by light, sexual development, and the presence of certain chemicals. Blue light and sexual stimulation increase in parallel the content of carotene and the content of mRNAs for genes carRA and carB, dedicated to the synthesis of β-carotene from geranylgeranyl pyrophosphate (GGPP). However, the gene for the biosynthesis of GGPP, GGPP synthase (GGPPS), has not been characterized in Phycomyces. GGPPS is duplicated in the Phycomyces genome (ggsA and ggsB). The proteins have the typical structure and domains of GGPPS but each gene has different regulation. The ggsA gene is activated by light and repressed by sexual stimulation while ggsB shows the opposite regulation as it is repressed by light and activated by sexual interaction suggesting opposite roles in Phycomyces biology. Mutants in the carC gene are whitish and the carC product has been proposed to act as a regulator of the β-carotene pathway. However, we have found that all the strains with mutations in carC have mutations in the ggsA gene, suggesting that the ggsA gene is carC. The mutations alter the splicing sites of introns 1 or 5, or change serine 92 for phenylalanine. Strains with carC mutations obtained after genetic crosses have inherited the mutations in the ggsA gene. The protein CarC provides GGPP synthase activity to E. coli which confirms that CarC is a GGPP synthase. The residual beta-carotene accumulation observed in carC strains could be due to the residual activity of the mutant CarC proteins, or to the activity of the other GGPPS encoded by ggsB.

313 THE GENES INVOLVED IN OCHRATOXIN A BIOSYNTHETIC PATHWAY ARE LOCATED IN A CLUSTER IN ASPERGILLUS STEYNII

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The ability of Aspergillus steynii to produce ochratoxin A (OTA) has been widely studied in our group. This species was revealed as the main OTA producing-species included in Aspergillus section Circumdati. There is little information on the genes involved in OTA biosynthesis by Aspergillus species and only a few gene sequences have been reported, such as those encoding polyketide synthases (PKS) in A. westerdijkiae and A. carbonarius, a non-ribosomal peptide synthetase (NRPS) in A. carbonarius and a cytochrome p450 monooxygenase (P450) in A. westerdijkiae. In this work, we have unravelled three contiguous genes putatively involved in the OTA biosynthetic pathway in A. steynii. In previous studies we had obtained the genomic complete sequence of a gene (p450ste), encoding a P450 using 5'-RACE technique. Subsequently, we followed a genome walking approach to obtain the two flanking sequences. After 18 rounds we obtained a region of more than 20 kb of fungal genome. The 2.5 kb region upstream p450ste, revealed no similarities with any sequence available on databases. The 17 kb downstream sequenced region uncovered two putative genes, nrpsste and pksste, encoding a NRPS and a PKS, respectively. These genomic sequences were compared with the corresponding cDNA obtained from RNA isolated from OTA-producing cultures. These two genes showed high identity when compared with the genome of A. niger. However, the identity was lower than 50% when compared with those putative homologous genes reported as involved in OTA biosynthesis in A. westerdijkiae and A. carbonarius. The expression of the three A. steynii genes was studied by real-time RT-PCR using new specific protocols described in this work. In all cases, OTA-producing strains consistently expressed p450ste, nrpsste and pksste and their expression patterns correlated with OTA production. No expression was detected in OTA non-producing strains. Additionally, Agrobacterium tumefaciens mediated transformation using OSCAR technology was developed to construct a deletion mutant of p450ste in A. steynii. This mutant showed a modified ability of OTA production. In conclusion, three contiguous genes involved in OTA biosynthesis have been described in A. steynii. This the first report of an OTA biosynthetic cluster in Aspergillus species.

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314 THE ROLE OF ATFA IN CONIDIA STRESS TOLERANCE AND ITS REGULATORY PATHWAY IN PATHOGENIC FUNGUS ASPERGILLUS FUMIGATUS

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Conidia are reproductive structures that are important for both distribution and survival for fungi. In general, conidia are tolerant structures of abiotic stresses including desiccation, high temperature, and oxidative stress. One crucial mechanism for the stress tolerance is accumulation of trehalose in conidia. However, studies on molecular mechanisms underlying the trehalose regulation were limited in A. fumigatus. In the present study, we put our focus on the AtfA transcriptional regulator, which was thought to be regulated by SakA MAPK cascade. In several filamentous fungi, deletion of the atfA gene led to decreased levels of stress tolerance in conidia. We investigated if A. fumigatus AtfA played a major role in conidial stress tolerance through a regulation of trehalose biosynthesis. We first constructed an atfA gene deletion strain (ΔatfA) and the complemented strain. Conidia from ΔatfA showed a marked sensitivity to heat stress (55°C, 15min) and oxidative stress (200mM H_2O_2, 15min) treatment. In the control strain, expressions of the catalase gene catA and the dehydlyn-like protein gene dprA, both of which are related to oxidative stress response in conidia, were induced during asexual development stage, but not in ΔatfA. This result suggested that the sensitivity to oxidative stress in ΔatfA conidia was caused by lack of CatA and DprA. The amount of trehalose in the ΔatfA conidia was significantly lower than that in the control strain. However, there was no difference in the expression of tpsA,B,C,D genes, which encode proteins responsible for the crucial step of trehalose biosynthesis, between ΔatfA and the control strain during asexual development. This unexpected result presented a possibility that when conidia were produced, trehalose was biosynthesized via unknown pathway and AtfA might regulate it. We then asked if upstream regulators of AtfA played a certain role in the conidia stress tolerance. The deletion mutants of SskA response regulator and PbsB MAPKK, which are upstream components of SakA, showed decreased levels of trehalose accumulation in conidia and tolerance to heat stress. However, deletion mutant of the SakA MAPK showed normal phenotypes. We then constructed a double deletion mutant of SakA and MpkC, a paralog of the SakA MAPK, and the sakA/mpkC double mutant showed the phenotypes similar to sskA or pbsB mutants. This suggested that SakA and MpkC redundantly or cooperatively regulate AtfA function during conidiation.

315 THE ROLE OF CARBON STARVATION IN INDUCTION OF HYDROLYTIC ENZYMES DURING EXPOSURE OF ASPERGILLUS NIGER TO WHEAT STRAW

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The saccharification of lignocellulosic biomass for the production of second generation biofuels requires cheaper and more effective enzyme mixtures. Understanding the mechanism behind the efficient degradative response of saprophytic fungi generates clues for the improvement of fungal enzyme mixtures. We recently identified the hydrolytic enzymes and accessory proteins produced by the industrially important fungus Aspergillus niger in response to wheat straw. Importantly, induction of transcripts encoding hydrolytic enzymes was sequential (Delmas et al., 2012). Carbon catabolite derepression was responsible for the initial induction of three genes that were induced early on straw. We presented a model of how fungi respond to lignocellulose, whereby starvation induced enzymes may produce a molecule that induces expression of the full suite of hydrolytic enzymes required for deconstruction of a particular substrate. To further elucidate this mechanism, we now investigated the early events in the response of A. niger to straw and carbon starvation using genome-wide RNA sequencing. The influence of carbon starvation during lignocellulose degradation was demonstrated by a substantial overlap in transcripts encoding carbohydrate active enzymes induced during early carbon starvation and during early exposure to straw. The majority of these enzymes are predicted to be active on plant-derived polymers. Filtrate from carbon starved cultures indeed contained enzymes active on plant-derived polymers. Our current investigation of the activities of these enzymes towards simple and complex plant polymers, as well as identification of the products formed by these activities, could yield information on transcriptional inducers of genes that encode these enzymes.

316 THE TRANSCRIPTIONAL ACTIVATORS ARAR AND XLNR FROM ASPERGILLUS NIGER REGULATE EXPRESSION OF PENTOSE CATABOLIC AND PENTOSE PHOSPHATE PATHWAY GENES

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The pentose catabolic pathway (PCP) and the pentose phosphate pathway (PPP) are required for the conversion of pentose sugars in fungi and are linked via xylulose-5-phosphate. Previously, it was shown that the D-xylose and L-arabinose release, as well as the PCP is regulated by the transcriptional activators XlnR and AraR in Aspergillus niger. In this study we assessed whether XlnR and AraR also regulate the PPP to evaluate to how deep into central carbion metabolism the influence of these regulator extents. Expression of two PPP genes, rpiA and talB, was reduced in the ΔaraR/ΔxlnR strain and increased in the xkiA1 mutant on D-xylose and/or L-arabinose, similar to what was found for PCP genes. This expression profile indicates that at least these two genes are under direct regulation of AraR and/or XlnR. It also demonstrates the strong regulatory connection between sugar release and sugar catabolism, that continues into the central part of carbon catabolism in Aspergillus.

317 THE TRANSCRIPTIONAL ACTIVATORS XLNR AND ARAR AFFECT PHYSIOLOGY OF ASPERGILLUS NIGER ON SOLID CULTURES WITH WHEAT BRAN AS CARBON SOURCE

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In nature Aspergillus niger degrades plant material using extracellular enzymes. One of the main regulatory factors controlling the production of these enzymes is the (hemi-)cellulolytic regulator XlnR. In the presence of D-xylose XlnR activates genes involved in cellulose and hemicellulose degradation, as well as some genes of the pentose catabolic pathway. We have also identified de L-arabinose responsive transcriptional activator AraR in A. niger, which controls activation of 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. In this study we analyze the influence of XlnR and AraR in A. niger during growth on solid media with wheat bran as carbon source. A. niger wild type and disruptants for both transcriptional activators (AraR, XlnR) were analyzed for enzyme activity, gene expression and protein production in 5 concentric zones of the colony from the centre (oldest part of the colony) to the periphery (youngest part of the colony).
Clear differences were visible between the mutants and the wild type, in particular with respect to genes involved in plant biomass degradation.

318 THE VELVET COMPLEX PROTEIN, VEA, REGULATES PRODUCTION OF NOVEL ASPERGILLUS FLAVUS SECONDARY METABOLITES INVOLVED IN MORPHOGENESIS AND SURVIVAL.

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Analysis of the Aspergillus flavus genome has identified 55 putative secondary metabolic gene clusters predicted to encode metabolites derived from polyketide synthases (PKSs), non-ribosomal peptide synthetases (NRPSs), hybrid PKS-NRPS and prenyltransferases (PTRs). Very few of the metabolites capable of being produced by these gene clusters have been characterized. By whole genome microarray transcript profiling we confirmed that expression of a number of genes associated with these putative secondary metabolic gene clusters in A. flavus are regulated by veA, a global regulator of fungal development and secondary metabolism. Focusing on two of these veA-dependent gene clusters, 23 and 27, we created gene deletion and overexpression strains and used comparative metabolomics to identify these gene cluster-associated metabolites. Cluster 27 harbors a PKS involved in the production of the anthraquinone, asparasone A (358 Da), as well as three related anthraquinones of molecular weight 374, 340, and 316 Da. The 316 Da anthraquinone is particularly interesting because it is most likely formed by incorporation of 7 malonyl-CoA units rather than the 8 units required for biosynthesis of asparasone A. Inactivation of the cluster 27 pks (pks27) affected sclerotial pigmentation but not conidial pigmentation. Sclerotia produced by pks27 mutants were significantly more susceptible to insect predation and damage by ultraviolet light and heat than were the sclerotia produced by the isogenic control. Cluster 23 is one of only two gene clusters in A. flavus that harbor a hybrid PKS-NRPS, the other being the cyclopiazonic acid (CPA) cluster. While no significant differences in phenotype or metabolic profiles were observed in a pks-nrps deletion mutant compared to the isogenic control strain, overexpression of one of three cluster 23 $Zn^{(2)}$-$Cys^{(6)}$ transcription factors resulted in transformants producing a novel 335 Da pyridone, subsequently identified as demethoxyleporin A by mass spectral and nmr analysis and by comparison to leporin A from Aspergillus leporis. Like leporin A, demethoxyleporin inhibits insect feeding. Our results show that biosynthesis of metabolites required for protection of A. flavus sclerotia, like the production of the sclerotia themselves, is inhibited in veA mutants, suggesting that a major role of VeA is its affect on sclerotial development and survival.

319 TRACKING THE BEST REFERENCE GENES FOR QPCR DATA NORMALISATION IN FUNGI

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As part of our project that aimed at deciphering the expression of glycoside hydrolases-encoding genes in the industrial relevant filamentous fungus, Penicillium funiculosum, we searched for putative reference genes for reliable qPCR data normalisation in a wide variety of samples of interest (~30 conditions covering stress, nature of carbon sources or development stages). We first used a RNA-seq based transcriptomics study of P. funiculosum exposed to glucose (control) and wheat straw (induction) to identify stable genes. A dozen of functionally unrelated candidates were then thoroughly analysed through qPCR assays and certified by GeNorm as being stable in our 30 P. funiculosum samples. For a more general contribution of this work, we then checked whether these reference genes could be applicable more widely for qPCR data normalisation in other model fungi relevant in biotechnology, human or animal health, as well as in agronomy as pathogenic or symbiotic organisms. To this end, another dozen of genes such as the actin or the glyceraldehyde-3P-dehydrogenase, which were frequently used for normalisation purposes, was included in this study. The behaviour of all of these genes was then analysed in nearly 100 RNA-seq samples available in the public, GEO database, which included stress and nutritional conditions, differentiation stages or hosts interactions. Even for our selection of reference genes, we could find exceptionally high fold changes between the control and few samples of interest (outliers), reminding about the necessity to formally check the stability of selected references before their use. However, when considering the whole set of RNA-seq samples, half of our selected reference genes but none of the classical ones, followed normal distribution of the logarithm of fold-change values, and successfully passed the statistical tests for mean and median close to zero (fold change equal to one). We therefore propose that this core subset of reference genes shall be used as universal reference genes for expression normalization of qPCR data, which will not preclude their validation in the samples/fungi of interest.

320 TRANSCRIPTOME OF THE WHEAT PATHOGEN ZYMOSEPTORIA TRITICI DURING A COMPLETE INFECTION CYCLE

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Zymoseptoria tritici (aka Mycosphaerella graminicola) is a hemibiotrophic fungus belonging to the Dothideomycetes, the largest class of ascomycetes that includes many plant pathogens. Like other hemibiotrophic pathogens Z. tritici uses different strategies for obtaining nutrition during its life cycle. For the first 10 days post inoculation (dpi) the pathogen survives as a biotroph without causing visible symptoms. The necrotrophic phase lasts until the affected plant cells have died. Depending on the strain-cultivar interaction, complete plant cell death occurs from 18 to 20 days after penetration. Z. tritici concludes its life cycle by surviving as a saprotroph on dead leaves for several months. Thus Z. tritici presents a powerful system to study host-pathogen interactions during different stages of disease development. Illumina sequencing technology was used to analyze changes in transcription during the complete infection cycle of Z. tritici on wheat across three biological replicates. Total RNA was extracted from inoculated plants at seven time points (3-, 7-, 11-, 13-, 14-, 21- and 56- dpi). The expression profile of 10,251 genes was analyzed. About 14% and 34% of the genes showed statistically significant differences in expression from the biotrophic to necrotrophic and from the necrotrophic to saprotrophic stages of infection, respectively. We identified five novel putative effector genes. Putative effector genes were preferentially transcribed at 11 dpi during the transition between biotrophy and necrotrophy. We also investigated the expression of gene clusters involved in the biosynthesis of non-proteinaceous metabolites. Two clusters of genes (PKS4 and PKS5-related genes) showed expression patterns similar to the putative effectors. A putative effector, PKS5 and a hemicellulase under diversifying selection were further characterized, using Agrobacterium-mediated transformation to determine their role in pathogenicity. From the functional analyses of the knockout strains we showed that eliminating the putative effector delayed the onset of necrosis by 48h. This study shows how next generation sequencing techniques can be used to screen the transcriptome during plant-pathogen interactions to identify novel virulence factors for further functional analyses.

321 TRANSCRIPTOMIC RESPONSE OF THE HUMAN PATHOGEN ASPERGILLUS FUMIGATUS TO THE PRESENCE OF 1,3-B-GLUCAN SYNTHASE INHIBITOR ANIDULAFUNGINA

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The fungal cell wall is a complex structure composed typically of chitin, 1,3-β- and 1,6-β-glucan, mannan and proteins. The cell wall integrity (CWI) signaling pathway regulates shape and biosynthesis of the cell wall, and CWI is essential for virulence, viability of fungal pathogens and is an important antifungal drug target. For a comprehensive evaluation of the CWI signaling pathway genes we performed a large-scale analysis of gene expression in Aspergillus fumigatus using microarray hybridization approach. Mycelia were incubated with anidulafungina, an echinocandin class member inhibitor of 1,3-β–glucan synthase. By comparing the expression of genes between the addition of anidulafungina and the control without the antifungi, we identified 920 genes differentialy expressed in the wild-type strain. To validate the expression of some of these genes during exposure to anidulafungina, we analyzed 08 genes showing higher expression in the presence of anidulafungina by quantitative PCR. In the microarray analysis we have observed the change in the mRNA expression level of genes involved in a variety of cell function, such as metabolism, cell rescue, defense and virulence, cellular transport, transcription, development, and cell cycle and DNA processing. masA, a gene that encodes a protein with unknown function and a signal peptide is among the highly expressed genes in the presence of anidulafungina and was also found as highly expressed in a transcriptome analysis of A. fumigatus exposed to voriconazole, an antifungal agent that blocks the ergosterol biosynthesis pathway by inhibiting the enzyme 14-α-demethylase. MasA ortholog in Magnaporthe grisea is involved in the apressoria formation. Despite A. fumigatus has no apressoria formation, masA gene is in its genoma and highly expressed in the presence of cell stress induced by antifungal agents that disturb the fungal cell wall and membrane. In A. fumigatus masA has no influence on virulence and additional work is necessary to elucidate the roles of this protein to this filamentous fungus.
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322 TROJAN HORSE STRATEGY AND FAIR TRADE AMONG SYMBIOSES: HOW ONE FUNGAL SPECIES CAN INVADE THOUSANDS OF PLANT SPECIES

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Arbuscular Mycorrhizal (AM) symbiosis, the oldest mutualistic plant-fungal symbiosis, concerns 80% of land plants. AM fungi (Glomeromycota) can indeed associate with non-vascular plants (liverworts, hornworts), early diverging vascular plants (ferns) or seed plants. Following the presymbiotic molecular dialog, the mycelium invades roots/thallus and grows in the cortical/parenchymal tissues where it develops intercellular highly branched hyphae - arbuscules – allowing bidirectional nutrient exchanges. The fungus forms a network of extraradical mycelium (ERM) in the soil that recruits water, nitrogen and phosphorus. Nutrients are efficiently taken up by high and low affinity transporters, then translocated to the Intra Radical Mycelium (IRM) where they will be exchanged in arbuscules against plant hexoses. The recent release of the gene repertoire of the AM fungal model species Rhizophagus irregularis (Tisserant et al., 2012; Tisserant, Malbreil et al., 2013) opens the way toward the description of the fungal processes involved in the establishment of the AM symbiosis. We addressed the question of host invasion by Rhizophagus irregularis. Like other AMF, R. irregularis has the ability to invade all mycotrophic plant species, implying different tissues to colonize (either thallus or true roots). Based on RNAseq approaches, we compare the fungal transcriptomes in symbiotic tissues from three host species (one liverwort and two flowering plants, a legume and a grass). Comparing IRM/ERM gene expression, we defined a set of specifically or highly up-regulated genes shared in the different interactions that can be hypothesized as the common fungal symbiotic toolkit. It includes nutrient transporters, metabolic genes and a panel of secreted proteins potentially involved in host manipulation. A part of those symbiosis-specific genes has been chosen for qPCR validation in an extended number of hosts, covering the green lineage. It will give the first glimpse of the gene set involved in symbiosis establishment regardless of the host colonized.

323 WHAT MAKES RUT-C30 AN APPEALING PARENT OF INDUSTRY STRAINS?

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Trichoderma reesei is used for industry scale production of enzymes, in particular cellulases of which the main one is the cellobiohydrolase I (encoding by cbh1). The ancestor of most currently used industry strains is Rut-C30, which was isolated after two rounds of mutagenesis by screening for high secretion of cellulases and was consequently described as cellulase hyperproducing strain. Later it was found that Rut-C30 bears just a truncated version of the Carbon catabolite repressor 1 (Cre 1)-encoding gene, which is responsible for repression of both, xylanase- and cellulase-encoding genes. This finding was assigned as a major reason for the Rut-C30 phenotype. After having genomes of the wild-type QM6a and Rut-C30 at disposition, it became clear that the two strains differed genetically in many ways. One striking finding was 85kb region that is missing in Rut-C30. However, it remained unclear what exactly enhances cellulase production in Rut-C30. During recent investigations we found that carbon catabolite repression is only partly released in Rut-C30. Interestingly, the Cre1-sites within the upstream regulatory region (URR) of cbh1 showed stronger protection/ hypersensitivity signals in Rut-C30 than in the wild-type according to in vivo footprinting analyses. This prompted us to investigate if the remaining part of cre1 is transcribed into mRNA and further translated into a protein, and if this protein is still able to bind the URR of cbh1. The native Cre1 was replaced by the Cre1-truncated version of Rut-C30 in the wild-type. Together with a wild-type strain bearing the above-mentioned 85kb-deletion and another bearing a cre1 deletion, a comparative analysis of the cellulase expressing phenotype was performed. As Cre1 was suspected to influence the chromatin packaging, we also investigated the DNA accessibility of the cbh1 URR in these strains.

324 A FORWARD GENETICS APPROACH FOR THE IDENTIFICATION OF PATHOGENICITY FACTORS IN PENICILLIUM DIGITATUM

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Penicillium digitatum is the main postharvest pathogen of citrus fruit all over the world, causing green mould on harvested fruit. It is a specialized necrotrpoh pathogen that only infects citrus fruit through wounds inflicted during fruit harvesting and handling. Despite the high economic impact of this fungus for the fresh fruit industry there is almost a complete lack of knowledge on its pathogenicity / virulence factors. The recently sequenced genome offers a first glimpse into the vast array of genes putatively involved in pathogenicity. In the present work, we have undertaken a forward genetic approach in order to identify pathogenicity factors through the generation of a collection of T-DNA tagged mutants using Agrobacterium tumefaciens-mediated transformation. We have constructed a binary plasmid containing the gene encoding a modified green fluorescent protein (sGFP) under the control of the P. digitatum tef (translation elongation factor) gene promoter. In order to facilitate the rescue of the inserted T-DNA as a plasmid, we included in the T-DNA the origin of replication and the ampicillin resistant marker from Bluescript. A collection of 4000 transformants resistant to hygromycin were obtained. Fluorescence levels were highly variable among these transformants. The average number of T-DNA fragments inserted in the genome was determined by qPCR in a subset of transformants. In 65% of them there was only one T-DNA copy integrated in the genome, which facilitates downstream analysis of selected transformants. Pathogenicity assays were conducted for 1920 of them using in vivo inoculation of citrus fruits. After two rounds of infection assays nine transformants showed a reduction in virulence. In all of them there was a single T-DNA integration. As preliminary experiments for recovering the T-DNA as a plasmid had limited success, we decided to sequence the genome of those nine transformants in order to locate the sites of T-DNA integration. Equal amounts of DNA from each transformant were pooled and sequenced using Illumina HiSeq200 2 x 100 bp pair-end sequencing. Mapping the reads against the T-DNA and the genome of P. digitatum allowed a precise localization of the T-DNA integration site as well as the determination recombination events that occurred during integration.

325 A METABOLOMIC APPROACH TO STUDY THE RHIZODEPOSITION IN THE TRITROPHIC INTERACTION: TOMATO, POCHONIA CLAMYDOSPORIA AND MELOIDOGYNE JAVANICA

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A combined chemometrics-metabolomics approach (EEM fluorescence spectroscopy, NMR and HPLC-MS) was used to analyse the rhizodeposition of the tri-trophic system: tomato, the plant-parasitic nematode Meloidogyne javanica and the nematode-egg parasitic fungus Pochonia chlamydosporia. Exudates from M. javanica roots were sampled at root penetration (early) and gall development (late). EMM indicated that late root exudates from M. javanica treatments contained more aromatic amino acid compounds than the rest (control, P. chlamydosporia or P. chlamydosporia and M. javanica). 1HNMR showed that organic acids (acetate, lactate, malate and succinate) and two (peaks no. 22 and 23) unassigned aromatic compounds (7.68 and 8.11 ppm chemical shifts, respectively) were the most relevant metabolites in root exudates. Robust PCA grouped early exudates for nematode (PC1) or fungus presence (PC3). PCA found (PC1, 73.31%) increased acetate and reduced lactate and an unassigned peak no. 22 characteristic of M. javanica root exudates resulting from nematode invasion and feeding. An increase of peak no. 22 (PC3, 4.82%) characteristic of P. chlamydosporia exudates could be a plant “primer” defence. In late ones in PC3 (8.73%) the presence of nematode grouped the samples. HPLC-MS determined rhizosphere fingerprints of 16 (early) and 25 (late exudates) m/z signals, respectively. Late signals were exclusive from M. javanica exudates confirming EEM and 1HNMR results. A 235 m/z signal reduced in M. javanica root exudates (early and late) could be repressed a plant defense. This metabolomic approach and other rhizosphere –omics studies could help to improve plant growth and reduce nematode damage sustainably.

326 A NOVEL SIAMYCIN DERIVATIVE POTENTIATES THE ANTIFUNGAL ACTIVITY OF CASPOFUNGIN AGAINST ASPERGILLUS FUMIGATUS BY BLOCKING THE HOG SALVAGE PATHWAY

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Aspergillus fumigatus is one of the most important opportunistic fungal pathogens, which causes life-threatening invasive aspergillosis with high mortality rates. The increase of these fatal fungal infections, coupled with the emergence of antifungal drug resistant strains, makes it an urgent task to search for new therapeutic strategies. A drug applied to therapy is the echinocandin derivative caspofungin (CAS). However, there is increased resistance and a so-called paradoxical effect leading to attenuated activity of echinocandin antifungals at high concentrations. Therefore, we aimed at identifying novel compounds enhancing the CAS effect by inhibition of putative salvage pathways. As shown here, the discovery of novel compounds displaying synergism with commercially available antifungals represents a promising strategy. By screening 20,000 microbial natural extracts we identified a novel siamycin-like peptide, named humidimycin, which is able to enhance 4.5 times the CAS effect against A. fumigatus. Humidimycin showed no antifungal effect when used alone. Transcriptome analyses, phenotypic characterization of A. fumigatus mutant strains and western blot analyses indicated that CAS affects the cell wall integrity pathway (CWI) and the high osmolarity glycerol response pathway (HOG), which acts as a salvage pathway. Although humidimycin alone also increaes the HOG pathway, when used in combination with caspofungin it silenced the HOG pathway and thus increases the activity of caspofungin.

327 A TIMECOURSE OF LACCARIA BICOLOR MYCORRHIZA DEVELOPMENT

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This study characterizes the transcriptomic coordination between the ectomycorrhizal mutualistic fungus Laccaria bicolor and two host plants, Populus trichocarpa and Pseudotsuga menziesii. In order to understand the molecular hallmarks of the early stages of mycorrhizal root development as compared to mature mycorrhizal root tips, we performed transcriptomic analyses of the host and the colonizing fungus on P. trichocarpa roots in contact with L. bicolor for 2 and 12 weeks. To serve as a host comparison, we also analyzed the transcriptome of mycorrhizal root tips of P. menziesii colonized by L. bicolor. We found that, in response to L. bicolor colonization, there were differential waves of gene regulation related to signaling perception and transduction, defense response and the induction of nutrient transfer in P. trichocarpa tissues. Within the transcriptome of L. bicolor we identified a set of genes that were coordinately regulated throughout colonization of P. trichocarpa and Pseudotsuga menziesii. We conclude that this set of genes, comprised mostly of mycorrhizal induced small secreted proteins (MiSSPs) and of L. bicolor-specific orphan genes, form the basic set of genes that has evolved in the Laccaria lineage that is necessary for the interaction with plant tissues and may help define the host range of L. bicolor.

328 ABC TRANSPORTERS CONTRIBUTE TO AZOLE TOLERANCE AND VIRULENCE IN FUSARIUM GRAMINEARUM

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Fusarium Head Blight (FHB) is a disease of small-grain cereals that is caused by Fusarium graminearum and closely related species. FHB leads to yield losses and contamination of harvested grains with mycotoxins. In wheat production, FHB is often controlled by application of azole fungicides during anthesis. However, the sensitivity of field populations to azoles is slowly declining. To elucidate mechanisms leading to resistance, we established an in vitro system simulating prolonged exposure to sublethal concentrations of triazoles. We recovered two types of isolates (P1 & P2) that diverged by morphology, the degree of resistance to tebuconazole, cross-resistance against other fungicides, vegetative fitness, virulence and nivalenol levels in wheat heads. Transcriptomic responses to tebuconazole treatment were studied using a custom Agilent microarray. In the wildtype, transcript abundances of ergosterol biosynthesis genes were significantly increased and amongst others also of those encoding certain ABC transporters. Analogous analyses with P1 and P2 mutants revealed diverging responses. In a P1-mutant, significant transcriptional differences to the wildtype occurred only after treatment with tebuconazole. In contrast, a P2-mutant showed constitutively increased expression of the responding genes. Sequence analysis of the three Cyp51 genes, encoding the molecular target of azoles, did not indicate any difference in six mutants analysed when compared to the wildtype progenitor. We thus deleted four genes encoding ABC transporters that exhibited responses in the microarray experiment. Compared to wildtype, deletion mutants of FgABC3 and FgABC4 were significantly more sensitive to triazoles and fenarimol but not to other fungicide classes tested. Deletion of FgABC1 and FgABC3 rendered the corresponding transformants significantly less virulent on wheat, barley and maize. Thus, ABC transporters of F. graminearum play prominent roles in fungicide tolerance and virulence.

329 AGROBACTERIUM TUMEFACIENS MEDIATED TRANSFORMATION OF THE ANTHRACNOSE FUNGUS COLLETOTRICHUM AS A TOOL TO STUDY THE MECHANISMS OF PATHOGENICITY

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Anthracnose diseases caused by Colletotrichum spp are very common and destructive for numerous crops and ornamental plants worldwide1. C. gloeosporioides and C. acutatum cause major economic losses, especially in fruits, vegetables, and ornamentals. Many investigations showed differences in the biology, epidemiology, pathogenicity and fungicide sensitivity of these phytopathogenic fungi however, little is known about the genetic basis of these differences3. Fungal transformation technology is considered essential for the study of fungal pathogenicity genes at the molecular level, and it is used to create targeted or random gene disruptions. Agrobacterium tumefaciens - mediated transformation (ATMT) of fungi has become a common technique for the study of a wide variety of different fungal species over the past 14 years. The possibility of performing the fungal transformation using A. tumefaciens provides an efficient tool for those fungi that are difficult to transform by traditional methods. One of the principal advantages of ATMT over conventional transformation techniques is the versatility it provides in choosing which starting material to transform (protoplasts, hyphae, spores, and blocks of mushroom mycelial tissue2). A. tumefaciens produce larger numbers of stable transformants, and more single copy T-DNA insertions than conventional transformation methods4. As the transformants are tagged with T-DNA, it is easy to identify the insertion site. Moreover, ATMT is simple, and suitable for both random insertional mutagenesis and targeted mutagenesis. In this report, we have developed and optimized ATMT to generate a library of hygromycin B-resistant transformants of the two Colletotrichum species, C. gloeosporioides and C. acutatum, from which we have screened for mutants defect in pathogenicity.

1 Cai Z, et al. Identifying pathogenicity genes in the rubber tree anthracnose fungus Colletotrichum gloeosporioides through random insertional mutagenesis, Microb. Research 2013, 168: 340-350.

2 De Groot, et al. Agrobacterium tumefaciens mediated transformation of filamentous fungi. Nat Biotechnol 1998, 16:839–42.

3 Lin CH, et al. The use of T-DNA tagging to isolate mutants of Colletotrichum gloeosporioides and Colletotrichum acutatum with reduced virulence against Hevea brasiliensis. For. Path. 2013, 43: 289-296.

4 Weld RJ, et al. Approaches to functional genomics in filamentous fungi. Cell Res 2006, 16: 31–44.

330 ANALYSIS OF DIFFERENTIAL EXPRESSION DURING THE EARLY STAGES OF THE PHASEOLUS VULGARIS – COLLETOTRICHUM LINDEMUTHIANUM) INTERACTION

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The common bean (Phaseolus vulgaris L) is a plant native to Central America and one of the most important legumes both in Latin America and worldwide. Beans are a staple food as a source of protein and can often replace meat in the diet. They are also of socioeconomic importance and an aspect of cultural identification. Bean production is facing serious problems such as low productivity, drought and low-nutrient soils in addition to the effects of multiple pests and diseases. In Mexico the bean crop ranks second in terms of national acreage and also represents the second most important agricultural activity in Mexico based on the number of producers. The effect of fungal diseases on bean production can be devastating when environmental conditions favor the pathogens and are one of the most important factors leading to losses in yield. Colletotrichum lindemuthianum (Sacc & Magnus), is the pathogen responsible for causing the disease known as Anthracnose, which can cause up to 90 % loss in the total production of the crop. The percentage of crop loss depends on the degree of resistance of the cultivated bean variety and the environmental conditions during the growing season. Successful colonization of host tissues by a pathogen depends on its ability to overcome host defenses and the interaction between P. vulgaris and C. lindemuthianum is considered to be of the gene-for-gene type, where several resistance genes have been identified, however introgression of resistance genes is time consuming and the genetic plasticity of the pathogen eventually leads to the resistance gene being overcome by new forms of the pathogen. More knowledge on common defense mechanisms and how pathogenic strains of C. lindemuthianum avoid detection by the plant in the initial stages of infection are necessary in order to develop durable strategies for resistance that can be applied to a wide variety of bean cultivars. In order to address this we have analyzed differential gene expression patterns based on RNAseq during the early stages of the P. vulgaris-C. lindemuthianum interaction by comparing compatible and incompatible isolates of C. lindemuthianum during the infection of the same bean cultivar. We hope to determine the very early genes involved in the determination of a resistant or susceptible response and in parallel we are documenting histologically the initial stages of infection using GFP tagged C. lindemuthianum isolates.

331 BINDING AND DEGRADATION OF COLLAGEN AND ELASTIN BY PATHOGENIC YEASTS, CANDIDA PARAPSILOSIS, CANDIDA TROPICALIS AND CANDIDA KRUSEI

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The development of infections caused by pathogenic fungi from the genus Candida is closely related to specific properties of pathogen's cell surface and its extracellular hydrolytic activity. Although Candida species belong to the human endogenous microflora, they can cause serious diseases (candidiases), especially in patients with severe immunodeficiency. Currently, the main medical problem associated with candidiases is the increasing incidence of infections caused by the so-called "non-albicans" Candida species, predominantly C. parapsilosis, C. tropicalis and C. krusei. Essential role for the initial stages of infections and eventual dissemination of pathogens within the bloodstream is played by fungal ability to adhere and destroy natural host barriers such as thin layer of epithelial and endothelial cells, as well as the fibrous proteins that are the main components of basement membrane (BM) and extracellular matrix (ECM), i.e., collagen type IV and elastin. Collagen is a triple helix with unusual amino acids, hydroxyproline and hydroxylysine which participate in formation of fibrillar aggregates. Elastin consists of highly hydrophobic tropoelastin moieties, cross-linked through their lysine residues to form insoluble complex. Together, these proteins are responsible for the strength and flexibility of tissues. In this work, with the use of biotin-labeled human proteins and a microplate-formatted ligand-binding assay, the interactions of collagen type IV and water-soluble elastin to the surface of unicellular yeast-like forms as well as (pseudo)hyphal forms of investigated "non-albicans" Candida species were detected. The relative strength of binding of both human proteins by these three species decreased in the order C. tropicalis > C. parapsilosis > C. krusei. Moreover, it was found that proteolytic enzymes secreted by C. krusei, C. parapsilosis and C. tropicalis had the ability to degrade collagen and elastin after several hours of incubation with substrates in acidic environment. Described features, mainly those displayed by C. parapsilosis and C. tropicalis, may contribute to more efficient spreading of pathogens in the organism of human host. This work was supported in part by the National Science Centre, Poland (the grant No. 2012/07/B/NZ1/02867 to A.K.) and the Jagiellonian University (statutory funds of the Faculty of Biochemistry, Biophysics and Biotechnology No. BMN 7/2013, K/DSC/001817/ to J. K.-K.).

332 BIOFUMIGATION FOR EARLY BLIGHT CONTROL IN POTATOES - IN VITRO ASSAY FOR SENSITIVITY OF ALTERNARIA SOLANI TO ISOTHIOCYANATES AND OTHER VOLATILES

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Early blight of potatoes is caused by the fungal pathogen Alternaria solani and can cause yield loss of up to 30 percent. Although fungicides for early blight control are still effective, the recent history of fungicide application has shown that fungal isolates can adapt to active agents that act as single site inhibitors within a few years. Therefore we are investigating alternative ways for early blight control in potatoes. Biofumigation, which means the suppression of soil-borne pathogens, pests, or weeds by hydrolysis products of Brassica-derived glucosinolates, could be a valuable tool amongst others for organic farming. Ongoing research has shown that Brassicaceae as well as other plant families contain volatiles with antifungal potential. We compared two different in vitro assays for evaluating the effect of several biofumigant agents. First, we observed the effect of the biofumigants on hyphal growth of A. solani isolates, when applied to the headspace of the respective petri dishes. In another attempt we added biofumigants to the growing medium and observed spore germination. This method resulted in a lower variance of results and was preferred for further testing. In vitro assays with serial dilutions of biofumigant agents have shown high sensitivity of A. solani isolates to allyl isothiocyanate (ITC), moderate sensitivity to ethyl, benzyl and 2-phenylethyl ITC and only low sensitivity to diallyl disulfide, a major antifungal compound of garlic. In addition to field trials, in which we observed reduced early blight development in the biofumigant treatments, our in vitro assays provide evidence for the antifungal effect of ITCs towards A. solani. Quantification of soil inoculum by qPCR is still to be established and shall substantiate our findings.

333 BIOLOGICAL CONTROL OF VERTICILLIUM WILT OF OLIVE BY TRICHODERMA HARZIANUM

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Verticillium wilt of olive tree (Olea europaea L.) (VWO), caused by the soil-borne fungus Verticillium dahliae Kleb., is considered one of the most devastating diseases affecting this crop in many areas, particularly in the Mediterranean Basin. It can only be effectively confronted by implementing an integrated disease management strategy. Preventive measures such as the treatment of the root systems of pathogen-free certified propagation/planting material at the nursery stage with microbial antagonists emerges as a promising alternative. Species of the soil-borne fungus Trichoderma have been widely described as biocontrol agents (BCAs) of several phytopathogenic fungi by means of different mechanisms, including the ability to colonize root tissues endophytically and to elicit systemic resistance in plants. In this study we aimed to evaluate the potential of T. harzianum CECT 2413 as effective BCA of VWO. Strain CECT 2413 has shown to be able to in vitro overgrow different representatives of V. dahliae pathotypes infecting olive: i.e., highly-virulent, defoliating (D) and midly-virulent non-defoliating (ND) isolates. Inhibition of Verticillium growth in media where strain CECT 2413 had previously grown showed that this ability might be due to the excretion of secondary metabolites or cell wall degrading enzymes. Furthermore, monitoring of in vitro direct confrontation assays between an eyfp-labelled derivative of V. dahliae and a gfp-labelled transformant of CECT 2413 by confocal laser scanning microscopy (CLSM) revealed that mycoparasitism is one of the mechanisms involved in this antagonism. Indeed, typical coiling structures of T. harzianum around V. dahliae hyphae were clearly observed. When a suspension of conidia of T. harzianum CECT 2413 were applied by irrigation to roots of nursery-propagated, three-month-old olives (cv. Picual) prior to the treatment with a D isolate of the pathogen, disease symptoms were significantly reduced compared to that observed in control plants. Moreover, increase in the number and size of leaves in plants treated with Trichoderma was also reported. Finally, colonization of olive roots surface by T. harzianum CECT 2413 during a prolonged period of time was demonstrated by CLSM. In summary, T. harzianum CECT 2413 is an efficient colonizer of olive roots and an effective BCA against the D pathotype of V. dahliae. [This work was supported by grant P10-AGR-6038 of Junta de Andalucía, Spain]

334 BOVINE PANCREATIC TRYPSIN INHIBITOR (BPTI) INHIBITS THE GROWTH OF YEAST BY A NOVEL MECHANISM THAT INVOLVES PERTURBATION OF CELLULAR MAGNESIUM HOMEOSTASIS

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Antimicrobial peptides (AMPs) are promising agents for control of bacterial and fungal infections. Traditionally, AMPs were thought to act through membrane disruption but recent experiments have revealed a diversity of mechanisms. Here we describe a novel antifungal activity for bovine pancreatic trypsin inhibitor (BPTI). BPTI has several features in common with a subset of antimicrobial proteins in that it is small, cationic and stabilized by disulphide bonds. BPTI inhibits growth of Saccharomyces cerevisiae, the human pathogen Candida albicans and the plant pathogen Fusarium graminearum. Screening of yeast deletion collections identified the magnesium transporter Alr1p as a potential BPTI target. BPTI treatment of wild type cells resulted in lowering of cellular Mg^{2+} levels. Mutants in the ECSRT pathway, which functions in recycling membrane proteins, were resistant to BPTI possibly due to higher levels of Alr1p in the membrane. Populations treated with BPTI had fewer cells in S-phase of the cell cycle and a corresponding increase of cells in G0/G1 and G2 phases. The same patterns of cell cycle arrest and resistance in the ESCRT mutants obtained with BPTI were also obtained with the magnesium channel inhibitor hexamine(III)cobalt chloride. Inhibition of a magnesium channel by BPTI represents a novel mechanism of action for AMPs. Current work is focused on confirming the mechanism of resistance in ESCRT mutants and expanding our studies on the pathogens C. albicans and F. graminearum.

335 CELL CYCLE MEDIATED PLANT INFECTION BY THE RICE BLAST FUNGUS, MAGNAPORTHE ORYZAE

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The rice blast fungus, Magnaporthe oryzae, elaborates a specialized dome-shaped infection structure called an appressorium. Initiation of appressorium development requires a switch from apical to isotropic growth which is coupled to a single round of mitosis in the germ tube and regulated by a G1 to S phase cell cycle transition. One of the resulting daughter nuclei moves to the developing appressorium while the other daughter nucleus migrates back to the conidium. A septum then rapidly forms and separates the incipient appressorium from the conidium which starts to collapse due to autophagy-dependent programmed cell death. Completion of mitosis is necessary for appressorium maturation. During maturation, the appressorium generates enormous turgor and this leads to septin-dependent re-orientation of the cytoskeleton at the base of the appressorium. This leads to penetration peg formation and plant cell invasion. How plant infection is linked to cell cycle progression is not fully understood. In this study, we present evidence to suggest that penetration peg formation is an S-phase dependent process and that progression of the appressorial nucleus into G2 is necessary for re-polarisation. Moreover, we show that DNA checkpoint kinases are key players in regulating appressorium formation and maturation.

336 CHARACTERISATION OF PROTEINS SECRETED BY LEPTOSPHAERIA MACULANS DURING INFECTION OF BRASSICA NAPUS AND IN CULTURE

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Leptosphaeria maculans causes blackleg disease of canola which is the major cause of yield losses worldwide. As a hemibiotroph, L. maculans enters the leaf through stomatal openings during its biotrophic phase, proliferates in the mesophyll, and then becomes necrotrophic causing leaf lesions. Meanwhile the leading edge of the mycelium grows asymptomatically down the petiole into the stem tissue where it eventually proliferates, constricts water uptake and causes stem cankers. The relationship between L. maculans and B. napus is initially governed by a gene-for-gene interaction, and although several avirulence genes have been cloned, their roles in this interaction are not clear. We sought to identify proteins secreted by L. maculans during infection of B. napus during the biotrophic, and the necrotrophic stages of infection and to determine their roles in the disease cycle. We generated an RNA-seq dataset comprised of infected cotyledons at the early biotrophic stage, and the late necrotrophic stage and compared this to the transcriptome during growth in culture. L. maculans genes were screened for those with a predicted signal peptide, a translation of less than 300 amino acids length and increased expression in planta. Select candidate genes were cloned into vectors for transient expression in planta via Agrobacterium tumefaciens or via biolistic transformation. The model secreted protein L. maculans Secreted protein 1 (SP1), a 137 aa protein, which is secreted in abundance in culture and induces cell death and autofluorescence when infiltrated into canola leaves, was used as a positive control to set up our in planta transient expression assay system. Several genes predicted to be secreted during the biotrophic stage are being expressed in planta to determine their contribution to evasion of host defence or lesion development.

337 CHARACTERIZATION OF A CIRCADIAN CLOCK IN BOTRYTIS CINEREA AND ITS ROLE IN PATHOGENESIS USING ARABIDOPSIS THALIANA AS A PLANT MODEL

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Botrytis cinerea is a necrotrophic fungus that infects over 200 plant species, ranking as the second most important phytopathogen according to its scientific-economic importance. Although, it has been suggested that the outcome of a plant-pathogen interaction could have daily oscillations, the importance of a circadian clock has only been addressed in the plant, but not in the pathogen. Therefore, we have started to characterize the B. cinerea clock, which is composed of the BcFRQ1 protein and a transcriptional complex formed by BcWCL1 and BcWCL2. Our results indicate that bcfrq1 mRNA presents daily oscillations in a light-dark cycle and in constant darkness (DD), rhythms which are lost in a bcwcl1 KO strain. We have observed oscillatory levels of the BcFRQ1 protein under temperature cycles and DD. Both the bcfrq1 mRNA and BcFRQ1 protein anticipate cyclical-environmental changes, a key characteristic of circadian behavior. Importantly, we have observed an impaired infection process using bcfrq1 and bcwcl1 KO strains. Moreover, we demonstrate that the outcome of the plant- fungal pathogen interaction using Arabidopsis thaliana and B. cinerea as working model varies with the time of day. These results provide the first evidence indicating the existence of a circadian clock in this necrotrophic pathogen, putting forward the concept that fungal clocks can synchronize key elements of pathogenesis.
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338 CHARACTERIZATION OF A SECRETED NUCLEOSIDE PHOSPHORYLASE IN COLLETOTRICHUM GRAMINICOLA

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The maize pathogen Colletotrichum graminicola is the causal agent of leaf anthracnose and stalk rot. C. graminicola secretes effector proteins that disable plant defense components and promote successful infection. We performed a genome-wide survey of C. graminicola proteins that are predicted to encode a secretion signal peptide and have evidence of nuclear localization and/or DNA binding domains. One of the proteins identified in this screen, GRLG_00879, is predicted to encode a secretion signal, a nucleoside phosphorylase domain, and a highly basic C terminal domain. Nucleoside phosphorylase domains are typically involved in nucleoside modification processes and repair of DNA damage and are usually found in the nucleus. The basic C terminal region has an isoelectric point of 10.74, and has a characteristic arginine pattern that may define a DNA interaction domain. This protein has no similarity with previously described pathogenicity factors and there are no homologs in public databases. The presence of these two very different domains led us to suggest that it could have evolved as a fusion of two distinct proteins. We prepared transcriptional fusion constructs with the promoter and a GFP reporter gene for use in microscopic studies. We also performed qPCR assays to determine the expression pattern of this gene. The expression assays show that the gene is expressed during the early stages of the infection process and it was confirmed with the microscopic studies. We hypothesize that this protein is localized to the host nucleus during the early staged of infection, where it modulates the host immune system. To test this hypothesis, we are preparing translational RFP (mCherry) fusion protein constructs as well as constructing null mutants for use in pathogenicity assays. The study of this gene and the encoded protein will reveal its role, if any, in the regulation of the host's immune system during the infection process.

339 CHARACTERIZATION OF NADPH OXIDASE AND ITS TARGETS IN F. GRAMINEARUM

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The multicomponent NADPH oxidase enzyme complex (Nox) converts molecular oxygen in a stepwise reduction to superoxides leading to the production of H2O2. This study characterized the role of the catalytic subunit (gp91phox) of Nox, NoxA, and NoxB and identified their potential targets in. We show that Nox homologues have non-redundant functions in F. graminearum. Targeted deletion of the genes NoxA and NoxB show that they differentially regulate the production of superoxides during mycelial development. The analyses also revealed that NoxA, but not NoxB, is involved in perithecia development and ascospore production. Pathogenicity tests confirmed that these two genes act synergistically to promote virulence. Proteomics analysis identified redox-modification of several target proteins. Modification of a cysteine residue in one of the target proteins confirmed that the target protein is likely a genuine substrate of the Nox enzyme complex.

340 CHARACTERIZATION OF PUTATIVE SECRETED PROTEINS AFFECTING PATHOGENESIS IN MAGNAPORTHE ORYZAE

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The repertoire of secreted proteins defines the nature of interaction between microbes and their hosts at the molecular level. Thus, characterizing the set of secreted proteins from a given pathogen is a pivotal step in understanding the molecular mechanism of pathogenesis. Unlike bacterial and oomycete phytopathogens, only a limited number of secreted proteins has been identified and analyzed in plant pathogenic fungi. Here new secreted proteins were identified and characterized in the rice blast fungus. SingalP program predicted a total 1,573 genes encoding secreted proteins in M. oryzae. Fourteen genes, which have T-DNA mutants already available were prioritized for in-depth analysis. To reveal their roles in pathogenicity, gene deletion mutants were generated and their functionalities characterized. Deletion of MoSPE3, MoSPE6, and MoSPE15 resulted in reduction of virulence. There were no observable developmental defects (vegetative growth, conidiation, germination, appressoria formation) in the deletion mutants except in ΔMospe3, which showed reduced growth on complete media. Rice sheath inoculation of ΔMospe15 showed that defects in pathogenicity could be attributed to the inability to grow inside plant tissues. Reactive oxygen species were known as factors affecting invasive growth. However, it is not the reason of reduced growth in case of MoSPE15 because ΔMospe15 didn't showed ROS sensitive phenotype in vitro. It suggests that MoSPE15 affects invasive growth as ROS independent manner. On the other hands, deletion of MoSPE6 didn't confer defect on early invasive growth. It is regarded that MoSPE6 affects late infection stage. Furthermore, proteins encoded by MoSPE6 and MoSPE15 were shown to be secreted in the yeast secretion trap system. This work would reveal novel function of these secreted proteins, providing new insight into fungal pathogenesis.

341 CHARACTERIZATION OF THE PENICILLIUM DIGITATUM PRTT GENE, A UNIQUE REGULATOR OF EXTRACELLULAR PROTEASE ENCODING GENES

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Penicillium digitatum is the causal agent of green mould rot of citrus fruit and represents the major postharvest pathogen of citrus fruit in Mediterranean regions. Previous results in our laboratory based on suppression subtractive hybridization showed the over representation of P. digitatum genes encoding proteases in infected orange fruits compared to the in vitro growth. RNA-Seq results also highlighted the high expression level of some protease encoding genes during infection. Proteases have been implicated as virulence factors in fungal pathogenesis. The transcription factor PrtT has been shown to control the expression of multiple secreted proteases in Aspergillus niger. In this work, we evaluated the role of PrtT in the virulence of Penicillium digitatum. To this aim, prtT gene was deleted in a P. digitatum strain by targeted gene replacement using Agrobacterium tumefaciens-mediated transformation. Loss of prtT did not lead to a reduced virulence in the citrus-pathogen interaction compared with the wild type and an ectopic mutant. However, the protease activity was reduced in the deleted strain when growing in minimum media supplemented with casein as the only nitrogen source. The expression of prtT and two genes coding for a serine protease (aor1) and an aspartic protease (asp1) were analyzed during the infection process. The results suggest that PrtT is not a significant virulence factor in P. digitatum.

342 CHARACTERIZATION OF THE SIALIDASE (KDNASE) KNOCKOUT MUTANT OF THE OPPORTUNISTIC FUNGAL PATHOGEN, ASPERGILLUS FUMIGATUS

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Aspergillus fumigatus is a filamentous fungus with worldwide distribution that produces airborne spores. A. fumigatus is the most common cause of life-threatening invasive mould infections in immunosuppressed individuals. Previous studies in our laboratory have shown that A. fumigatus has alpha 2,6-linked sialoglycoconjugates on the spore surface, and that sialic acids are involved in adhesion of spores to the basal lamina. More recently, we have cloned and characterized a sialidase from A. fumigatus and shown that this enzyme is really a KDNase as it has a marked preference for the sialic acid, 2-keto-3-deoxynononic acid (KDN). AfKDNase appears to be localized in the cytosol though some enzyme is detected extracellularly. A. fumigatus can use KDN but not Neu5Ac as a carbon source; however, little is known about the role of this enzyme in A. fumigatus during normal fungal development or during pathogenesis in vivo. Therefore, the aim of our current research is to determine the role of the AfKDNase in A. fumigatus by generating a mutant strain deficient in KDNase. A KDNase knockout strain of A. fumigatus was constructed by inserting a hygromycin resistance cassette in place of the AfKDNase gene via homologous recombination using Agrobacterium tumefaciens-mediated transformation. The knockout was confirmed by Southern blot and sequencing. DAfKDNase grown on minimal media supplemented with glucose revealed a unique phenotype compared to wild type and formed high density mats of swollen, hyper-branched hyphae which could be partially reversed by treatment with amphotericin B. Interestingly, in the presence of Congo Red, a diazo dye that binds to polysaccharide components of the cell wall including beta glucan and chitin, the growth of the parental strain was strongly inhibited whereas the KDNase knockout strain was relatively unaffected. The knockout strain was more susceptible than wild type to osmotic stress. These data indicate that KDNase plays an important role in both A. fumigatus metabolism and development. KDNase deficiency affects cell permeability though the molecular basis by which this occurs is as yet unknown.

343 CLASS III PEROXIDASES SECRETED BY TOMATO ROOTS TRIGGER HYPHAL CHEMOTROPISM IN FUSARIUM OXYSPORUM

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Soil-inhabiting fungal pathogens and symbionts exhibit directed hyphal growth towards plant roots, but the chemoattractant signals are currently unknown. Using a plate assay, we found that germ tubes of Fusarium oxysporum display a significant chemotropic response towards root exudates from the host plant tomato (Solanum lycopersicum). Fractionation of root exudate by size exclusion and anion-exchange chromatography followed by liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) analysis, identified class III peroxidases as the chemoattractant compounds secreted by tomato roots. Class III peroxidases exist as large multigene families in plants and have been associated with a variety of processes such as cell elongation, cell wall construction and differentiation, and pathogen defense. Importantly, commercial class III peroxidase from horseradish also triggered a robust chemotropic response in F. oxysporum, while specific peroxidase inhibitors abolished the chemoattractant activity of tomato root exudates and horseradish peroxidase. Collectively, these results suggest that secreted class III peroxidases may represent a general means for chemotropic sensing of plant roots by soil-inhabiting fungi. Knowledge of the mechanism through which the peroxidase signal is sensed by the fungal cell will advance our understanding of the molecular events triggering fungus-root interactions.

344 COMPARATIVE PROTEOMIC ANALYSIS OF PHYTOPHTHORA PISI AND PHYTOPHTHORA SOJAE IN GERMINATED CYSTS AND MYCELIA

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Phytophthora pisi, a recently described species, causes root rot on pea and faba bean. Phytophthora sojae is the causal agent of soybean root and stem rot. Despite being closely related species, P. pisi and P. sojae evolved different repertories of pathogenicity factors that enable them to have distinct host specificity towards pea and soybean, respectively. Using tandem mass spectrometry we carried out a comparative global proteome study of P. pisi and P. sojae in mycelium and germinating cysts. The germinated cysts were exposed to pea exudate with the hypothesis that germinating cyst of P. pisi can recognize host factors and induce a specific set of pathogenicity factors when exposed to pea exudate, which will not be induced in P. sojae. Fifty-eight orthologous proteins were induced in germinating cyst of both pathogens and thus identified as candidate proteins for the infective stage. Twenty-three orthologous proteins were induced in mycelia of both pathogens and thus were identified as candidate proteins for vegetative growth. In the germinated cysts, proteins associated with lipid transport and metabolism, and energy production were up-regulated. In mycelia, proteins involved in transport and metabolism of carbohydrates and proteins involved in secondary metabolites biosynthesis, transport and metabolism were up-regulated. Based on our data, we hypothesize that germinating cysts catabolize lipid reserves through the beta-oxidation pathway to drive the protein synthesis necessary to produce the germ tube and initiate infection. Once inside the host cells, the pathogen switches to vegetative growth where energy is derived from carbohydrates from the host. Analyzing the orthologous proteins within these closely related species, we identified many species specific proteins. Thirty-seven proteins were induced in the germinating cyst of P. pisi while no orthologs of these proteins were identified in P. sojae or they were not expressed, and thus were identified as candidates for pea specificity. Among such proteins are serine proteases, ABC transporters and dioxygenases. In addition, nineteen proteins were uniquely expressed in germinating cysts of P. sojae. These results significantly expand the knowledge on the expressed proteome in these important pathogens and shed light on the active metabolic processes during cyst germination and mycelial growth. In addition, many unique, putative pathogenicity factors for each species were also identified.

345 CONTROLLING MYCOTOXIN CONTAMINATION IN MAIZE VIA HOST-INDUCED GENE SILENCING

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Aspergillus flavus and Fusarium verticillioides are important ear rot pathogens of maize. Although A. flavus and F. verticillioides reduce yields and grain quality, the principal concern associated with these pathogens is the production of mycotoxins. Aflatoxins (produced by A. flavus) and fumonisins (produced by F. verticillioides) are distinct groups of polyketide-derived mycotoxins that pose significant health risks to humans and livestock. Despite the health and economic concerns associated with aflatoxins and fumonisins, few effective tools are available to prevent grain contamination. Host-induced gene silencing (HIGS) was recently demonstrated as a means of manipulating gene expression in pathogens through the expression of pathogen-specific hairpin RNA (hpRNA) in the host plant. The goal of this research was to generate transgenic maize plants expressing pathogen-specific hpRNA to inhibit mycotoxin accumulation. To this end, a pipeline was created to develop and evaluate HIGS vectors. First, fungal genes that regulate mycotoxin biosynthesis were selected for silencing. Second, a high-throughput cloning process was developed in which a single ligation step was used to generate constructs containing a sense and antisense fragment of the target gene separated by an intron from the Magnaporthe oryzae cutinase gene. The constructs were then transformed into A. flavus or F. verticillioides and evaluated for reduced toxin accumulation. Finally, hpRNA constructs that significantly lowered mycotoxin levels were used to generate plant expression vectors that were then transformed into maize. Currently, vectors have been created to silence expression of aflatoxin biosynthesis-specific genes in A. flavus, including the polyketide synthase gene (aflC), a fatty acid synthase gene (aflA), and a regulatory gene (aflS). A vector was also created to silence expression of the polyketide synthase gene (FUM1) required for fumonisin biosynthesis in F. verticillioides. In addition, silencing vectors have been generated to target non-cluster genes that regulate mycotoxin biosynthesis, such as genes encoding hexokinase (hxkA) in A. flavus and ±-amylase (AMY1) in F. verticilliodes. Through the creation and evaluation of transgenic maize plants expressing pathogen-specific hpRNA, this research will advance the current understanding of HIGS and potentially provide a novel means of controlling mycotoxin contamination in maize.

346 DEFECTS IN CONIDIATION DO NOT LEAD TO REDUCED VIRULENCE IN THE VASCULAR PATHOGEN VERTICILLIUM DAHLIAE

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Verticillium dahliae, the causal agent of Verticillium wilt, has a worldwide distribution and a plethora of hosts of agronomic value. Differentiation processes in fungal pathogens are critical to complete the disease cycle. V. dahliae produces resistant structures, the microsclerotios, that can survive in the soil for years and are the main source of inoculum. Germination of the microsclerotios produces hyphae that penetrate the root cortex. Upon entering the vascular vessels, it is thought that the fungus produces large amounts of conidia capable of passive transport within the xylem, allowing for fast systemic colonization. We are using forward and reverse genetic approaches to analyze the regulation of these morphogenetic processes. One of the genes selected as part of the candidate gene approach, VDAG_08656 (vst1) which codes for an APSES transcription factor, has been found to be a key regulator of development. Thus, strains deleted for vst1 lacked microsclerotia and produced aberrant conidiophores that did not exhibit the characteristic verticillate structure on solid media. V. dahliae do not form verticillate conidiphores in the xylem or liquid media, in those cases conidia germinate to produce phialides that generate new conidia; absence of vst1 also led to a drastic reduction in conidiation rates in liquid cultures. However, when two different hosts, tomato and Nicotiana benthamiana, were inoculated with vst1- mutants, the temporal expression of symptoms and disease severity were most similar to those induced by the parental strain. Additional mutants exhibiting a drastic reduction in conidiation rates in liquid media were produced by random insertion of T-DNA in Agrobacterium tumefaciens mediated transformations, to further analyze the role of this process in virulence. Nine out of ten selected mutants were as virulent as the parental strain on tomato plants. Therefore our results do not support the proposed model of rapid systemic invasion through the generation of a unicellular form. Interestingly, mutants with defects in conidiation often exhibited other morphological alterations, like absence of microsclerotia. This finding suggests that different developmental processes that involve changes in mode of growth share some regulating mechanisms. We are currently using vst1- strains as a tool to analyze global gene expression patterns associated to morphogenesis in V. dahliae, and characterizing vascular colonization by mutant strains.

347 DEFENSE RESPONSES OF MULTICELLULAR FUNGI TOWARDS FUNGIVOROUS NEMATODES

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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 reductionistic and experimental approaches to identify and characterize these mechanisms at molecular level. Special emphasis is put on fungal defense mechanisms against fungivorous nematodes as these predators are prevalent in many habitats of multicellular fungi. Based on toxicity assays in which we employed model organisms from different fungivore-containing phyla, 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. Genome-wide gene expression studies by next generation RNA sequencing in the coprophile model mushroom Coprinopsis cinerea revealed that the genes coding for these 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 Aphelenchus 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 employing microfluidics and fluidic force microscopy to characterize this response. Preliminary results using a C. cinerea reporter strain expressing a red fluorescent protein under control of the A. avenae-inducible cgl2 promoter show that (1) the defense response is confined to sites of direct contact between the fungivorous nematode and the fungal mycelium; (2) the response can spread over mm distances from these sites along leading hyphae both in anterograde and retrograde direction but not along branching hyphae and (3) the response appears to go on and off under conditions of continuous feeding. 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 additional 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.

348 DETECTION OF ASPERGILLOSIS BY GLIT-SPECIFIC IGG ELISA

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Aspergillus fumigatus is the predominant causative agent of Aspergillosis and Invasive Aspergillosis (IA), indeed the latter exhibits a notoriously high mortality rate, largely due to poor diagnosis. The critical condition of many IA patients necessitates non-invasive serological diagnostic methods, which require standardisation. To this end, antibody prevalence against GliT (AFUA_6G09740), an oxidoreductase, normally required for gliotoxin production and resistance in A. fumigatus, was investigated. Recombinant GliT was expressed at high level in Escherichia coli and a robust indirect enzyme-linked immunosorbent assay (ELISA), for the detection of GliT-specific antibodies was developed. Efficacy of this ELISA has been demonstrated for diagnosing Aspergillosis in a canine model system whereby Receiver Operator Characteristic (ROC) analysis confirmed that the assay is highly sensitive (90%) and specific (86%) using 59 sera (n = 10 positive; n = 49 negative) from canines with Sinonasal Aspergillosis. Moreover, analysis of immune-competent human sera has revealed a range of GliT-specific antibody titres, in all samples tested (n = 42). We have also identified GliT-specific IgE in selected human sera and purified the GliT-specific IgG which is immunoreactive with both native and recombinant GliT. Others have suggested GliT-specific IgG detection as an immunodiagnostic for Aspergillosis, but clearly the occurrence of anti-GliT IgG in immune-competent individuals necessitates further investigation of this proposal. To summarise, the high GliT-specific IgG prevalence observed herein indicates that exposure to A. fumigatus spores is common and that GliT is highly antigenic, as widespread antibody responses are not evident against other A. fumigatus proteins. We suggest that this response is initiated in the upper respiratory tract or alveoli, whereby inhalation and germination of spores may expose individuals to the gliotoxin oxidoreductase, GliT. Ultimately, this research supports GliT-specific IgG as a promising diagnostic marker for A. fumigatus infection in humans, however, further assay validation is necessary.

349 DEVELOPMENT OF A RECYCLABLE MARKER SELECTION SYSTEM FOR PENICILLIUM DIGITATUM

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The fungus Penicillium digitatum, the causal agent of the citrus green mould rot, is the most destructive postharvest pathogen of citrus fruit worldwide. The application of synthetic fungicides constitutes the most common method used to control postharvest diseases in citrus fruit. However, the emergence of resistant strains, the more restrictive legislation and the growing public concern on the negative effects of fungicides have led to an increased interest in developing new methods to control postharvest diseases. Knowledge of the mechanisms underlying pathogenicity could lead to the identification of novel fungal factors that can be good candidates to be targeted in rational antifungal approaches. Recently, we have published the genome sequence of P. digitatum and have identified some genes putatively involved in pathogenesis. Obtention of gene knock out mutants is the main approach to study the role that these genes play in pathogenesis. However, the simultaneous deletion of more than a gene usually requires the use of different selectable markers, thus limiting the number of gene that can be deleted to the available selection markers. The use of a recyclable marker that allows repetitive rounds of gene deletion would overcome this limitation. Recently, a self-excising marker cassette using a codon-optimized prokaryotic small β-serine recombinase acting on six recognition sequences has been successfully established in several filamentous fungi. Expression of the β-recombinase encoding gene is under the control of the P. chrysogenum xylP gene. In this work we describe the application of this β-rec/six based system for generating consecutive deletions in P. digitatum using Agrobacterium tumefaciens mediated transformation. We have developed a binary vector that includes the hygromycin resistance cassette and the β-rec/six recombination system. The β-rec/six cassette was amplified from plasmid pSK485 and the amplified fragments were cloned into plasmid pRFHU2. In order to test the functionality of the system in P. digitatum, two polygalacturosase encoding genes (pg1 and pg2) were chosen for consecutive deletion. The results confirm that the designed β-rec/six recyclable marker system allows consecutive gene deletions in P. digitatum. Virulence assays of single Δpg1 and Δpg2 and double Δpg1Δpg2 knock out mutants confirm the role of these pectin degrading enzymes in the pathogenicity of P. digitatum towards citrus fruit.

350 DYNAMICS OF COLONIZATION OF COMMON BEAN BY HIGHLY VIRULENT AND WEAKLY VIRULENT STRAINS OF F. OXYSPORUM: MICROSCOPIC ANALYSIS AND GENE EXPRESSION PATTERNS

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Fusarium oxysporum f.sp. phaseoli is the causal agent of Fusarium wilt of Phaseolus vulgaris. In former works we have shown that pathogenic strains of this forma specialis can be classified as weakly, highly and super virulent. The aim of the present study was to compare the colonization dynamics of weakly and highly virulent strains in a susceptible P. vulgaris cultivar and to find correlations with fungal and plant-induced gene expression patterns, in an effort to understand the genetic basis of virulence in the F. oxysporum species complex. The highly virulent strain FOP-SP1 and the weakly virulent strain FOP-SP4 were selected to get transformants that express GFP under the control of GPDH constitutive promoter. Also, FOP-SP1 was engineered to express GFP under the control of FTF1 promoter. FTF1 is a virulence gene that encodes a transcription factor exclusive of highly and super virulent strains. Confocal laser scanning microcopy (CLSM) was used to compare colonization patterns of both strains. Quantitative analysis of colonization and gene expression were carried out by means RT-qPCR. CLSM analysis showed that FOP-SP1 penetrates the root epidermal cells, advances through intercellular spaces of the root cortex and reaches the central root system. At equivalent times, FOP-SP4 massively colonizes the root cortex but hardly grows inside the central root system. Fungal development inside the lower zone of hypocotyl is critical in plant colonization: FOP-SP4 needs more time to heavily colonize this region while FOP-SP1 quickly takes over xylem vessels and progresses upward the stem. Transcriptomic analysis of several fungal genes involved in virulence and plant genes involved in the defense response have revealed different expression patterns for both strains. It is worth to highlight that expression of FTF1 takes place only inside the xylem vessels of hypocotyl and stem. We conclude that virulence of strain FOP-SP1 can be specifically attributed to the ability to reach the central root cylinder and the fast progression inside the hypocotyl and stem xylem vessels, and is not related to the degree of global colonization of the root (higher in FOP-SP4 interaction than in FOP-SP1 one). We propose a main role in xylem colonization for the transcription factor FTF1, the first gene of Fusarium that is restrictively expressed in the stage of growth inside the vascular system whose colonization is the proof of concept of vascular pathogens.

351 EFFECT OF HOST SIGNALS ON THE EPIDEMIOLOGY OF THE FUNGUS RAMULARIA COLLO-CYGNI

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Being hardly recognized until only a decade ago the hemibiotrophic fungus Ramularia collo-cygni has become a major pathogen in barley production of temperate climates. Its epidemic shows some particularities which can explain why its presence in the crop has been overlooked for a long time. Being detected by molecular methods throughout the entire plant development the epidemic appears only late in the growing season, not until past flowering. Typically, epidemic starts with unspecific spotting leading to rapid loss of green leaf area and heavy sporulation on upper leaves. Unlike the epidemic of similar necrotising fungi this phenomenon is decoupled from the general plant maturity moving upwards from the lower canopy. The effect of plant age dominates differences in tolerance. Sporadic sporulation on senescent leaves and molecular detection from asymptomatic leaves early in the season give evidence of endophytic stages and a hemibiotrophic life style. The movement from seed into shoot and leaves could be shown. The present study uses Realtime PCR to quantify fungal DNA in different plant parts throughout the growing season and compare them to the visually and microscopically assessed disease development. The influence of plant senescence is observed under natural epidemiological conditions in field plots of the same spring barley variety differing in plant development due to different sowing dates. DNA quantities generally correlate with the observed symptoms and sporulation on the leaves. While there is clearly a signal being related to host senescence which causes the fungus changing from endophytic to pathogenic lifestyle, the direct effect remains difficult to investigate. This could be partly due to the influence of environmental factors in a complex interaction with host and pathogen. The fungus produces host nonspecific, phytodynamic toxins which can be related to symptom development and explain an influence of light. In a detached leaf assay it was possible to show the release of toxins into plant tissue and the effect of electrolyte efflux into the apoplast on fungal growth. The efflux is frequently related to cell death and senescence. In vitro trials showed the influence of UV light and nutritional salts on sporulation and toxin production. The influence of plant senescence on epidemics will be discussed in respect to the conclusions on the life cycle of Ramularia collo-cygni.

352 EFFECTOR HUNTING – NECROSIS AND CHLOROSIS INDUCING PROTEIN(S) OF ZYMOSEPTORIA TRITICI

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The dothideomycete Zymoseptoria tritici (previously named Mycosphaerella graminicola) is a major wheat pathogen, inflicting significant yield losses worldwide. Little is known about the biochemical mechanisms underlying its pathogenicity despite intensive efforts over several decades. Our laboratory has been investigating substances secreted by Zymoseptoria tritici which induce wheat leaf chlorosis and necrosis in the absence of the pathogen. These substances are secreted by the fungus under specific in vitro growth conditions and induce disease-like necrosis and chlorosis (NC) symptoms in planta. Size exclusion and protease treatment experiments have shown that the causative agent is a small protein. Current data does not suggest a specific gene-for-gene mechanism as a wide range of wheat cultivars, both susceptible and resistant to the pathogen, as well as other plants such as barley, develop similar levels of symptoms. The activity has successfully been enriched using ion exchange chromatography. Mass spectrometry (MS) analysis of this enriched fraction has revealed candidates of which a gene is being tested for activity in the created knock-out mutants of the pathogen. Currently RNA sequencing (RNAseq) experiments are also being undertaken from activity and non-activity producing cultures to analyse the spectrum of further candidates. Together with a detailed MS analysis of the active anion exchange fraction, a pool of candidate effectors is being generated. The putative effector is relatively small, its activity is light dependent and it appears to be threshold dependent. This research is expected to establish a foundation to understand the molecular mechanisms underlying Zymoseptoria tritici infection of wheat.

353 EVALUATION OF THE EFFECT OF POCHONIA CHLAMYDOSPORIA WORLDWIDE ISOLATES ON GROWTH AND DEVELOPMENT OF TOMATO AND A. THALIANA

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The fungal parasite of nematode eggs Pochonia chlamydosporia (= Verticillium chlamydosporium) has great potential as a biocontrol agent of plant parasitic nematodes. Colonization of root system by P. chlamydosporia is known to reduce disease caused by fungal root pathogens and in turn promote growth of barley and tomato plants. In this work we have evaluated the effect of the root colonization by nine different isolates of P. chlamydosporia on the development of seedlings (petri dish experiments), plantlets (growth chambers) and tomato plants (Greenhouse conditions). Growth parameters included (Maximum Shoot length, Maximum Root length, Fresh Shoot weight, Dry Shoot weight and Fresh root weight) were evaluated at the end of the experiments. Some isolates were able to promote root and shoot growth whereas others do not promote. All isolates were able to colonize roots; as determined by plating and qPCR. Flowering of tomato plants inoculated by different P. chlamydosporia isolates in greenhouse conditions showed differences respect to the control (Uninoculated plants). The effect of P. chlamydosporia on growth promotion and flowering time were also evaluated in growth chambers using several A. thaliana mutants (35S::pep, pep-4, flk2, fca9, ft10) respect to wild type Col-0.

354 FUNCTIONAL CHARACTERIZATION OF A COLLETOTRICHUM GRAMINICOLA FUNGALYSIN WITH A ROLE IN VIRULENCE

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Plants produce a wide array of pathogenesis-related (PR) proteins to defend themselves against microbial invaders. Some of these PR proteins are hydrolytic enzymes that target fungal cell walls, such as chitinases. Fungi also secrete a wide range of effector proteins to manipulate the plant immune system. Recently, fungal effectors have been identified that interfere with chitin-triggered immunity, protecting the fungal hyphae against hydrolysis by chitinases or sequestering the chitin oligosaccharides and preventing chitin binding to the receptor. In this study, we describe CgEP2 (Colletotrichum graminicola Effector Protein 2), a 640 aa secreted protein in the maize hemibiotroph Colletotrichum graminicola, with an important role in virulence. CgEP2 is a fungalysin highly conserved in diverse fungi, that binds plant produced class IV chitinases and directly catalyzes their post-translational modification by enzymatic activity. Using transcriptional fusions of the gene promoter with GFP as well as qPCR assays at different time points during infection, we show that the gene expression is activated specifically when the fungus switches from biotrophic to necrotrophic growth. We constructed null mutants by gene replacement and performed pathogenicity assays in maize and observed reduced lesion sizes on leaves and reduced colonization of roots, showing that CgEP2 has a role in C. graminicola virulence. Biochemical analysis of chitinase activity with the null mutant confirms the lack of ability to degrade this substrate. Our results show that CgEP2 plays a role in plant infection and host colonization and could be important in the lifestyle change of C. graminicola.

355 FUNCTIONAL DIVERSITY OF ECTOMYCORRHIZAL FUNGI: TRANSCRIPTOME ANALYSES REVEAL THAT HOST PLANTS INTERACT DIFFERENTIALLY WITH CENOCOCCUM GEOPHILUM AND OTHER SIMULTANEOUSLY ASSOCIATED ECTOMYCORRHIZAL FUNGI

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Most trees in temperate and boreal forests live in symbiosis with hundreds of ectomycorrhizal fungi. What is the significance of this diversity for the host tree? Is the plant-fungus interaction specific to each ectomycorrhizal partner and do they confer different benefits to their host plant? Cenococcum geophilum, for example, which is a widespread and often dominant mycorrhizal symbiont belonging to the Dothideomycetes, is thought to enhance drought resistance of its host plant whereas other mycorrhizal species might be more efficient in delivering nutrients. However, clear evidence for differential interaction is scarce. To tackle these questions we studied the gene expression in mycorrhizal root tips of greenhouse grown Scots pine seedlings that were inoculated with more than one mycorrhizal species simultaneously. The specific questions were: 1) Are Scots pine genes in mycorrhizal roots differentially expressed depending on which fungal partner is present? 2) Which genes are specifically expressed when interacting with C. geophilum and what might be the functional meaning? 3) Which genes are differentially expressed in C. geophilum when interacting with the host plant? We used microarray analyses, RNA Seq and qRT-PCR using RNA extracted from mycorrhizal root tips of Scots pine/C. geophilum, as well as of Suillus granulatus and Rhizopogon roseolus either of which was simultaneously inoculated. The transcriptome analyses revealed that Scots pine differentially interacted with C. geophilum than with the two other mycorrhizal species even though mycorrhizal root tips existed side by side. Interestingly, many genes involved in pathogen resistance were up to 20x higher expressed in C. geophilum mycorrhizas than in the other two. The most differentially expressed gene was a CC-NBS-LRR resistance like protein which has been shown to be involved in drought tolerance of plants and which indicated that biotic and abiotic stress signalling pathways may share nodes. We also found an aquaporin, which transports water and small solutes, to be upregulated in Scots pine/C. geophilum mycorrhizas. On the fungal side, among the four most differentially expressed genes compared to free-living mycelium were also two aquaporins. These findings support a special role of C. geophilum in the water uptake and drought tolerance of the host plant and emphasize the functional significance of a species-rich association of host plants with ectomycorrhizal fungi.

356 FUNGI CHALLENGE GLOBAL 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 resilience (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 the emergence of new pathotypes affecting crop yields and to fungi and oomycetes decimating our natural and managed landscapes. I shall review some of our recent work looking at the movement of fungi polewards in a warming world (Bebber, Ramatowski and Gurr, 2013 Nature Climate Change 11 985-989), at the global distributions of crop pests and pathogens (Bebber et al., 2014 New Phytologist (in press) and at the global saturation rate of crops by such organisms (Bebber, Holmes, Gurr (at review)). I shall conclude with some thoughts on the emergence of fungi in natural and crop ecosystems, of future threats and challenges and discuss policy and disease mitigation.

357 FUSARIUM GRAMINEARUM IS ABLE TO MANIPULATE ETHYLENE PRODUCTION

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Production of ethylene is a response of plants to various endogenous signals and also plays a role in resistance to (necrotrophic) fungal pathogens. In case of Fusarium graminearum contradicting evidence is reported for the role of ethylene in disease development. On the one hand ethylene signalling is generally presumed to be associated with induction of plant genes playing a role in disease resistance. On the other hand it was reported that ethylene insensitive Arabidopsis mutants (ein2) and also a wheat line with silenced EIN2 show increased Fusarium resistance and reduced mycotoxin accumulation (Chen et al., 2009), suggesting that the fungus can exploit ethylene signalling to increase susceptibility. We observed that F. graminearum (PH-1) can release ethylene when grown on media with high methionine content. In plants ethylene is produced from methionine via the precursor ACC (1-aminocyclopropane-1-carboxylic acid). Homology searches revealed that F. graminearum not only possesses 3 putative homologs of ACC-synthases, but also 2 candidate ACC deaminase genes. ACC deaminase is used by various microbes to reduce ethylene production and consequently responses to ethylene in host plants. We have cloned all the predicted ORFs into E. coli expression vectors and also started to disrupt the candidate genes in Fusarium. Results of in vitro tests with the purified proteins and virulence testing of knock out strains will be presented.

358 GENE EXPRESSION AND HISTOPATHOLOGICAL CHARACTERIZATION OF TWO FORMAE SPECIALES OF SPORISORIUM REILIANUM IN MAIZE AND SORGHUM REVEALS DIFFERENCES IN FUNGAL COLONIZATION AND PLANT DEFENSE RESPONSES

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Smut fungi are biotrophic plant pathogens with a narrow host range. Sporisorium reilianum causes head smut in maize and sorghum. The fungus exists in two host-adapted formae speciales (SRS and SRZ) that either produce spores on sorghum (SRS) or on maize (SRZ). To characterize the differences leading to host specificity in S. reilianum, we infected sorghum and maize with SRS and SRZ and microscopically followed fungal plant colonization. Both varieties were able to penetrate and multiply in sorghum and maize leaves. In sorghum, SRS entered the vascular bundles and reached the apical meristems, while SRZ did not show a preference for vascular bundles and was not detected in apical meristems. In maize, both varieties were able to grow from inoculated leaves to the nodes containing the floral meristems, but SRS did not produce spores. To confirm these microscopic observations, we quantified fungal genomic DNA during biotrophic plant colonization. In sorghum, DNA concentration of SRS was prominent in inoculated leaves, nodes and apical inflorescences, but decreased with increasing distance from the inoculation site for SRZ, being non-detectable in nodes. In maize, DNA of SRZ was prominent in all parts of the plant, while it decreased to very low amounts in nodes for SRS. To explain this opposing colonization behavior, we investigated the occurrence of different plant defenses responses. In sorghum, hyphae of SRZ induced a local response of increased H2O2 at 1 day after inoculation (dai), at 2 dai the plant deposited callose in the cell walls of infected cells, and at 3 dai phytoalexins were observed, together with the expression of several resistance and defense genes. For SRS on sorghum, the production of H2O2 and callose was very low, no phytoalexins were detected and resistance and defense genes were not up regulated. Maize reacted similarly to both SRS and SRZ with a very weak production of H2O2 and callose, and no prominent variation in marker gene expression was found. These results indicate that host colonization by S. reilianum leads to different defense responses in maize and sorghum.

359 GENETIC AND IMMUNOLOGICAL CHARACTERIZATION OF THE PUTATIVE EXO-1,3-β GLUCANASE-1 (PINSEXO1) OF THE PATHOGENIC OOMYCETE PYTHIUM INSIDIOSUM

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Human pythiosis is endemic in Thailand resulted from Pythium insidiosum which is a causative agent of a fatal infectious disease, called pythiosis, in both human and animals. The organism is normally found in tropical and subtropical countries, especially in swampy area. According to fungus-like the morphology, this organism was misdiagnosis to other fungi which leading to the poor prognosis and also ineffective treatment. Previously study, the 74-kDa immunodominant antigen of P. insidiosum was discovered by western blot analysis Additionally, mass spectrometry and molecular genetic analysis revealed the partial protein (ADI86643) encoding PinsEXO1 sequence match to putative exo-1,3-beta glucanase protein (AAM183483) of the plant pathogenic oomycete Phytophthora infestans. In order to understand and better know the P. insidiosum glucanase, full length of glucanase gene was demonstrated by RACE and adaptor PCR techniques to obtain 2,229 ORF and then phylogenetic analysis of the sequences against various glucanase gene sequences from fungi and oomycetes revealed that PinsEXO1 was group within the same clade of oomycetes. Interestingly, this PinsEXO1 was closely related to Saprolegnia parasitica which is animal pathogen. Immunoreactivity by ELISA method of PinsEXO1 was shown that one of three synthesize peptides resulted from B-cell epitope prediction and then immunized in two rabbits to obtain rabbit anti-peptide sera revealed strong signal than others. Moreover, cellular localization of PinsEXO1 was performed by western blot analysis and immunofluorescence labeling method (Alexafluo568) was demonstrated positive bands about 80 kDa only in cell lysate but not presented in secreted CFA interpreting the protein was produced in cell but cannot secret out of the cell. Composing of the result from program prediction by BlastP, domain search, TMHMM, InterProScan and signal reveal that this gene contained signal peptide and transmembrane protein. Transmembrane protein was supported the information from result of western blot above that the protein was anchored in cell membrane instead of secret out to cell wall. Moreover, from blastP result reveal that this protein presented many band in western blot result related to molecular weight prediction resulted from this protein represented more than one isotype. For the precisely result in immunolocalization, this method was optimized by antigen pre-absorption based on BlastP and transcriptome data was shown the result as expected.

360 GENOTYPE ANALYSIS AND ANTIMICROBIAL PHOTODYNAMIC THERAPY OF SCYTALIDIUM DIMIDIATUM AND S. HYALINUM CLINICAL ISOLATES FROM DERMATOMYCOSES AND ONYCHOMYCOSIS

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Scytalidium dimidiatum is a saprophytic fungus and pathogen found in soil and vegetation of tropical climate. The habitat of S. hyalinum is unknown, and is considered a hyaline variant of S. dimidiatum. Infections by Scytalidium sp. affects immunocompromised and immunocompetent patients, with special attention to dermatomycoses and onychomycosis. Considering the resistance to antifungal agents against opportunistic fungi and the growing commercial use of antimicrobial photodynamic therapy (APT), the aim of this study was to evaluate the effectiveness of APT using commercial photosensitizers (PS) against both, S. dimidiatum and S. hyalinum. Additionally, the sensitivity against antifungal drugs was tested. Scytalidium sp. strains were isolated from biological samples and classical and molecular identification were carried out. The APT test was done with methylene blue (MB), Ortotoluidine Blue (TBO), New Methylene Blue (NMB), and methylene blue derivative named S137. These PS were distributed with arthroconidia of S. dimidiatum and S. hyalinum in RPMI culture media and incubated in the dark for 30 minutes and then exposed to light for final doses of 10 and 20 J cm-2. The minimum fungicidal concentration (MFC) was determined after the incubation at 37°C for 96 hours. NMB and S137 showed higher efficiency for growth inactivation of S. dimidiatum, and even increasing light dose, S. dimidiatum strain displays a refractory phenotype to APT with FS, MB and TBO. Low values of MFC is shown to S. hyalinum to all PS tested. Thus, even though FS, MB and TBO displayed refractory effect to S. dimidiatum possibly justified by the presence of melanin-like pigment, the APT proved to be a good option to inactivate Scytalidium sp.

361 HETEROGENEOUS MECHANISMS UNDERLYING BEHAVIORAL MANIPULATION BY FUNGAL PARASITE OPHIOCORDYCEPS UNILATERALIS

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Fungal insect pathogens are an important group with over 700 species spanning all major taxa. Some of these, such as species of the genus Ophiocordyceps, are able to interact with the host's nervous system, changing behavior. One of the most dramatic examples is the fungus Ophiocordyceps unilateralis s.l. infecting Camponotus species, where ants bite into vegetation at an elevated position before dying to facilitate spore dispersal. To establish this, the fungus not only overcomes the immune system, but also manipulates the brain and atrophies the mandible muscles. We recently succeeded in moving this model system into the lab, allowing us to start unraveling the proximate mechanisms underlying this phenomenon. By combining metabolite profiling with ex vivo insect tissue culturing, compounds secreted in different areas of the host were studied. Using this technique we established that generalist and specialist fungal entomopathogens react differently to the same insect tissues. Next to that, these entomopathogens react heterogeneously to brain and muscle tissue by secreting a significantly different array of metabolites. Furthermore, ~70% of the metabolites O. unilateralis significantly employs when presented with brains differ when these brains are derived from different ant species. This is in line with infection studies performed under laboratory conditions: while some ant species display the manipulated biting behavior prior to death, other species do not suggesting this phenomenon is species specific. As part of this interdisciplinary dataset, we also identified two metabolites significantly related to O. unilateralis growth on ant brains that, according to literature, are involved in various neurological diseases and cancers. This suggests that these are possibly involved in establishing manipulation. To move further towards unraveling the fungal parasitic mechanisms, we are now developing the protocols, involving laser capture microscopy, to study fungal gene expression of cells surrounding ant brains during manipulated biting behavior.

362 HETEROLOGOUS EXPRESSION OF TRI5 GENE IN TRICHODERMA HARZIANUM: EFFECT OF TRICHODIENE PRODUCTION ON TRICHODERMA ENVIRONMENTAL INTERACTIONS

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Terpenes form a large class of compounds that have a variety of roles in mediating antagonistic and beneficial interactions among organisms. The trichothecenes are phytotoxic sesquiterpenoid compounds that have been extensively studied, mainly in the genus Fusarium, that can act as virulence factors in plant diseases caused by these fungi. One interesting exception is harzianum A (HA), a non-phytotoxic trichothecene produced by T. arundinaceum strains. The first step in the biosynthesis of HA is the conversion of farnesyl diphosphate to trichodiene, catalyzed by a terpene synthase encoded by the tri5 gene. Heterologous expression of tri5 gene in the well-characterized biocontrol strain T. harzianum CECT 2413 resulted in production of trichodiene and a reduction in the level of ergosterol produced. In addition, trichodiene from this strain affected the expression of tomato defense related genes and Botrytis cinerea 05.10 virulence genes. Together, these results suggest that trichodiene has a signaling role in interactions between Trichoderma and plants and microorganisms in its environment.

363 HOW TO COMPROMISE THE FUNGAL VIRULENCE REMOVING JUST ONE MANNOSE

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Glycosylation consists of the addition of sugar residues to specific target proteins. This slight post-translational modification is however crucial for the activity of most secreted proteins. In fact, previous studies in the corn smut fungus Ustilago maydis have shown as a defective glycosylation process leads to non-pathogenic strains (Schirawski et al., 2005; Fernandez-Alvarez et al., 2009; Fernandez-Alvarez et al., 2013). The glycosylated virulence factors behind these phenotypes have not been identified. In order to disclose this problem, we carried out a screening of glycoproteins purified by affinity chromatography specifically induced by the virulence program triggered by the b-dependent zinc finger protein biz1 (Flor-Parra et al., 2006). Using this approach, we identified new glycosylated target proteins of the O-mannosyltransferase Pmt4. The characterization of these virulence factors and the relevance of Pmt4 for their function in planta will be shown. We think that our new results add relevant data to the puzzle of the complex pathogenic interaction between U. maydis and Z. mays.

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364 IDENTIFICATION OF A MYCOVIRUS IN FUSARIUM OXYSPORUM F. SP. DIANTHI WHICH AFFECTS PHENOTYPIC TRAITS OF THE FUNGAL HOST

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Determinants of virulence in plant pathogenic fungi can be associated to both chromosomal and extrachromosomal factors. Extrachromosomal double-stranded RNA (dsRNA) molecules have been associated to viruses infecting fungal cells (mycoviruses), some of which are known to induce hypovirulence against the host plant in phytopathogenic species. Nowadays, integrated pest management approaches to control plant diseases demand the development of new strategies, including biological control, which are environmentally friendly. Mycoviruses that induce hypovirulence have a great potential as biological control tools, hence the importance of identifying and characterizing these extrachromosomal determinants of virulence in phytopathogenic fungi. Fusarium oxysporum f. sp. dianthi (Fod) is the causal agent of Fusarium wilt of carnation, the most important disease of this crop worldwide. Fod populations show a high level of genetic and pathogenic diversity, that includes the existence of 10 pathogenic races and, moreover, of virulence groups among isolates of the same race. Analysis of total nucleic acid extracts from a large collection of Fod isolates, obtained from the carnation growing areas in southern Spain, identified a dsRNA extrachromosomal element in one race 2 isolate (Fod 116). Molecular characterization of this element indicated that it was a novel mycovirus, designated FodV1, with a genome composed by 4 dsRNAs segments, and phylogenetically related to the family Chrysoviridae. To study the phenotypic effects of this novel mycovirus on the fungal host we obtained a "cured" version of Fod 116. Comparison of the virus-containing isolate and the virus-free version showed that FodV1 affects colony morphology, pigmentation and conidiation. What is more, preliminary data from a pathogenicity test on 5 different carnation cultivars (1 resistant and 4 susceptible to Fod race 2), indicate that FodV1 causes hypovirulence in Fod, although this effect is cultivar-dependent.

365 IDENTIFICATION OF A VIRULENCE EFFECTOR VAG2 OF THE MAIZE HEAD SMUT PATHOGEN SPORISORIUM REILIANUM

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Sporisorium reilianum and Ustilago maydis are closely related biotrophic smut fungi that cause different symptoms on maize. Following fungal inoculation of seedlings, S. reilianum 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. In contrast, U. maydis leads to symptoms within one week after plant penetration, forming tumors containing fungal spores near the penetration site. The genomes of both fungi are highly syntenic but contain regions with weakly conserved genes mainly encoding secreted proteins [1]. To test whether these genes are involved in symptom formation of S. reilianum, we deleted the largest divergence region of about 30 genes in S. reilianum, which resulted in strains showing dramatically reduced virulence and inducing 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, and this reduction can be complemented by re-introduction of vag2. Deletion strains show a similar growth pattern inside in the host as wild-type strains. qRT-PCR analysis revealed that vag2 is highly upregulated during biotrophic growth of S. reilianum. Plants inoculated with deletion strains show an increased gene expression of pathogenesis related (PR) genes compared to wild-type inoculations. 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.
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366 IDENTIFICATION OF MICROORGANISMS WITH ANTIFUNGAL ACTIVITY AGAINST T. AGRRESIVUM AND OTHER "WEED" FUNGI IN MUSHROOM COMPOST

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The cultivation of mushrooms is susceptible to unwanted growth of fungi, like Trichoderma aggresivum and Penicillium. The growth of these fungi causes substantial decrease of yields to the growers or even destroy the harvest completely. In this study we have identified microorganisms present in compost that are able to inhibit growth of fungi like T. aggresivum and Penicillium. The antifungal compounds are secreted in the medium and in a bioassay the effect of the presence of these antifungal producing bacteria on the growth of the commercial mushroom Agaricus bisporus and T. aggresivum is studied.

367 IDENTIFICATION OF THE ARBUSCULAR MYCORRHIZAL FUNGI EFFECTOR REPERTOIRE

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Arbuscular mycorrhiza fungi (AMF) form an ecologically important symbiosis with more than two-thirds of studied land plants. This relationship persisted morphologically unchanged throughout the evolutionary development within the plant phylum from haploid gametophytes to diploid sporophytes. The mechanism of plant infection and colonization while avoiding plant defence is ancient and conserved within AMF. Recent studies of plant-microbe interactions, involving mutualistic ectomycorrhizal fungi, exhibit striking similarities during infection and point in the same direction: genes encoding effector proteins were shown to play a key role in host colonization by controlling the plant immune system. Pathogen effector proteins are released to alter host cell structure and function, allowing successful infection, and suppressing the host defence response. There are indications that symbiotic effectors may similarly suppress the plant immune system and affect plant colonization. We hypothesise that also in AM symbiosis effectors play an important role. The conservation level of effector proteins between AMF species may be indicative whether they play a fundamental role or have more supplemental function during fungus-plant interaction. We predicted the effector repertoire of the AMF genus Rhizophagus as well as assigned their conservation level between two species, R. irregularis and R. clarus. The putative secretome was identified from the R. irregularis genome. The effectome was predicted based on the observation that known effector proteins from fungal and oomycete plant pathogens are secreted and fulfil at least one of the following criteria: contain a nuclear localization signal (NLS), are small and cysteine rich, contain internal repeats or show similarity to haustorial (arbuscular) expressed proteins. Subsequently, we generated a genome draft of R. clarus and searched for effector candidate homologs present in both Rhizophagus species. Based on similarity level we divided the candidates into conserved (>90%) and non-conserved ones. Members of the NLS group are highly enriched among the conserved candidates and NLS effectors also show the highest conservation level (62%) in comparison to the other groups. Therefore, plant nucleus localized effectors might play a fundamental role during AMF symbiosis.

368 IDENTIFICATION OF THE VOLATILE ORGANIC COMPOUNDS (VOCS) INVOLVED IN MEDICAGO TRUNCATULA GROWTH PROMOTION AND ANTIFUNGAL TRAITS AGAINST THE PHYTOPATHOGEN BOTRYTIS CINEREA IN PSEUDOMONAS FLUORESCENS STRAINS

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Rhizobacteria exhibit direct and indirect mechanisms to beneficiate plant growth promotion. Direct mechanisms include the synthesis of phytohormones, while indirect mechanisms comprise antagonism toward phytopatogens. Very few works in literature report strains containing both mechanisms. Here, we present four novel strains containing both, direct and indirect mechanisms of plant-growth promotion. By sequencing the complete ribosomal 16S ribosomal genes, strains were characterized as Pseudomonas fluorescens species (UM16, UM240, UM256 and UM270). Pseudomonas strains exhibited differential growth promotion of Medicago truncatula plants, either by synthesis of diffusible compounds or by volatile organic compounds (VOCs) emission. Chromatographic analysis showed that strains produce indolacetic acid (AIA) at different concentrations. Plant-bacteria interacting experiments revealed that strains produce diverse VOCs, including the dimethylhaxadecylamine, which has been previously assigned in our work group as a plant-growth promoting compound. Pseudomonas strains also exhibited antagonism toward the fungi Fusarium oxysporum, Rhizoctonia solani, Diaporthe phaseolorum,Coletotricum linthemutianum and Botrytis cinerea. Respect to the latter, all strains showed strong mycelial inhibition in Petri dish assays, covering the plates with a red color pigment which has been related to the synthesis of 2,4-diacetylphloroglucinol (DAPG). PCR amplification showed the presence of the complete operon phlACBD, responsible of DAPG synthesis in all strains. As expected, we also detected genes related to phenazines, HCN, as well as the synthesis of siderophores and protease activity. Interestingly, B. cinerea induced phlD expression in Pseudomonas strains, which suggest bacterial strains are sensing and defending from the pathogen trough synthesis of this antibiotic. Finally, we observed biocontrol activity against B. cinerea by protecting M. truncatula plants from fungal infection. Therefore, these Pseudomonas strains have a high potential to be used as either biocontrol or biopromoting agents in crops.

369 IMMUNOASSAY FOR THE DETECTION OF FUSARININE C PRODUCED BY ASPERGILLUS SPP.

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Aspergillus fumigatus is an opportunistic pathogen responsible for a number of clinically relevant diseases in immunocompromised individuals, including invasive aspergillosis (IA). IA is the most lethal type of infection with mortality rates ranging from 30 – 90 % depending on the patient primary condition causing the immunosuppression. Conventional diagnosis of IA can be laborious, subject to poor sensitivity or specificity of detection, or unable to differentiate between live versus dead organisms. Detection of siderophores (triacetylfusarinine C or fusarinine C) which may be produced by infectious microorganisms represents an alternative method to diagnose fungal infections. We have developed a fungal-siderophore ELISA to detect fusarinine C (FusC) as a biomarker of IA in animals. FusC was purified from culture supernatants of A. fumigatus ATCC46645 by passage through Sep-Pak C18 cartridges. Quantification of FusC was measured photometrically using a molar extinction co-efficient of 2996 M-1 cm-1 at 435nm. FusC-KLH immunogen (6 mg) was generated using a UV crosslinking method. As this cross-linker had not previously been used for immunogen synthesis several molar ratios of hapten to carrier protein and UV exposure times were evaluated in order to maximise conjugation. Western blotting, spectrophotometry and RP-HPLC were utilised to confirm the formation of these conjugates. These techniques were also employed to investigate the hapten densities attached to the carrier protein. Rabbit immunisations using the FusC-KLH immunogen were carried out by commercial arrangement. The resulting polyclonal antisera contained highly specific antibodies against FusC. Purified FusC was then used to develop a fusarinine C-specific competitive ELISA, based on the competition between immobilized and free FusC for IgG [anti-FusC]. Availability of the FusC ELISA facilitated evaluation of analyte presence in normal-state and disease-state specimens of animal origin. FusC concentration in specimens were calculated by comparison to a FusC calibration curve (0 – 50 µg/ml). FusC was detectable in the urine and sera of immunocompromised guinea pigs while no detectable FusC was present in uninfected guinea pigs. In summary we have developed the first fungal-siderophore specific ELISA to detect siderophores as biomarkers of IA in animals.

370 IN VIVO VISUALISATION OF CANDIDA ALBICANS PERSISTENCE UNDER ANTIMYCOTIC THERAPY

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Candida albicans is a normal commensal on human mucosal surfaces. However, surgical interventions, immunosuppression regimens and antibiotic therapy in ICU patients predisposes for the development of life-threatening disseminated candidasis. To study pathogenesis of disseminated candidiasis and efficacy of therapeutic approaches the mouse model of intravenous infection is commonly used. However, conventional monitoring of disease progression is limited to snapshots of the infections process since animals are generally sacrificed at pre-defined time points and, therefore, no longer available for subsequent investigations. To overcome these limitations, we developed a real-time non-invasive bioluminescence imaging technique that allows visualisation of disseminated infection with Candida albicans in internal organs such as the kidney. Using C. albicans reporter strains expressing a synthetic codon-optimised luciferase, we could detect less than 5000 Candida cells per entire kidney and, additionally, identified individual animals with foci of infection in the brain and translocation of C. albicans to the urinary bladder. Quantification of renal bioluminescence correlated well with determination of fungal burden as colony forming units (Spearman correlation 0.92, $p < 0.0001$), suggesting that this method is suitable to monitor disease progression and efficacy of therapeutic intervention. As prove of principle, we monitored persistence of C. albicans during consecutive treatment with caspofungin and fluconazole: While therapy significantly improved clinical outcome and mediated the expected clearance of C. albicans from kidneys, we made the unexpected discovery that C. albicans can persist and withstand antifungal therapy in cryptic niches. Our data imply that these niches might provide a reservoir for de novo colonisation of a host after discontinuation of antifungal therapy and, thus, might lead to recurrence of C. albicans infections.

371 INFECTION PROCESS RESTORES DEFECTIVE DON PRODUCTION IN A F. GRAMINEARUM HETEROCHROMATIN PROTEIN 1 (HEP1) MUTANT

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Heterochromatic marks are known to be involved in the regulation of secondary metabolite gene clusters in saprophytic fungi. To test the influence of chromatin-level regulation on metabolite production and virulence in a plant pathogenic fungus, we deleted the heterochromatin protein-1 homologue, called Hep1, in F. graminearum, a species causing head blight disease of wheat. In axenic culture the hep1 deletion mutant showed a strongly altered secondary metabolite profile including significant reduction in levels of deoxynivalenol (DON) and its acetylated derivative 15ADON. Interestingly, the addition of a simple plant-derived DON/15ADON inducer (ornithine) to synthetic media could not restore the defective trichothecene production in the mutant. In contrast, on killed what heads, the most similar saprophytic substrate for the pathogenicity assays, DON/15ADON production already was higher in the hep1 deletion mutant compared to the Ph-1 wild type. This indicates that pre-existing plant metabolites exist in killed wheat heads which are able to counteract the negative effect on DON/15ADON production in the heterochromatin mutant. In the following infection assays on living wheat heads, the hep1 deletion strain significantly exceeded the wild type levels of DON/15ADON production. Consistently the mutant also showed stronger disease symptoms and a ~ 1.5 fold higher overall infection rate on the wheat cultivar Remus. These results indicate that the plant response to infection is upregulating the production of metabolites able to overdrive a repressive genetic network responsible for suppression of DON/15ADON production in the hep1 deletion strain. RNA-seq based transcriptome analysis of the saprophytic and pathogenic growth states to identify genes with potential function in chromatin-related pathogenicity control is on the way.

372 INTERACTION BETWEEN THE FUSARIUM COMPLEX OF BARLEY AND AGRONOMICAL AND ENVIRONMENTAL FACTORS

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Fusarium head blight is known as a destructive disease in all cereal growing areas of the world. In this regard, monitoring results suggest differences concerning the impact between years, cultivars and varieties. As in other small grain cereals, the infection of barley implies yield loss, mycotoxin contamination and modification of enzyme patterns in grain. But the disease is caused by a complex of several Fusarium species differing in their toxin spectra and epidemiologies. Field trials, green house experiments and in vitro approaches were carried out to compare F. graminearum, F. culmorum, F. avenaceum, F. tricinctum, F. sporotrichioides and F. langsethiae as barley pathogens due to their epidemiology. In this context, the influence of environmental factors like weather conditions and the use of agronomic tools like nitrogen fertilization and fungicide application were of special interest. Results indicate species-specific characteristics and behavior referring to inoculum distribution, infestation on various plant organs and spike infection strategies. Artificial inoculation experiments were conducted to investigate the consequences of the impact of different Fusarium species on yield and quality parameters, focusing especially on malting quality factors e.g. sorting, germination capacity and proteomics. Subsequent gene expression studies and the observation of species-specific DNA and toxin contents throughout the malting process gave further hints in regard to host-parasite interactions. On the one hand, the present study detected differences in the epidemiology of single Fusarium species and could show effects of agronomic treatments. On the other hand, investigations of artificially inoculated barley plant material gave more detailed information about the influence of single species on proteomics and other malting parameters. First and foremost, the generated information could support the choice of single factors to prevent from Fusarium infestation. Over and above, a more detailed knowledge about Fusarium-barley interactions could contribute to improvements in the malt production process.

373 INTERPLAY OF HYDROPHOBINS IN TRICHOLOMA VACCINUM LIFE CYCLE

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Hydrophobins are small secreted proteins with low sequence homology and eight highly conserved cysteines which form disulfide bridges. Two classes of hydrophobins are distinguished, depending on their solubility. Their broad range of functions includes cell wall integrity, coating and adhesion in pathogenic or ectomycorrhizal interactions. In addition, hydrophobins are differentially expressed in the mutual ectomycorrhiza symbiosis. We showed an up-regulation for the hydrophobin hyd1 in the Hartig'net during interaction of Tricholoma terreum with pine. We investigate hydrophobins in T. vaccinum, a widely spread basidiomycete (Agaricales - Tricholomataceae) forming ectomycorrhiza with compatible host Picea abies and low-compatible Pinus sylvestris. The formation of a compatible mycorrhiza needs about one month in co-culture. In contrast, a low compatibility mycorrhizal interaction with a different host takes about four months. For T. vaccinum, a preliminary genome sequence has been annotated allowing us to compare all hydrophobin genes for phylogeny and differential expression. So far, we investigate nine T. vaccinum hydrophobins and compare them to 17 hydrophobins available from the genus Tricholoma in total. Via qRT-PCR we can show regulation of hydrophobin genes in various stages of the life cycle, like the fruit body specific h3. Moreover, the mycorrhiza specific hydrophobin h5 is differentially expressed in compatible and low-compatible hosts. In addition, we identified the role of heavy metal stress for hydrophobin regulation. In silico analyses identified protein-protein complexes for rodlet layer formation and in comparison with our phylogenetic investigations, we thus are able to predict functional and evolutionary association.

374 INTRACELLULAR AND POTENTIALLY EXTRACELLULAR ROLES OF THE USTILAGO MAYDIS ACYL-COA-BINDING PROTEIN ACB1

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Conventionally secreted fungal effectors play a crucial role during the biotrophic interaction between the smut fungus Ustilago maydis and its host plant Zea mays. In the last decade it has been well established that proteins without a signal peptide can also be targeted to the outside of the cell in an ER/ Golgi independent manner. Many of these unconventionally secreted proteins have been shown to be 'moonlighting' proteins with an extracellular function distinct from their intracellular function. One candidate for an unconventionally secreted protein in U. maydis is the UmAcb1 protein, previously detected in the apoplastic fluid of infected maize. UmAcb1 is an ortholog to the Dictyostelium discoideum acyl-CoA-binding protein AcbA, which is unconventionally secreted as full-length protein and extracellularly processed into a small peptide (SDF-2) that triggers terminal spore differentiation upon interaction with a membrane receptor (Duran et al., 2010). We could show that hyphal culture supernatants, as well as apoplastic fluid extracted from U. maydis infected plants, trigger spore formation in D. discoideum indicating the secretion of an SDF-2-like peptide in U. maydis. We are currently investigating the role of the UmSDF-2 peptide during the U. maydis life cycle. Deletion of the acb1 gene in U. maydis leads to a significant axenic growth phenotype most likely due to the lacking intracellular acyl-CoA-binding function. This growth defect is spontaneously suppressed by point mutations in central fatty acid metabolism genes. Using lipidomic profiling and cell biological approaches we aim to understand the mechanism that makes the Acb1 protein dispensable in the suppressor strains.

Duran et al., 2010. Unconventional secretion of Acb1 is mediated by autophagosomes. J Cell Biol. 2010 Feb 22; 188(4):527-36

375 INVESTIGATING SPORULATION OF ZYMOSEPTORIA TRITICI, A PATHOGEN OF WHEAT

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Zymoseptoria tritici is an ascomycete fungus which causes Septoria Tritici Blotch, a major disease of wheat. A potential way to control this pathogen is to prevent asexual sporulation by inhibiting development of the asexual fruiting body and spores. The recent sequencing of the Z. tritici genome has provided useful insights into the pathogen, and can be used identify candidate genes for investigation. Comparative genomic analyses have been used to identify potential genes involved in asexual sporulation in Z. tritici. Key Genes already known to be important for this process in other model ascomycete fungi were BLAST searched against the Z. tritici database to find potential homologues. Of the 84 genes analysed, 21 potential homologues were found in Z. tritici. Molecular genetic analyses were conducted on the candidate genes to elucidate their temporal and spatial expression patterns across the time course of infection. Of these, 5 have been selected for further study to assess their role in asexual sporulation in Z. tritici. Reliable sporulation of Z. tritici has also been successfully established in vitro. This will help future research into the fungus, particularly in the identification of knock-out mutants. The most recent results from these ongoing investigations will be presented.

376 INVESTIGATING THE ROLE OF TYROSINE CATABOLISM AND PYOMELANIN PRODUCTION DURING IN VIVO GROWTH IN THE HUMAN PATHOGEN PENICILLIUM MARNEFFEI

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For pathogens to successfully infect a host, two equally important events must be achieved; the pathogen must be able to evade or tolerate the host's defence systems and it must be able to acquire and utilise the available nutrient sources within the host in order to grow. These factors are significant hurdles for most pathogens, but especially so for intracellular pathogens which have to evade or tolerate the cytotoxic machinery of innate immune cells and scavenge nutrients from this relatively nutrient poor environment. In a screen aimed at identifying genes which are important determinants of pathogenicity in the dimorphic fungus Penicillium marneffei, a number of metabolic genes were found which are specifically expressed in the pathogenic yeast cells and not in the saprophytic hyphal cells. Preliminary characterisation showed that one of these genes, designated hpdA, encodes 4-hydroxyphenlypyruvate dioxgenase (4HPPD) which catalyses the conversion of 4-hydroxyphenlypyruvate to 2,5-dihydroxyphenylacetate (homogentisate), a step in the tyrosine catabolic pathway. Tyrosine is metabolized via a conserved pathway to provide the fungus with both nitrogen and carbon. In addition, the oxidation and polymerization of a tyrosine metabolic intermediate, homogentisate, can generate the brown pigment pyomelanin which can protect against oxidative stress and is therefore an important survival and pathogenicity determinant. Genes required for the catabolism of tyrosine are located in a conserved gene cluster. This study describes the deletion of genes of the tyrosine catabolism cluster and the characterization of their role in growth, pyomelanin production and pathogenicity in P. marneffei.

377 LEPTOSPHAERIA MACULANS EFFECTOR AVRLM4-7 SUPPRESSES THE PLANT DEFENCE RESPONSES IN SUSCEPTIBLE CULTIVAR OF BRASSICA NAPUS

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Leptosphaeria maculans interacts with its host Brassica napus according to gene-for-gene concept, where the recognition of the only molecule (pathogen´s effector) by its corresponding partner (product of plant resistance gene) protects the plant against disease development. However, the fungus is able to avoid recognition by mutation events, partial or complete deletion of the avirulence gene. Eleven L. maculans avirulence genes were genetically mapped and at least 3 were cloned up to date, however the functions of the effector proteins for which they code are still unknown. AvrLm4-7 belongs among the few cloned effectors and is special for its dual specificity to products of B. napus resistance genes Rlm4 and Rlm7. By a single amino acid change AvrLm4-7 escapes recognition by Rlm4, but is still recognized by Rlm7. Moreover, absence of the functional AvrLm4-7 allele is linked with decreased aggressiveness suggesting that the effector is important for L. maculans fitness. The question remains: why? After the recognition of invading agent plant signal transduction pathways mediated by plant hormones play a pivotal role in induction of appropriate defence responses. Although more is known about bacterial effectors, recently has been shown that also fungal effectors can affect the plant defence signalling in favour of the pathogen. We investigated the effect of AvrLm4-7 on defence signalling in oilseed rape. Using RT-qPCR we examined the level of transcription of previously characterized marker genes of salicylic acid, jasmonic acid, abscisic acid and ethylene signalling pathways in a susceptible cultivar of B. napus inoculated by L. maculans isolates carrying or lacking AvrLm4-7. We found that salicylic acid and ethylene-specific marker genes are supressed during the infection by the isolate carrying the AvrLm4-7 effector gene. Moreover, the presence of AvrLm4-7 seems to influence the formation of reactive oxygen species in infected cotyledons. Although it is still long way to go to uncover the function of AvrLm4-7 effector, we contributed to narrow the field of search for possible AvrLm4-7 target proteins.

378 METABOLIC RESPONSE OF EUCALYPTUS SHOWS EARLY HOST DIFFERENTIATION BETWEEN PATHOGENIC AND MUTUALISTIC FUNGAL PARTNERS

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Belonging to the Myrtaceae, the economically important Eucalyptus genus is made up of more than 700 individual species. Due to their fast rate of growth and high level of stress resistance, Eucalypts have become industrially important and are currently cultivated throughout the southern hemisphere and as far north as the southern United States. Their migration from their native Australia and surroundings has not been alone; microbiota including both pathogenic and mutualistic fungi normally associated with the roots of Eucalyptus seedlings have also followed suit. The co-migration with mutualistic fungi such as Pisolithus microcarpus has had a positive impact on the establishment of Eucalypt plantations. In this interaction, Pisolithus provides Eucalyptus roots with scarce, growth-limiting nutrients, physical protection from root herbivory and chemical protection from toxic compounds in return for 10-20% of host photosynthetically derived carbon. Competing with mutualistic fungi for nutrients provided by roots are pathogenic fungi such as Sclerotinia which devastate the root growth of seedling Eucalypts. Given the fact that Eucalypt seedlings encounter both mutualistic and pathogenic fungi in natural soils, we were interested in determining how the metabolic response of the plant host differed depending on the lifestyle of the fungus present in the rhizosphere and how early this difference became evident. We demonstrate that the plant exhibits a very different response in sugar transport, the metabolism of aromatic compounds and in the production of defensive compounds between different fungal lineages spanning the mutualism-parasitism continuum. This difference in response on the part of the plant host was found far earlier than expected and suggests that some of the earliest signals exchanged between fungi and plants is enough to alert the plant to the 'intentions' of the colonizing microbe.

379 MULTITROPHIC BEHAVIOUR OF ENTOMOPATHOGENIC AND NEMATOPHAGOUS FUNGI MAKES THEM VERSATILE BIOCONTROL AGENTS

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Nematophagous (NF) and Entomopathogenic fungi (EF) infect invertebrate hosts (nematodes and insects) which cause directly or indirectly (usually as vectors) important pathologies (with associated enormous economic losses) in both plant and animal hosts. NF and EF have therefore a direct application as biological control agents in sustainable agriculture. NF and EF are usually not obligate pathogens. This means that they can have, apart from nematodes and insects which we call their canonical or normal hosts, other non-canonical o alternative hosts. NF and EF can, for instance, colonise plants (mono and dicots) acting as endophytes. As endophytes NF and EF modify plant physiology and development by modulating their defences and promoting growth. Our group is currently exploring using metabolomics the signals involved in the multitrophic interactions of plants, nematodes and NF. This topic is important for the application of biocontrol fungi as plant inoculants. Consequently NF and EF have also a role in plant health independently of the presence of pest or disease causing agents. When infecting their canonical hosts NF and EF must degrade their barriers (eg. egg-shells/cuticles). Chitin is a main structural component of these barriers. Its deacetylated form, chitosan, has higher solubility and is known to have interesting biological properties. Chitosan permeabilises the membrane of plant and human fungal pathogens killing them or compromising their growth. On the contrary NF and EF are resistant to chitosan. Chitosan in both NF and EF activates fungus development and expression of pathogenicity factors such as proteases involved in degradation of host barriers. In our group we are using molecular, cell and agronomical approaches to understand the multimodal action of EF, NF and chitosan to fully exploit it in sustainable agriculture and health applications. We have recently sequenced the genome of the NF Pochonia chlamydosporia (Larriba et al., 2013 under revision). This has lead us to gain a better understanding of the multitrophic behaviour of this biocontrol agent. A phylogenomic analysis showed, for instance, that the genome of the fungus is closest to that of EF but it is also related to that of fungal plant pathogens.

380 MYCOLOGICAL MONITORING OF CROPS IN CENTRAL AREAS OF RUSSIAN FEDERATION

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Microscopic fungi can play an essential role in the etiology of living organisms' diseases. Mycotoxicological quality control of crops and forage is highly recommended therefore it became the aim of our investigation. The samples investigated were from the central areas of Russian Federation. The samples were studied for toxicity and exposed to mycological analyses. The fungi cultures were investigated for toxicity also. Our investigation demonstrated that 36.4% forage crops samples had toxic and weak toxic effect, food crop samples were less poisonous (4.5% samples). Mycological investigation identified the isolates of Aspergillus, Penicillium, Fusarium, Trichoderma, Mucor, Rhizopus, Alternaria, Stachybotrys, Cladosporium, Claviceps, Ustilago genera. Also straw was revealed to contain Aspergillus niger, A. ochraceus, A.fumigatus, Trichoderma lignorum, Stachybotrys alternans, Cladosporium sp. In rolled and siloing-based preserved crops had A.niger, A.fumigatus, A.flavus, A.nidulans, A.candidus, A.glaucus, Penicillium cyclopium, P.viridicatum, P.notatum, P.chrisogenum, Fusarium moniliforme, F.oxysporum, F.avenaceum, F.sporotrichiella, Mucor sp., T.lignorum, S.alternans species. In forage crops (wheat, oats, barley, rye) the species of A.niger, A.fumigatus, A.flavus, A.nidulans, A.ochraceus, A.candidus, A.clavatus, P.cyclopium, P.citrinum, P.urticae, P.rubrum, P.notatum, F.graminearum, F.moniliforme, T.lignorum, Rhizopus sp., Alternaria sp., Ustilago sp, Claviceps purpurea were isolated. Mixed fodders contained A.fumigatus, A.flavus, A.nidulans, A.candidus, P.cyclopium, P.citrinum, P.urticae, P.chrisogenum, P.rubrum, P.notatum, Mucor sp., Rhizopus sp., Alternaria sp. Research of fungi isolates having toxic effect revealed that the greatest toxicogenic capacity was shown by the species of Aspergillus (37 %), Penicillium (17 %), Fusarium (21 %), Trichoderma (7 %), Mucor (10 %), Stachybotrys (3 %). Therefore, mycotoxicological analyses allow rejection of bad forages in-time and reveal the etiological factors of the disease rate.

381 NUCLEAR EFFECTORS IN FUNGI: MANY PROTEINS ESSENTIAL FOR PATHOGENICITY AWAITING TO BE DISCOVERED

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Through a comparative genomic analysis using seven pathogenic fungal species (Colletotrichum graminicola, Colletotrichum higginsianum, Magnaporthe oryzae, Ustilago maydis, Sporisorium reilianum, Cochliobolus heterostrophus and Fusarium graminearum), we uncovered 263 putative effector proteins likely to target the host nucleus as they simultaneously contain sequence signatures for secretion and nuclear localization. Using the pathosystem maize-Colletotrichum graminicola as a model system, we demonstrate that one of those putative proteins is a novel class of pathogenicity factor, CgEP1. This protein is synthesized during the early stages of disease development and is necessary for fungal hyphae to grow within the epidermal layer of the host. Genetic, molecular and biochemical studies confirmed that this novel effector targets the host nucleus, and is a novel class of DNA-binding protein with regulatory properties that is expressed during pathogenesis. From sequence analysis and allelic variation studies, we discovered that CgEP1 has undergone an intense evolution and selection process that led to duplications of complete internal repeats and has undergone episodes of positive selection. This effector family is also highly conserved in other monocot-infecting Colletotrichum species, affecting important crops such as sorghum, wheat and sugar cane. Our work functionally demonstrates the impact of effectoromics in the identification of novel/unknown effector protein in fungal species, and their potential use in the development of novel strategies for crop disease management.

382 PATHOGENICITY CHROMOSOMES IN HOST-SPECIFIC TOXIN-PRODUCING ALTERNARIA SPECIES

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Alternaria alternata plant pathogens consist of seven variants (pathotypes), all of which produce host-specific (selective) toxins (HSTs); all cause necrotic diseases on different plants. We have shown that all strains of A. alternata pathotypes harbour small and extra chromosomes, whereas nonpathogenic isolates do not have these small chromosomes. Based on biological and pathological observations, those small chromosomes were termed conditionally dispensable chromosomes (CDCs) and pathogenicity chromosomes. HST biosynthetic genes have been isolated from five pathotypes (apple, Japanese pear, strawberry, tangerine, and tomato) of A. alternata and found to be clustered on the CDCs. Sequencing of the entire CDCs of the apple, strawberry and tomato pathotypes which produce AM-, AF- and AAL-toxins, respectively, revealed that the CDC of each consists of CDC-specific and repetitive sequences related to the HST production and pathogenicity. The CDC in the tomato pathotype strains from different geographical origins was identical although the genetic backgrounds of the strains differed. The results imply that CDCs have a different evolutionary history from the essential or core chromosomes in the same genome. A hybrid strain between two different pathotypes was shown to harbour the CDCs from both parental strains and had an expanded pathogenicity range, indicating that CDCs could be transmitted from one strain to another and stably maintained in the new genome. We propose a hypothesis whereby the ability to produce HSTs and to infect a plant is distributed among A. alternata strains by horizontal transfer of an entire pathogenicity chromosome (CDC). This could provide a possible mechanism by which new pathogens arise in nature. The chemical structure of the AAL-toxin resembles to that of a mycotoxin fumonisin produced by Fusarium (Gibberella) spp., and both toxins are classified as sphinganine-analog mycotoxins due to their structural similarity to sphinganine. The AAL-toxin biosynthetic (ALT) gene cluster consists of at least 13 genes homologous to the fumonisin biosynthetic (FUM) genes in F. verticillioides. The FUM (ALT) cluster homologues are found in Fusarium spp., Aspergillus niger and Cochliobolus heterostrophus. On the other hand, only the tomato pathotype of A. alternata has the cluster among Alternaria spp.. The ALT cluster in the tomato pathotype also might be acquired by horizontal transfer of the entire cluster genes from those pathogenic fungi.

383 PRODUCTION OF A NEW TYPE A TRICHOTHECENE BY ISOLATES OF FUSARIUM GRAMINEARUM

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Production of deoxynivalenol (DON) is a virulence factor of Fusarium graminearum on wheat and most likely on other host plants. A large survey of F. graminearum (sensu strictu) in the northern United States revealed the existence of strains which - based on molecular markers - belong to the 3-acetyl-DON (ADON) chemotype, but do not produce known DON or nivalenol derivatives. With a LC-MS/MS multitoxin method we confirmed that the isolates do not produce known trichothecenes. Yet, GC-MS headspace analysis revealed presence of the precursor trichodiene. A compound occurring in the “Northland” (N) strains was purified and its structure elucidated. The novel trichothecene, termed NX2, is identical to 3-ADON with the exception that it lacks the keto group at C-8, which is a hallmark of the typical type B trichothecenes. During colonisation of wheat the new toxin NX2 is deactylated to DNX2, like 3-ADON to DON. The toxicity of DNX2 is very similar to that of DON in plant and animal derived in vitro translation systems (wheat germ extract, rabbit reticulocyte lysate). We have started to investigate the molecular basis of NX2 production. The TRI1 gene product introduces an oxygen function only at C8 in F. sporotrichioides, while it is is responsible for both the C7 and C8 oxygenation in normal F. graminearum strains. We are testing the following hypotheses: Either the TRI1 gene in N strains is non-functional and the oxygenation at C7 is performed by a different cytochrome P450 oxygenase, or, a modified TRI1 product of NX-2 producing strains might hydroxylate at C7 only. We found 14 amino-acid changes in all N strains investigated compared to the sequenced strain PH-1. Inactivation of the TRI1 gene in an N strain led to production of trace amounts of NX2. To test whether the N-allele of TRI1 is responsible for NX2 production we have swapped the TRI1 alleles of PH-1 and N-strains. It is unclear at present how prevalent and toxicologically relevant this new mycotoxin is, which escapes conventional detection techniques, and whether its production provides a selective advantage on certain host genotypes.

384 PRODUCTION OF A NOVEL IMMUNOMODULATORY COMPOUND BY A PROTEIN KINASE A REGULATED GENE CLUSTER OF ASPERGILLUS FUMIGATUS

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Aspergillus fumigatus is a common soil inhabiting mould but also an opportunistic human pathogen that can cause life threatening infections in immunocompromised individuals. Its survival in these entirely different habitats depends on effective mechanisms for signal perception and transduction. One of these signal transduction cascades is the cAMP/protein kinase A pathway that represents also an important virulence determinant of A. fumigatus. In a transcriptomics approach putative targets of this signaling cascade were identified. Among these were several transcriptional regulators and a transcription factor, which showed highest upregulation by PKA. This transcription factor is part of a secondary metabolite gene cluster. Deletion of the corresponding gene as well as a deletion of the non-ribosomal peptide synthetase (NRPS) encoding gene in the cluster led to reduced fungal growth and sporulation. Overproduction of the transcription factor resulted in formation of a brown substance, whose structure was elucidated. The novel identified compound was named fumipyrrole. Remarkably, fumipyrrole inhibits interleukin-1β secretion by human peripheral blood mononuclear cells, suggesting a role in pathogenicity of A. fumigatus.

385 QUANTITATIVE PROTEOMICS WITH CUSTOM DATABASE SEARCHING TO REVEAL DIFFERENCES IN THE HAUSTORIAL PROTEOMES OF THREE RACES OF WHEAT LEAF RUST, PUCCINIA TRITICINA

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The fungus Puccinia triticina (Pt) is an obligate parasite that causes leaf rust on wheat, leading to significant annual yield losses worldwide. Rust populations consist of races, with only subtle differences in their genomes. Phenotypically these small differences can enable a rust race to overcome the host's defences and cause disease. Thus wheat bearing the Lr1 resistance (R) gene is resistant to Pt Race 1, but not to Pt Race 9. The Pt genes responsible are termed avirulence (avr) genes, and the way in which they interact with the host R genes is well understood at the genetic level, but not at the biochemical level – indeed the identity of the vast majority of rust avr genes remains unknown, in spite of their tremendous economic importance. Pt enters the host leaf through stomata and ultimately forms intracellular feeding structures called haustoria, which direct the import of nutrients from the host into the fungus and synthesize and secrete proteins, including avr proteins, which are critical for pathogenesis. We have purified haustoria from three Pt races, all with full genomic sequences available, to near-homogeneity using monoclonal antibodies. These races are Race 1 which was sequenced in 2009 and Race 9 and Race 161, for which we have access to sequences. This has enabled us to create custom databases which we can query with MS spectra created from haustoria containing a single unique race of Pt. A high-resolution mass spectrometer and a combination of iTRAQ labelling and spectral counting were used to measure quantitative differences in the three proteomes. Comparative analysis of the mass spectra from the three races is expected to identify variation at the protein level and possibly provide candidate avr proteins. This project is in progress and the latest results will be presented, highlighting the use of custom databases and quantitative mass spectrometry to detect subtle differences in closely related pathogens.

386 RECOGNITION AND RESPONSE TO BACTERIAL NON SELF IN PODOSPORA ANSERINA

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Recognition and response to non self, whether conspecific (between individuals form the same species) or heterospecifc (individuals form another species) is essential to many aspects of life including development, symbiosis and protection against pathogens. However distinction between these modes of recognition and responses is somehow blurred and can overlap. For instance in plants and animals Pathogen Recognition Receptors (PRRs) can occasionally lead to auto-immune diseases in absence of pathogens. The NLR and NBS-LRR STAND proteins (a class of signal transduction proteins) are major PRRs in plants and animals, but these receptors remain largely unidentified in fungi. In Podospora anserina vegetative incompatibility (VI), a conspecific non self recognition process (between individuals of the same species), leads to cell death and autophagy. VI is determined by interaction of het-c, encoding a glycolipid transfer protein, with members of the hnwd gene family encoding for STAND proteins that appear analogous to plant and animal PRRs. We hypothesized that these genes are involved in pathogen recognition and that recognition of heterospecific non self would initiate a response similar to the VI reaction. We undertook the task of deciphering the response of P. anserina to heterospecific non self, focusing our efforts on the description of the cellular response and the identification of fungal PRRs. We first screened bacterial species able to initiate a response in P. anserina that is altered in mutants affected for the VI reaction and e selected species from the genus Serratia and Pseudomonas. When confronted to S. fonticola or S. marcescens, P. anserinas response overlap the VI response at all levels investigated so far, including cellular morphology and cytology, requirement of autophagy and induction of the expression of a specific set of genes. TEM observation of P. anserina confronted to Serratia species revealed the presence of vesicles in and around P. anserina cells, suggesting a possible role for bacterial Outer Membrane Vesicles (OMVs) in the initiation of a response. Indeed addition of partially purified S. fonticola OMVs in the growth medium in absence of any bacteria initiates a fungal reaction, and we are now attempting to identify fungal receptors to these OMVs.

387 REGULATION OF INNATE IMMUNITY TO THE FUNGAL PATHOGEN FUSARIUM OXYSPORUM BY MICRORNAS IN TOMATO

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MicroRNAs (miRNAs) are important regulators of growth and development in plants. Several miRNA families target genes encoding nucleotide binding site–leucine-rich repeat (NBS-LRR) plant innate immune receptors. The fungus Fusarium oxysporum f.sp. lycopersici causes vascular wilt disease in susceptible tomato plants. We explored a possible role for miRNAs in tomato defense against F. oxysporum using comparative miRNA profiling of susceptible (Moneymaker) and resistant (Motelle) tomato cultivars infected with F. oxysporum. The results revealed that slmiR482f and slmiR5300 were repressed during infection of Motelle with F. oxysporum. Northern analysis confirmed that two predicted mRNA targets each of miR482f and miR5300 exhibited increased expression in Motelle and the ability of these four targets to be regulated by the miRNAs was confirmed by co-expression in Nicotiana benthamiana. A virus-induced gene silencing approach in the resistant Motelle cultivar revealed a role in fungal resistance for all four targets. All four genes encode proteins with full or partial nucleotide-binding (NB) domains. One miR5300 target, Solyc09g018220, is tm-2, a susceptible allele of the Tomato Mosaic Virus resistance gene. Our findings reveal microRNAs that mediate resistance to F. oxysporum in tomato, implicate miR5300 in plant immunity and support functions for tm-2 in resistance to a fungal pathogen.

388 RESPONSES TO THE BRASSICACEOUS INDOLIC PHYTOALEXIN BRASSININ IN THE PHYTOPATHOGENIC FUNGUS ALTERNARIA BRASSICICOLA

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Chemical defence mediated by host antimicrobial secondary metabolites is one of the key elements of the plant immune system. Phytoalexins are biosynthesized de novo by plants in response to microbial attack and other abiotic stresses, and are secreted to the infectious site. They have been demonstrated to have activity in vitro against plant pathogens, such as bacteria and fungi, and numerous studies indicate that induced antimicrobial chemicals confer protection against disease. Despite the paucity of studies in their mechanism of action, it appears that generally phytoalexins act as plasma membrane disruptors of microbial pathogens. Due to their infection strategy, fungal necrotrophs are exposed to several antimicrobial defense metabolites during host infection. For successful colonization of host tissues, these fungal pathogens may have therefore developed efficient adaptation responses to overcome such chemical stress. Alternaria brassicicola, a typical necrotrophic fungal pathogen responsible for the black-spot disease of Brassicaceae, is thus exposed to several preformed and neo-synthesized indolic metabolites produced by its host plants. To date, more than forty phytoalexins have been identified from cultivated and wild Brassicaceae. They share typical structural features and contain an indole ring, derived from (s)-tryptophan, and potential additional nitrogen and sulfur atoms. The phytoalexin brassinin is the major indolic phytoalexin in cultivated Brassica species. Analysis of the transcriptomic response of A. brassicicola to short-time exposure to brassinin suggested that the phytoalexin targets mitochondria and provokes ROS accumulation and mitochondrial membrane defects. Activation of several cellular signaling pathways were observed in response to these oxidative and membrane stresses. In particular the unfolded protein response and cell wall integrity pathways were quickly activated upon brassinin exposure suggesting that cell wall and membrane strengthening constitute a major protection mechanism for the fungus. We then tested the possibility that inhibitors of these signaling pathways could act synergistically with brassicaceous phytoalexins and thus constitute relevant candidates for the development of new plant protection strategies.

389 RNA-SEQ ANALYSIS OF THE INTERACTION OF ASPERGILLUS FUMIGATUS WITH HUMAN NEUTROPHILS REVEALED NITRIC OXIDE AS NEGATIVE FEEDBACK REGULATOR OF NEUTROPHIL EXTRACELLULAR TRAPS

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Aspergillus fumigatus is a saprophytic mold that can cause life-threatening infections in immunocompromised patients. In the lung, conidia are challenged by immune effector cells. Among them, neutrophils attack fungal hyphae using various mechanisms including production of antimicrobial proteins, degranulation or neutrophil extracellular trap (NET) formation. Moreover neutrophils form nitric oxide (NO) and reactive nitrogen intermediates (RNI) whose function in defence against A. fumigatus is still a matter of debate. By applying a dual transcriptomics approach, we were able to identify genes expressed during the interaction of A. fumigatus with human neutrophils. Here, we show that A. fumigatus produces several enzymes potentially involved in RNI detoxification, namely two flavohemoglobins, FhpA and FhpB, and the S-nitrosoglutathione reductase GnoA. To elucidate the role of these enzymes, single and double deletion mutants of FhpA, FhpB and GnoA encoding genes were generated. Our data indicate that FhpA and GnoA are the primarily responsible enzymes in A. fumigatus to counteract RNI. Interestingly, based on the finding that human neutrophil granulocytes showed a significant reduction in NETosis when either confronted with hyphae of A. fumigatus mutants deficient for RNI-detoxification or by addition of exogenous NO donors, we strongly suggest that NO derivates play a role as potential regulator of NET formation.

390 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. In addition, we have identified defects in Mgslt2 null mutants such as reduced mycelium hydrophobicity and complete lack of melanisation that were not frequently reported in other fungal slt2 up to now, while classical defects such as hypersensitivity to calcofluor was not observed in Mgslt2 mutants. Therefore, M. graminicola SLT2 pathway has likely evolved to control gene networks in part different from those of other fungal SLT2 pathways. To identify these networks using transcriptomics, we have constructed strains expressing an active MAPKK allele either under its native promoter or under the control of the nitrate reductase promoter. These strains allow the induction of the SLT2 pathway in controlled conditions. Differential genome wide expression analyses are currently performed to identify genes whose expression requires an active SLT2 MAPK. The role of these genes in development and infection will be studied by reverse genetics.

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391 ROLE OF OXYLIPINS, PRODUCED DURING THE INTERACTION BETWEEN THE ENDOPHYTIC FUNGUS PARACONIOTHYRIUM VARIABILE AND THE PHYTOPATHOGEN FUSARIUM OXYSPORUM

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1UMR 7245, MCAM, CNRS, Muséum National d'Histoire, 63 Rue Buffon, 75005 Paris, France; 2UPMC, Université Pierre et Marie Curie, 4 Place Jussieu, 75005 Paris, France. Endophytic fungi have been shown to establish mutualistic associations with their host plants and conferring fitness benefits such as tolerance to biotic stresses. In many cases, compounds isolated from endophytic fungi are fungicidal or antibacterial thus supporting the idea that they play a role in the host plant defence. We studied and identified the fungal diversity of Cephalotaxus harringtonia, an Asian conifer tree of medical importance. We focused on the isolated endophyte Paraconiothyrium variabile, as it showed a strong antagonism towards Fusarium oxysporum known as an ubiquitous fungal phytopathogen. We identified competition-induced oxylipin production, which provoked a negative modulation of beauvericin biosynthesis, one of the most potent mycotoxins of F. oxysporum (Combès et al. 2012). We started to study the exact role of these oxylipins, identified as 13-oxo-9,11-octadecadienoic acid and 13-hydroperoxy-9,11-octadecadienoic acid, in the antagonistic interaction between F. oxysporum and P. variabile by constructing oxylipin mutants in the endophyte P. variabile. Study of these mutants will clarify the origin of the competition induced oxylipins, their role in the observed antagonism and the negative regulation of beauvericin production in the pathogen F. oxysporum.

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392 ROLE OF THE NADPH OXIDASES OF TRICHODERMA ATROVIRIDE IN HOST INTERACTION

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One of the most important problems in agriculture is the diseases caused by fungi. These plant deseases brings low productivity, important economic losses and, in most cases environmental damage due to the use of fungicides and agrochemicals. Moreover the ability to control these diseases has been difficult due to their soilborne nature, wide host range, and its ability to adapt to new conditions. In the last years mechanism of biocontrol have been employed as alternative to control phytopathogenic fungi with the advantage that they are a friendly alternative to the environment. One of these soilborne pathogens is Rhizoctonia solani J.G. Kuhn and its different anastomosis groups (AGs). This specie include some of the most devastating plant pathogens. These fungi are geographically distributed worldwide and are capable to infect most of the vascular plants. Since long time species of Trichoderma have been employed as a biological control agent due to its ability to antagonize phytopathogenic fungi, one of this is Trichoderma atroviride, wild-type isolated IMI206040, a filamentous fungus with a cosmopolitan life style that associates with economically important crop plants. Although the molecular mechanism of interaction with plants and biocontrol are still not entirely clear, the synthesis of Reactive Oxygen Species (ROS) by NADPH oxidases (nox) plays a key role in the biological control of these fungi and in the modulation of symbiosis with plants. The overall aim of this research is to understand the molecular role of nox genes of T. atroviride in biocontrol and symbiosis with plants. Mutants for the nox genes in T. atroviride, nox1, nox2 and noxR were generated. We have observed loss, and partial loss in the ability to control R. solani and R. solani AG5 in this mutants, and the induction of plant defense. The next step for this research is the analysis of the differencial gene expression at three stages of interaction, before contact, during the initial contact and when the interaction has been established. We hope to determine the genes involved in response of this interaction and whether their interaction with plants is modified.

393 SEARCHING FOR MYCOVIRUSES IN CHALARA FRAXINEA, THE CAUSAL AGENT OF ASH DIEBACK

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The ascomycete fungus Chalara fraxinea (teleomorph Hymenoscyphus pseudoalbidus) is a new invasive pathogen causing severe dieback of Fraxinus spp in Europe. The disease was first recorded in the 1990s in eastern Poland and Lithuania and has since then spread across the continent. Options for management of this novel forest disease are currently very limited and mainly directed to search for resistance in the host trees. Mycoviruses are commonly found in all major groups of plant pathogenic fungi. Some of these viruses were found to cause debilitating disease or reduce virulence in their fungal host and thereby have the potential to be used as biological control agents (e.g. hypoviruses in the chestnut blight fungus, Cryphonectria parasitica). The main aim our study is to identify mycoviruses, describe their phylogenetic position, and assess their biological control potential against ash dieback. To do so, we sampled ash stands both in Switzerland and Lithuania. In Switzerland, the disease was first reported in 2008 and is still expanding, while Lithuania experiences a post-epidemic chronic dieback with only a small fraction of asymptomatic trees. Using Illumina Miseq we sequenced 2 isolates of this fungus and with the help of bioinformatic tools we could identify a RNA virus sequence within each of the fungal isolates. Phylogenetic analyses of our contig (AA open reading frame) and 15 members of the pfam05919 (mitovirus RNA-dependant RNA polymerase) revealed closest relationship with Chryphonectria cubensis mitovirus 1c and Helicobasidium mompa mitovirus 1-18. Both are members of the genus Narnaviridae, which also hosts mitoviruses relevant for biological control in other tree species (e. g. chestnut blight). In order to confirm this relationship, and to identify additional mycoviruses, we are currently Sanger Sequencing our contig and running an additional set of 120 Swiss and Lithuanian isolates on a Illumina HiSeq machine.

394 SEED-BORNE FUNGI OF BARLEY GRAINS AND THEIR IMPACT ON SEED GERMINATION

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Eleven Barley seeds samples collected from different storage locations in the Jazan province and examined for fungal seed-borne fungi by using the standard PDA method described by the International Seed Testing Association (ISTA). Five seed- borne fungi viz., Aspergillus niger, A. flavus, A. parasiticus, Alternaria alternata and Drechslera sp. Were isolated from 11 barley seed samples, Aspergillus spp., was predominately with an infection range from 42.5 - 46.5. The highest seed germination was noticed by sample no. 2 and the other parameters i.e. shoot and root length of seedlings recorded different measurements along with the tested barley seed- borne fungi. Cluster analysis was a reliable method to differentiate between 14 isolates belonging to the closely related Alternaria alternata. A similarity coefficient matrix of Alternaria alternata, isolates, involved in barley seedling damping-off showed a few significant correlations between the tested isolates of Alternaria alternate

395 SIDEROPHORE BIOSYNTHESIS IN PATHOGENIC MUCORALES

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Iron is essential for growth, and in low iron environments such as in serum, some microorganisms secrete ferric iron-chelating molecules called siderophores. A few species of Mucorales fungi have been shown previously to secrete the polycarboxylate siderophore, rhizoferrin. The overall aim of our research is to investigate the role of rhizoferrin in survival of pathogenic Mucorales in vitro and in vivo. Using HPLC, we examined the supernatants of 6 common Mucorales pathogens after growth in low iron medium containing human serum, and showed that all 6 species secreted rhizoferrin. Iron replete conditions suppressed production of rhizoferrin. Using the chrome azurol S assay, we determined the rhizoferrin levels in serum-containing media to be 32 +/- 3 μM after 15 h at 30°C. To identify the gene(s) responsible for rhizoferrin biosynthesis, we examined the genome of Rhizopus delemar 99-880 for a homologue to the bacterial gene, sfnaD, shown to be responsible for biosynthesis of staphyloferrin A, a carboxylate siderophore produced by Staphylococcus aureus. Recently, it was shown that SfnaD, a protein belonging to the non-ribosomal peptide synthetase-independent synthase (NIS) family catalyzes the first step in the biosynthesis of staphyloferrin A. A homologue of sfnaD was identified which we called rfs. Blastp showed a 22% identity and a 37% similarity between Rfs and SfnaD. The predicted protein is 634 amino acids and contains an N-terminal IucA/IucC family domain, responsible for biosynthesis of the carboxylate siderophore, aerobactin. There is also a C-terminal conserved ferric iron reductase FhuF-like transporter domain. We cloned and expressed the putative rfs gene from R. delemar in a bacterial expression host. An IPTG-inducible protein was detected that corresponds to the predicted molecular weight of 72kDa. Siderophore biosynthesis from citrate and diaminobutane was observed in whole cell assays and confirmation of rhizoferrin production by cell lysates and purified protein is under investigation using HPLC.

396 SIMULTANEOUS TRANSCRIPTOME ANALYSIS OF COLLETOTRICHUM GLOEOSPORIOIDES AND TOMATO FRUITS RESPONSE AT DIFFERENT STAGES OF PATHOGENICITY REVEAL FRUIT-FUNGAL ARM AND DEFENSE STRATEGIES

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Colletotrichum gloeosporioides is a widely distributed fungus that causes economically significant fruit disease. C. gloeosporioides breach the unripe fruit cuticle whence the fungi adopt a biotrophic-like morphology and remain quiescent until fruit ripening. The 'awakened' fungus grows necrotrophically and causes anthracnose disease. Each stage, appressorial, quiescent and necrotrophic was morphologically characterized and simultaneous transcriptomes of the fungus and tomato fruit were obtained. Analysis of the appressoria stage, prior to fungal penetration, showed activation of several genes and pathways as cAMP, MAPK kinase, melanin biosynthesis, TCA cycle, and glycerolipid metabolism. The energy source appears to be degradation of fatty acid reserves. Concomitantly, the fruit activates a major resistant response involving JA, ethylene and ABA pathways, lipid-turnover, cuticle synthesis and phenylalanine pathway. The quiescent stage is characterized by two distinct fungal structures: a dendritic like structure which appears in the fruit cuticle and swollen hyphae which colonizes the first epidermal cell. C. gloeosporioides was found to alkalinize the surrounding tissue during the quiescent stage this alkalization regulate cuticle degrading enzymes. Chromatin remodeling activities and cell cycle arrest are up-regulated and may assist in the global down-regulation of genes expression during quiescent stage. In contrast, the tomato transcriptome reveals intensification of active defense pathways including phytoalexins and lignification. When the fruit ripens, necrotrophic growth ensues and the fungus activates an arsenal of pathogenicity factors including proteases and cell wall degrading enzymes. At this stage the fungal energy source is through glycolysis. In contrast to the defensive positions shown at the appressoria and quiescent stage, the ripe fruit activates a salicylic acid mediated suicidal response, the ensuing cell death enhances susceptibility resulting in anthracnose disease. Altogether, simultaneous RNA-seq analysis describes complex synchronized arms and defenses strategies for C. gloeosporioides and tomato fruit interaction.

397 THE ADENYLYL CYCLASE CONTROLS THE MORPHOGENETIC SWITCH FROM VEGETATIVE TO PATHOGENIC LIFESTYLE OF FUSARIUM GRAMINEARUM ON WHEAT

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In previous studies, we identified infection structures of the phytopathogenic ascomycete Fusarium graminearum. Now, we present evidence that the formation of such structures is prerequisite for successful penetration and that their development is controlled by cyclic 3′,5′-adenosine monophosphate (cAMP) signaling. The ubiquitous second messenger cAMP is a nucleotide derived from adenosine triphosphate. Intracellular cAMP levels are synthesized by a large membrane-bound protein, the adenylyl cyclase. In order to analyze the function of this gene and the importance of cAMP in the life cycle of the cereal pathogen Fusarium graminearum, the adenylyl cyclase gene (FGSG_01234) was deleted by gene replacement (deltaFgac1). Phenotypic characterization of deltaFgac1 revealed its importance for vegetative growth, as well as sexual and asexual reproduction. Pathogenicity towards wheat was drastically reduced in ΔFgac1 compared to the wild type. Point-inoculated spikelets showed only small lesions but no typical head blight disease symptoms. Fluorescence microscopy using dsRed-expressing strains revealed that the deltaFgac1 strain was unable to develop any complex infection structures like lobate appressoria and infection cushions. Instead, hyphal anastomosis occurs frequently. Scanning electron microscopy demonstrated the lack of any fungal penetration. Hyphae on flower leaves massively produced new conidia, thereby circumventing the infection cycle. This abundant sporulation on wheat epidermis was never observed in the wild type. Intriguingly, the Fgac1 deletion mutant was able to infect maize cobs similar to wild-type, indicating that cAMP signaling is not important for maize infection. The deltaFgac1 mutant was unable to produce the mycotoxin deoxynivalenol both in vitro and during wheat infection. In this study, we show that cAMP signalling controls important cellular processes such as development of infection structures, pathogenicity, secondary metabolite production and sexual reproduction. For the first time, we show that cAMP regulates the switch from vegetative to pathogenic lifestyle of F. graminearum on wheat, which is most fundamental for a pathogen. Furthermore, this study proved the importance of appressoria formation for the successful penetration of the plant by F. graminearum.

398 THE FUNGAL PATHOGEN COCHLIOBOLUS HETEROSTROPHUS RESPONDS TO MAIZE PHENOLICS BY ACTIVATING DIFFERENT PATHWAYS FOR STRESS AND METABOLISM

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Phenolics and related compounds found in plants can provide a signal and a nutrient source to plant pathogens, but may also act as a toxic stress. The transcription factor ChAP1 of Cochliobolus heterostrophus responds to oxidative stress by retention in the nucleus, up-regulating the expression of genes for proteins involved in antioxidant responses. Phenolics also promote nuclear localization of ChAP1, but without triggering a strong antioxidant response [1]. By microarray hybridization and candidate gene approaches, we searched for genes whose expression is regulated by phenolic compounds. The C. heterostrophus genome contains a cluster of genes for metabolism of phenolics (�-ketoadipate pathway). Intradiol dioxygenase (CCHD1), which is at the start of the �-ketoadipate pathway, was strongly induced by caffeic, coumaric and other phenolic or aromatic acids. The toxicity of a series of phenolic compounds correlated well with GFP:ChAP1 nuclearization. Phenolics that induced CCHD1 expression did not cause strong ChAP1 nuclearization, and those causing ChAP1 nuclearization did not induce CCHD1 strongly. The structure-activity relationships of a series of phenolics showed that the phenolic hydroxyl is required for induction of expression of CCHD1. A conjugated system is required for nuclear retention of ChAP1, but the phenolic hydroxyl is not [2]. Specific requirements for different cellular responses suggest specific receptors for these compounds, and set the stage for their identification. The pathogen thus detects phenolics by at least two signaling pathways: one causing stress and nuclear retention of ChAP1, and another triggering induction of the �-ketoadipate pathway and phenolic degradation. Focusing on the group of compounds that cause a stress response, we found that ferulic acid caused damage to the membrane as shown by permeability to trypan blue, and hyphal shrinkage to nearly half the normal diameter. Mutants in the MAPK Hog1 are hypersensitive to growth on toxic phenolic compounds, and exposure to some of these compounds caused dephosphorylation of Hog1, showing involvement of the Hog1 pathway in the stress response to phenolics.

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399 THE MYCOTOXIN PATULIN CONTRIBUTES TO PATHOGENICITY OF PENICILLIUM EXPANSUM

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Penicillium expansum, the causal agent of blue mold rot, causes severe postharvest fruit maceration through secretion of D-gluconic acid (GLA) and secondary metabolites such as the mycotoxin patulin in colonized tissue. While GLA involvement in pathogenicity has been suggested, the mechanism of patulin accumulation and its contribution to P. expansum pathogenicity remain unclear. The roles of GLA and patulin accumulation in P. expansum pathogenicity were studied using: i) GOX2-RNAi mutants exhibiting decreased GOX2 expression, GLA accumulation and reduced pathogenicity ii) IDH-RNAi mutants exhibiting down regulation of IDH (the last gene in patulin biosynthesis), reduced patulin accumulation and no effect on GLA level; and iii) PACC-RNAi mutants exhibiting down regulation of both GOX2 and IDH, that reduced GLA and patulin production. Present result indicate that conditions enhancing the decrease in GLA accumulation by GOX2-RNAi and PACC-RNAi mutants, and not low pH, affected patulin accumulation, suggesting GLA production as the driving force for further patulin accumulation. Thus, it is suggested that GLA accumulation may modulate patulin synthesis as a direct precursor under dynamic pH conditions modulating the activation of the transcription factor PACC and the consequent pathogenicity factors, which contribute to host-tissue colonization by P. expansum.

400 THE SFP-TYPE PHOSPHOPANTETHEINYL TRANSFERASE, PPTA, IS CRITICAL FOR THE VIRULENCE AND IMPORTANT IN HOST DETECTION OF ASPERGILLUS FUMIGATUS

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Aspergillus fumigatus is the leading cause of invasive aspergillosis (IA), a fungal disease which is increasing annually on a global scale. IA poses as a common threat to patients with a weakened immune response due to disorders such as leukaemia, HIV, AIDS and also persons undergoing chemotherapy treatments. The ability of A. fumigatus to produce a wide array of secondary metabolites is thought to contribute to the pathogenicity of this organism. We have identified an enzyme, PPTA that plays a key role in secondary metabolism in A. fumigatus. PPTA is a sfp-type phosphopantetheinyl transferase and is required to activate non-ribosomal peptide synthases, polyketide synthases and a protein required for lysine biosynthesis aminoadipate reductase (AARA). Disruption of pptA renders the fungus avirulent in both insect and murine infection models. To investigate which aspects of pptA activity are essential to virulence a series of knock out mutant strains were generated; ΔaarA, ΔpksP and ΔsidA. These genes play a vital part in lysine, melanin and siderophore biosynthesis pathways respectively. The sidA gene proved vital to virulence in the insect model whereas the ΔaarA and ΔpksP mutants were unaffected. The pathogenicity of both the pptA and sidA knock out strains was restored by co-injecting larvae with iron. We postulate that, at least in the larval model, it is PPTAs role in siderophore biosynthesis and not the activation of other secondary metabolism pathways that is critical for the virulence of A. fumigatus. The loss of pptA appears to affect the immune recognition of spores by human and murine cells which may be due to the role it plays in melanin biosynthesis. In vivo studies challenging human dendritic cells against fixed ΔpptA conidia lead to an increase in the release of proinflammatory cytokines Il-1B and Il-6. Furthermore, intranasal challenge of the ΔpptA mutant in an immunocompetent murine model leads to a reduction in neutrophil recruitment in the lung which suggests a more rapid clearance of the fungus by macrophages.

401 THE SMUT FUNGUS THECAPHORA THLASPEOS INFECTS THE BRASSICACEAE ARABIS CILIATA

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Smut fungi are a wide-spread group of plant pathogens that infect agriculturally important crop plants such as maize or potato. During the major part of their life cycle, these Basidiomycetes grow as endophytes inside the host without causing disease symptoms. Only at late stages of infection they interfere with seed development by producing large amounts of fungal spores. To date, no infection system of Brassicaceae with Basidiomycetes is studied at the molecular level. Therefore we set out to investigate the interaction between Thecaphora thlaspeos and Arabis species aiming at a smut infection system, in which both partners are genetically tractable. The initial aim was to collect spores from natural infections. In three consecutive years, we have collected spores from seven different populations in Europe in Arabis hirsuta and the novel host Arabis ciliata. The next step was the characterization of the germination process. Interestingly, in contrast to other smut fungi, T. thlaspeos germinates only in the presence of a yet uncharacterized plant signal. The resulting filaments insert retraction septa and are able to infect germinating seedling of the original host. At present, it is unclear whether these filaments are dikaryotic and formed as result of a mating reaction during the early phase of infection as described for other smut fungi. Upon successful infection, T. thlaspeos can overwinter as mycelium in the vegetative tissue of the perennial host and gives rise to spores when the plant is flowering the following year. Currently, we are in the process to establish culture conditions.

402 TRANSCRIPTIONAL REGULATION OF PUTATIVE SECONDARY METABOLITE CLUSTER GENES IN THE ESCA RELEVANT STRAINS PHAEOACREMONIUM ALEOPHILUM AND PHAEOMONIELLA CHLAMYDOSPORA

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Grapevine trunk diseases including young Esca (Petri disease) and Esca proper (Black Measles) have become one of the most important diseases wherever grapevine is cultivated. Esca is caused or at least supported by the fungal species Phaeoacremonium aleophilum (Togninia minima) and Phaeomoniella chlamydospora whose genome drafts have been published by Blanco-Ulate and Rolshausen, 2013 (P. aleophilum) or have been sequenced, assembled and annotated by us (P. chlamydospora, unpublished). Pathogenicity of many fungi including Fusarium grammeniarum, Fusarium fujikuroi, Leptosphaeria maculans or Ustilago maydis was found to be dependent or at least supported by secondary metabolite (SM) production. These compounds are produced by enzymes which derive from genes usually physically linked on the genome and depending on the complexity of the biosynthetic pathway - forming small to large, multi-kilobase SM gene clusters. Esca disease displays severe foliar symptoms (271tiger stripes) but no pathogenic fungi could be detected directly in diseased leaves or green twigs. To explain these paradoxes, it was suggested that toxic (secondary-) metabolites could be responsible for the symptoms produced by the pathogens in the trunk and transported to the leaves where they may then cause the observed symptoms. By bioinformatics analysis we detected 12 (P. aleophilum) or 11 (P. chlamydospora) putative secondary metabolite gene cluster based on prediction of polyketide synthases, nonribosomal peptide synthases and prenyltransferases in both strains and established qPCR assays to monitor their transcript levels. We here present results of this transcriptional analysis of secondary metabolite genes from axenic cultures using different media compositions as well as from co-incubations of both pathogens in the same culture. In these assays, we detect culture-specific as well as interaction-specific transcripts. In addition, we also studied fungal SM transcript levels from infected grapevine trunks and present here their correlation to SM gene expression levels in axenic cultures. This knowledge will improve the understanding of grapevine trunk diseases and shed some novel aspects into our knowledge of how endophytic/pathogenic fungi interact with their host.

403 TRANSCRIPTOME OF TRICHODERMA VIRENS IN INTERACTION WITH MAIZE OR TOMATO ROOTS: EVIDENCE FOR PLANT HOST SPECIFICITY

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Trichoderma spp. 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 co-cultured Trichoderma virens with maize or tomato in a hydroponic system. 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 (1). Transcripts corresponding to 43 gene models differed significantly in their expression levels between maize and tomato, with ratios of transcript abundance on tomato compared to on maize ranging from 60.9 to 0.005. The differentially expressed genes encode proteins belonging to several functional classes including enzymes, transporters and small secreted proteins. Secreted proteins released in response to plant signals might have a role in inducing systemic resistance in the plant (2). The abundance of glycosyl hydrolases and transporters suggests that Trichoderma can break down the host cell walls during colonization of the outer root layers. Reporter genes chosen from the microarray data will be useful to follow the time course and localization of the response of the fungal cell to plant signals, and will provide an entry point to the signaling pathways. One of the glycosyl hydrolase genes induced in co-culture with roots, 90504, is nearly identical to CBH1 of T. virens T87, induced in contact with tomato roots (3). We introduced a vector for GFP expression under control of the predicted upstream regulatory region of this gene into T. virens, and observed strong fluorescence after 2 days of co-culture with maize root sections. The tomato and maize – induced transcriptomes differ from each other and from that in saprophytic growth, consistent with the wide host range of T. virens.

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(2) Rubio et al. (2012) Microbiology 2012, 158:119-128.
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404 TRANSFER AND STABILITY OF LINEAGE-SPECIFIC CHROMOSOMES IN FUSARIUM OXYSPORUM

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The F. oxysporum species complex consists of many host specific lineages. Host specificity is determined by Lineage Specific (LS) chromosomes that carry effector genes required for virulence. LS chromosomes are not required for vegetative growth and differ from the core chromosomes in repeat content, gene content and possibly histone modifications. LS chromosomes can be transferred between strains causing gain of pathogenicity in a non-pathogenic recipient. Although transfer of LS chromosomes has been described in a number of species it is not known which features and cellular processes contribute to transfer. It is also not known if LS chromosomes are as mitotically stable as core chromosomes and whether or not this is dependent on the strain carrying the LS chromosome. To determine which chromosomes of a tomato-pathogenic strain of F. oxysporum are capable of transfer a screen was performed and combined with a targeted approach for individual chromosomes. Around 200 strains were created with a marker integrated at a random location. These were then tested for transfer. Four strains were identified that consistently showed transfer. In each of these the marker co-segregated with effector genes residing on an LS chromosome that had already been shown to be capable of transfer. In some cases another, smaller LS chromosome co-migrated, but this chromosome was never observed to have transferred by itself. Several larger LS chromosomes were not found to have transferred in the screen. To determine if the smaller chromosome can be transferred independently it was targeted with a marker and tested for transfer. The same was done for the three smallest of the core chromosome. No transfer was observed. From this we conclude that only small LS chromosomes are amenable for transfer. In addition to gain of pathogenicity by chromosome transfer it has also been shown that Fusarium solani can lose LS chromosomes and pathogenicity. We are currently expressing fluorescent proteins from LS chromosomes and screening for loss of fluorescence by Fluorescence Assisted Cell Sorting (FACS). In this way we can compare the stability of LS chromosomes with core chromosomes and the stability of LS chromosomes in donor and recipient background.

405 UNRAVELING THE MYSTERY OF SPORISORIUM REILIANUM'S HOST SPECIFICITY BY COMPARATIVE GENOMICS OF MIXED-VARIETY SEGREGANTS

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Sporisorium reilianum is a biotrophic fungal pathogen of agronomically important crop plants and causes head smut disease on its hosts maize and sorghum. The fungus exists in two varieties with different host ranges: S. reilianum f.sp. reilianum (SRS) is only able to produce spores on sorghum and S. reilianum f.sp. zeae (SRZ) can solely complete its life cycle on maize. In the quest for identifcation of the factor(s) determining host specificity, we made use of the great possibilities offered by S. reilianum: apart from well-annotated genomes and established molecular genetics tools, classical genetics can be used to generate a population of cross-variety segregants. Individual segregants were phenotyped in sorghum infection assays by back-crossing to the sorghum-variety parent (SRS). The segregants turned out to exhibit the whole range of possible phenotypes, from no spore formation over medium up to full virulence. This indicates that host specificity is a multi-genic trait. To map the responsible genes, we chose three markers on each chromosome and performed species-specific PCRs on highly virulent as well as on avirulent segregants. Using this rough mapping approach, we could identify regions on the genome segregating with the disease-phenotypes. One of them contains gene hsc1 (host specificity candidate 1) which only exists in SRZ and whose expression in SRS leads to a reduction in disease incidence – confirming our approach. In order to not miss important markers, we sequenced 16 different segregants using Illumina. De-novo assemblies of each of the segregants' individual quality-trimmed read-files confirmed the genome sizes of the parental strains and the high quality of DNA-libraries obtained from the segregants. Using processed read files of each segregant, we performed mappings against both of the parental genomes separately, as well as against a mixed-genome reference. Both separate mappings were inconclusive whereas the latter approach led to clearly cut borders of base coverage depicting the previously happened meiosis events of each segregant. We present results of this dataset that allows us to mine for regions segregating with the disease phenotypes. This fully unbiased approach contributes to unraveling the mystery of host specificity of S. reilianum.

406 USING SUBSTRATE CONSERVATISM TO SCREEN FOR POTENTIAL LIFESTYLE DEVIATIONS IN FUNGAL-ALGAL SYMBIOSES

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Fungi involved in algal symbioses have long been thought to represent autonomous and autotrophic systems. However, the summary categorization of these interactions as lichens may mask complex trophic interactions. We examine substrate-dependent lifestyles as a guide to flagging potential, unrecognized trophic interactions in the first part of a joint phylogenetic-genomic study of metabolisms in the subclass Ostropomycetidae of the Lecanoromycetes. Using a combined analysis of ancestral state and adaptive evolution modeling, we report high levels of substrate-related niche conservatism connected to close physical contact with specific organic and non-organic substrates.

407 USTILAGO MAYDIS TRIGGERS TUMOR FORMATION IN MAIZE BY ORGAN-SPECIFIC EFFECTOR PROTEINS

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Ustilago maydis infects all aerial organs of its host plant maize. It establishes a biotrophic interaction which ultimately results in formation of plant tumors. Formation of these structures thought to result from activity of effector proteins, which are secreted by the fungus to the host tissue. In light of the huge differences between the maize organs that are transformed to tumors, we hypothesized that U. maydis deploys organ specific effectors to manipulate physiology and development of infected 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 set of nine U. maydis effectors which contribute to virulence in an organ-specific manner. Of these, seven effectors are only required for tumor formation in leaves, while two are specifically required in floral infections. A novel effector with strictly leaf-specific expression and function is See1 (Seedling efficient effector 1). U. maydis deletion mutants for see1 are not impaired in saprophytic growth, are fully virulent in ears and anthers, but show a strong reduction of tumor formation in maize seedlings leaves. Laser scanning confocal microscopy shows that the mutant hyphae successfully enters the leaf tissue but is blocked during proliferation stages in the mesophyll and bundle sheath cells of the leaf. By labeling of replicating plant DNA using 5-ethynyl-2-deoxyuridine (EdU), we observed that maize leaves colonized by Δsee1 do not show mitotic activity during infection, while host cell division in leaves is strongly induced in wild type infected host cells. In contrast, the Δsee1 mutant induces normal tumor formation in tassels and also shows wild type level cell division rates in colonized anthers. To understand the organ-specific role of See1, interacting plant proteins have been identified. Molecular genetics, imaging and biochemical analyses are used to unravel how See1 triggers tumorigenesis in maize leaves.

408 VERTICILLIUM TRANSCRIPTION ACTIVATOR OF ADHESION VTA2 SUPPRESSES MICROSCLEROTIA FORMATION AND IS REQUIRED FOR SYSTEMIC INFECTION OF PLANT ROOTS

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Six transcription regulatory genes of the Verticillium plant pathogen, which reprogrammed non-adherent budding yeasts for adhesion, were isolated by a genetic screen to discover control elements for early plant infection. Verticillium transcription activator of adhesion Vta2 is highly conserved in filamentous fungi but not present in yeasts. The Magnaporthe grisea ortholog Con7 controls the formation of appressoria which are absent in Verticillium species. Vta2 was analyzed by using genetics, cell biology, transcriptomics, secretome proteomics and plant pathogenicity assays. Nuclear Vta2 activates the expression of the adhesin encoding yeast genes FLO1 and FLO11. Vta2 is required for fungal growth of Verticillium where it is a positive regulator of conidiation. Vta2 is mandatory for accurate timing and suppression of microsclerotia as resting structures. Vta2 controls expression of 270 transcripts including 10 putative genes for adhesins and 57 for secreted proteins. Vta2 controls the level of 125 secreted proteins including putative adhesins or effector molecules and a secreted catalase-peroxidase. Vta2 is a major regulator of fungal pathogenesis, controls host-plant root infection and H2O2 detoxification. Verticillium impaired in Vta2 is unable to colonize plants and induce disease symptoms. Vta2 represents an interesting target to control growth and development of these vascular pathogens.

409 WORKING TOGETHER: PROTEINASE INHIBITORS ENHANCE THE ANTIFUNGAL ACTIVITY OF THE PLANT DEFENSIN, NAD1

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Plant defensins with potent antifungal activity have the potential to be used in both transgenic plant and human pharmaceutical applications. NaD1, a plant defensin from the flowers of Nicotiana alata, is active against filamentous fungi and appears to act via a three-step process beginning with interaction with the fungal cell wall, followed by permeabilisation of the plasma membrane and subsequent entry of the protein into the cytoplasm. Permeabilisation occurs via a novel mechanism that may involve a receptor located in the proteinaceous layer of the cell wall. The kinetics of membrane permeabilisation by NaD1 is significantly different to the kinetics of antimicrobial peptides that act solely through membrane permeabilisation such as CP-29 and BMAP-28. This has led to the hypothesis that interaction with an intracellular target(s) is required for the complete antifungal activity of NaD1. NaD1 also allows entry of other molecules such as proteinase inhibitors into the cytoplasm of fungal hyphae. Together these molecules display synergistic activity which suggests that innate immunity may involve a complex interaction of antimicrobial peptides and that we need to look beyond the direct antimicrobial activity of individual peptides in order to develop effective, robust therapeutics.

410 ZEBRAFISH: A NEW HOST SYSTEM TO STUDY RNAI FUNCTION IN MUCOR CIRCINELLOIDES PATHOGENESIS.

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Mucor circinelloides is an emerging opportunistic pathogen in immunocompromised patients, recently isolated in some otherwise healthy individuals. Besides its emerging pathogenicity, M. circinelloides also outstands for being one of the first fungi in which endogenous small RNAs (esRNAs) with putative regulatory functions have been identified. The main class of M. circinelloides esRNAs derives from exons (ex-siRNAs) and regulates the expression of the protein coding genes from which they are produced. M. circinelloides mutants affected in genes involved in the production of ex-siRNAs present defects in general developmental processes such as growth and sporulation, besides other processes related to stress response such as oxidative and heat stresses. These phenotypic changes may suggest a role for ex-siRNAs in pathogenesis, since spore size and growth at high temperature have been identified as virulence factors in this fungus. M. circinelloides pathogenesis has been studied using inmunocompromised mice, larvae of the wax moth (Galleria mellonella) and macrophage cell cultures. Here, we present zebrafish (Danio rerio) as a new study model for fungal pathogenesis. Techniques and methods have been developed to establish a new infection system based on the injection of fungal spores in the abdominal cavity of zebrafish adults. Survival of both fungal spores and fishes has been studied after the infection with pathogenic and non-pathogenic M. circinelloides strains, as well as histological and molecular processes occurring during the infection. Our work is currently focused on finding new ex-siRNAs and target mRNAs specifically regulated during zebrafish infection by M. circinelloides. This work was funded by the Spanish MICINN (BFU2009-07220) and MINECO (BFU2012-32246) co-financed by FEDER.

411 GENOMIC ANALYSES OF MORTIERELLA ELONGATA AND ASSOCIATED BACTERIAL ENDOSYMBIONT (CANDIDATUS GLOMERIBACTER SP.)

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Collaborative efforts to characterize the Populus root microbiome have resulted in numerous isolates and genome sequences of its beneficial rhizospheric fungi and bacteria. One of these fungi is Mortierella elongata (Mortierellales, Zygomycota), a rapidly growing coenocytic, multinucleate fungus with an interesting yet cryptic ecology. Traditionally considered a soil dwelling saprotroph, M. elongata can also be isolated from the rhizosphere of plants. In fact, greenhouse studies show that plants inoculated with Mortierella grow faster and tolerate greater heat stress than non-inoculated control plants. Mortierella species are unique in their high production of unsaturated fatty acids, making them relevant to commercial and biological applications. Recent sequencing of the M. elongata genome revealed the presence of a bacterial endosymbiont, identified as a relative of the genus Burkholderia. Further analyses, including full genome sequencing of the Mortierella endosymbiont, indicate its close relationship with other fungal endosymbionts, namely the Candidatus Glomeribacter lineage associated with arbuscular mycorrhizal fungi in the genera Gigaspora and Scutellospora. Genomic signatures of the fungal endosymbiosis mirror those documented in other Eurkaryotic-endosymbiont systems, including genomic reduction when compared to facultative and free-living relatives, degradation of select metabolic pathways, increased interdependence between bacteria and host, and control of reproductive fitness. By clearing the endosymobiont from several M. elongata strains we observed fitness costs to the host under specific conditions. Several lineages of fungi and bacteria presented here exhibit congruent cophylogenies suggesting an ancient origin for this co-evolved symbiosis and begging the question; what, if any benefit do endosymbiotic bacteria offer their fungal host, and in turn, plant associates? Aspects of the Mortierella endosymbiont genome and functional host-endosymbiont relationship hypotheses will be discussed further.

412 A NEW TYPE OF HELITRON IN FUNGI

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Helitrons are a class of transposons with a distinct replication mechanism and the ability to capture gene fragments. Autonomous Helitrons encode a protein that contains a rolling circle replication initiator (rep) domain and a helicase (hel) domain. Helitrons occur in a wide range of plants, animals and fungi. In some cases they constitute a significant portion of a genome, e.g. 5% in Drosophila virilis. In fungi, the characteristics of Helitrons are less well documented. The availability of genome sequences of 10 different strains within the F. oxysporum species complex (FOSC.), allows us to study fungal Helitrons in great detail. We identified candidate Helitrons in FOSC genome sequences based on sequence similarity with known Helitrons. Next we aligned all putative Helitron sequences including 1000 bp flanking the coding sequence, and clustered these sequences into 5 distinct groups. Analyses of the flanking regions revealed that FOSC Helitrons do not possess the classical structural features of Helitrons, namely the 3' hairpin and the CTRR terminal motif. Instead, we find that most FOSC Helitrons possess two hairpins, one at the 3' end and one at the 5' end, combined with an inverted repeat and distinct 5' and 3' ends. FOSC Helitrons transposition occurs between the 5'-T4 and 3'-A5 of the target site 5'-TXATA-3' (where 'X' stands for any nucleotide). Using these structural features, we scanned FOSC genomes for non-autonomous Helitrons – which possess the structural features but lack the full protein coding sequence – and identified two families of non-autonomous Helitrons within the FOSC. Finally, we surveyed ~ 200 publically available Pezizomycotina genomes for proteins that contain both hallmark Helitron domains and the distinct structural features described above and find that this type of Helitron occurs in multiple species, dispersed throughout the Pezizomycotina. The fact that non-autonomous Helitrons with two hairpins and an inverted repeat have recently also been identified in the green alga Chlamydomonas reinhardtii warrants further research into the age and origin of this new type of Helitron.

413 ANALYSIS OF CARBON SOURCE UTILIZATION DATA COMBINED WITH A MULTIGENE PHYLOGENY OF MORTIERELLALES

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Members of the order Mortierellales are zygomycetes fungi can be isolated most from soil where – as many other organisms – they act as decomposers of various organic materials. One species (Mortierella wolfii) is also known as an opportunistic animal pathogen causing fetal abortion or infections in the respiratory tract. On the other hand, several species are considered as promising sources of polyunsaturated fatty acids (e.g. M. alpina) for the biotechnological industry. In this study, a phylogeny of this fungal group inferred by using a combined data set of two protein coding genes (tef and RPB1) and three ribosomal sequences (the nrSSU, nrLSU genes and the complete ITS region). Carbon assimilation patterns of the isolates involved in the phylogenetic study were also determined using 67 different compounds as sole carbon sources. The resulting data could not only help in the differentiation of the isolates by their utilization patterns but also to study the changes in the utilization of substrates with various chemical structures in a phylogenetic context. Carbon sources were clustered by three different approaches: a strict binary (inhibiting, 0 or utilizable, 1), and two more permissive ternary (inhibiting, 0; weak background growth, 1 and utilizable, 2) and quaternary (inhibiting, 0; weak background growth, 1; utilizable, 2 and strongly utilizable, 3) matrices. Among the tested carbon sources, 39 compounds showed larger differences in their effects. We combined the phylogenetic data and the results of the carbon source utilization tests by mapping the characters onto the tree. Our results suggest that utilization capabilities appeared independently in many cases. We also found that members of the closely related Mucorales show significant differences in their carbon source utilization patterns. This research was supported by the grant TÁMOP-4.1.1.C-12/1/KONV-2012-0014 and OTKA NN106394. For Cs.V. this research was realized in the frames of TÁMOP 4.2.4. A/2-11-1-2012-0001 „National ExcellenceProgram – Elaborating and operating an inland student and researcher personal support system" The project was subsidized by the European Union and co-financed by the European Social Fund.

414 ANALYSIS OF FUSARIUM FUJIKUROI GENETIC STRUCTURE BY SSR MARKERS

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Fusarium fujikuroi Nirenberg (teleomorph: Gibberella fujikuroi Sawada) is an heterothallic ascomycete, agent of Bakanae of rice, a disease that has become of increasing economical concern in the last years in the major rice-producing areas in the world and a threat for the Italian rice cultures. A collection of 315 isolates of F. fujikuroi from 8 Italian rice-cultivated areas has been established and analysed for genetic variation at 19 specific polymorphic Simple Sequence Repeats (SSR) loci and for mating types frequencies. The estimated average number of alleles over all 19 SSR loci was 5.25. Overall, 107 different haplotypes were detected with 80 private haplotypes distributed in all populations. The Nei's genetic diversity ranged from 0.40 to 0.59, while the clonal fraction ranged from 8% to 57% among populations. All the isolates were characterized by a small number of private alleles. The four most frequent haplotypes encompassed 40% of the all collected isolates, suggesting that clonal reproduction plays an important role. Nevertheless, the potential of sexual recombination was highlighted by the 1:1 mating type ratio in 5 out of the 8 populations analysed and by the high value of genotyping diversity (He = 0.90, by stepwise model). AMOVA has shown a small level of genetic polymorphism among populations while the majority of genetic variation occurred within populations (about 98%). The data suggest that sexual reproduction may play a role in generating variation in F. fujikuroi. This could represent also a threat for Italian rice cultivation in terms of development of fungicide resistance and of development of more virulent isolates of F. fujikuroi that could overcame the resistance to this species introgressed into new rice varieties. To this aim, the screening for resistance of a collection of 63 Italian rice genotypes has been performed. Overall results showed significant differences of plant response to the pathogen and a number of cultivars that could represent an interesting genetic source for the resistance/tolerance to Bakanae.The work was supported by Progetto AGER, grant n° 2010-2369.

415 BIOLOGICAL AND GENETIC CHARACTERISTICS OF COLLECTIONS OF SEVERAL POLYPORE MUSHROOMS (BASIDIOMYCOTA)

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Polypore mushrooms (Basidiomycota, Polyporales) are wood inhabiting fungi with significant biotechnological potential. Many polypores, such as species of genera Ganoderma, Fomes and Fomitopsis produce different bioactive substances (polysaccharides, terpenoids, phenolics, etc) with immune-modulating, antimicrobial and other activities. Polypores are also known as producers of lignin and cellulose degrading enzymes (laccases, peroxidases), and proteolytic enzymes, applicable in biotechnological processes. Establishment of culture collections of polypores with different geographical origin are of great importance to study their biological (morphological, ecological, physiological, biochemical) and genetic characteristics, as well as to estimate their biotechnological potential. In our study variability in morphological, physiological and ecological characteristics of mycelial collections of several polypores with different origin, particularly species of Ganoderma (G. lucidum, G. applanatum, G. adspersum, G. resinaceum), Fomitopsis (F. annosa, F. pinicola) and Fomes (F. fomentarius) at different temperatures and pH was revealed. Colony morphology and growth parameters on different agar media were described. Mycelial microstructures, such as hyphal clamps, loops, cystidia, anamorphic sporulation (oidia), chlamydospores (oval, round) and others were observed in polypores' cultures. Formation of pellets and their morphology (filamentous, smooth) during submerged cultivation of studied collections were described. Taxonomic significance of observed cultural characteristics was evaluated. The highest proteolytic (milk-coagulating) activity was detected in cultural liquid of F. fomentarius and G. resinaceum, whereas activity of others was weaker. High antifungal/antagonistic activity against different test-fungi (plant pathogenic and their antagonists, potentially pathogenic for humans/animals) was observed in G. resinaceum. The genetic analysis of Ganoderma collections from Armenia, France, Iran, Italy and China show that the sequences of G. lucidum collections from European, Trans-Caucasian and Iranian regions are closely related and phylogenetically separated from the East-Asiatic sequences. DNA-markers based genetic analysis of Russian collections revealed higher polymorphism in G. applanatum, rather than F. fomentarius and F. pinicola isolates.
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416 CHARACTERIZATION OF A NEW PARTITIVIRUS ISOLATE IN VERTICILLIUM DAHLIAE PROVIDES FURTHER EVIDENCE OF THE SPREAD OF THE HIGHLY VIRULENT DEFOLIATING (D) PATHOTYPE THROUGH NEW INTRODUCTIONS

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Verticillium dahliae is a cosmopolitan soil fungus which causes important vascular diseases in a variety of crops, including olive trees, cotton and horticultural crops. The widespread of a highly virulent defoliating (D) pathotype has greatly increased the threat posed by V. dahliae in certain crops like olive trees. In olive orchards in Spain and Turkey, it has been proposed that the spread of the D pathotype can be connected to the previous existence in the same areas of infected cotton crops in which the D pathotype was frequently found. Since V. dahliae has been, until now, described as an asexually-reproducing fungus, vegetative compatibility is a prerequisite for genetic exchange. Compatible isolates are placed within the same vegetative compatibility group (VCG). Isolates from the D pathotype are all placed in the VCG1A. Extracromosomal double-stranded RNA (dsRNA) molecules (mycoviruses) have been detected in all major taxonomic groups of filamentous fungi. In this study, we have identified two dsRNAs in a Turkish D isolate of V. dahliae infecting olive. Sequencing and phylogenetic analysis of these dsRNAs confirmed that they corresponded to a micovirus and clustered it with members of the family Partitiviridae, being most closely related to a partitivirus identified in a V. dahliae cotton isolate from China (VdPV1). Sequence identities between these two viral isolates are 94% and 91% at the nucleotide level for RNA1 and RNA2, respectively, and 96% and 93% at the deduced amino acid sequence levels for RNA1 and RNA2, respectively. This high similarity indicates that the new virus identified in the Turkey isolate could correspond to a strain of the established species VdPV1. For this reason, we have designated it VdPV1-ol (from olive). Our results support the hypothesis that the spread of the D pathotype in different regions could be associated to new introductions from distant geographical areas, and that once introduced in a crop, it can affects other crops in the same region. Although no information is available about the VCG (or pathotype) of the cotton isolate in which VdVP1 was identified, our results indicate that it is probably a D isolate (VCG1A). We propose that the characterization of micoviruses can serve as fingerprints to study the geographical flow of V. dahliae isolates.

417 COEVOLUTION AND LIFE CYCLE SPECIALIZATION OF PLANT CELL WALL DEGRADING ENZYMES IN A HEMIBIOTROPHIC PATHOGEN

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Zymoseptoria tritici is an important fungal pathogen on wheat that originated in the Fertile Crescent. Its closely related sister species Z. pseudotritici and Z. ardabiliae infect wild grasses in the same region. This recently emerged host–pathogen system provides a rare opportunity to investigate the evolutionary processes shaping the genome of an emerging pathogen. Here, we investigate genetic signatures in plant cell wall degrading enzymes (PCWDEs) that are likely affected by or driving coevolution in plant-pathogen systems. We hypothesize four main evolutionary scenarios and combine comparative genomics, transcriptomics, and selection analyses to assign the majority of PCWDEs in Z. tritici to one of these scenarios. We found widespread differential transcription among different members of the same gene family, challenging the idea of functional redundancy and suggesting instead that specialized enzymatic activity occurs during different stages of the pathogen life cycle. We also find that natural selection has significantly affected at least 19 of the 48 identified PCWDEs. The majority of genes showed signatures of purifying selection, typical for the scenario of conserved substrate optimization. However, six genes showed diversifying selection that could be attributed to either host adaptation or host evasion. This study provides a powerful framework to better understand the roles played by different members of multigene families and to determine which genes are the most appropriate targets for wet laboratory experimentation, for example, to elucidate enzymatic function during relevant phases of a pathogen's life cycle.

418 COLLETOTRICHUM PLANT-LIKE SUBTILISIN (CPLS): A FAMILY OF PROTEINS ACQUIRED BY HORIZONTAL TRANSMISSION FROM PLANT TO FUNGUS

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Horizontal gene transfer (HGT) in one possible mechanism by which fungi can acquire new capabilities and in the case of pathogenic fungi, may represent a means of obtaining genes that are important for infecting and surviving in the host. Inter-Kingdom HGT into fungal genomes has been reported in the past but knowledge about HGT between plants and fungi is particularly limited. We show here a gene in the genome of several species of the genus Colletotrichum with a strong resemblance to subtilisins typically found in plant genomes. Subtilisins are an important group of serine proteases, widely distributed in all of the kingdoms of life. Our hypothesis is that the gene was acquired by Colletotrichum spp. through HGT from plants to a Colletotrichum ancestor. We provide evidence to support this hypothesis in the form of phylogenetic analyses as well as a characterization of the similarity of the subtilisin at the primary, secondary and tertiary structural levels. The remarkable level of structural conservation of CPLSs with plant subtilisins and the low similarity with the rest of Colletotrichum subtilisins suggests the possibility of molecular mimicry. Our phylogenetic analysis indicates that the HGT event would have occurred approximately 150-155 million years ago, after the divergence of the Colletotrichum lineage from other fungi. Gene expression analysis shows that the gene is modulated during the infection of maize by C. graminicola suggesting that it has a role in plant disease. Furthermore, the upregulation of the CPLS coincides with the downregulation of several plant genes encoding subtilisins. Based on the known roles of subtilisins in plant pathogenic fungi and the gene expression pattern that we observed, we postulate that the CPLSs have an important role in plant infection.

419 DEVELOPMENT OF A SIMPLE MOLECULAR TOOL TO RESOLVE PHYLOGENETIC RELATIONSHIPS AMONG FUSARIUM OXYSPORUM F. SP. DIANTHI ISOLATES

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Fusarium wilt, caused by Fusarium oxysporum f. sp. dianthi (Fod), is the most important carnation disease worldwide. Fod populations show a high level of diversity, that includes the existence of 10 pathogenic races and of virulence groups (molecularly distinguishable) among isolates of the same race. The objective of the present study was to clarify the phylogenetic relationship among Fod isolates, and to develop a tool to quickly analyze population structure in order to select suitable resistant carnation cultivars for specific growing areas. For that, we used selected Fod isolates obtained from the most important growing areas in Spain during the period 1998-2011, along with some representatives from Italy, and analyzed polymorphisms at three different nuclear loci. These included non-coding DNA sequences derived from partial β-TUB and EF-1α genes, and full-length sequences of the IGS region. Phylogenies obtained from the sequence data of the three different gene regions produced congruent topologies, but bootstrap support for the grouping was only strong for the IGS tree, showing a perfect clustering of isolates from the same molecular group (virulence grouping) and vegetative compatibility group (VCG). Furthermore, the phylogenetic relationships reconstructed in the concatenated dataset were also driven by the IGS phylogenetic signal. Analysis of the ClustalW nucleotide alignment showed that the number of parsimony informative sites was limited for β-TUB and EF-1α sequences, but high along the IGS region. Thus, the use of the IGS sequence seemed the perfect choice for the phylogenetic studies of Fod isolates. However, the amplification and sequencing of the 2600 bp IGS region is not a practical approach. Therefore, we determined if a shorter IGS sequence would allow us to resolve intraspecific relationships in Fod. Interestingly, we identified a fragment of approximately 300 bp, located between two conserved regions, that accumulated a high number of informative sites. The tree generated with this short region showed an identical clustering and well-supported branches than the one generated with the full-length IGS sequence. Moreover, the "condensed" alignment of this region showing only the informative positions, revealed the existence of group-discriminating positions. It can be concluded that the short length and sequence variation nature of this IGS region make it a useful choice for a simple phylogenetic analysis of Fod isolates.

420 DIVERSE S. CEREVISIAE STRAINS HAVE DIFFERENT EVOLUTIONARY POTENTIALS

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Since the completion of the Saccharomyces cerevisiae reference genome in 1996 and the advent of high-throughput sequencing, the genomes of many wild and industrial strains have been completed, allowing deeper understanding of selection pressures, trait variation, population structure, and molecular mechanisms in this key model organism. However, little is known about the evolutionary potential of these different lineages. In this study, we used a collection of seventeen strains representing diverse lineages of S. cerevisiae from different geographical and ecological niches (Malaysian, West African, North America, European, and Sake). We characterized their initial growth abilities and their capacity to increase fitness when subjected to hundreds of generations of experimental evolution in a new, challenging environment. We found that the initial fitness of these strains showed a high degree of intra-species variability in both sulfate-limited batch and continuous cultures, with relative fitness ranging from -25% to +27% versus the reference strain. We selected 10 of the strains representing the whole range of initial growth abilities, and subjected them to 200 generations of growth in sulfate-limited chemostat cultures to quantify their short-term evolvability. We found that despite the broad initial growth ability of the diverse lineages, all the evolved clones were more fit than the parental strains by at least +10%, with the fittest evolved strains showing fitness improvements of +70%. Interestingly, evolutionary potential was anti-correlated with initial fitness, with the strains starting out as the poorest performers improving the most during the evolution timecourse. All evolved strains converged at a similar improved fitness value relative to the reference, indicating a strain-independent peak in the fitness landscape. We are currently testing if the divergence in the strains' performances can be explained by variation in the sequence or expression level of SUL1, the major high affinity sulfate transporter. Our results introduce a powerful new system to study fitness landscapes within a species and support the notion that deleterious mutations may cause proportional increases in evolutionary potential.

421 EFFECTS OF ACQUISITION, LOSS, AND NEOFUNCTIONALIZATION OF TRICHOTHECENE BIOSYNTHETIC GENES ON VARIATION IN TRICHOTHECENE STRUCTURE, BIOSYNTHETIC PATHWAY REGULATION, AND SELF-PROTECTION MECHANISMS IN THE HYPOCREALES

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Trichothecenes are secondary metabolites produced by multiple fungi in the order Hypocreales, including species of Fusarium, Myrothecium, Stachybotrys, and Trichoderma. These metabolites are of concern because they are toxic to humans and other animals, can contribute to pathogenicity in Fusarium, and are required for full biological control activity in Trichoderma. All trichothecenes have the same core structure, a three-ring skeleton with an epoxide function, but differ in patterns of oxygenation and acylation. In this study, comparison of trichothecene biosynthetic gene (TRI) clusters in hypocrealean genera and functional analysis of individual genes provide evidence that variation in trichothecene structure among genera can result from acquisition, loss, and neofunctionalization of TRI genes. Gene loss and acquisition have also contributed to fundamental changes in TRI gene regulation, as is evident by a transcription factor gene that is unique to the TRI cluster in one Fusarium lineage, and by the presence of the regulatory genes TRI6 and TRI10 in all genera examined except Beauveria. Analysis of phylogenetic relationships of TRI genes and of trichothecene-producing genera provide evidence for the order in which such changes in the TRI cluster have occurred. Together, the results provide insight into the evolutionary history of biochemical and regulatory pathways as well as self-protection mechanisms responsible for trichothecene production in the Hypocreales.

422 ELUCIDATING THE QUANTITATIVE BASIS OF VIRULENCE IN THE WHEAT PATHOGEN ZYMOSEPTORIA TRITICI.

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Zymoseptoria tritici (aka Mycosphaerella graminicola) causes Septoria tritici blotch, a damaging disease on wheat worldwide. Symptoms include chlorotic and necrotic lesions containing asexual fruiting bodies called pycnidia. The reduced photosynthetic capacity caused by leaf lesions can reduce yields by up to 40%. Despite its significance, little is known about virulence mechanisms in this pathogen. A QTL mapping approach was used to elucidate the genetic basis of virulence using ~700 progeny from two crosses between four parents differing in their virulence phenotypes. RADseq was used to generate ~8500 informative SNP genetic markers in each cross. A high throughput phenotyping method based on automated digital image analysis was developed to accurately measure the percentage of leaf area covered by lesions (PLACL) as well as pycnidia size and number. The data were obtained by inoculating seedlings of two Swiss winter wheat cultivars with each offspring. Pycnidia size and density were found to be quantitative traits that showed a continuous distribution in the progeny. There was a weak correlation between pycnidia density and size and between pycnidia density and PLACL. There were significant differences in PLACL and pycnidia density on resistant and susceptible cultivars. Over 20% of the offspring exhibited significantly different pycnidia density on the two cultivars, consistent with host specialization. QTLs underlying all measured virulence traits were identified. In some cases, the same QTLs were associated with PLACL and pycnidia production, but in other cases they mapped as separate characters. Different QTLs were found between different mapping populations and different wheat cultivars. Based on the QTL mapping results, several candidate genes associated with virulence were identified.

423 EXTENSIVE TRANS-SPECIFIC POLYMORPHISM AT THE MATING TYPE LOCUS OF THE ROOT DECAY FUNGUS HETEROBASIDION

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Incompatibility proteins play a fundamental role in structuring genotypes in natural populations. In systems where allelic diversity is favoured, rare alleles have an advantage through negative frequency selection. Negative frequency selection retards allele loss by genetic drift and in doing so may extend coalescence times of alleles deeper than the divergence times between species, causing trans-specific polymorphism of allelic lineages. The basidiomycete tree pathogen Heterobasidion annosum sensu lato (s.l.) have been used to study intra and interspecific allele inheritance among multiallelic MAT loci. The five Heterobasidion species that were formerly all considered H. annosum are closely related and have speciated both allopatrically and sympatrically, with species restricted to either North America or Eurasia on a specific set of host taxa. Heterobasidion annosum s.l. is heterothallic and bipolar with a single MAT locus with a structure and gene content similar to that of other multiallelic bipolar species with two pairs of divergently transcribed homeodomain genes, that are presumably redundant in function. However, the DNA binding homeodomain of the HD2 homologue appeared to be abolished or highly divergent. Here we use DNA sequences to show that the extended coalescence time of MAT alleles greatly predates speciation in the group. Loci outside of MAT show divergences largely consistent with the species phylogeny contrasting those of MAT which show rampant trans-species polymorphism. We observe a roughly six-fold greater genealogical depth and polymorphism of MAT compared to non-MAT which argues for the maintenance of balanced polymorphism for greater than 24 million years based on a molecular-clock calibrated species phylogeny. As with other Basidiomycete MAT genes, balancing selection appears to be concentrated at the specificity-determining region in the N-terminus of the protein based on identification of codons under selection and the absence of recombination within the region. However, the elevated polymorphism extends into the non-specificity determining regions as well as a neighboring non-MAT gene, the mitochondrial intermediate peptidase. In doing so, increased divergence should decrease recombination among alleles and as a by-product create incompatibilities in the functional domains not involved in allele recognition but in regulating sexual development.

424 FIFTY SHADES OF BLUE: INSIGHTS INTO PENICILLIUM ROQUEFORTI GENETIC DIVERSITY

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Blue-veined cheeses are manufactured and consumed in numerous countries worldwide. Each blue-veined cheese type (i.e. Gorgonzola, Roquefort, Stilton) is obtained by different manufacturing methods leading to specific organoleptic and texture properties. The typical appearance and flavor of blue-veined cheeses are due to the development of Penicillium roqueforti used as a starter culture worldwide. Yet, as this species can grow under harsh conditions including cold temperatures, low oxygen and high carbon dioxide levels and/or in the presence of preservatives, it is also a common spoilage organism in numerous substrates like refrigerated stored foods, meats, wheat products or silage. From a taxonomic point of view, a single species name is currently recognized, P. roqueforti, although great morphological differences have been observed amongst isolates. Moreover, several technological names have been used in the past such as Penicillium stilton, P. italicum, P. gorgonzola, P. aromaticum or P. glaucum. This study investigates a large worldwide P. roqueforti collection including 145 isolates from 116 blue-veined cheeses and 17 other substrates in order to (i) determine whether P. roqueforti includes cryptic species by applying the Genealogical Concordance Phylogenetic Species Recognition criterion (GC-PSR) using five genes (tsr1, mcm7, cct8, ^{2}-tub, cmd) and (ii) assess the P. roqueforti intraspecific diversity and population structure using SSR markers developed by searching microsatellite motifs within the P. roqueforti FM164 genome sequence (Cheeseman et al., 2014). Based on preliminary GC-PSR analyses, the 20 morphotypes seem to constitute distinct cryptic species. The utility of 24 SSR markers for studying P. roqueforti diversity was then evaluated on 8 representative isolates. Amongst these SSR markers, four were selected according to their ability to discriminate representative isolates and allowed to distinguish 27 haplotypes. The population structure analysis (Structure 2.3.4 program) suggests that P. roqueforti encompasses genetically differentiated populations. By creating a worldwide P. roqueforti collection (numerous blue ripened cheeses and diverse substrates), by using GC-PSR, and by developing specific and discriminant SSR markers, we obtained new insights into P. roqueforti genotypic diversity. In the future, these data will be associated to strain specific functional properties including metabolic traits such aroma or mycotoxin-production.

425 GENETIC POPULATION STRUCTURE OF EPIDEMIC AND POST-EPIDEMIC POPULATIONS OF THE ASH DIEBACK PATHOGEN CHALARA FRAXINEA

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The ascomycetous fungus Chalara fraxinea (teleomorph: Hymenoscyphus pseudoalbidus) is an invasive fungal pathogen, which causes a severe ash (Fraxinus spp.) dieback across Europe. The disease was first recorded in mid 1990's in eastern Poland and has since then spread across the continent. Exact origin of C. fraxinea and mechanisms governing the outbreak of the disease are still unknown and are the subject of ongoing investigations. In Lithuania, ash dieback has started around 1995-1996, and, to date, nearly all Fraxinus excelsior stands are affected, with the disease being in its post-epidemic (chronic) phase. On the contrary, in Switzerland, ash dieback was first observed only in 2008, and the disease here is still in its epidemic and expanding phase. The main objectives of this study were to: [(1)] test the hypothesis that epidemic populations of the pathogen are less diverse than its post-epidemic populations, [(2)] determine the extent of gene flow between Lithuanian and Swiss populations of the fungus, and [(3)] check for genetic differences between isolates of C. fraxinea originating from necrotic lesions and from infected leaf petioles. A total of 1,850 samples of necrotic twigs and shoots and more than 1,000 infected dead (shed) leaf petioles were collected in five Lithuanian and five Swiss stands of F. excelsior. In each country, a distance between the sampling sites (subpopulations) was at least 50 km. At each site, necrotic lesions were sampled from 60 trees and petioles were collected under 40-60 trees, which were at least 5 m apart from each other. More than 1,300 C. fraxinea isolates (872 from lesions and 444 from petioles, representing 451 and 444 trees, respectively) were obtained in total. More than 500 selected isolates (each subpopulation was represented by approx. 50 isolates, about a half of which originated from lesions and half – from petioles) were genotyped at 11 polymorphic microsatellite loci. Results of the ongoing study will provide new information on genetic population structure of C. fraxinea at different geographical scales in Europe, and on evolutionary potential of the fungus. Furthermore, a better understanding of how genetic diversity may vary across C. fraxinea populations and in different substrate types (lesions vs. petioles), and how gene flow can affect population structure of this pathogen will be gained.

426 GENOME SEQUENCE OF SACCHAROMYCES CARLSBERGENSIS, THE WORLD'S FIRST PURE CULTURE LAGER YEAST

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Lager yeast beer production was revolutionized by the introduction of pure culture strains. The first established lager yeast strain is known as the bottom fermenting Saccharomyces carlsbergensis, which was originally termed Unterhefe No.1 by Emil Chr. Hansen and used in production in since 1883. S. carlsbergensis belongs to group I/Saaz-type lager yeast strains and is better adapted to cold growth conditions than type II/Frohberg-type lager yeasts, e.g. the Weihenstephan strain WS34/70. Here, we determined the draft genome sequence of S. carlsbergensis based on Illumina sequencing of 8 kb mate-pair libraries. Lager yeasts such as S. carlsbergensis are descendants from hybrids formed between a S. cerevisiae parent and a parent similar to S. eubayanus. Accordingly, the S. carlsbergensis 19.5 Mb genome is substantially larger than the 12 Mb S. cerevisiae genome. Based on the sequence scaffolds, synteny to the S. cerevisae genome, and by using directed PCRs for gap closure we generated a chromosomal map of S. carlsbergensis consisting of 29 unique chromosomes. We present evidence for genome and chromosome evolution within S. carlsbergensis via chromosome loss and loss of heterozygosity specifically of parts derived from the S. cerevisiae parent. Based on our sequence data and via FACS analysis we determined the ploidy of S. carlsbergensis. This inferred that this strain is basically triploid with a diploid S. eubayanus and haploid S. cerevisiae genome content. In contrast the Weihenstephan strain is essentially tetraploid composed of two diploid S. cerevisiae and S. eubayanus genomes. Evolutionary implications for type I and type II lager yeasts are discussed.

427 HSP104 AS STRESS INDICATOR IN HYBRIDS OF THE SACCHAROMYCES SENSU STRICTO COMPLEX

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The Saccharomyces sensu stricto complex is a group of different yeast species, which are used in fermentation processes. This group includes S. cerevisiae, S. bayanus, S. paradoxus, S. mikatae, S. kudriavzevii and S. cariocanus. The members of this group are closely related and are able to mate and produce viable hybrids. The hybrids propagate by mitotic divisions, but produce only few viable spores. Hybridization between two different species forms a hybrid harboring two genomes, which expresses both proteomes and thus harbor characteristics of both parental strains. The expression of both proteomes will lead to the formation of hybrid protein complexes which could affect the function of these complexes and thus generate hybrid specific responses to environmental changes. We generated diploid hybrids of S. cerevisiae and other members of the Saccharomyces sensu stricto group and analyzed their stress tolerance. For this we employed a chromosomally integrated ScHSP104-GFP as an in vivo stress indicator. Hsp104 is a heat shock protein, which localizes to the cytoplasm and the nucleus in unstressed cells. Under different stress conditions Hsp104 binds to denatured and aggregated proteins to promote their refolding. These aggregates are visible as HSP104-GFP foci in the cell and the number of formed foci can be used as an indicator of the stress level in the different hybrids. We present results of heat-stressed and ethanol stressed cells that show differential responses of these hybrids to environmental stresses.

428 IDENTIFICATION OF HORIZONTAL GENE TRANSFER IN THE GENUS COLLETOTRICHUM REVEALS A CONSTANT FLUX OF GENE TRANSFER FROM BACTERIA TO FUNGI

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Horizontal gene transfer (HGT) is the stable transmission of genetic material between organisms without vertical inheritance. The increasing number of sequenced fungal genomes gives us the opportunity to detect HGT events in new species. We investigated the impact of the HGT in the genus Colletotrichum. This genus includes many phytopathogenic species that are responsible for important losses in agriculture around the world. To detect HGT events a strict methodology was developed based on gene-by-gene phylogenies and several steps of manual curation. The result was a list of eleven homology groups, genes present in at least one species of Colletotrichum that are of putative bacterial origin. These genes are predicted to be involved with the metabolism of amino acids, lipids and sugars as well as pathogenicity. The putative minimal dates of the HGT events were calculated based on the molecular clock hypothesis, by calibrating a phylogenetic tree using fossil evidence. This analysis reveals a constant flux of genes from bacteria to fungi throughout the time and the evolution of the Pezizomycotina. At the same time a complex pattern of transferred gene losses was deduced from the reconstructed species tree. For that reason we propose that the HGT events could be important for the niche adaptation of species but not indispensable for the survival. This study, in agreement with other studies of HGT in the Ascomycetes reveals a complex pattern of evolution in the Pezizomycotina. These results also show the importance of lateral transmission and gene losses in the history of this subphylum.

429 INVESTIGATING COLLETOTRICHUM TELEOMORPHIC STRAINS FROM ANTHRACNOSE LESIONS ON COMMON BEAN PLANTS IN BRAZIL

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Colletotrichum lindemuthianum is one of the most important pathogen in common bean culture causing anthracnose disease and leading to significant loss of production worldwide. The origin of high genetic diversity found in C. lindemuthianum populations is unknown, but sexual recombination is one possibility. Colletotrichum strains that produce a Glomerella teleomorph in culture are frequently recovered from anthracnose lesions on common bean plants in the field. In this study, a large collection of Glomerella isolates was characterized. The isolates were separated into two groups based on phylogenetic analysis of the internal transcribed spacer of the ribosomal DNA and on the high mobility group (HMG)-encoding sequence of the MAT1-2-1 mating type gene sequences, colonial growth, germination rates in culture, and pathogenicity to beans. Individual ascospores recovered from Glomerella perithecia gave rise to either fertile (perithecial) or infertile (conidial) colonies. Some pairings of perithecial and conidial strains resulted in induced homothallism in the conidial partner, while others led to apparent heterothallic matings. Conidia efficiently formed conidial anastomosis tubes (CATs), but ascospores never formed CATs. The Glomerella strains formed abundant appressoria and hyphae on the plant surface, but were unable to penetrate or form infection structures within the tissues. These same Glomerella strains produced thick intracellular hyphae, and eventually acervuli, when localized plant cell death was induced by the application of dry ice. When Glomerella was co-inoculated with C.lindemuthianum, it readily invaded the anthracnose lesions. The results led to the conclusion that these sexual Colletotrichum strains are not G. lindemuthiana, and instead represent at least two nonpathogenic species that survive epiphytically on the bean tissue.

430 MITOCHONDRIAL GENOME OF TAPHRINA BETULINA SHARES FEATURES SIMILAR TO BOTH ASCOMYCETES AND BASIDOMYCETES

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Historically the phylogenetic position of Taphrina has been controversial and incongruities still exist between morphological and molecular data. Currently, the genus Taphrina has been classified as early diverging Ascomycota. Complete mitochondrial genome of Taphrina betulina, which is a biotrophic pathogen of Betula sp, has been determined with de novo 454- and PacBio RS II sequencing. The mitochondrial sequence forms a circle of 44 771 bp encoding; three ATP synthase subunits (ATP6, 8, 9), three cytochrome c oxidase subunits (COX1, 2, 3), seven nicotinamide adenine dinucleotide ubiquinone oxidoreductase subunits (NADH1, 2, 3, 4, 4L, 5, 6) and cytochrome b (CYTb), altogether 14 orfs with known function. Ribosomal protein (RPS3) commonly found from Ascomycota mitochondrial genomes was not identified from the T. betulina assembly. Introns were found from five protein coding orfs (Cox1,2,3, Atp9 and Nadh5) most introns (group I) encoded Laglidadg homing endonucleases (8/9). Majority of tRNAs encoding sequences were clustered within two 2 kb clusters containing altogether 22 tRNAs. tRNAs for tyrosine, asparagine and valine were dispersed in the genome. tRNAs representing all essential amino acids were found and multiple codons were found for serine, arginine leucine (two codons) and methionine (three codons). Large and small subunits of ribosomal genes (rnls) were identified on basis of similarity to Pneumocystis rnls genes. Protein coding genes displayed contrasting similarities towards both Ascomycetes and Basidiomycetes. In Blastp analysis COX1,2, NADH,2,3,4, CYTb were more similar to Basidiomycetes (e.g. Ustilago maydis and Rhizoctonia solani) whereas COX3 Nad1,5,6,4L ATP6, 8,9) shared higher similarity to Ascomycetes (e. g. Ajellomyces dermatitidis ER-3 and Phialocephala subalpina). Clustering of orfs with similarity to different fungal division was apparent and partial orf with similarity to Ascomycetes Cytb was found in flanking region of the clusters. Overall the similarities were low to both divisions and even to other Taphrinomycota species (S. pombe and Pneumocystis). In the presentation the evolutionary position of Taphrina sp. will be re-evaluated with state of art phylogenetic method benefiting from the first complete mitochondrial genome in Taphrinomycetes class.

431 MORPHOLOGIC, PHENOTYPIC, PATHOGENIC AND MOLECULAR CHARACTERIZATION OF MACROPHOMINA PHASEOLINA ISOLATES FROM MAIZE AND PEANUT

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Twenty nine isolates of Macrophomina phaseolina from peanut and maize plants collected from 5 different locations of Eastern Mediterranean Region of Turkey. All the isolates were subjected to growth rate tests at 15, 20, 25, 30, 35 and 40°C. Optimum growing temperature was found to be 30ºC but there was no correlation between location or host plants with optimum growing temperature. Chlorate phenotype of each isolate was determined after growing on a minimal medium containing 120 mM potassium chlorate. Three chlorate phenotype; Feathery, Dense and Restricted; were differentiated by their chlorate sensitivity. Mostly maize isolates were chlorate resistant, whereas peanut isolates were chlorate sensitive. Chlorate sensitive isolates were phenotyped as restricted and fathery. In the pathogenicity tests, dense isolates were more virulent on maize, whereas restricted and fathery isolates more virulent on peanut seedlings. Polymorphic bands with the RAPD primers were 40% but 75% with SSR primers. We found no correlation between location and ssr groups but there was highly correlation between host selectivity and ssr groups. Peanut isolates grouped in the same cluster, and maize isolates grouped in the second cluster. In the molecular tests, SSR technique was more efficient than RAPD.

432 NATURAL DIVERSITY OF PHYCOMYCES

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Our scant knowledge of Phycomyces in nature hinders our understanding of the abundant results acquired in the laboratories in the last century and a half. Fungi of this genus are uniform in appearance and clearly distinct from all other Mucorales. We gathered 80 strains from around the world, 18 of them from Spain. The morphology of their spores and the sequences of their loci for sex and for ribosomal DNA establish two clear groups that correspond with the P. nitens and P. blakesleeanus species defined by Burgeff (1925) and confirmed by Benjamin and Hesseltine (1959). The considerable genetic distance to other Mucorales justifies the acceptance of the family Phycomycetaceae. The DNA sequences above differ in the two species, but are conserved in strains of the same species, even from different continents. This is particularly surprising since the spores of Phycomyces are not dispersed easily by wind or rain. The two main components of the variance of 32 restriction fragment length polymorphisms in 40 strains of P. blakesleeanus, which explain 56 per cent of the total variance, lead to the recognition of four distinct groups, two of them with strains from North America and two from Europe. This allows us to suggest the origin of some strains in the collections. The homogeneity of the species does not preclude a degree of diversity related to geographic expansion.

433 POPULATION GENETIC DIVERGENCE AND EXPRESSION PROFILES IN A VIRULENCE-RELATED CANDIDATE GENE OF THE COFFEE OBLIGATE PATHOGEN, HEMILEIA VASTATRIX

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Hemileia vastatrix is a complex biotrophic fungal pathogen that causes coffee leaf rust (CLR), a disease that has been a permanent threat to Arabica coffee production for more than a century. Frequent shifts in pathotypes, driven by the dynamic system of host-pathogen co-evolution, have proved to be a critical limitation for achieving durable CLR resistance, resulting in a gradual breakdown of many of the successfully improved varieties worldwide. Under the constant threat of new pathotypes emerging under a strong selective pressure and becoming epidemically spread on a continental scale, a better understanding of the adaptive genetic variation of H. vastatrix populations across large geographic areas, different coffee hosts and periods of time, is an immediate priority. Since genes involved in coffee-rust interaction are expected to evolve under strong selection, the analysis of genetic and expression differences in putative candidate genes could provide insights on the pathogen virulence evolution. In this study, we analyzed a candidate gene activated during early stages of H. vastatrix infection, retrieved from a 454-transcriptomic dataset, under a population framework. Sequence data was obtained for 75 H. vastatrix isolates comprising different geographic origins, virulence profiles, collection years and coffee hosts, and used for the analysis of genetic diversity, differentiation and detection of selection signatures. Although a low genetic diversity was found, more than 50% of polymorphic sites identified on the fragment sequence, corresponded to non-synonymous mutations. A pattern of very divergent haplotypes/alleles was found from a phylogenetic reconstruction, where the most basal and distinct group corresponded to rusts isolated from diploid coffees. Interestingly, in heterozygous isolates, the respective alleles clustered apart in two independent and divergent groups, revealing a clear structuring of genetic variation with a probable adaptive significance. Moreover, preliminary results of neutrality tests indicated signs of selection. Complementary assessment of gene differential expression was initiated for 6 isolates representing contrasting rust virulence profiles, in the early-stages of the infection process of compatible interactions. This study opens the way to further tracing population divergence and the context in which it occurs, aiming ultimately to start unveiling rust adaptation. Funded by FCT (project PTDC/AGR-GPL/119943/2010).

434 POPULATION GENETICS ANALYSIS IN PUTATIVE HOUSEKEEPING GENES IN ORDER TO INVESTIGATE GENETIC DIVERSITY OF RAMULARIA COLLO-CYGNI

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Ramularia collo-cygni is now recognized as an important pathogen of barley in Northern and Central Europe, New Zealand and South America and has also been reported recently on oats and wheat. It is the cause of Ramularia leaf spot (RLS), a disease which occurs late in the season. It induces necrotic spotting and premature leaf senescence, leading to loss of green leaf area in crops, and can result in substantial yield losses. The fact that the fungus can remain latent in barley plants until flowering, coupled with its very slow growth in vitro, makes it difficult to detect it in crops. As a result, the epidemiology of this pathogen remains poorly understood. To know more about its epidemiology, having the knowledge of its genetic structure and diversity is important. In this study, we tried to have a first look at the population genetics of Ramularia collo-cygni. Since Ramularia genome sequences were not yet available, a gene fishing strategy was performed to select putative housekeeping genes. We used the sequences of several housekeeping genes in Cercospora zeae-maydis and Mycosphaerella graminicola, reported to be two related species to Ramularia. After testing primers for these genes on Ramularia, five putative housekeeping genes were selected. To carry out the study, genes fragments had to reach a minimum size of 500 bp. To reach this minimum size, we performed Thermal Asymmetric Interlaced (TAIL) PCRs on these genes. Once the right size was reached and after making sure that the amplified fragments are homologous to their corresponding genes on other fungi, we amplified and sequenced them on 20 Ramularia collo-cygni isolates. We performed classic population genetics analysis (Theta-W, Theta-Pi, Tajimas D, Fst) to uncover genetic variability and population structure.

435 POPULATION HISTORY AND PATHWAYS OF SPREAD OF THE PLANT PATHOGEN PHYTOPHTHORA PLURIVORA

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Human activity has been shown to considerably affect the spread of dangerous pests and pathogens worldwide. Therefore, strict regulations of international trade exist for particularly harmful pathogenic organisms. Our study organism, Phytophthora plurivora, which is not subject to regulations, is a plant pathogen frequently found on a broad range of host species, both in natural and artificial environments. It is supposed to be native to Europe while resident populations are also present in the US. We characterized a hierarchical sample of isolates from Europe and the US and conducted coalescent-, migration, and population genetic analysis of sequence and microsatellite data, to determine the pathways of spread and the demographic history of this pathogen. We found P. plurivora populations to be moderately diverse but not geographically structured. High levels of gene flow were observed within Europe and unidirectional from Europe to the US. Coalescent analyses revealed a signal of a recent expansion of the global P. plurivora population. Our study shows that P. plurivora has most likely been spread around the world by nursery trade of diseased plant material. In particular, P. plurivora was introduced into the US from Europe. International trade has allowed the pathogen to colonize new environments and/or hosts, resulting in population growth.

436 RESURRECTION OF ANCESTRAL OXIDOREDUCTASES: AN IN SILICO APPROACH

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The evolution of gene function is a central issue in molecular evolution and ancestral sequence reconstruction is a powerful tool in these studies. It has the advantage of inferring ancient gene sequences that act as hypothesis that can be tested in laboratory by resurrection of those sequences. During the past years the resurrection of genes had help to prove several hypothesis about how was life on Earth, and how some enzymes had evolved and obtained the functions they have today. The basis of this technique is the use of extant proteins to infer the ancestral sequences of interest, which will be expressed and characterized in vitro. Resurrected proteins are of interest not only because the basic information about evolution that they give us, but also because these proteins have great biotechnological potential: the resurrected proteins catalyzed reactions in a planet where the conditions were very different from actual Earth, with different temperature, pH, oxidation conditions, etc. Recently, it has been published the Paleozoic origin of lignin degradation using 31 genomes, where 6 genomes of Polyporales were used, reconstructing the ancestral state of discrete characters in ligninolytic and generic peroxidases, such as the appearance or disappearance of the oxidizing sites that defines the catalytic activities of these peroxidases. This study has been amplified and concreted using 10 genomes of Polyporales. In this way, we propose the reconstruction not only of those discrete characters, but the whole protein by inferring the ancestral sequences using the information of extant basidiomycete peroxidases. Thereby, by resurrection and characterization of those sequences in the laboratory we will be able to determine the mechanisms that lead the ancient proteins to the functions and properties they have today including the ability to degrade the recalcitrant lignin polymer, a key issue for development of land ecosystems. Here we present the preliminary results based on in silico resurrection and modeling of the ancestral PODs, with special interest in the oxidizing sites and their modification through the evolution.

437 SEQUENCES SUITABLE FOR DNA BARCODING ANALYSIS OF CRYPTIC DIVERSITY OF A BOTRYTIS CINEREA AS COMPLEX SPECIES

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The necrotrophic fungus Botrytis cinerea Pers. Fr. is the causal agent of grey mould, a disease affecting many economically important crops. At the present, B. cinerea is considered a species complex consistent of two phylogenetic or cryptic species, B. pseudocinerea (also called group I) and B. cinerea sensu stricto (group II). It is extremely difficult to be distinguished by classical taxonomic criteria. For identification of two cryptic species of B. cinerea, vegetative compatibility analyses, genealogical concordance of the phylogenetic species recognition using multiple gene sequences and selected several molecular markers are commonly required which takes a lot of effort and is time-consuming. Screening an appropriate DNA barcode for identification of the two cryptic species is essential. DNA barcoding aims to provide an efficient method for species-level identifications. Our purpose was to select useful molecular markers as candidates, to investigate the possible DNA barcode for Botrytis cinerea species complex. Although ITS is the most widely used marker for species identifications in mycology, its sequence has earlier stated not suitable for differentiation among members of the genus Botrytis as providing limited phylogenetically informative characters. Two other sequences have been tested for differentiation of Botrytis cinerea species complex (i) beta-tubulin sequence, and (ii) cyanide resistant alternative oxidase (AOX). The beta-tubulin sequences has been used earlier for differentiate B. cinerea from B. pseudocinerea. The parsimony analysis of the beta-tubulin sequences clearly separated the two B. cinerea cryptic species. A short sequence fragment has been chosen for DNA barcoding with nucleotide differences in eight positions. The AOX sequences nicely correlated with the phylogenetic connection of the studied Ascomycetous fungi. It also proved to be suitable for differentiate B. cinerea from B. pseudocinerea. Acknowledgement: Anikó Szojka is supported by TÁMOP 4.2.4. Her research was realized in the frames of TÁMOP 4.2.4. A/2-11-1-2012-0001 National Excellence Program - Elaborating and operating an inland student and researcher personal support system convergence program The project was subsidized by the European Union and co-financed by the European Social Fund. The research was supported by the EU and co-financed by the European Social Fund under the project ENVIKUT (TÁMOP-4.2.2.A-11/1/KONV-2012-0043).

438 SEX MODULATES LONG DISTANCE DISPERSAL AND ANTAGONISTIC POTENTIAL OF TRICHODERMA REESEI

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The genetic improvement of biotechnologically important fungal strains is largely based on mutagenesis, however the controlled sexual reproduction may considerably facilitate this technique. Trichoderma reesei (teleomorph Hypocrea jecorina, Ascomycota), an industrially important cellulase producing filamentous fungus possess a bipolar heterothallic sexual system which requires the two mating type genes, mat1-1 and mat1-2. This property, and strategies for its further genetic improvement, have recently regained strong interest because of the attempts to produce second generation biofuels. However, T. reesei was historically stigmatized as weak to moderate mycoparasite, which defense mechanisms and strong competition for nutrition against other fungi are its main weapon. We characterized numerous T. reesei wild type and progeny strains derived from two mating sets. For this purpose we tested them for mating type dependent differences in macromorphological characters, conidiation, carbon utilization, mycelial growth and antagonistic potential. In this study we reveal that although conidiation in T. reesei is carbon source depended ait is also strongly altered by mating, yet not determined by the mating type. We also show that in the conditions resembling T. reesei natural ecosystem and its opponents, this fungus provides all the evidence to render a strong mycoparasite and that this feature depends on recombination events rather than on mating types. Furthermore, the master regulator of cellulase expression, XYR1 but not LAE1 and several polyketide synthase genes (PKSs) are differentially involved in T. reesei defense mechanisms and mycoparasitic attack.

439 SIDEROPHORE UTILIZATION DETERMINES FUNGAL COMPETITIVENESS

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Nature abounds with a rich variety of altruistic and competitive strategies. The most common form of microbial cooperation is the secretion of enzymes and metabolites to the environment, e.g. saprobic decomposition. This benefits not just to the secretor, but anyone in close proximity. Another well-studied altruistic trait is iron mobilization through secreted low molecular-mass iron chelators, termed siderophores. On the other hand however, here we demonstrate that siderophores and their utilization, respectively, determine fungal competiveness. We have previously shown that the mold Aspergillus fumigatus secretes the siderophores fusarinine C (FSC) and triacetylfusarinine C (TAFC). In this study, we found that the growth of Aspergillus terreus, secreting the siderophores coprogen and ferrichrysin, is inhibited by desferri-TAFC induced iron deprivation, indicating an inability to utilize TAFC-chelated iron. Subsequent phylogenetic analysis indicated the lack of two putative siderophore transporters in A. terreus compared to A. fumigatus, termed Afu-MirB and Afu-MirD. Heterologous expression of the gene encoding Afu-MirB, but not Afu-MirD, rendered A. terreus resistant to desferri-TAFC, revealing its substrate specificity. In cocultivation with A. fumigatus, Afu-MirB-mediated TAFC uptake increased the fitness of A. terreus as indicated by significantly increased production of conidial offspring. These data underline that, with respect to competiveness, the optimal strategy is to produce siderophores unusable by competitors, while at the same time accepting xenosiderophores. In line, most fungal species encode multiple siderophore transporters (including A. fumigatus and A. terreus), even siderophore non-producers such as Saccharomyces cerevisiae and Cryptococcus neoformans.

440 SIGNALLING ROLE OF MYCOTOXINS IN PATHOGEN – PATHOGEN INTERACTIONS

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The ability of fungal plant pathogens to exude bioactive compounds is an important element of competition in a changing environment. The balance between compound producers and divergent non-producers plays an important role in the infection process. Thus the filamentous fungi usually retain a number of adaptations related not only to the production of toxic compounds but also to the adaptation to the mycotoxin presence in the environment. We examined a distinct effect of toxins on morphology, growth patterns and gene expression after stimulation in both mycotoxin producing and non-producer isolates of divergent Fusarium species. Toxigenic capability of examined strains was confirmed by sequencing based on cross-species specific degenerate primers and HPLC/GC-MS. Traditionally mycotoxin molecules are investigated as harmful compounds, detrimental to organism survival. We investigated the potential adaptation of toxins as molecular signals in the interactions between pathogens with different susceptibility and toxigenic potential. By comparing the gene expression profile and growth patterns of plant pathogenic Fusarium isolates, we obtained evidence that some trichothecene compounds can act as potential signalling molecules. In particular, the non-producing Fusarium verticilioides/Fusarium proliferatum isolates show a direct reaction to deoxynivalenol. The toxin present in the medium has strong effects on the mycelial growth rate, number of viable fungal cells in medium (luminogenic ATP assay) and early gene expression, in particular genes related to biosynthesis and transport of fumonisins (fum1, fum19, fum21).

441 SIGNIFICANT DIFFERENCES IN SENSITIVITY TO DMI FUNGICIDES WITHIN FIELD POPULATIONS OF FUSARIUM GRAMINEARUM

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Fusarium head blight caused by Fusarium graminearum sensu stricto (Fg ss) is a destructive disease on cereals all over the world. The damage is due to both yield losses and mycotoxin production. Intensive application of fungicides on cereals beginning in the 1970s selected for fungicide resistance in many cereal pathogens. But this is the first report of fungicide resistance in field populations of Fg ss. 250 single spore Fg ss isolates originating from 15 field populations were tested in vitro to measure their resistance to the DMI propiconazole. A split plot design was used with two biological repeats each composed of three technical repeats. Each isolate was exposed to eight sub-lethal concentrations to calculate the EC50. EC50 values ranged from 0.78 to 62.2 mg.l-1 and showed a high broad-sense heritability (h2= 0.97). Significant differences ($P<0.001$) were found among the isolates. The variance component within populations was higher (76.8%) than the variance among populations (15.82%), indicating that propiconazole resistance is not geographically restricted. The entire coding sequence of CYP51 (a known target for DMI fungicides) was obtained for 20 isolates representing extreme EC50 values. Only six point mutations were detected, all encoding synonymous amino acids. This suggests that the observed DMI resistance in Fg ss is not due to mutations in the coding region of CYP51. Future analyses will use RADseq (Restriction site Associated DNA Sequencing) to conduct GWAS to detect candidate QTLs associated with the observed resistance.

442 SPECIES AND CHEMOTYPE CHARACTERIZATION OF FUSARIUM HEAD BLIGHT AND FUSARIUM ROOT ROT DISEASES ON WHEAT IN SARDINIA (TYRRHENIAN ISLANDS)

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Fusarium spp. can cause foot and root rot (FRR) as well as Fusarium head blight (FHB) on wheat. Depending on climate conditions the these diseases have different diffusion and prevalence. As trichothecene mycotoxins can be transported from roots upward to the heads, it is important to understand the toxigenic potential of Fusarium spp. causing the two diseases. In Sardinia (Tyrrhenian Islands, Italy) both FRR and FHB are present, hence from 2001 to 2013 a study was carried out to determine their causal agents. A total of 74 strains were collected from symptomatic plants in different areas. Species was determined morphologically and by sequencing of elongation factor EF-1α. Prevalent species was Fusarium culmorum (W.G. Smith) Sacc. followed by Fusarium graminearum Schwabe. Also a supposedly southern-emisphere isolate of F. cortaderiae O'Donnell, T. Aoki, Kistler & Geiser was identified. EF1-α polymorphisms analysis highlighted two populations of F. culmorum characterised by a single base mutation in the EF1-α gene. Fungal chemotype were determined by a multiplex PCR based on TRI12 polymorphism. Prevalent chemotype in F. culmorum was 3-ADON (87%) while in F. graminearum it was 15-ADON (100%). Nivalenol (NIV) chemotype isolates were significantly associated to FHB disease (80%). The single isolate of F. cortaderiae belonged to NIV chemotype. This study suggests that, compared to disease situation in continental Europe, where F. graminearum is reported to be the major cause of concern in wheat, F. culmorum is constantly prevailing in Sardinian wheat. Moreover, it seems that the NIV chemotype is less favoured as a cause of FRR. § The first two authors have equally contributed to the present work. Research funded by Regione Autonoma della Sardegna (Legge Regionale 7 agosto 2007, n. 7 “Promozione della ricerca scientifica e dell’innovazione tecnologica in Sardegna”) and by the Ministry of University and Research (PRIN 2010: Cell wall determinants to improve durum wheat resistance to Fusarium diseases). B.Scherm acknowledges support by P.O.R. SARDEGNA F.S.E. 2007-2013 - Obiettivo competitività regionale e occupazione, Asse IV Capitale umano, Linea di Attività l.3.1 (research project Identification of natural and natural-like molecules inhibiting mycotoxin biosynthesis by Fusaria pathogenic on cereals).

443 THE (NOT SO) SECRET HISTORY OF NON-REDUCING POLYKETIDE SYNTHASE DIVERSITY

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In recent years, the influx of newly sequenced fungal genomes has enabled sampling of secondary metabolite biosynthesis on an unprecedented scale. However, the evolutionary basis of variability taking into account both large-scale phylogeny reconstructions and knowledge gained from multiple genome projects is still largely unprobed. Available resources aiming to put large data sets (polyketide and terpene synthases, major families of accessory genes) in context of evolutionary origins of their diversity (e.g. duplication, speciation, loss and horizontal transfer) are scarce. We reconciled NR-PKS (non-reducing polyketide synthase) phylogeny based on conserved ketoacyl synthase/acyl transferase core domains, with species phylogeny based on single-copy orthologous housekeeping genes. This allowed for batch annotation of evolutionary events (duplication, speciation, transfer and loss) on a set of NR-PKS from over 140 model fungal genomes. For the purpose of detecting monophyletic patterns in synteny, domain architecture and gene structure, we devised and assessed a novel statistical approach to validating and pinpointing conserved traits positively correlated with tree topology. Thus we were able to both provide points of origin for trait gain/loss (strongest correlations with monophyletic clades), chains of inferred evolutionary events and their relative (topological) dating. Major results indicate that: (i) evolutionary root of extant non-reducing polyketide synthases predates split between Basidiomycetes and Ascomycetes (ii) capability to biosynthesise derivatives of orsellinic acid, through C2-C7 cyclisation, is a likely ancestral trait of all fungi (iii) gain of NAD-dependent reductase domain (e.g. tropolone, fusarubin biosynthesis) involved in cyclic aldehyde biosynthesis occurred twice in the history of Ascomycetes (iv) gain of independent Mn-dependent lactamase involved in termination is a late evolutionary development in filamentous fungi (v) maximally parsimonious reconciliations allow consistent prediction of transfer events for a range of costs, including transfers previously indicated by research focused on individual gene transfer events (e.g. history of aflatoxin/sterigmatocystin/dothistromin biosynthetic cluster).

444 A COMPLEX INTERPLAY OF ASPERGILLUS FUMIGATUS WITH THE SOCIAL AMOEBA DICTYOSTELIUM DISCOIDEUM SUPPORTS THE VIRULENCE SCHOOL CONCEPT

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Aspergillus fumigatus can be regarded as a classical example of an environmentally acquired pathogen with a broad host range covering humans and various animals. The disease pattern may vary greatly depending on the immune status or other predispositions of the patient, ranging from the sole mucus colonization in the lungs of cystic fibrosis patients to a deleterious, tissue invasive growth in immunocompromised individuals. Originally focused on the identification of single virulence factors, research in the last years has established that virulence is rather driven by multifactorial processes on the host and pathogen side. However, the evolutionary basis between these complex interplays are yet to be identified. It has long been proposed that the general mechanisms of fungal virulence must have emerged long before the appearance of innate immune systems and fostered the hypothesis that it could at least partially result from the selection pressure imposed by amoeba predation. A central question is therefore how the interaction with predatory amoeba could also have stimulated the development of virulence mechanisms. We have exploited the social amoeba Dictyostelium discoideum as a model organism to study its interaction with conidia of A. fumigatus. Although not a regular food source, fungal conidia were readily taken up by the amoeba within the first hours of exposure, as seen by transmission electron microscopy and discriminant fluorescence of intracellular conidia. Interestingly, non-melanized conidia of a pksP-deletion mutant of A. fumigatus were taken up at much higher rates than the wild type, leading to a massive intracellular accumulation. These results are also in agreement with the well documented role of melanin in the protection against macrophage killing. Despite the observation of phagolysosomal fusions, the viability of the fungal conidia was not impaired after phagocytosis and intracellular germination initiated after 24 hours. Up to this time point amoeba also remained fully viable and only fungal germination coincided with an increased number of dead amoeba. We hypothesize that both, fungus and amoeba also interact chemically and present first results on the cross inhibition of the two species.

445 A HOMOLOGY BASED SEARCH FOR NEUROSPORA REC GENES

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rec 1+, rec 2+ and rec 3+ are dominant trans-acting genes that suppress meiotic recombination in specific regions of the Neurospora crassa genome. For example, up to 1% of progeny from a rec 2 by rec 2 cross experience recombination in his 3 but only 0.005% when one or both parents carries rec 2+. rec 2+ resides in a 10 kb stretch of DNA absent from rec 2 strains, which have instead a 2.8 kb stretch of unique DNA. Several pieces of evidence show that meiotic silencing at this large insertion is responsible for the dominance of rec-2+. If meiotic silencing is blocked by sad-1, recombination at his 3 is increased substantially in rec 2+ by rec 2 heterozygotes, a rec 2 deletion behaves like rec 2+ as a dominant suppressor of recombination and, finally, inserting rec-2 at the same location in a mating pair yields a high recombination frequency at his-3. Indeed, meiotic silencing is responsible for the apparent dominance of all three rec+ genes. In the absence of meiotic silencing, recombination frequencies at his 1 in rec 1+/rec 1 heterozygotes and at am in rec-3+/rec-3 heterozygotes are indistinguishable from those of rec-1 and rec-3 homozygotes respectively. Thus, the products of rec 1, rec 2 and rec 3 act to promote recombination in specific regions of the genome and are probably all genes located within indels that are regulated by meiotic silencing. We have sequenced the genome of a rec-1 strain by PacBio technology to search for large indels in the appropriate genomic region. Of three candidate indels, a 4.7kb insertion unique to the rec-1 strain has homology to the predicted rec-2 gene.

446 A NEW METHOD FOR FUNGAL GENETICS: FLOW CYTOMETRY OF MICROENCAPSULATED FILAMENTOUS MICROCOLONIES

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Genetic analysis of organisms can be achieved by the isolation of consistent, well-defined colonies on solid media. In the case of non-filamentous microorganisms genetic analysis is also facilitated by the handling of individual cells by flow cytometry. In contrast, some filamentous fungi are hard to be analyzed by flow cytometry. In this work we have employed the combination of single spore microencapsulation and large particle flow cytometry as an alternative for the analysis of filamentous fungi. Mycelium proliferation inside the microcapsules can be detected using either microscopy or COPAS™ large particle flow cytometry.
Here we show the successful application of the Flow Focusing® technology to the microencapsulation of filamentous fungi in monodisperse alginate microspheres, using Aspergillus and Trychoderma as model systems. Using a Cellena® Flow Focusing microencapsulator, we managed to produce monodisperse microparticles containing individual spores and to develop microcolonies of these fungi upon germination in the appropriate conditions. Proliferation inside the particles was monitored by microscopy and large particle flow cytometry without requiring fluorescent labeling. Auxotrophic mutants were utilized to demonstrate the feasibility of the method. This procedure allows for the handling, screening and analysis of clonal colonies in liquid culture. Examples of genetic analysis with conditional mutants are provided.

447 ADENINE METHYLATION IN THE DNA OF FUNGI

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The presence of modified bases in the DNA of almost every organism analysed is known for more than fifty years now. While m5C (5-methyl-citosine) methylation has been shown and broadly studied in bacteria, fungi, other lower eukaryotes, plants, and animals, the investigation on DNA m6A (6-methyl-adenine) methylation in eukaryotes has been relatively ignored, probably by the thinking of it being absent, irrelevant, or difficult to accomplish. The idea that m6A has a modest (or not at all), role in the eukaryotic genomic structure, organisation, and regulation contrasts with what it happens in bacteria, in which more than one important cellular and epigenetic mechanisms of regulation are mediated by m6A DNA methylation. Anyhow, several lower and higher (human, rat and plant) eukaryotes have been investigated in relation to their m6A presence and content, with some interesting results. A gap in these studies is the fungal kingdom, which probably could give mixed (and/or relevant), information on what happened from prokaryotes to eukaryotes in this matter. Our first approach to answer this question was based on m6A sensitive digestion with restriction enzymes, and in silico analyses of DNA from more than ten fully-sequenced fungi. The data obtained in these two kind of experiments gave us new ideas to find out the presence, importance, and possible function of m6A DNA-methylation in fungi. Here we present recent results based in HPLC-MS, the isolation and characterization of sequences with an m6A-end from Phycomyces blakesleeanus, and the isolation of two putative N6-DNA adenine methyl transferases from Mucor circinelloides.

448 ASPERGILLUS NIGER AS EXPRESSION PLATFORM FOR SECONDARY METABOLITE PRODUCTION

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Filamentous fungi can each produce dozens of different secondary metabolites which are attractive to become exploited as therapeuticals, drugs, antimicrobials, flavour compounds, and other high-value chemicals. Although the genomes of filamentous fungi contain numerous gene clusters that encode for enzymes involved in the biosynthesis of so far uncharacterized secondary metabolites (SM), many of these gene clusters are silent under laboratory growth conditions. Where known, SM production is regulated by complex networks and involves intricate multi-step biosynthetic machineries, as well as major reorganization of primary metabolic fluxes to redirect cellular metabolic resources towards their biosynthesis.

As SM expression varies considerably with the host and enzymes catalyzing SM are often difficult to express, we aim to establish the filamentous fungus Aspergillus niger as heterologous host for SM production. We used a multifunctional non-ribosomal peptide synthetase (NRPS), catalyzing the formation of a cyclic peptide and expressed its encoding gene under control of a bacterial-fungal hybrid promoter in A. niger. The peptide was isolated and purified from A. niger shake flask cultures using EtOAc extraction and liquid chromatography. The purity of the peptide was proven by MS/MS and NMR analyses. The initial yields of 1 mg / litre were increased at least 100 fold using a DOE approach, which addressed feeding conditions and the morphology of A. niger. This peptide titre was found to be significantly higher than that reached in bacterial expression hosts, demonstrating that A. niger is a promising host for NRPS expression.

449 BIOSYNTHETIC POTENTIAL FOR POLYKETIDES IN TALAROMYCES ATROROSEUS

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The genus Talaromyces produces interesting secondary metabolites (SM) of polyketide nature, including yellow and red azaphilone pigments belonging to the mitorubrins and the Monascus pigments. Especially the potential involvement of SM in the pathogenicity of the pathogenic dimorphic Talaromyces marneffei (former Penicillium marneffei) is of significant interest. The two genome sequenced species of Talaromyces show a promising potential for polyketide production, Talaromyces stipitatus has 18 PKS and 2 PKS-NRPS genes and Talaromyces marneffei has 23 PKS and 2 PKS-NRPS genes. The potential for polyketide production in T. marneffeis is therefore much higher than other dimorphic pathogenic fungi such as Histoplasma capsulatum (1 PKS) and Coccidioides immitis (9 PKS / 1 PKS-NRPS) (Collemare et al., 2008). In Talaromyces the only polyketides linked to their genes are stipitatic acid (tropA, T. stipitatus), mitorubrinic acid and mitorubrinol (pks11 and pks12, T. marneffei) and melanin pigment (alb1, T. marneffei). In order to study the distribution and potential evolutionary cluster dynamics of PKS clusters in the Talaromyces genus, we sequenced the genome of T. atroroseus IBT11181. Using the SMURF algorithm we identified 16 putative PKSs and 5 putative PKS-NRPS genes. Recent progress in the evolutionary dynamics of PKS clusters will be presented.

450 CELL SURFACE DISPLAY OF CORIOLOPSIS RIGIDA LACCASE IN PICHIA PASTORIS

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Lignocellulosic materials are considered as a sustainable environmental alternative to produce bioethanol. Lignin is a recalcitrant polymer that acts as cementing agent between cellulose fibers, but removal of this material is necessary to improve the rate of enzymatic hydrolysis of cellulose or hemicelluloses to fermentable sugars. Steam-explosion is one of the most commonly used methods for destructuration of plant cell wall but this process generates inhibitors, including furfural, organic acids and phenolic compounds, which affect the yield of the process. Laccase is a copper-containing blue oxidase that catalyzes the oxidation of phenolic lignin units and a wide number of phenolic compounds and aromatic amines, with molecular oxygen as the electron acceptor, which is reduced to water1. These enzymes have been used to reduce the toxicity of industrial mill effluents with high content of free phenolic compounds2. It has been reported that laccase participates also in detoxification of steam-exploded wheat straw, increasing 2G ethanol production3. However, although these results are promising, the addition of a new enzyme in the process seems not to be economically feasible. As a preliminary study we have achieved the heterologous production of Lcc1 C. rigida laccase in the cell wall of P. pastoris, as a model yeast expression system. This gene was fused to alpha secretion factor and Cwp2 cell wall binding domain from S. cerevisiae. Laccase activity was detected in yeast cells using ABTS as enzyme substrate and the presence of the protein in the cell surface was confirmed using anti-laccase antibodies conjugated with fluorescently labeled antibodies. These results open the possibility to express successfully laccase in cell wall of commercial yeast used in 2G bioethanol for increasing the process yield without additional production cost.

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451 CHARACTERIZATION OF A MYCOVIRUS LEV-FMRI0339 IN THE CULTIVATED MUSHROOM, LENTINULA EDODES FMRI0339 STRAIN

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The dsRNA was first found in the malformed cultures of Lentinula edodes strain FMRI0339, one of three most popular sawdust cultivating commercial strains of shiitake and was also found in the healthy-looking fruiting bodies and actively growing mycelia. Curing L. edodes FMRI0339 of the mycovirus infection was carried out by plating small hyphal fragments from an aged mycelia and protoplasting followed by regeneration to study the viral effect of LeV-FMRI0339 on mushroom biology and develop virus-free commercial lines. More than 20% of colonies were free of LeV-FMRI0339 dsRNA element. The mycelial growth and density of the virus-cured and -infected isogenic strains were measured in eleven kinds of fungal culture media, including a sawdust medium. Eliminated LeV-FMRI0339 resulted in increased mycelial growth and mass. Growth of virus-cured strains was especially enhanced more than 30% on potato dextrose agar, V8, and czapex dox media. Interestingly, LeV-FMRI0339 virus was constantly detected in mycelia of L. edodes FMRI0339 from the solid-medium culture condition, however, no viral productivity appeared culturing mycelia in the submerged culture. Viral titer of LeV-FMRI0339 changed over culture condition. Output of fruiting bodies in the virus-cured and -infected isogenic strains was investigated in the malformed cultures to study the efficacy of virus curing and biological funtion of mycovirus LeV-FMRI0339.

452 CHARACTERIZATION OF A PUTATIVE ANTIFUNGAL PROTEIN IN THE CITRUS POSTHARVEST PATHOGEN PENICILLIUM DIGITATUM

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Antimicrobial peptides (AMPs) are widely found in nature and are considered as novel antifungal alternatives in medicine, agriculture or food industry. Our group has worked in the identification and characterization of small synthetic AMPs to be used in crop protection, including peptides with inhibitory activity against the citrus postharvest pathogen Penicillium digitatum. It has been described the production and secretion of small antifungal proteins (AFPs) by certain filamentous fungal species, generally from the genera Aspergillus and Penicillium. These AFPs are small, cationic, rich in cysteine, contain disulfide bridges, fold into stable structures, and are secreted into the culture medium by the producer fungus. AFPs inhibit the growth of specific fungi and are thus considered a specific class of AMP with biotechnological potential. The role of AFPs in the biology of the producer fungus is unclear. The sequencing of the P. digitatum genome has allowed the identification of a number of putative small cysteine-rich open reading frames (ORFs) that are AFP candidates. Phylogenetic analyses, sequence homology, signal peptide identification, and molecular modeling of one of these ORFs, PDIG_68840, confirmed its identity with the previously described AFPs from P. chrysogenum Pc12g08290 and Pc24g00380. The expression of the gene coding for PDIG_68840 was determined and results showed that it was highly induced during growth in rich liquid medium and during fruit infection. In addition, it was demonstrated the production of a small thermostable protein in the supernatant of liquid cultures of P. digitatum. Synthetic peptides derived from the PDIG_68840 amino acid sequence were synthesized, and some of them shown to be AMPs active against different fungi including P. digitatum. The disruption of the gene coding for PDIG_68840 was accomplished in order to determine the functional role of this protein.

453 CHEMICAL GENOMICS SCREENS TO IDENTIFY DRUG MECHANISM OF ACTION IN ASPERGILLUS FUMIGATUS

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Current options for the treatment of Aspergillus infections are limited and suffer from a variety of shortcomings. Chemical genomics supports the identification of drug mechanism of action as a diploid strain deficient in a single copy of a drug's target is hypersensitive to that drug. A high-throughput targeted gene KO method for A. fumigatus has been established by employing fusion-PCR to generate targeted gene disruption cassettes, optimizing the common transformation protocol for A. fumigatus high-throughput gene disruption, and utilising a diploid Ku80-/Ku80- mutant to facilitate more reliable homologous recombination. Preliminary efforts have produced 46 heterozygous KO strains and subsequently, the feasibility of chemical genomic studies in filamentous fungi has been demonstrated with several compounds.

To facilitate high-throughput chemical genomics, the heterozygote collection has been pooled into a single culture and grown competitively in the presence of sub-MIC levels of drug. Individual strain fitness has been determined by next generation, illumina sequencing which facilitates multiplexing of samples and enables vast scalability. Itraconazole and brefeldin A have been used to validate this method and results demonstrate a decreased abundance and relative fitness of the erg11a and arf2 heterozygote mutants, the known targets of these drugs. This process with enable high-throughput methods for surveying the genome of A. fumigatus for new drug targets and supports unveiling the mechanisms of action of antifungal drugs.

454 CLASSICAL STRAIN DEVELOPMENT USING THE COLONY PICKER QPIX2

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The aim of the project is the stepwise increase of the α-amylase activity of the filamentous fungus Aspergillus oryzae by classical mutagenesis of the production strain. To find a high performing mutant strain, a huge amount of mutants has to be picked and analyzed which is very time consuming if done manually. Furthermore the viscous growth of the strain leads to difficulties in cultivation and less reproducibility of amylase activity. On this account a technically feasible screening-process to increase the amylase activity had to be developed. The use of the colony picker QPix2 (Genetix) enabled us to pick the required number of mutant strains in a fast and efficient way and analyze the mutant strains in a 96 microtitre plate format. Based on the fact that the colony picker was originally designed for picking bacteria or yeast we had to overcome different obstacles regarding e.g. the design of the picking-pins, the density of plated spores, or the size of the colonies. Additionally, the redesign of the cultivation medium leading to less viscous growth of the strain was essential to increase the comparability of the results. Several experiments will be shown in which we try to define the optimal values for the picking parameters as well as the optimization of the cultivation medium.

455 CONTROL OF PENICILLIUM EXPANSUM PATHOGENESIS AND PATULIN PRODUCTION BY PEARS OF SARDINIAN GERMOPLASM

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The aim of this research was to correlate the natural resistance of different Sardinian pears accessions against P. expansum and its capability of patulin production. Experiments were carried out in vivo and in vitro with seven accessions of pears (Sarmentina, Vacchesa, De Puleu, De su Duca, Natalina, Oliena, Laconi5) belonging to the CNR-ISPA ex-situ collection and one national cultivar (Abate) used as control. P.expansum was isolated from blue mould-decayed Sardinian pear fruit and selected for its aggressiveness and patulin production. Mycelial growth of P.expansum were evaluated in vivo on wounded fruits and in vitro on a standard growth medium (Potato Dextrose Agar, PDA) and on Pear Puree Agar Medium (PAM), for 7 days at 23° C. P.expansum growth was evaluated by means of daily measurement of the colony diameter and by observations made through the scanning electron microscope (SEM). Patulin production was detected by high-pressure liquid chromatography-mass spectrometry (HPLC-MS) and the expression of the isoepoxydon dehydrogenase gene (idh), involved in patulin biosynthesis was monitored by real time PCR. Mycelial growth of P. expansum on Sardinian PAMs was inhibited in comparison to Abate PAM and PDA. In particular the accessions Sarmentina and Vacchesa showed the maximum inhibitory activity both in vitro and in vivo. Patulin concentration on PAMs from Sardinian accessions was lower than patulin detected in PDA showing that there is a positive correlation between fungal growth and patulin production. Otherwise the idh gene was downregulated in presence of PAMs medium, suggesting a post trascpitional regulation. Sardinian pear accession seem to have a high effect on the development of mold decay and inhibition of patulin production.

456 EFFICIENT GENERATION OF ASPERGILLUS NIGER KNOCK OUT STRAINS BY COMBINING NHEJ MUTANTS AND A SPLIT MARKER APPROACH

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To generate gene deletion mutants in Aspergillus niger, we combined the use of Non-Homologous End Joining (NHEJ) mutants (ku70 mutant) and the split marker approach. The combination of both tools resulted in efficient PCR amplification because of the reduced length of the PCR fragments and efficient homologous recombination frequencies. A set of five selection markers, two dominant selection markers (hph; hygromycin B resistance and BLE; phleomycin resistance) and three auxotrofic markers (pyrG, argB and nicB) were successfully used in a split marker approach to obtain amyR knock outs with high efficiency. AmyR encodes a transcription factor that is required for the expression of starch degrading enzymes and disruption of amyR results in the inability to grow on starch. The strategy to generate the gene deletion constructs is such that with one set of four gene specific primers, a gene deletion mutant can be generated with either one of the five selection markers. The strategy is based on fusion PCR and omits the necessity for cloning the disruption cassettes. This accelerates the process of generating gene deletion cassettes which can now be accomplished within eight hours. The split marker approach can also be used to make gene deletions in a wild-type background in stead of a Δku70 background. In this chapter, we present protocols and considerations that we used to generate gene knock out constructs by fusion PCR and to obtain and verify gene knock outs with any of the five marker genes using the split marker approach. The method is easily transferable to other filamentous fungi.

457 ENDOLYSOSOMAL MEMBRANE TRAFFICKING COMPLEXES DRIVE NUTRIENT-DEPENDENT TORC1 SIGNALING TO CONTROL CELL GROWTH IN SACCHAROMYCES CEREVISIAE

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The Target of rapamycin (TOR) kinases are conserved across eukaryotes and orchestrate myriad cellular processes to control growth in response to nutrients and environmental signals. The Tor kinases form two evolutionarily conserved multi-protein complexes known as TORC1 (Tor complex) and TORC2. TORC1 is sensitive to the immunosuppressive and antiproliferative drug rapamycin, and in Saccharomyces cerevisiae is populated by Tor1 (or to a lesser extent Tor2), Kog1, Lst8, and Tco89. TORC1 is physically associated with endomembranes and controls cell growth when nutrients such as amino acids are abundant and serves to maintain robust nutrient transport, ribosome biogenesis, and protein synthesis, and concomitantly inhibits autophagy. We have shown that mutations in Class C Vps (Vps-C) complexes are synthetically lethal with tor1 mutations and confer rapamycin hypersensitivity in Saccharomyces cerevisiae, suggesting a role for these complexes in TORC1 signaling. Vps-C complexes are required for vesicular trafficking and fusion, and comprise four distinct complexes; HOPS and CORVET and their minor intermediaries i-CORVET and i-HOPS. We show that at least one Vps-C complex is required to promote TORC1 activity, with the HOPS complex having the greatest input. The vps-c mutants fail to recover from rapamycin-induced growth arrest and show low levels of TORC1 activity. TORC1 promotes cell growth via Sch9, a p70S6 kinase ortholog. Constitutively active SCH9 or hyperactive TOR1 alleles restored rapamycin recovery and TORC1 activity of vps-c mutants, supporting a role for the Vps-C complexes upstream of TORC1. The EGO GTPase complex (EGOC) and its homologous Rag-GTPase complex convey amino acid signals to TORC1 in yeast and mammals, respectively. Expression of activated EGOC GTPase subunits, Gtr1-GTP and Gtr2-GDP, partially suppressed vps-c mutant rapamycin recovery defects, and this suppression was enhanced by increased amino acid concentrations. Moreover, vps-c mutations disrupted EGOC-TORC1 interactions. TORC1 defects were more severe for vps-c mutants than those observed in EGOC mutants. Taken together, our results support a model in which distinct endolysosomal trafficking Vps-C complexes promote rapamycin-sensitive TORC1 activity via multiple inputs, one of which involves maintenance of amino acid homeostasis that is sensed and transmitted to TORC1 via interactions with EGOC.

458 ENGINEERING OF NEUROSPORA CRASSA FOR THE PRODUCTION OF HETEROLOGOUS PROTEINS

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The Fraunhofer ITEM is developing alternative microbial expression systems in collaboration with the Technische Universität Braunschweig to meet an increasing demand for the production of heterologous proteins. Among different eukaryotic organisms, the filamentous fungus Neurospora crassa was chosen because it had already demonstrated its high potential in protein expression. The system is genetically well characterized and exhibits high protein secretion capacity. In order to obtain high-yield production strains, several engineering steps need to be accomplished. The first and most important step in developing this expression system is the elimination of protease activity in the culture supernatant leading to a degradation of future products. Various mutants from the N. crassa knock out collection carrying deletions of protease genes were tested for lacking protease activity in the culture supernatant. Additionally, a set of different temperature-sensitive morphological mutants was screened for increased secretion levels in comparison to the wild type. The focus was set on phenotypes showing hyperbranching or apolar growth because these phenotypes were reported to result in the secretion of higher protein amounts compared to the wild type. The future challenge is to further develop and engineer this system and the corresponding production processes towards GMP compliance.

459 ENHANCING OF THE SUGAR CANE BAGASSE HYDROLYSIS ADDING A COMMERCIAL CBHI IN A COCKTAIL OF FILAMENTOUS FUNGI HETEROLOGOUS HEMICELLULASES AND ESTERASES

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The plant cell wall polysaccharides represent a potential source of renewable energy to biofuels and chemical production. Its fractions can be divided in cellulose, hemicellulose, lignin and pectin. Due to this complexity a large set of enzymes are necessary to degrade the primary or secondary plant cell wall. Aiming to analyze the potential of enzymes directly involved in the sugar cane bagasse depolimerization, the purpose of this study was demonstrate a comparative investigation of a filamentous fungi heterologous expressed enzymes set (arabinases, xylanases, β-xylosidases and esterases), followed by addition of commercial cellobiohydrolase, acting in sugar cane bagasse hydrolysis. The genes encoding interesting proteins were cloned in the pEXPYR vector, which allowed the expression and secretion drived by a glucoamylase promoter and signal peptide (GAp). The analysis of reducing sugars and enzymatic synergism were performed by HPLC and by 3,5-dinitrosalicylic acid (DNS) assays for reducing sugars. Recombinant hemicellulases were used individually or in combination in sugarcane bagasse (SCB) and SCB submitted to steam pre-treatment to try to elucidate the mechanisms involved in the biomass hydrolysis. All results showed a strong correlation between the used enzyme and the biomass composition and its molecular structure. It was observed that the enzyme sequence applied in the enzymatic biomass pre-treatment is important in its degradation. It could be verified that the recombinant xylanases were the most important acting enzymes on the integral SCB. However, on the steamed SCB, individual hemicelulases/esterases and the most of combinations were positive in sugar release, indicating the mechanical pre-treatment exposed the sugar cane cell wall and enhanced the enzymatic attack.

460 EXPRESSION AND PURIFICATION OF HYDROPHOBIN FUSION PROTEINS TARGETED TO INTRACELLULAR PROTEIN BODIES IN TRICHODERMA REESEI

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Insufficient production levels and the lack of efficient purification methods are the two major bottlenecks hindering the recombinant protein production. The need for novel production platforms is growing together with the number of new applications for recombinant proteins. The filamentous fungus Trichoderma reesei is an excellent producer of hydrolytic enzymes that are exploited by the enzyme industry. However, heterologous protein production in T. reesei is often suffering from low product yields due to protease degradation and inefficiency in heterologous protein secretion. We have previously demonstrated a novel intracellular recombinant protein production system for T. reesei using hydrophobin, a small and amphipathic fungal protein, as a fusion tag for purification and ER retention signal for targeting the produced protein to protein bodies. Using this system, the model GFP-HFBI fusion protein is produced in high yield and can be extracted from total protein lysate by surfactant-based aqueous two-phase separation system (ATPS). We have now demonstrated the applicability of this production concept for additional proteins, e.g. glucose oxidase (GOX) and tissue plasminogen activator (tPA). Effect of C- and N-terminal hydrophobin fusion on productivity and extraction by ATPS will be discussed. The new production concept is aiming at widening the spectrum of recombinant proteins that can be produced efficiently in T. reesei.

461 GENETIC AND GENOMIC INSIGHTS INTO THE SEXUAL REPRODUCTIVE SYSTEMS IN RED YEASTS

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Sexual reproduction is ubiquitous and extant in all of the major groups of the eukaryotic tree of life and defines a key evolutionary innovation by which species transmit and admix genetic material. Although the core features of sexual reproduction seem to be conserved, namely ploidy changes and cell-cell recognition between compatible mating partners, the evolution of sex-determining systems in the fungal kingdom is highly dynamic. Within Fungi, basidiomycetes have developed a complex mating system involving two sets of genes: lipopetide pheromones and their cognate receptors (P/R) that coordinate cell-cell recognition leading to cell fusion, and compatible homeodomain transcription factors (HD) that regulate the progression through the sexual cycle. When both MAT genes are determining sexual identity, their genomic organization defines the mating system as bipolar (if P/R and HD are located the same chromosome and genetically linked) or tetrapolar (if the genes are unlinked, e.g. located in different chromosomes). Transitions between the two systems have been observed in at least two of the major lineages of basidiomycetes and are assumed to be influenced by the relative importance of inbreeding and outbreeding in the lifestyle. In addition to these two mating systems, we proposed a third mating configuration referred to as pseudo-bipolar in the saprobic red-pigmented yeasts Sporidiobolus salmonicolor, which belongs to the earliest derived lineage within the basidiomycetes (Pucciniomycotina). This system was suggested based on two main observations: firstly, we found multiple HD alleles in natural isolates of these species, but each allele was always associated with only one of the two P/R alleles; and secondly, we found that recombination may occur (albeit rarely) between P/R and HD genes as assessed in the progeny of a laboratory cross between S. salmonicolor compatible strains. Using genetic and genomic approaches, we are currently expanding the characterization of the pseudo-bipolar system in S. salmonicolor by investigating the genetic structure and chromosomal localization of the MAT locus. Finally, to address if this deviation from the bipolar-tetrapolar mating paradigm represents a represents a stable or an otherwise short-lived transitional system, the mating system of two close related red yeast species (Rhodosporidium babjevae and Rhodosporidium toruloides) and a more distantly related species (Leucosporidium scottii) are also under study.

462 GENETIC BASIS OF FUNGICIDE RESISTANCE IN PHYTOPATHOGENIC FUNGI

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Fungicides are a key component in the integrated management of plant diseases and the increasing resistance of key fungal pathogens has become an important factor limiting their effectiveness and usefulness. The selection pressure resulting from regular, long-term fungicide application, leads to the emergence and spread of new strains with increased resistance to whole groups of compounds. There are several resistance mechanisms: alteration of target site towards reduced affinity to fungicide molecules, complementation by an alternative enzyme taking over target function, overexpression of the fungicide target, active efflux or reduced uptake of the fungicide. Available information on the molecular basis of fungal resistance, allow the design and deployment of molecular diagnostics procedures to analyse environmental samples. As part of the research we conducted bioassays, PCR and gene expression experiments on fungicide sensitive (e.g. Fusarium graminearum, F. culmorum) and resistant (e.g. Alternaria alternata, Trichoderma atroviride) fungal strains. Our experiments aim to correlate morphological changes in mycelia with polymorphism of candidate resistance genes and their expression in stress conditions (fungicide treatment). To confirm the participation of selected genes in the development fungicide resistance - susceptible strains were subjected to selection pressure due to fungicide application and the resulting changes in expression of potential resistance genes were examined. We demonstrate polymorphisms and changes in expression in CYP51 sterol demethylase and in two ABC transporter genes from Fusarium graminearum (FGSG_02865 and FGSG_05318) which appear to provide one of the best candidate genes for wide specificity MDR pumps capable of exporting fungicides.

463 GFP RECOMBINATION REPORTER ANALYSIS OF NEUROSPORA ΔMSH-2 OCTADS REVEALS HIGH FREQUENCY SYMMETRIC HETERODUPLEX.

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Recombination generates heteroduplex (hDNA), mismatched parental DNA strands that can be used to infer the mechanisms involved. In an eight-spored fungus such as Neurospora, hDNA can be detected by marker segregation in octads. For example, aberrant 4+:4M segregation (such as ++++M+MMM) indicates symmetric hDNA, arising from Holliday junction migration, while 5+:3M is an indicator of asymmetric hDNA, a result of DNA synthesis initiated at a recombination hotspot. Since mismatch repair (MMR) often removes such evidence before sporogenesis, we have deleted msh-2 in a cross in which GFP is inserted close to the recombination hotspot cog. Scanning the MMR-deficient octads for recombination events under a fluorescent microscope revealed the wide range of recombination outcomes at his3 normally hidden by the activity of msh-2. In contrast to recombination events in yeast, symmetric hDNA is common at his-3 in the absence of MMR and occurs with equal frequency to asymmetric hDNA. Although much less frequent than either 5+:3M or aberrant 4+:4M, the frequency of 6+:2M octads is little changed by deletion of msh-2, suggesting an additional MMR mechanism that makes a substantial contribution when the msh-2 pathway is inactivated. msh-2 deletion increases his-3 allelic recombination by a factor of 1.5-1.7 when the recombination initiator cog+ is in cis to the closer his-3 allele, but has no effect if the only copy of cog+ is in cis to the more distant his-3 allele. Since His+ spores are more likely to result from asymmetric hDNA initiated at cog in the former and symmetric hDNA in the latter arrangement, we conclude that repair of asymmetric hDNA exhibits a strong bias in the direction of restoration rather than conversion, while symmetric hDNA may be repaired without bias.

464 HETEROLOGOUS EXPRESSION OF NOVEL ACCESSORY ENZYMES IN TRICHODERMA REESEI

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Most of the carbohydrates in plants are in the form of lignocellulose, which essentially consists of cellulose, hemicellulose, pectin and lignin. Lignocellulose can be converted into fermentable sugars via chemical and/or physical pretreatment followed by enzymatic hydrolysis usually performed at moderate to high temperatures. After saccharification sugars can be fermented into bioethanol and other biochemicals. Lignocellulose degradation usually requires a broad range of various enzymes acting sequentially or simultaneously. The cost of these enzymes is the major factor restricting the extensive use of biological hydrolysis processes for biomass conversion. Optimization of the components in enzyme complexes, e.g. by supplementing with synergistically acting enzymes, is needed to improve the overall hydrolytic performance and reduce the total enzyme dosage required. Search for auxiliary enzymes with unique characteristics is an attractive approach due to their potential also in other applications. In this study, two lytic polysaccharide monooxygenase (LPMO) and two ferulic acid esterase (FAE) genes isolated from thermophilic fungi were expressed in a proprietary Trichoderma reesei strain with a low-cellulase and -protease background. Efficacy of these accessory enzymes in hardwood and bagasse liquefaction and other high-temperature applications will be discussed.

465 HETEROLOGOUS PRODUCTION OF LIPOXYGENASES DERIVED FROM DIFFERENT SOURCES IN TRICHODERMA REESEI AND THEIR APPLICATION

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Fatty acids and their derivatives are very important raw materials in the chemical industry. Typical applications are paints, coatings, adhesives, lubricants, detergents and plasticizers. Lipids are also used as raw materials for polymer synthesis, e.g. for manufacture of polyurethanes and specialty nylons. The most valuable plant oil for the oleochemical industry is castor oil, which contains an untypical hydroxyl-allylic group in its structure enabling easy functionalization. Low production levels and relatively high cost of castor oil are however limiting its wider use in oleochemistry. Cheap biomass derived unsaturated fatty acids could be transformed to products resembling structural features of castor oil by oxidizing the unsaturated structure to reactive peroxides. Lipoxygenase (LOX) is an enzyme able to carry out this reaction on different types of unsaturated fatty acids. The resulting reactive hydroperoxides can be further chemically converted to different valuable lipid-derived functional chemicals to be used as monomers, additives and building blocks in various large-volume oleochemical products. Chemical technologies to produce these types of intermediates from conventional lipid raw materials are not well developed and contain hazardous steps such as ozone oxidation or environmentally harmful intermediates e.g. bromoacids. Therefore, LOX catalyzed functionalization of unsaturated cheap fatty acids has huge potential for oleochemistry provided that suitable LOXs can be efficiently produced and the reactions controlled to the desired intermediates. LOXs have also the potential to bleach baking products and/or cotton which could render them useful for purposes in bread making and textiles. Today, the availability of LOXs is rather limited and only enriched soy flour LOX is commercially available. This product is a very crude soy preparation with relatively low activity and thus not suitable for the oleochemical applications but it is used for baking applications. Fungal and bacterial LOXs have been reported, but so far these enzymes have not been produced in a larger scale. Plant LOXs could also be used as an industrial bulk enzyme provided that the enzymes can be efficiently expressed and produced in microbial hosts. Successful expressions of LOXs derived from a diverse range of organisms together with some application tests will be shown.

466 HIGH-LEVEL PRODUCTION OF MONO-COMPONENT AND ENZYME MIXTURES IN MYCELIOPHTHORA THERMOPHILA

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The thermophilic ascomycetous fungus Myceliophthora thermophila C1 was developed into an efficient and versatile platform for high-level production of industrially relevant enzymes. By means of strain development strategies, such as random mutagenesis and targeted disruptions, we obtained two strain lineages that are currently being developed and exploited as enzyme production hosts. One strain lineage (HC-strains) is able to produce and secrete high amounts of enzyme mixtures that contain large amounts of (hemi-) cellulases. The other strain lineage (LC-strains) is impaired in its cellulase producing capability, resulting in low background-protein production. For that reason the LC-strains could be very suitable for the production of individual enzymes. The LC strain has been further developed for high-level production of homologous enzymes. An open reading frame encoding an endoglucanase was introduced at multiple copies by multiple rounds of transformation. After fermentation optimization, protein levels of up to 45 g/L were reached of which ~ 80% was the overexpressed endoglucanase. These results indicate that the LC strain is capable of producing mono-component enzymes at high levels in a relatively pure form. By transforming the LC strain with selected C1 genes, a wide collection of strains was obtained, each of which produced mainly one enzyme. This has ultimately led to an enzyme library of over 100 functional enzymes of which many have been purified and characterized in detail. In addition to producing relatively pure mono-component enzymes, the LC strain can also be used for the production of combinations of enzymes. To this end, the LC strain can be designed and constructed in such a way that it produces only those enzymes that are functional under application conditions. The resulting enzyme products are very efficacious and cost-efficient; in addition they don't show undesirable side effects. In conclusion, M. thermophila C1 was developed into a high-level protein-production platform. The HC strain is successfully applied to produce enzymes for the production of biofuels and biobased-chemicals. The LC strain is being used to produce single enzymes and defined combinations of enzymes. The obtained C1-enzyme library is a rich source for academic and industrial research. The properties of M. thermophila C1 make this fungus a highly suitable alternative for traditional fungal protein production hosts.

467 IDENTIFICATION AND CHARACTERIZATION OF PLANT CELL WALL DEGRADING ENZYMES FROM ANALYSES OF GENOMIC LIBRARIES OF BJERKADERA ADUSTA

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The use of lignocellulosic biomass as raw material in various industrial processes, has received much interest. Among the enzymes that degrade biomass, which are produced by different organisms, carbohydrate esterases (CE) and xilanases (Xil) are required for complete degradation of plant material. Hemicellulose shows modifications (presence of acetyl groups) that may be eliminated by the CE. In addition, Xil catalyse the depolymerisation of hemicellulose, being this an essential step for the conversion of lignocellulosic biomass. Bjerkandera is a genus that groups several Bacidiomycetes causing white rot of wood, and their enzymes have great potential for biotechnology applications. Performing an analysis of sequences from a cDNA library of Bjerkandera grown in the presence of crude oil, sequences with similarity to CE and Xil were found. Knowing the roles of these proteins, it will be interesting to characterize and express these genes in a heterologous system, allowing us to characterize biochemically. To predict the size of the transcript, Northern blot was performed, and full genomic and cDNA sequences were obtained using G.Walker and RLM-RACE kit, respectively. The cDNA was cloned into the pPICZ-αA vector, for expression in P. pastoris and the transformant strains was analysed. The complete gene for CE has an ORF of 470 aa and high identity with family 4 CE. The protein was partially characterized and shows a temperature and a pH optimum of 28°C and 6.0 respectively, and the substrate specificity was better for the 2-naphthyl acetate and 4-ethyl-p-Nitrophenol as compared to other substrates tested. Acetic acid release was measured from natural substrates, confirming the activity of deacetylase. These results correlate with the possible function of the protein predicted by in silico analysis. On the other hand, characterization of a Xyl gene demonstrated that this sequence was incomplete. Genome Walker and 5'RACE allowed progress in sequence from genomic DNA and cDNA. The sequence of the gene from genomic DNA is comprised of 1500 bp. Blastx with this sequence showed homology with fungal glycosyl hydrolases. Currently, we are working on the cloning and expression of this gene in P. pastoris, for the subsequent functional characterization of the enzyme. This study demonstrates that the application of genomic tools for characterization of new genes is a novel approach to study the physiology of poorly characterized organisms.

468 INFLUENCE OF HYPOXIA ON ANTIFUNGAL SUSCEPTIBILITY, STEROL PATTERN AND BIOMARKER RELEASE OF ASPERGILLUS SPP.

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Invasive aspergillosis (IA) is a major life-threatening disease in immunocompromised patients, with mortality rates from 40% up to 90% in high-risk populations. The most common species causing aspergillosis is Aspergillus (A.) fumigatus, accounting for approximately 90% of infections. Depending on regional distinctions, A. flavus and A. terreus are frequently reported. During infection, fungal pathogens must adapt to microenvironmental stresses, including hypoxia as well as high CO2 levels. Such oxystress conditions are usually not taken into account in current in vitro models of infection, the assessment of antifungal sensitivities or the release of biomarkers used for diagnosis. Therefore, we compared the in vitro activity of amphotericin B (amB), different azoles and echinocandins in hypoxic conditions (1% O2, 5% CO2) to their activity in normoxic conditions against isolates of A. fumigatus and A. terreus and other aspergilli. Using Etest strips, we found a reduction of the minimal inhibitory concentration (MIC) for amB for all aspergilli in hypoxic conditions. Similarly, a significant reduction in the MIC for all tested azoles was demonstrated for A. terreus isolates, while for A. fumigatus isolates differences were less pronounced. For echinocandins, little or no change in the MEC (minimal effective concentration) was detected between hypoxic and normoxic conditions for all aspergilli. Most interestingly, A. terreus strains, that are resistant to amB in normoxia, exhibited sensitivity to amB in hypoxic conditions, defining a breakpoint of > 2 µg/ml. Furthermore we investigated if changes in the sterol pattern or the amount of ergosterol contribute to these changes in antifungal susceptibility in hypoxia. The detection of circulating fungal antigens in serum for Aspergillus galactomannan or β-D-glucan has become an accepted diagnostic strategy. However, sensitivity and specificity vary extremely and the reasons are only partially clear. We demonstrated that the release of such biomarkers is elevated within the first 24 h of growth under hypoxic conditions.

469 INNOVATIVE STRATEGIES FOR DEVELOPING CELL WALL CANDIDA VACCINES

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The dramatic increase in Candida infections in recent years can be mainly attributed to increased aggressiveness of antibiotic therapy in healthcare settings. Developing Candida vaccines represents a new hope for combating invasive Candida infections. Conjugate Vaccine β-glucan & Laminarin provides excellent protection for both systemic and vaginal candidiasis. Passive vaccination with a β-glucan monoclonal antibody resulted in protection against candidiasis (Brito et al., 2011). β-Mannan and Peptide Conjugates vaccine induced strong to moderate antibody-mediated protection against murine disseminated and mucosal candidiasis (Xin et al., 2008). Monoclonal antibodies specific for peptide epitope was protective when used in passive transfer (Xin & Cutler, 2011). Recombinant Cell Wall Protein-Based Vaccine rAls3p-N vaccine protects mice from lethal disseminated candidiasis. It reduces fungal vaginitis model and a steroid-treated oropharyngeal candidiasis model through IFN-γ and IL-17A cytokine production (Hennessey et al., 2011).Phase I clinical trial in humans showed a robust production of IgG and IgA antibodies in the majority of the subjects and Increased production of IFN-γ and IL-17A (Hennessey et al., 2011). Hyr 1 Vaccine: a cell surface protein expressed on hyphae is a promising vaccine for disseminated and mucosal candidiasis (Luo et al., 2010). Candida Heat Shock Protein 90 Vaccine has powerful immunostimulatory effects on dendritic cells in a TLR2- and TLR4-dependent fashion and induces antibody and cellular immunity (Brown et al., 2010). Mycograb against HSP90 is a genetically recombinant human monoclonal antibody against the Candida heat shock protein 90. Mycograb conferred protection in cases of murine systemic candidiasis in mice (Brown et al., 2010). Killed Panfungal Vaccine: a heat-killed Saccharomyces cerevisiae which induces IFN-γ, IL-6 and IL-17A cytokine production (Capilla et al., 2009; Liu et al., 2011a, b; Stevens et al., 2011). Therefor, development of vaccines targeting human fungal pathogens is now realistic and achievable goal.

470 MASS PRODUCTION OF PHLEICHROME FROM THE PHYTOPATHOGENIC FUNGUS, CLADOSPORIUM PHLEI, USING SYNTHETIC INDUCERS AND PHOTODYNAMIC ROS PRODUCTION BY PHLEICHROME

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Two different diketopiperazines, cyclo-(L-Pro-L-Leu) and cyclo-(L-Pro-L-Phe), which were identified from the culture filtrate of Epichloe typhina, were chemically synthesized and evaluated for use in the mass production of phleichrome from wild-type and UV-mutagenized strains (M0035) of Cladosporium phlei. When supplemented with PDA and V8 juice agar media, both inducers showed significant increases in the production of phleichrome. Phleichrome production was increased in a dose-dependent manner up to concentration of 100 uM for both inducers. No further induction was observed at 150 uM and 200 uM. Among the two inducers, cyclo-(L-Pro-L-Phe) showed better inducing capability than cyclo-(L-Pro-L-Leu). The maximum yield was observed from the M0035 strain grown on V8 juice media supplemented with 150 uM cyclo-(L-Pro-L-Phe), which was estimated to be 232.6 mg of phleichrome per gram of mycelia and 10.2 mg of secreted phleichrome per 20 agar-plugs. Superoxide production by purified phleichrome was dramatically stimulated upon illumination, suggesting photodynamic action of phleichrome.

471 MGMFS1 IS THE MAJOR MEMBRANE TRANSPORTER INVOLVED IN MULTIPLE DRUG RESISTANCE IN MYCOSPHAERELLA GRAMINICOLA FIELD ISOLATES

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Multidrug resistance (MDR) is a common trait developed by many organisms to counteract chemicals and/or drugs used against them. The basic MDR mechanism is relying on an overexpressed efflux transport system that actively expulses the toxic agent outside the cell. In fungi, MDR (or PDR) has been extensively studied in Saccharomyces cerevisiae and Candida albicans, but also plant pathogenic fungi, e.g., Botrytis cinerea, Oculimacula yallundae and Mycosphaerella graminicola are concerned by this phenomenon. In agriculture, the occurrence of MDR strains may threaten the efficacy of current fungicide treatments MDR strains were detected in septoria leaf blotch (M. graminicola) field populations since 2008. These strains are cross-resistant to fungicides with different modes of action. The identification of the molecular mechanism explaining the MDR phenotype in two isolated strains (MDR6 and MDR7) was the main goal of this study. Measuring the intracellular accumulation of a radiolabeled fungicide demonstrated increased fungicide efflux in both MDR strains in comparison to sensitive strains. RNA-sequencing led to the identification of several overexpressed transporter genes, out of which MgMFS1 encoding an MFS (major facilitator family) transporter had particularly abundant mRNA in both MDR strains. Crosses between both MDR strains showed that mdr6 and mdr7 loci are closely linked. We applied bulk-progeny sequencing to progeny of the crosses MDR6 x sensitive and MDR7 x sensitive in order to map the genomic regions co-segregating with the MDR phenotypes. SNP frequency analysis in sensitive and resistant bulks showed a clear co-segregation between phenotypes and the left arm of chromosome 7. This region harbors several genes including the MgMFS1 gene mentioned above with a 514 bp promoter insertion in both MDR strains. Gene disruption of MgMFS1 in the MDR6 strain abolished resistance to several fungicides and reduced the resistance to others, demonstrating the importance of MgMFS1 in the MDR phenotype in M. graminicola. Studies are underway to demonstrate if the promoter insertion by its own is sufficient or if an additional mutation is necessary to drive MgMFS1 overexpression and hence MDR in M. graminicola. Financial support: Arvalis Institut du Vegetal, BASF Agro SAS, Bayer SAS, DuPont de Nemours SAS, Syngenta Crop Protection AG.

472 MOLECULAR DETERMINANTS OF THE HOMOTHALLIC SEXUAL BEHAVIOR OF THE BASIDIOMYCETE YEAST PHAFFIA RHODOZYMA

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Sexual behavior in Fungi can be self-sterile (heterothallic, requiring genetically distinct and compatible partners) or self-fertile (homothallic, requiring no partner). In most basidiomycetous yeasts the heterothallic sexual cycle is initiated by mating of two compatible strains of distinct mating types. This first step is regulated by pheromones and G protein-coupled pheromone receptor genes (P/R) that mediate cell-cell recognition leading to cell fusion. The later progression through the sexual cycle is then controlled by homeodomain (HD) transcription factors. In homothallic basidiomycetes the presence/absence and function of these genes has not hitherto been fully characterized at the molecular level. Using as a model the basidiomycete yeast Phaffia rhodozyma, this study aims to provide a better understanding of homothallic behavior in basidiomycetes. Using a draft genome sequence from P. rhodozyma strain CBS 7918, we identified putative mating type genes, representing both MAT loci. Two putative pheromone (MFA1 and MFA2) and pheromone receptor genes (STE3-1 and STE3-2) were identified, as well as a pair of divergently transcribed homeodomain genes (HD1 and HD2). Deletion mutants of HD and P/R genes where constructed and their ability to undergo sexual reproduction was evaluated. The first results indicate that the putative MAT genes identified play an active role in the homothallic life cycle of P. rhodozyma. The two pheromone receptor genes seem to be redundant but necessary for sporulation, while the homeodomain proteins encoded by the two HD genes appear to be able to form a functional Hd1-Hd2 complex. Interestingly the sole presence of the HD1 transcription factor may be sufficient to support completion of the sexual cycle albeit very inefficiently. The presence of only one pair of pheromone and receptor genes (STE3-1 and MFA1 or STE3-2 and MFA2) renders the mutant unable to sporulate. These results provide the first insight into the molecular determinants of the homothallic life cycle of P. rhodozyma.

473 MOLECULAR SYSTEMS DEVELOPMENT FOR STUDYING NATURAL PRODUCT BIOSYNTHESIS

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A recent advancement to the more traditional single gene heterologous expression is the use of multi-gene expression vectors to elucidate natural product pathways. The explosion of fungal genome sequencing has revealed many untapped gene clusters that are cryptic or orphan, as well as clusters responsible for known natural products. To elucidate the pathways behind these natural products, and importantly, to produce high titres, it can be useful to express the genes responsible in a heterologous host. The multi-gene expression system that we have developed can be widely applicable to a range of filamentous fungi, as it simply relies on PCR to clone the genes responsible, and uses strong promoters for expression in the heterologous host Aspergillus oryzae.

A. oryzae has been successfully used as a heterologous host to express single genes from natural product pathways. It has additionally been used to express more than one gene from a pathway via sequential or co-transformation of several individual plasmids. However this approach is limited by the number of dominant and auxotrophic selectable markers.

We have developed a multi-gene expression system that can be used to rapidly clone up to twelve genes on three separate plasmids, and includes a technology for reassembling megasynthase genes (which typically synthesise the natural product backbone), which can be too large to clone via a single PCR amplification. The multi-gene expression system and megasynthase reassembly technology rely on homologous recombination in the yeast Saccharomyces cerevisiae. Only 30 bp of homology is sufficient to bring about recombination, which makes this technology incredibly flexible and versatile as the homology can be designed into primers. Reassembly of a megasynthase, cloning of putative tailoring genes into the multi-gene expression vector, and finally transfer of the megasynthase into the vector by Gateway recombination can be done in under a week.

474 NEW FERULOYL ESTERASES AS BIOTECHNOLOGICAL TOOLS

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The feruloyl esterase enzyme (FAE) catalyses the release of phenolic compounds from plant cell walls, which, apart from ferulic acid, include others such as p-coumaric, caffeic and synaptic acids, since it hydrolyses the ester bond between phenolic and polysaccharide acids. FAEs have generated a great deal of interest in biotechnological processes with applications in the industry as well as in medicine. However, up to now on the market there are neither enough stocks of these enzymes nor totally pure FAEs enzymes. This enzymatic activity has been found not only in fungi but also in bacteria with different molecular weights, isoelectric point and optimal conditions for enzyme activity. This variation lets us conclude that FAEs can act on a wide range of plant substrates and that the phenolic acids releasing efficiency might vary depending on the substrate. Ferulic acid is a potent antioxidant able to neutralise free radicals with applications in food, cosmetic and pharmaceutical industry. Nowadays, fungi are the group more used for FAE production, as FAE activity has been found in different species of the genus Penicillium. Following this line, an analysis of FAE activity in selective solid medium was made in different Penicillium strains and, specifically, in the species Penicillium chrysogenum. The results show a higher activity in the strains NRRL-1951 and Wisconsin-54-1244. Both strains were fermented in liquid medium in presence of sugar beet pulp in order to quantify FAE activity through the measurement by HPLC of the ferulic acid released. Results evidence that P. chrysogenum Wisconsin 54-1244 is the strain that shows a higher FAE activity in submerged fermentations and in presence of sugar beet pulp. Besides, enzymatic extracts from this strain were collected in the highest peak of FAE activity determining, in vitro, the optimal reaction conditions for the efficient release of ferulic acid.

475 NOVEL ENDOGLUCANASES FOR TREATMENT OF CELLULOSIC MATERIAL

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Production of biofuels i.e. bioethanol from lignocellulosic material is a promising alternative technology for using biomass as a renewable and clean source of energy instead of consuming limited natural resources e.g. fossil fuels, and releasing increasing amounts of CO2. Enzymatic hydrolysis is considered to be the most promising technology for converting cellulosic biomass into fermentable sugars. Enzymatic total hydrolysis of (ligno)cellulosic substrates requires at least cellobiohydrolases, endoglucanases, beta-glucosidases and hemicellulases. We have cloned two novel glycoside hydrolase family 7 endoglucanases (EG_A and EG_B) from a thermophilic fungus Acremonium thermophilum. In order to study the effect of carbohydrate binding module (CBM) on the properties of the Cel7/EG enzymes, sequences encoding the CBMs from T. reesei cel7a/cbh1 and cel7b/egl1 genes were fused to the A. thermophilum endoglucanase genes. CBMs are non-catalytic domains of carbohydrate-acting enzymes, and are known to promote the association of enzyme with its insoluble substrates. The genes encoding the native A. thermophilum EGs and their fusion proteins were expressed in Trichoderma reesei and their physicochemical properties and hydrolysis performances were determined. The temperature and pH profiles of the native A. thermophilum EG_B and its fusion proteins were similar with each other and to those of native T. reesei EGI. Instead, the physicochemical properties of A. thermophilum EG_A and its fusion proteins were different from those of native T. reesei EGI and A. thermophilum EG_B. The hydrolysis studies showed that both of the A. thermophilum EGs and their fusion proteins, as part of the enzyme mixture, had enhancing effect on hydrolysis yield compared to the Trichoderma reesei enzymes.

476 PENICILLIUM CHRYSOGENUM ANTIFUNGAL PAF PROTEIN VARIANTS: A STEP TOWARDS UNDERSTANDING THE STRUCTURE-FUNCTION RELATION

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The small (55 amino acids) and cationic (pI 8.9) protein PAF from Penicillium chrysogenum shows antifungal activity against numerous filamentous fungi. It is harmless for mammalian cells [1,2] and promises great potential for medical and agricultural applications, e.g. prevention and treatment of fungal infection in humans, animals and plants. We have recently determined the solution structure of PAF by Nuclear Magnetic Resonance (NMR) analysis [3], which revealed a Greek-key secondary structure containing five antiparallel beta-strands. Three disulfide bonds stabilize the hydrophobic protein core. Protein motifs that might be structurally important and play a role in regulating antifungal toxicity were identified, for example a gamma-core motif (GXCX3-9C) [4] and cationic motifs on the protein surface. To investigate in detail the role of the postulated motifs for the structure and function of PAF we generated distinct protein variants by PCR-based site-directed mutagenesis and high-yield expression in a P. chrysogenum paf deletion strain that does not produce wild-type PAF [5]. The folding of the PAF variants was assessed by NMR analyses and the minimal inhibitory concentration was determined in growth inhibition assays to evaluate the antifungal activity. Single amino acid exchanges had dramatic impact on the antifungal activity, but only little or no influence on the solution structure of PAF. In vitro protein-lipid overlay experiments (PIP Strip assays) gave first insights into motifs involved in the interaction of PAF with membrane lipids and/or lipids relevant for intracellular signalling that might regulate the PAF-susceptibility of sensitive molds. Interestingly, the increase of the hydrophobicity and positive net charge of the gamma-core motif (PAF T8Y/S10K/E13I) enhances PAF binding to phosphatidylserine, whereas mutation of the hydrophobic core (PAF F31N, PAF F25A/I26N) weakens the interaction of PAF with phosphatidylinositol(3,4)-bisphosphate compared to the wild-type PAF, respectively. Data from functional and structural analyses will be presented.

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477 PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF TRICHOPHYTON RUBRUM STRAINS RESISTANT TO ANTIFUNGAL DRUGS FLUDIOXONIL AND AMBRUTICIN

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Dermatophytes are the most causative agents of cutaneous infections of skin and nails worldwide, causing severe lesions in immunossupressed patients and impairment of living standards of the infected individuals. Treatment of dermatophytoses includes the use of topic and systemic antifungal drugs that display several side effects in humans, which may lead to therapy interruptions, thus favoring the selection of resistant strains. Therefore, researchers are searching for new compounds with antifungal properties having little or no effects in humans. The antifungal compounds Fludioxonil and Ambruticin present a unique mode of action, interfering with the fungal osmotic signaling pathway. Here, we evaluated the effect of these drugs on Trichophyton rubrum, in which low doses inhibit growth of this dermatophyte, leading to hyphal-tip swelling, rupture of cell wall, and leakage of cell contents. We isolated ambruticin/ fludioxonil resistant mutants that presented different phenotypic changes related to conidiation, pigmentation, growth rates, and sensitivity to osmotic and cell wall stress suggesting that more than one gene may be involved in resistance to these drugs. Then, we searched for mutations in two mutant strains by whole genome sequencing using the Illumina MiSeq technology. Several types of mutations were found, including non-synonymous and synonymous single nucleotide variation, frame shift insertions and deletions, stop codon gain, in genes from different cellular pathways, including the histidine kinase, which is the most probable target of these drugs. These mutations might be responsible not only for the cross resistance to fludioxonil and ambruticin, but also for the phenotypic changes observed in these mutants. Although these drugs present a potential use as antifungal agents against dermatophytes, the relatively high percentage of resistant strains selected by exposure to high concentrations of these drugs and the high amount of mutations accumulated in the genome of resistant strains is a concerning point of these drugs, which may be further evaluated.
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478 PREDICTED SECRETOME ANALYSIS OF BASIDIOMYCETE PLEUROTUS OSTREATUS

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Most eukaryotic proteins are synthesized in the cytosol, and many need to be sorted to different subcellular locations. Extracellular proteins contain an N-terminal targeting sequence that is recognized by the secretory pathway, and these signal peptides (SPs) are responsible for targeting proteins to the endoplasmic reticulum for subsequent transport through the secretory pathway. In most cases, SPs are cleaved off by specific signal peptidases. Secreted or extracellular proteins are found in the in vitro growth medium, and to reach the outer surface of the organisms, these proteins have to travel though the cell wall. Fungi possess a huge capacity to secrete complex and highly dynamic mixture of proteins with a broad spectrum of activities that has been widely exploited by industry. Lignocellulose, the major reservoir of organic carbon on Earth, is recalcitrant to turnover and resistant to microbial and enzymatic attack because of the combination of the protective action of lignin and the crystalline structure of cellulose. Pleurotus ostreatus is a filamentous fungus of the phylum Basidiomycota, the only organisms known to degrade lignocellulose at a global scale. This degradation is carried out by a complex portfolio of extracellular enzymes whose expression and export are modulated in response to environmental and substrate variations sensed by these fungi. To perform the in-silico secretome study, genome sequence of two P. ostreatus haplotypes (PC15 and PC9) were subjected to analysis by several programs including SignalP, TargetP, PredGPI, TMHMM and WolfPsort using the web based pipeline called SECRETOOL (Cortázar et al., in press). Furthermore, a comparative analysis of P. ostreatus predicted secretome was carried out against phylogenetically related basidiomycetes in order to assign a putative function for each of the selected proteins, and to reveal the specificity of secreted proteins between species and lifestyles. Orthology relationships were found based on the reciprocal best hits of each proteome against the others, but several of these proteins did not show an annotated function reflecting limitations in the currently available genome annotations. Finally, transcriptomic studies of P. ostreatus were carried out in lignocellulose or glucose containing media to support the secreted protein predictions and adding valuable data to identify the function of the proteins determined by the previous methods.

479 RAPID EVALUATION OF CYCLOPIAZONIC ACID PRODUCTION IN ASPERGILLUS ORYZAE BY MULTIPLEX PCR AND HPLC

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Cyclopiazonic acid (CPA) is a toxic secondary metabolite produced by genus Aspergillus and Penicillium. Aspergillus oryzae are used widely as a koji starter to fermented soybean products in East, but their evaluation for CPA production is rare. A multiplex PCR method was developed for the rapid evaluation of the CPA production in A. oryzae used as koji starters or isolated from various fermented foods. The three sets of multiplex PCR primers were designed for the maoA, dmaT, pks/nrps genes in the CPA gene cluster. When multiplex PCR was applied to A. oryzae NBRC 4177 (CPA producer) and A. oryzae RIB 40 (CPA non producer), two strains were distinguished by different amplification patterns, validating the developed mulpiplex PCR as a tool for the evaluation of the CPA gene cluster. Among 23 A. oryzae, all three genes were amplified in 15 strains and the pks/nrps gene was not amplified in two strains as in A. oryzae RIB 40. Interestingly, no amplification was observed in six strains, which suggest that the most of CPA gene cluster had been deleted in those strains. CPA was detected as expected in eight strains which showed PCR amplification pattern of A. oryzae NBRC 4177 by HPLC. However, CPA was not detected in seven strains even with an amplification pattern of a CPA producer. These strains might produce CPA at undetectable level in our conditions or have some mutations in regulatory regions of the CPA gene cluster. The developed multiplex PCR and HPLC will be applied for the rapid screening of CPA non-producers from strains isolated from various fermentation products and a CPA non-producer will be developed to be a starter of industrial fermentations.

480 RECOMBINANT PRODUCTION OF CLASS II HYDROPHOBINS FROM TRICHODERMA VIRENS BY THREE MICROBIAL CELL FACTORIES

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Hydrophobins are amphiphilic proteins with high biocompatibility, which are apt to form strong and ordered assembly onto a broad range of materials. These intrinsic physical-chemical properties make hydrophobins suitable for surface modification and biomolecule immobilization purposes. Many of these applications, however, require a production quantity that exceeds the capacity of the native hosts. We have therefore initiated a comparison of recombinant overproduction of two class II hydrophobins from Trichoderma virens (HFB4 and HFB7; Espino-Rammer et al. 2013) in three popular cell factories: Escherichia coli BL21 DE3, Pichia pastoris CBS7435 and Trichoderma reesei QM9414. Inducible promoters (lacZ, AOX1) were used for the former two, whereas expression in T. reesei was performed under the constitutive promoter cdna1 enabling high expression (Uzbas et al. 2012). The expressed hydrophobins were compared with respect to yield, protein properties, final location (extracellular fluid, cell wall, inclusion bodies), and biological activity (i.e. ability to influence the hydrophobicity/hydrophily of surfaces). In case of Escherichia coli the proteins were localized in inclusion bodies, in contrast to that they were found in Trichoderma reesei bound to the cell wall and intracellular in Pichia pastoris.

481 ROLE OF AN ATP-BINDING CASSETTE PLEOTROPIC DRUG TRANSPORTER IN XENOBIOTIC TOLERANCE AND ANTAGONISM IN FUNGAL BIOCONTROL AGENT CLONOSTACHYS ROSEA

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ATP-binding cassette (ABC) transporters mediate active efflux of natural and synthetic toxicants, and are considered to be important for drug tolerance in microorganisms. In biological control agents (BCAs), ABC transporters can play important roles in antagonism by providing protection against toxins derived from the fungal prey, and by mediating the secretion of endogenous toxins. The fungus Clonostachys rosea is a ubiquitous soil borne ascomycete known for its antagonistic abilities against a wide range of plant pathogens, and also has entomopathogenic and nematophagous behaviour. In addition, C. rosea can tolerate diverse groups of fungicides when exposed to doses similar to those recommended for controlling plant pathogenic fungi. Recently, we identified a putative ABC transporter gene abcG5 in the fungal BCA C. rosea that was induced by the Fusarium spp. mycotoxin zearalenone (ZEA), through a suppression subtractive hybridization based transcriptome approach. In the present study, by generating abcG5 deletion and complementation strains, we characterize the function of abcG5 in C. rosea aiming to understand its role in xenobiotic tolerance and antagonism. The conserved domain analysis showed that the domain topology of the abcG5 protein is similar to the domain topology (NBD-TMS6)2 of typical pleiotropic drug resistance (PDR) ABC-G family transporter proteins. Gene expression analysis shows induced expression of abcG5 in presence of the Fusarium spp. mycotoxin ZEA, secreted metabolites of F. graminearum and different classes of fungicides. Phenotypic analysis of the abcG5 deletion and complementation strains showed that the deletion strains were more sensitive towards F. graminearum culture filtrates, ZEA and iprodione- and mefenoxam-based fungicides, thus suggesting the involvement of abcG5 in cell protection. The ΔabcG5 strains displayed reduced antagonism towards F. graminearum in a plate confrontation assay. Furthermore, the ΔabcG5 strains failed to protect barley seedlings from F. graminearium foot rot disease. These data shows that abcG5 is a PDR ABC-G transporter that is important for xenobiotic tolerance and biocontrol traits in C. rosea.

482 SPECIES IDENTIFICATION AND MOLECULAR BARCODES IN THE GENUS PLEUROTUS

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White-rot fungi of the genus Pleurotus include a number of economically important edible species such as P. ostreatus, P. pulmonarius, P. eryngii, P. djamor, P. citrinopileatus and others. Morphologically, mushrooms are very variable in shape which complicates species determination by fruiting body appearance. Likewise, vegetative mycelia of isolates of the same species can look very different from each other so that species identification is not reliably possible. To overcome the problems, molecular markers such as ITS sequences are used. ITS-based barcodes for species discrimination might be complete sequences or, technically more easily, deduced specific restriction enzyme digestion patterns. In not all cases, such barcodes distinguish different species. For example, Pleurotus ostreatus and Pleurotus sajor-caju have identical ITS sequences. However, mating tests performed with isolates from the Russian collection at Moscow State University clearly defines them as two different species (2012, Russ J Genet 48:1080-1088). In the literature, the position of P. sajor-caju is controversy and confusion with the species Lentinus sajor-caju exists. Grand, Hughes and Petersen (2011, Mycol Prog 10:399-413) defines ITS sequences for the distantly related L. sajor-caju. All strains in their study named P. sajor-caju turned out to be mating-compatible with P. ostreatus. In contrast, Li and Yao (2005, Mycotaxon 91:61-73) like us distinguish P. sajor-caju strains clearly as an own species different from P. ostreatus.
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483 STRATEGIC SCREENING OF FILAMENTOUS FUNGI FOR THEIR PRODUCTION OF LIGNOCELLULOSE DEGRADING ENZYMES

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Filamentous fungi possess a high unexplored potential for production of lignocellulose degrading enzymes. There are a few fungi, such as Trichoderma reesei and Aspergillus niger, which are widely used in industry. But there is a huge interest in discovery of novel and more efficient enzymes, and therefore other fungi should be evaluated. The goal of this study was to strategically screen selected filamentous fungi, which are potentially good producers of lignocellulose degrading enzymes, and to link their genomic information to the experimental data.
We picked 9 fungi for which genomic data is available and screened for their growth abilities on cellulose and lignocellulose (steam exploded spruce). The five best performing fungi were chosen for further cultivations for evaluation of their enzyme profiles and enzymatic hydrolysis efficiency. SDS-PAGE showed large differences between the secretomes of the different fungi. For enzyme screening different enzyme assays were designed based on the CAZymes in the genomes of these fungi and the characteristics of the growth substrates. Enzymes produced by the fungi were evaluated for their hydrolytic efficiency on the different substrates. All in all, our experimental approach provided insight into the mechanisms of enzymatic hydrolysis by different filamentous fungi.

484 SUSCEPTIBILITY OF TRICHOPHYTON RUBRUM CHALLENGED BY TERBINAFINE

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Dermatophytoses are superficial fungal infections caused by keratinolytic fungi from Microsporum, Trichophyton and Epidermophyton genera. These infections affect approximately 40% of the world population, representing 30% of the cutaneous fungal infections. Epidemiological records indicate that Trichophyton rubrum and Trichophyton mentagrophytes are the major causative agents of dermatophytoses in humans. T. rubrum genome is completely sequenced, allowing several studies on taxonomy, cellular and metabolic processes, providing better conditions and background for the search for more efficient therapies. Gene expression profile of pathogenic fungi in the presence of cytotoxic drugs, as well as resistant strains, has been conducted by several researchers to elucidate the molecular mechanisms involved in antifungal response and resistance. Previous studies from our research group revealed that salA gene, which encodes a salycilate 1-monooxygenase, a well-characterized naphthalene-degrading enzyme, is related to terbinafine resistance in Aspergillus nidulans. Since terbinafine is widely used in the treatment of dermatophytoses, the goal of this work was to evaluate whether salA plays a role in terbinafine resistance in T. rubrum. In silico searches identified the salA gene in the genome of T. rubrum. Transcriptional profile analyses showed upregulation of salA gene in response to terbinafine in T. rubrum, suggesting that this gene may play a role in resistance to this drug. The insertion of the salA heterologous gene (from A. nidulans) in T. rubrum genome conferred increased resistance to terbinafine. Our hypothesis is that the presence of multiple copies of the salA gene may be responsible for upregulation of this enzyme, which may act in terbinafine degradation. Our results suggest a role of the salA gene in T. rubrum susceptibility to terbinafine, a mechanism not yet described in dermatophytes, contributing for a better comprehension of the responses of this pathogen to a drug currently in use in the treatment of dermatophytoses. Financial support: FAPESP, CAPES, CNPq and FAEPA.

485 SUSTAINABLE CONTROL OF GRAPEVINE TRUNK DISEASES. COST ACTION FA1303

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Grapevine trunk diseases (GTDs) are among the most destructive diseases of vineyards worldwide. Fungicides with the potential to control GTDs have been banned and there are no highly effective treatments available. Developing sustainable alternatives to manage GTDs is therefore required. Currently, there is not a coordinated research approach in Europe, even though a strong demand for innovative disease management strategies is given. The goal of this COST Action FA1303 would be to develop a network of European expertise to improve understanding of GTDs by acquiring knowledge on occurrence of pathogens, vine-pathogen interaction, ecology of wood-inhabiting microorganisms, and to develop new management protocols and biocontrol approaches. This COST Action gathers leading multidisciplinary academic researchers and institutes within Europe to propose new recommendations for the management of GTDs and establish Europe as a world leader in GTD research to safeguard vineyards. This knowledge will be promoted in an effort to increase knowledge and awareness of the problem by disseminating information to end-users and authorities in the viticulture sector, and to the general public.

Presently, experts from nearly 50 institutions and companies in 20 European (AT, BG, CH, CZ, DE, GR, ES, FR, HR, HU, IL, IT, MT, NL, PL, PT, RO, SE, SI, UK) and near neighbour (Algeria) countries have already responded to join this Action that leads on the period 2013-2017.

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486 THE EFFECT OF HYDROPHOBINS ON WATER REPELLENCY OF SOIL AND ENZYME ACTIVITY

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Water repellency is an important characteristic of soils and a significant problem in the world. Several factors contributing to the water repellency of soils have been studied. However, the contribution of fungi to water repellency of soil is poorly understood. One way filamentous fungi might contribute to the water repellency of soil is by secreting hydrophobins. Hydrophobins are small secreted proteins, produced in large amounts by filamentous fungi. There are two classes of hydrophobins (class I and class II), which can self-assemble on hydrophilic-hydrophobic interphases and form an amphipathic membrane. Upon drying of soil, hydrophobins could coat hydrophilic soil particles, thereby making them hydrophobic. This could impair re-hydration of the soil and contribute to the water repellency of the soil. In addition to contributing to water repellency of soil, hydrophobins might have an effect on the immobilization of proteins to soil particles. For example, assembled hydrophobins could function as a scaffold for secreted enzymes like CAZymes. Growth of Schizophyllum commune in sorghum and compost is used as a model system to assess the effect of class I hydrophobins on water repellency and protein immobilization. Two highly produced class I hydrophobins, SC3 and SC4, have been inactivated in S. commune. Re-hydration capacity of sorghum colonized by the Δsc3Δsc4 strain of S. commune was 50% higher when compared to the wild-type. At the moment, we study the effect of hydrophobins on immobilization of secreted proteins to sorghum and compost particles.

487 THE ER SUBDOMAINS OF TRICHODERMA REESEI RESPOND TO PROTEIN OVEREXPRESSION

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In this work, we have focussed on ultrastructural characterisation of the ER architecture in a series of T. reesei strains that exhibit an increasing level of ER- related stress caused by protein secretion and overexpression. Ultrastructural and quantitative changes of the ER in T. reesei wild-type QM6a, a high protein-secreting mutant (Rut-C30) and a Rut-C30 transformant strain (BV47) overexpressing a BiP1-VenusYFP fusion protein were established. The strains displayed a progressively changing spatial organisation of the ER that was observed by transmission electron microscopy and quantified by stereological studies. The wild-type strain displayed a number of ER subdomains including parallel tubular/cisternal ER, ER whorls, ER-isolation membrane complexes with abundant autophagy vacuoles and dense bodies. The high protein-producing mutant T. reesei Rut-C30 and its transformant BV47 overexpressing the BiP1-VenusYFP fusion protein also contained parallel tub/cis ER, but no ER whorls, very few autophagy vacuoles and an increasing amount of punctate bodies, where particularly the recombinant BiP1-VenusYFP fusion protein was localised. These data indicate that the plasticity of the ER has a key role in accommodating protein overexpression in fungal hyphae. Ultrastructural analysis of the ER pleiomorphism and orchestration of autophagy under secretion stress have not been portrayed before for filamentous fungi.

488 THE FIRST SET OF EXPRESSED SEQUENCE TAGS (ESTS) IN THE MEDICINAL MUSHROOM AGARICUS SUBRUFESCENS PROVIDES OPPORTUNITIES TO DISCOVER PRODUCTION PATHWAYS OF BIOACTIVE COMPOUNDS AND DEVELOP MOLECULAR BREEDING

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Agaricus subrufescens Peck is one of the most important culinary-medicinal cultivable mushrooms with potentially high added-value products and extended agronomical valorization. It has been reported to produce various bioactive compounds that could have potential medicinal applications. However, no study dedicated to identify the underlying molecular mechanisms has been reported yet due, among others, to a lack of genomic resources. To fill this gap, we have developed the first set of expressed sequence tagged (EST) fragments available for A. subrufescens using 454 pyrosequencing technology. Using one quarter-plate pyrosequencing run for two tagged samples, we obtained about 30 Mb of raw data per sample. The assembly process resulted in two sets of 4989 and 5125 unigenes respectively. Using BLASTx program, most of the sequences (80%) found significant hit against non-redundant protein database and 87% showed similarity with A. bisporus predicted protein. The assignation of Gene Ontology (GO) terms was possible for 50 % of the sequences. We explored in silico the assembled sequences to identify putative genes potentially involved in production pathways of bioactive compounds. EST sequences were also used as a source of molecular markers and new microsatellite markers were developed. The new EST dataset provides solid genomic bases for decoding the metabolic engineering as well as efficient tools to foresee genetic studies and molecular breeding of A. subrufescens. It offers also opportunities to initiate comparative genomic studies in Agaricus species, and to a larger extent in basidiomycetes.

489 THE RELATION OF DICER-DEPENDENT RNA INTERFERENCE WITH INFECTION-RELATED DEVELOPMENT IN A PLANT PATHOGENIC FUNGUS COLLETOTRICHUM ORBICULARE

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RNA interference (RNAi) is a conserved eukaryotic gene silencing mechanism that uses small noncoding RNAs. Fungi generally possess RNAi components, although they are absent in some. Importantly, recent studies on RNAi of fungi are revealing that the fungal kingdom exhibits a remarkable diversity of RNAi pathways and functions. However, it is still largely unclear about RNAi in plant pathogenic fungi, especially its physiological roles for phytopathogenicity. In this study, we show the relation of RNAi with infection-related development of a phytopathogenic fungus, Colletotrichum orbiculare. Two dicer-like genes were identified in the genome sequence of C. orbiculare, and designated DCL1 and DCL2, respectively. We then generated the dcl1Δ and dcl2Δ mutants of C. orbiculare. Both dcl1Δ and dcl2Δ mutants grew normally on nutrient rich media as compared to wild type (WT), however, the dcl1Δ dcl2Δ double mutants exhibited a slightly reduced growth rate. The dcl1Δ mutants showed WT levels of pathogenicity on host cucumber plants. In contrast, the dcl2Δ mutants had severely reduced pathogenicity, suggesting the involvement of DCL2 in pathogenicity of C. orbiculare. Interestingly, conidia of the dcl2Δ mutants showed delayed germination, and also subsequent appressorium development. The DCL1 deletion in the dcl2Δ mutant enhanced the delay in germination. In addition, it was found that the dcl2Δ appressoria failed to develop invasive hyphae efficiently. To assess the roles of DCL1 and DCL2 for RNAi in C. orbiculare, we generated a GFP-silenced strain of C. orbiculare and then deleted DCL1 or DCL2 in the silenced strain. As a result, the DCL2 deletion largely recovered GFP fluorescence in conidia, appressoria and mycelia of the strain whereas the DCL1 deletion slightly recovered it, suggesting that DCL2 has a major role for RNAi in C. orbiculare. Together with the delayed germination in dcl2Δ, the results also suggest that the DCL2-dependent RNAi in conidia is probably important for efficient germination. To further elucidate the molecular link between DCL2-dependent RNAi and the infection-related development of conidia, we performed deep sequencing of small RNAs isolated from conidia of WT and dcl2Δ strains, and obtained approximately 13 million sequences and 19 million sequences, respectively, which perfectly matched the genome sequence of C. orbiculare. The comparative analysis of small RNAs in the WT conidia with those in the dcl2Δ conidia will be presented.

490 THE ROLE OF ERAD AND AUTOPHAGY IN THE DEGRADATION OF MISFOLDED PROTEINS IN ASPERGILLUS NIGER

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Folding of secretory and transmembrane proteins is tightly controlled by a stringent ER quality control system which only allows correctly folded proteins to leave the ER for subsequent delivery to their site of action. Misfolded proteins are sent back into the folding cycle or targeted for destruction via the ER-associated degradation (ERAD) pathway. It has been demonstrated that deletion of the ERAD gene derA in Aspergillus niger only slightly reduces growth and conidiation, even when the mutant strain contains multiple copies of a Glucoamylase-Glucuronidase (mcGlaGus) gene fusion (Carvalho et al., 2011). This present study investigates whether autophagy might serve as an alternative process to remove misfolded proteins from the ER. Autophagy is an intracellular degradation process functioning in the targeting of cytoplasmic content and organelles to vacuoles. We generated A. niger double knock-out mutants in which genes were deleted that are essential for ERAD (derA) or autophagy (atg1 and atg8) and assessed their growth both under normal and under ER stress conditions. It was observed that deletion of genes required for autophagy in combination with deleting the derA gene had no effect on growth, not even in the mcGlaGus background. Furthermore, sensitivity to the ER stressor dithiothreitol was not increased in the double knock-outs in comparison to the single knock-out mutants. Functional redundancy between ERAD and autophagy could thus not be observed in A. niger; deleting both processes did not affect the growth phenotype or conidiation in this species.

491 TOWARDS USING ASPERGILLUS NIDULANS AS CELL FACTORY FOR PRODUCTION OF MYCOPHENOLIC ACID

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Filamentous fungi have evolved a sophisticated and tightly regulated chemical arsenal of compounds to increase odds for survival in nature. In particular, secondary metabolites show tremendous variety and biological activities, some of which can advantageously be exploited in treatment of human disease. One significant example is mycophenolic acid (MPA) that inhibits inosine-5'-monophosphate dehydrogenase (IMPDH) activity. This activity is critical for purine biosynthesis in T- and B-lymphocytes and MPA can therefore be used an immunosuppressant during organ transplants. The MPA gene cluster was recently identified in Penicillium brevicompactum, and we are in the process of reconstructing the biochemical pathway by expressing the relevant genes in Aspergillus nidulans. Using this approach, we have previously demonstrated that the first steps towards formation of MPA are 5-methyl orsellinic acid (5-MOA) and 5,7-dihydroxy-4-methylphthalide (DHMP). Here we present our latest efforts towards producing this medically exciting compound in A. nidulans.

492 USE OF ACOUSTICS TO ANALYZE THE INFECTION OF THE RED PALM WEEVIL BY THE ENTOMOPATHOGENIC FUNGUS, BEAUVERIA BASSIANA.

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Acoustic detection has been used to detect hidden insects such as larvae of red palm weevil (RPW) by analysis of sounds they produce during movement and feeding activities. In this study, spectral and temporal patterns were analyzed in the trains of sound impulses produced in small palm trees by pairs of control (untreated) RPW larvae and pairs of (RPW) larvae infected with the entomopathogenic fungus, Beauveria bassiana (Bb 203). Signal processing software, Raven and Davis, identified a subgroup of sound impulse trains, denoted as bursts, containing more than 6 impulses which occurred frequently when larvae were present but only rarely when larvae were absent. Four new profiles which comprised of low frequency, low medium frequency and high frequency sound impulses produced by larvae red palm weevil can be used to distinguish the acoustic signal of the RPW larvae from the noise signal. The use of bursts impulses in the analysis process significantly improved the capability to monitor the activity of the larvae inside the palms. We provide evidences that acoustics can monitor activity of entomopathogenic fungi on their insects target.

493 ADAPTIVE ZN TOLERANCE IN THE ECTOMYCORRHIZAL FUNGUS SUILLUS LUTEUS IS ASSOCIATED WITH A HIGH EXPRESSION AND EXTENSIVE MULTIPLICATION OF A CDF TRANSPORTER GENE

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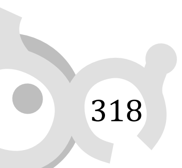
Zn is an essential micronutrient but can become toxic when present in excess. Elevated concentrations of Zn in the environment may cause a selective pressure on exposed biota and can lead to the evolution of tolerant ecotypes. Adaptive Zn tolerance has developed in populations of the ectomycorrhizal fungus Suillus luteus. A wide range of Zn detoxification mechanisms including exclusion, chelation, compartmentalisation, anti-oxidative response and repair is known and, may be involved. Gene expression and enzyme activities of key players in different detoxification pathways were measured and Zn content was determined in several Zn tolerant and sensitive isolates (i.e. genotypes) upon Zn exposure. Selection of a high transcript level of a CDF family transporter, ZnT2 occurred in the Zn tolerant group of isolates. The high transcript level is at least partially caused by gene expansion and potentially supported by differences in cis-regulation. Gene copy number varied within the tolerant group and different promoter genotypes are present. Anti-oxidative and repair mechanisms may be important in detoxification for individual Zn tolerant and sensitive isolates. Each isolate tends to have its own preferred approach to handle Zn related oxidative stress and damage. In general, a high intraspecific variation was detected and may contribute to the success of this species in adapting to disturbed sites.



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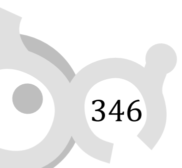

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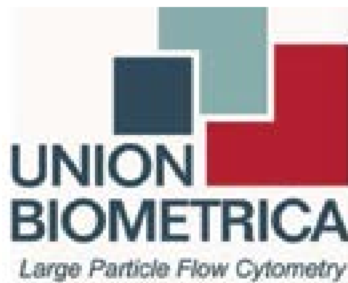

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- **Developing a novel method for the screening of fungal germinated spores using hydrogel microencapsulation and large particle flow cytometry:** Chavez, et al.; see QTN 015, www.unionbio.com

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