ABTRACT BOOK

WELCOME

The 13th European Conference on Fungal Genetics (ECFG13) is happy to welcome you in Paris.

The conference showcases all the recent advances in fungal genetics, molecular biology, cellular biology, evolutionary genomics, biotic interactions, systems and synthetic biology, eco-genomics and biotechnology. The conference includes three keynote lectures, six plenary sessions (with 18 lectures), nine concurrent sessions (with 72 lectures), and three poster sessions (with 550 posters). As you will experience, this conference will provide a “formidable” (in French in the text) forum for interdisciplinary exchanges among the 840 participants, and for discussing the latest trends in the field of fungal genetics and molecular biology. In addition to an *exciting scientific* program, the *conference* will provide ample networking opportunities.

The conference is taking place at the National Science Museum, La Cité des Sciences, in La Villette Park. We do hope that you will enjoy its surroundings and a lovely springtime in Paris. Please join us for the conference dinner at the Aquarium, a magical place.

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D. Wipf, Université Bourgogne, Agroécologie, Dijon

CO_ORGANIZING INSTITUTIONS

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SCIENTIFIC PROGRAM

SUNDAY 3rd APRIL 2016

16.00 - 22.00 **Registration at National Science and Industry Museum (La Cité des Sciences et de l'Industrie) in La Villette Park, 30 Avenue Corentin Cariou, 75019 Paris**

18.00 - 22.00 **Opening Mixer and cocktail (Wine and Cheese)**

MONDAY 4th APRIL 2016

Main amphi-theatre Gaston Berger

8.30 - 8.45 **Welcome & Opening Remarks** (**MH Lebrun, F. Martin and C. van Den Hondel**)

8.45 - 9.30 **Keynote Lecture** **R. Kahmann** (MPI and Marburg University, Germany)
How to colonize a plant: insights from secreted effectors

09.30 - 11.00 **Plenary Session 1: Developmental pathways**

Chairs**: P. Silar** (Paris-Diderot University, France) & **R. Fischer** (KIT, Germany)

09.30 - **A. Idnurm** (University of Melbourne, Australia). Reflections on fungal evolution based on the analysis of mirror mutants of *Sporobolomyces*
10.00 - **O. Etxebeste** (University of the Basque Country, Spain). Hyphal tips control conidiation in *Aspergillus nidulans*
10.30 - **J. Aguirre** (UNAM, Mexico). Fungal development and the mechanisms of sensing and responding to ROS

11.00 - 11.30 ***Coffee Break***

11.30 - 13.00 **Plenary Session 2: Fungal Biotic Interactions**

Chairs: **C. Veneault-Fourrey** (Lorraine University, France) & **M. Rep** (Amsterdam University, Netherlands)

11.30 - **C. d'Enfert** (Pasteur Institute, France). A functional genomic approach to understand *Candida albicans* colonization of biotic and abiotic surfaces
12.00 - **C. Roux** (Paul Sabatier University, France). Strategies developed by arbuscular mycorrhizal fungi (Glomeromycota) to manipulate host cells: outcomes, hypotheses and outstanding questions
12.30 - **P. Birch** (James Hutton Institute, UK). The host targets of *Phytophthora* effectors: pressure points to promote susceptibility

13.00 - 14.00 ***Lunch***

14.00 - 16.00 **Poster session CS1-2 and Coffee Break (15h30)**

16.00 - 18.40 **Concurrent Sessions**

CS1: **Cell biology and traffic** **Amphi-theatre Louis Armand Ouest**

Chairs: **D. Wipf** (Bourgogne University, France) & **G. Steinberg** (Exeter University, UK)

16.00 - **M. Penalva** (CSIC, Madrid, Spain). Golgi exit by maturation of TGN cisternae
16.20 - **N. Takeshita** (KIT, Germany). Super-resolution microscopy reveals a dynamic picture of cell polarity maintenance during hyphal growth
16.40 - **H. Wösten** (Utrecht University, Netherlands). Inter-compartmental streaming in *Aspergillus*
17.00 - **E. Perez de Nanclares** (University of Basque Country, Spain). Tip-to-nucleus migration of transcription factor FIbB controls conidiation in vegetative hyphae of *Aspergillus nidulans*

17.20 - **W. Tanner** (University of Regensburg, Germany). Plasma membrane micro-domains in *S. cerevisiae*: effect of de-energization on plasma membrane properties
17.40 - **S. Roberts** (Dartmouth College, USA). Clustered nuclei maintain autonomy and nucleo-cytoplasmic ratio control in a syncytium
18.00 - **M. Martin-Urdiroz** (University of Exeter, UK). Investigating the role of BAR domain proteins during plant infection by *Magnaporthe oryzae*
18.20 - **E. Oliveira Garcia** (Kansas State University, USA). Understanding plant cell effector uptake in rice-*Magnaporthe oryzae* interactions

SCIENTIFIC PROGRAM

MONDAY 4th APRIL 2016

CS2: **Pathogenesis and symbiosis** **Main amphi-theatre Gaston Berger**

Chairs: **J-P. Latge** (Pasteur Institute, France) & **A. Zuccaro** (MPI and University of Cologne, Germany)

16.00 - **A. Brown** (University of Aberdeen, UK). Environmental adaptation in *Candida* during colonisation and infection
16.20 - **M. Kretschmer (**University of Bristish Columbia, Canada). Modulating host carbohydrate metabolism: a silver bullet for impairing *Ustilago maydis* infection?
16.40 - **H. Jin** (UC Riverside, USA). Cross-Kingdom RNAi in plant-pathogen interactions
17.00 - **M. Nottensteiner** (TU Muenchen, Germany and University of Graz, Austria). ROPIP1 of the barley powdery mildew fungus is a retro-element-derived virulence effector targeting barley susceptibility factor RACB
17.20 - **C. Veneault-Fourrey** (Lorraine University, France). Mutualistic ectomycorrhizal fungi: a delicate line between saprotrophic and biotrophic plant-pathogenic fungi
17.40 - **S. Wawra** (University of Cologne, Germany). The novel fungal specific beta-glucan binding lectin FGB1 alters susceptibility to cell wall stress and prevents glucan-triggered immunity in plants
18.00 - **M. Paoletti** (IBGC, CNRS, France). NOD-like receptors (NLR) and non self-recognition in fungi: *Podospora* / *Serratia* interactions as a model for fungal innate immune response
18.20 - **S. Kind (**WWU Münster, Germany). Identification of fungal cytokinin biosynthetic *de novo* pathway and its relevance for virulence.

CS3: Environmental sensing, stress response **Amphi-theatre Louis Armand Est**

Chairs: **P. Simoneau** (Angers University, France) & A**. Fleissner** (Braunschweig University, Germany)

16.00 - I. Kronholm (Jyväskylä University, Finland). Contribution of epigenetic mechanisms to phenotypic plasticity in *Neurospora crassa*
16.20 - J. Schumacher (WWU Münster, Germany). Transcriptional responses to white light in *Botrytis cinerea* involve the GATA transcription factors BcWCL1 and BcLTF1 and the MAP kinase BcSAK1
16.40 - **A. Brand** (University of Aberdeen, UK). Protein complexes involved in hyphal steering in *Candida albicans*
17.00 - **S. Zeilinger** (University of Innsbruck, Austria). Host sensing and attack in the myco-parasite *Trichoderma atroviride*: role of the 7-transmembrane receptor Gpr1 and its putative interactor, a Sur7-family protein
17.20 - **A. Di Pietro** (University of Cordoba, Spain). A stress response pathway mediates host signal sensing in *Fusarium oxysporum*
17.40 - M. Schumann (TU Braunschweig, Germany). SIP-1 is essential for anastomosis formation in *Neurospora crassa* by promoting cell fusion competence
18.00 - M. Künzler (ETH Zürich, Switzerland). The defence response of *Coprinopsis cinerea* against fungivorous nematodes reveals differentiation and communication among vegetative hyphae
18.20 - **J. Bormann** (University of Hamburg, Germany). A novel virus response gene determines fitness in the cereal pathogen *Fusarium graminearum*

SCIENTIFIC PROGRAM

TUESDAY 5th APRIL 2016

Main amphi-theatre Gaston Berger

8.45 - 9.30 **Keynote Lecture.** **B. Dujon** (Pasteur Institute, France)
Inside the genomes of yeasts

09.30 - 11.00 **Plenary Session 3: Changing landscapes of fungal genetics and genomics**

Chairs: **C. D'enfert** (Pasteur Institute, France) & **N. Talbot** (Exeter University, UK)

09.30 - **J. Stajich** (UC Riverside, USA). Genome evolution in early diverging fungi from the 1000 fungal genomes perspective
10.00 - **G. Janbon** (Pasteur Institute, France). Introns in *Cryptococcus neoformans*
10.30 - **J. Taylor** (UC Berkeley, USA). Evolution and ecology meet development: Harnessing natural variation to inform studies of molecular function

11.00 - 11.30 ***Coffee Break***

11.30 - 13.00 **Plenary Session 4: Evolutionary genomics**

Chairs: **T. Giraud** (Paris-Saclay University, France) & **J. Spatafora** (Oregon State University, USA)

11.30 - **A. Branca** (Paris-Saclay University, France). The adaptive genomic toolbox of *Penicillium* molds: insights from between and within species comparisons
12.00 - **E. Stukenbrock** (MPI and Kiel University, Germany). Genome-wide recombination landscapes and adaptation in fungal plant pathogens
12.30 - **H. Johannesson** (Uppsala University, Sweden). Genomic conflict as a motor for evolutionary change: insights from the fungal spore killers

13.00 - 14.00 ***Lunch***
Workshop Fungal genomics and bioinformatics resources at JGI and FungiDB. **: Gaston Berger**

14.00 - 16.00 **Poster sessions CS3-5 and Coffee Break (15h30)**

16.00 - 18.40 **Concurrent Sessions**

CS4: Regulatory networks **Amphi-theatre Louis Armand Ouest**

Chairs: **S. Saupe** (IBGC, CNRS, France) & **L. Glass** (UC Berkeley, USA)

16.00 - **Y.-S. Bahn** (Yonsei University, Seoul, South Korea). Systematic functional profiling of patho-biological signalling networks in *Cryptococcus neoformans*
16.20 - **A. Sellam** (Université Laval, Québec, Canada). Systematic identification of biological circuits that couple cell growth and divisio in the opportunistic yeast *Candida albicans*
16.40 - **J.-R. Xu** (Purdue University, USA). Genome-wide A-to-I RNA editing occurs specifically during sexual reproduction independent of ADAR enzymes
17.00 - **M. Brunner** (Heidelberg University, Germany). Dynamics of light induced transcription in *Neurospora*
17.20 - **I. Fudal** (BIOGER, INRA, France). Transcriptional and chromatin-based control of effector gene expression in plant pathogenic fungus *Leptosphaeria maculans*
17.40 - **S. Wirth** (Friedrich Schiller University, Jena, Germany). Sexual development affects volatile production of *Schizophyllum commune*
18.00 - **Ph. Benz** (TUM, Friesing, Germany). Unravelling polysaccharide degradation signalling networks in *Neurospora crassa*
18.20 - **T. Pakula** (VTT, Espoo, Finland). Quantitative site-specific phosphor-proteomics of *Trichoderma reesei* signalling pathways upon induction of hydrolytic enzyme production

SCIENTIFIC PROGRAM

TUESDAY 5th APRIL 2016

CS5: **Applied genomics and biotechnology** **Main amphi theatre Gaston Berger**

Chairs: **M.-N. Rosso** (BBF, INRA, France) & **A. Martinez** (CSIC, Madrid, Spain)

16.00 - E. Master (University of Toronto, Canada). New approaches to uncovering function from fungal genomes
16.20 - R. Cox (Leibniz University, Germany). Investigating and exploiting fungal secondary metabolism
16.40 - B. Henrissat (CNRS, Marseille, France). Carbohydrate-active enzymes in fungal genomes
17.00 - M. Alfaro (Universidad Pública de Navarra, Spain). Comparative, transcriptional and proteomic analysis of secretome in the lignocellulose degrading basidiomycete *Pleurotus Ostreatus*
17.20 - I. Druzhinina (TU Wien, Austria). Horizontal gene transfers drove the myco-parasite *Trichoderma* to adapt to saprotrophy and cellulase production
17.40 - T. Vesth (Technical University of Denmark, Denmark). AspMine - Online comparative analysis of *Aspergillus* genera
18.00 - E. Shelest (HKI, Jena, Germany). CASSIS, a method for promoter-based prediction of secondary metabolite gene clusters in eukaryotic genomes
18.20 - N. Van Peij (DSM, Netherlands). Industrial strain construction: improving the toolbox for faster and more efficient strain engineering

CS6: **Ecological and population genomics** **Amphi-theatre Louis Armand Est**

Chairs: **R. Marmeisse** (EM, CNRS, France) & **E. Stuckenbrock** (MPI, University Kiel, Germany)

16.00 - P. Gladieux (BGPI, INRA, France). Population genomics of endemic and pandemic lineages of the rice blast pathogen
16.20 - N. Corradi (University of Ottawa, Canada). Genome and mating-type organization in the arbuscular mycorrhizal fungi
16.40 - T. Giraud (CNRS, Paris Sud University, France). Identifying candidate effectors using population genomics and by detecting selective sweeps in the plant pathogenic anther-smut fungi
17.00 - S. Branco (Paris Sud University, France). Continental-level population differentiation and environmental adaptation in *Suillus brevipes*
17.20 - C. Lemaire (Université d'Angers, France). Did domestication of apple tree promoted speciation of its fungal pathogen, *Venturia inaequalis*?
17.40 - C. Hann-Soden (UC Berkeley, USA). Sympatric speciation by an evolutionary ratchet
18.00 - T. Gabaldon (University of Barcelona, Spain). Impacts of genomic hybridization in fungi
18.20 - R. Vilgalys (Duke University, USA). Functional metagenomics of forest microbiomes

SCIENTIFIC PROGRAM

WEDNESDAY 6th APRIL

Main amphi-theatre Gaston Berger

8.45 - 9.30 **Keynote Lecture** **P. Bonfante** (New Phytologist Lecture) (Torino University, Italia)
Symbionts inside Symbionts: arbuscular mycorrhizal fungi and their intracellular microbiota

09.30 - 11.00 Plenary Session 5: Epigenetics

Chairs: **F. Malagnac** (Paris-Sud University, France) & **M. Freitag** (Oregon State University, USA)

09.30 - Y. Liu (UT Southwestern Medical Center, USA). Synonymous but not the same: new codes within genetic codons
10.00 - Z. Lewis (University of Georgia, USA). Heterochromatin is essential for normal chromatin architecture and genome function
10.30 - S. Saupe (IBGC, CNRS, France). Prion amyloid folds in NLR signalling in fungi

11.00 - 11.30 Coffee Break

11.30 - 13.00 Plenary Session 6: Systems and synthetic biology

Chairs: **A. Margeot** (IFPEN, France) & **M. Andersen** (Denmark Technical University, Denmark)

11.30 - V. Meyer (TU Berlin, Germany). *Aspergillus* in synthetic biology – where is it taking us?
12.00 - R. Koszul (Pasteur Institute, France). Redesigning chromosomes to optimize 3D conformation assays
12.30 - U. Mortensen (Technical University of Denmark, Denmark). CRISPR methods for product discovery and fungal cell factory construction

13.00 - 14.00 Lunch

14.00 - 16.00 Poster sessions CS 6-9 and Coffee Break (15h30)

16.00 - 18.40 Concurrent Sessions

CS7: **Metabolism and physiology** **Main amphi-theatre Gaston Berger**

Chairs: **M. Viaud** (BIOGER, INRA, France) & **H Arst** (Imperial College, UK)

16.00 - R. Wilson (University of Nebraska, USA). Nutrient sensing and metabolic responses governing rice cell invasion and proliferation by the blast fungus *Magnaporthe oryzae*
16.20 - O. Lastovetsky (Cornell University, USA). Central roles of lipid metabolism in an intimate fungal-bacterial interaction
16.40 - G. Diallinas (University of Athens, Greece). Genetics and structure of UapA transporter of *Aspergillus nidulans* reveal that gating elements and homo-dimerization are critical for function and specificity
17.00 - E. Fekete (University of Debrecen, Hungary). Spliceosomal twin introns: their evolution and possible relevance to post-transcriptional regulation
17.20 - A. Brakhage (HKI, Jena, Germany). Bacterial reprogramming of fungi by epigenetic manipulation leads to activation of silent gene clusters
17.40 - J. Strauss (BOKU University, Vienna, Austria). KdmB, an *Aspergillus* histone H3K4 demethylase, is a genome-wide chromatin regulator and activator of secondary metabolism
18.00 - S. Janevska (WWU Münster, Germany). Two separate key enzymes and two pathway-specific transcription factors are involved in fusaric acid biosynthesis in *Fusarium fujikuroi*
18.20 - A. Porquier (BIOGER, INRA, France). Specific regulation of botrydial and botcinic acid gene clusters in *Botrytis cinerea*

SCIENTIFIC PROGRAM

WEDNESDAY 6th APRIL

CS8: **Adaptation to xenobiotics** — **Amphi-theatre Louis Armand Ouest**

Chairs: **S. Perotto** (Torino University, Italia) & **G. Scalliet** (Syngenta Crop Protection, Switzerland)

16.00 - J. Ruytinx (Hasselt University, Belgium). Mechanisms of metal homeostasis and their adaptation in the ectomycorrhizal fungus *Suillus luteus*
16.20 - S. Daghino (University of Torino, Italy). Phenotypic and genomic adaptation of the ericoid fungus *Oidiodendron maius* to heavy metals
16.40 - M. Morel-Rouhier (Lorraine University, France). Evolution of detoxification systems in ligninolytic fungi
17.00 - N. Hawkins (RRES, UK). Mapping the adaptive landscape of azole resistance in *Zymoseptoria tritici*
17.20 - S. Fillinger (BIOGER, INRA, France). Several mutations of *Zymoseptoria tritici* field strains lead to MFS1 overexpression and multi-drug-resistance (MDR)
17.40 - M. Dubey (Swedish University of Agricultural Sciences, Sweden). ABC transporters are important for xenobiotic tolerance and biocontrol traits in the fungus *Clonostachys rosea*
18.00 - K. Pianalto (Duke University, USA). Cell-wall-mediated mechanisms of echinocandin resistance in *Cryptococcus neoformans*
18.20 - G. Scalliet (Syngenta Crop Protection, Switzerland). Resistance mechanisms to aniline-pyrimidines fungicides in *Botrytis cinerea*

CS9: **New tools for fungal biology** — **Amphi-theatre Louis Armand Est**

Chairs: **S. Baker** (PNNL, USA) & **V. Vleeshouwers** (Wageningen University, Netherlands)

16.00 - M. Seidl (Wageningen University, Netherlands). Mind the gap: Lessons from complete fungal genomes
16.20 - C. Voigt (University of Hamburg, Germany). 3D-Visualization of the fungal cell wall through super-resolution microscopy
16.40 - M. O'Malley (UC Santa Barbara). Deciphering the biomass-degrading behaviour of the anaerobic gut fungi (Neocallimastigales)
17.00 - J. Schiling (Minnesota University, USA). Space-for-time designs to resolve gene regulation and feedback as fungi degrade wood
17.20 - T. Schuetze (TU Berlin, Germany). The use of poly-cistronic gene expression to produce secondary metabolites in *Aspergillus niger*
17.40 - J. Carere (CSIRO, Brisbane, Australia). Discovery of *in planta* targets of pathogen enzymes
18.00 - L. Larrondo (Pontifical Universidad Catolica de Chile, Santiago, Chile). Synthetic biology of fungal systems: opto-genetic tools to manipulate gene expression for scientific and artistic purposes
18.20 - M. Legrand (Pasteur Institute, INRA, France). *Candida albicans*: a model organism for studying Loss-Of-Heterozygosity events in eukaryotes

Main amphi-theatre Gaston Berger

18.40 – 19.00	**Poster Prizes** **Chairs: S. Kamoun** (TSL, UK), **B. Horwitz (**Technion Institute of Technology, Israel**), L. Corrochano** (Sevilla University, Spain) **Next ECFG14 Meeting announcement, B. Horwitz** **Farewell remarks, M.-H. Lebrun and F. Martin**

Gala — **Trocadero Aquarium, 5 ave Albert du Mun, 75016 Paris**

20.00 - 22.00	**Cocktail, buffet dinner and free visit**
22.00 - 24.00	**Fungi rock circus**

KEYNOTE LECTURE ABSTRACTS

KEYNOTE LECTURE ABSTRACTS
Keynote 1

Monday 4th April
8:45 - 9:30
Gaston Berger

KAHMANN Regine
Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

How to colonize a plant: insights from secreted effectors

Plants can be infected by a variety of microorganisms with either beneficial or detrimental effects. While the mechanisms that allow bacteria to colonize plants are relatively well understood, knowledge how eukaryotic pathogens establish themselves in a plant host largely remains to be established. A successful colonization requires active effector-mediated suppression of plant defense responses and host tissue reprogramming to meet the needs of the pathogen. While bacterial pathogens use Type III secretion systems for injecting in the range of up to 50 protein effectors into plant cells, eukaryotic pathogens have an effector repertoire of several 100 proteins which display their activity either in the apoplast or translocate to host cells. How effectors are delivered to plant cells by eukaryotic plant pathogens remains elusive. In my presentation I will focus on smut fungi. These comprise a large group of biotrophic pathogens that mostly infect cereal crops and wild grasses. During host colonization, smut fungi establish an extended interaction zone with the plant in which fungal hyphae are completely encased by the host plasma membrane. The best studied member of this group, *Ustilago maydis*, infects maize and induces characteristic tumor formation and anthocyanin induction. Its secreted effector repertoire consists of about 300 completely novel proteins plus additional secreted proteins with known functional domains. Many of these effectors have critical virulence functions. In my presentation I will highlight where and how selected effectors function and why so many are needed.

KEYNOTE LECTURE ABSTRACTS
Keynote 2

Tuesday 5th April
8:45 - 9:30
Gaston Berger

DUJON Bernard
Institut Pasteur, Dept. Genomes and Genetics, CNRS UMR3525 & University P. M. Curie, UFR927, 25 rue du Docteur Roux, Paris, France

Inside the genomes of yeasts

Yeast genomics has reached a developed stage. Sequencing yeast genomes has become routine tool not only to explore novel evolutionary lineages - a rapidly accelerating trend - but also to follow experiments or even to simply validate novel technological methods. Reference genomes sequences are now available for numerous yeast species of distinct phylogenetic origin and ecological adaptation, and open the way to analyze the genomic bases for the diversity and evolution of natural populations. In this lecture, I will briefly review the major steps of the extraordinary development of yeast genomics over the last two decades from the original sequencing of *Saccharomyces cerevisiae*, back in 1996, paying particular attention to the emergence and progress of novel basic concepts. I will then summarize our present knowledge on the diversity of yeast genomes and, from these data, discuss our understanding of the mechanisms of their evolution. I will conclude by trying to illustrate phenomena that may suggest novel research directions.

KEYNOTE LECTURE ABSTRACTS
Keynote 3

Wednesday 6th April
8:45 - 9:30
Gaston Berger

BONFANTE Paola
Department of Life Science and Systems Biology, University of Torino, Torino, Italy

Symbionts inside Symbionts: arbuscular mycorrhizal fungi and their intracellular microbiota

Arbuscular mycorrhizal fungi (AMF) are a crucial component of plant microbiota: thriving both in the rhizosphere and inside root tissues, they play key roles in nutrient cycling and plant health. In addition to some peculiar biological features (they are obligate multinucleated biotrophs), many of them possess endobacteria in their cytoplasm. These microbes are usually transmitted following a vertical transmission along the fungal sporal generations. Diverse bacterial populations may coexist in the same fungal spore, offering an interesting example of a fungal intracellular microbiota. The development of -omics approaches has allowed us to sequence the genome of some endobacterial lines revealing a reduced genome and dependence on the fungal host. To understand the adaptive traits of this fungal-bacterial interaction, we applied transcriptomics and proteomics approaches to an isolate of *Gigaspora margarita* containing an endobacterial population identified as *Candidatus Glomeribacter gigasporarum* versus a cured line without endobacteria. RNA-seq analysis and iTRAQ quantitative proteomics of the AMF in the presence an absence of its endobacterium indicated that endobacteria have an important role in the fungal pre-symbiotic phase by enhancing fungal bioenergetic capacity, increasing its ATP production, and respiration. Carbonylated proteins indicated that the cured line has a higher oxidative stress levels, and surprisingly the same condition was confirmed in the host plants colonized by the cured line, opening the question whether the presence of endobacteria may have effects not only on the fungal host, but also on the plant. Fungal symbionts inside plant symbionts originate a complex interdomain network that probably affects fungal-plant interactions opening questions on the adaptation mechanisms and on the evolution of such interactions.

PLENARY LECTURE ABSTRACTS

PLENARY LECTURE ABSTRACTS
Plenary session 1: Developmental pathways

Monday 4th April
9.30 - 10.00
Gaston Berger

IDNURM Alexander
School of BioSciences, the University of Melbourne, Melbourne, Australia

Reflections on fungal evolution based on the analysis of mirror mutants of *Sporobolomyces*

The success of the fungi, in terms of their species diversity or absolute numbers of cells on the planet is due to many factors, one of which is the ability of fungi to produce and disseminate spores. While much research has focused on the genetics of spore production, little has been paid to the important role of the genes required to spread those spores in the environment. Species in diverse lineages have evolved multiple and independent mechanisms by which to disperse their spores. One unique mechanism that is found in the basidiomycetes is the production of ballistospores, made either asexually or sexually, that use a liquid fusion mechanism to force the spore into the air. Ballistospores enable the spread of mushrooms and rust fungi, cause allergies and asthma in humans, and are a major part of the bioaerosol implicated in the nucleation of rain in tropical rainforests. The genes involved in ballistospore formation and release have not been sought in depth. Using a species of basidiomycete yeast in the Pucciniomycotina, mutant strains have been isolated by different methods that are unable to «mirror» their colonies onto adjacent plates. Gene identification implicates cell wall biosynthesis, morphogenesis pathways and very long chain fatty acid biosynthesis in the process of making and then fired ballistospores. In addition to providing information about gene functions in *Sporobolomyces*, this research also provides insight more generally on fungal gene function and evolution.

PLENARY LECTURE ABSTRACTS
Plenary session 1: Developmental pathways

Monday 4th April
10.00 - 10.30
Gaston Berger

ETXEBESTE Oier
Biochemistry II laboratory, Department of Applied Chemistry, Faculty of Chemistry, University of The Basque Country (UPV/EHU), San Sebastian, Spain

Hyphal tips control conidiation in *Aspergillus nidulans*

Suboptimal growth conditions trigger genetic reprogramming of vegetative hyphae, resulting in the generation of asexual spores and allowing dissemination to new environments. Asexual development or conidiation in the model filamentous fungus *Aspergillus nidulans* is induced by the upstream developmental activation (UDA) pathway. UDA proteins transduce signals from the tip of hyphae to nuclei, where the expression of *brlA*, the central regulator of conidia synthesis, is induced. This talk will summarize the current knowledge on this tip-to-nucleus communication mechanism, which is based on the long-distance retrograde movement of the transcription factor FlbB. Future approaches to the topic will also be suggested, as stimulating elements contributing to the understanding of how apical signals are coupled with the transcriptional control of development in filamentous fungi.

PLENARY LECTURE ABSTRACTS
Plenary session 1: Developmental pathways

Monday 4th April
10.30 - 11.00
Gaston Berger

AGUIRRE Jesús
Departamento de Biología Celular y Desarrollo, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Mexico

Fungal development and the mechanisms of sensing and responding to ROS

We have proposed that life early confrontation with reactive oxygen species (ROS) led cells to evolve not only defense mechanisms but also and more importantly, the utilization of ROS as signaling molecules to regulate growth and development. Using the fungus *Aspergillus nidulans* we have shown that transcription factors (TF) *SrrA*, *NapA* and *AtfA* are individually required to survive oxidative stress and that their inactivation have profound effects in asexual and sexual development. *AtfA* is regulated by the stress MAPK SakA, which belongs to the Hog1/p38 family and is involved in transducing many types of environmental stresses. Recently, we characterized SrkA, a MAPK-activated protein kinase, as a novel component of the SakA pathway. *ΔsrkA* and *ΔsakA* mutants share a de-repressed sexual development phenotype. However, *ΔsrkA* mutants are not sensitive to oxidative stress and in fact, *srkA* inactivation partially suppresses the sensitivity of *ΔsakA* mutant conidia to H_2O_2, t-BOOH and menadione. In absence of stress, SrkA shows physical interaction with non-phosphorylated SakA in the cytosol. Notably, H_2O_2 induces a drastic change in mitochondrial morphology consistent with a fission process and the re-localization of SrkA to nuclei and mitochondria, depending on the presence of SakA. SakA-SrkA nuclear interaction is also observed during normal asexual development in dormant spores. Using SakA and SrkA S-tag pull-downs coupled to mass spectrometry, we have shown that SakA interacts with SrkA, the stress MAPK MpkC, the PPT1-type phosphatase AN6892 and other proteins involved in cell-cycle regulation, DNA-damage response, mRNA stability and protein synthesis, mitochondrial function and other stress-related responses. We propose that oxidative stress induces DNA damage and mitochondrial fission and that SakA and SrkA mediate cell-cycle arrest and regulate mitochondrial function during stress. These results provide new insights on the mechanisms by which SakA and SrkA regulate the remodelling of cell physiology during oxidative stress and development.

PLENARY LECTURE ABSTRACTS
Plenary session 2: Fungal biotic interactions

Monday 4th April
11:30 - 12:00
Gaston Berger

D'ENFERT Christophe
Fungal Biology and Pathogenicity Unit - INRA USC2019, Department of Mycology, Institut Pasteur, Paris, France

fA functional genomic approach to understand *Candida albicans* colonization of biotic and abiotic surfaces

The ability of *Candida albicans* to colonize biotic and abiotic surfaces plays crucial roles in its pathogenesis. Here, I will show how our use of signature-tagged overexpression screens has revealed new links between cell wall biogenesis and the formation of biofilms on abiotic surfaces or the colonization of the gastro-intestinal tract. First, I will present our identification and characterization of *C. albicans* cell wall proteins that contribute to the cooperative behavior between biofilm cells. Second, I will describe our identification of a novel regulator of the production of cell surface glycoproteins whose role in pH adaptation under oxygen-deprived conditions suggests that cell wall remodeling is central to *C. albicans* interaction with the gut environment.

PLENARY LECTURE ABSTRACTS
Plenary session 2: Fungal biotic interactions

Monday 4th April
12.00 - 12.30
Gaston Berger

ROUX Christophe (1)
KAMEL Laurent (2), TANG Nianwu (3), DELAUX Pierre-Marc (1), FREI DIT FREY Nicolas (1)
(1) Laboratoire de Recherche en Sciences Végétales, Université de Toulouse, CNRS, UPS, 24 chemin de Borde Rouge, Auzeville, BP42617, Castanet-Tolosan, France
(2) Agronutrition Laboratoire de biotechnologies, Labege, France, Labège, France
(3) State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan, China

Strategies developed by arbuscular mycorrhizal fungi (Glomeromycota) to manipulate host cells: outcomes, hypotheses and outstanding questions

Arbuscular mycorrhizal (AM) fungi (Glomeromycota) can establish symbiotic relationship with 80% of land plants, including non-vascular plants (liverworts, hornworts), early diverging vascular plants (ferns) and seed plants. Comparative phylogenomic approaches in plants revealed the presence of highly conserved genes required for the establishment of AM symbiosis. By contrast, the AM fungal «symbiotic gene toolkit» remains poorly characterized. This is of particular interest given that AM fungi do not display any host specificity. This feature, unique among plant-interacting fungi, is exemplified by the widely studied species *Rhizophagus irregularis* that can interact with all host-plant species tested so far. Following the publication of the first genomic assembly from *Rhizophagus irregularis* strain DAOM197198 (Glomerales)[1,2], we defined the gene repertoire of a phylogenetically distant species: *Gigaspora rosea* (Diversisporales). Comparative transcriptomics of these two fungi associated with multiple hosts revealed three patterns of expression. Two of these sets of genes were either fungal or host specific. The third set identified was formed by genes expressed by both fungal species in association with all the hosts tested. How these genes can be considered as the remnant of the ancient AM fungal symbiotic gene network will be discussed.

1. Tisserant *et al.* (2013) PNAS 110: 20117-22.
2. Lin *et al.* (2014) PLoS Genet, 10, e1004078.

PLENARY LECTURE ABSTRACTS
Plenary session 2: Fungal biotic interactions

Monday 4th April
12.30 - 13.00
Gaston Berger

BIRCH Paul
James Hutton Institute, UK

The host targets of *Phytophthora* effectors: pressure points to promote susceptibility

The oomycete *Phytophthora infestans* remains the most serious potato pathogen, and thus a threat to food security. During the biotrophic phase of its interaction with hosts, such as potato, it delivers RXLR effectors from haustoria to the inside of plant cells. Over recent years we have been investigating what RXLR proteins interact with and what they do to promote infection. Many RXLR effectors directly target and suppress the activity of immune regulators. However, I will report on the unexpected finding that several key RXLRs target susceptibility factors - host proteins whose activity is promoted or utilised by these effectors to enhance late blight infection.

PLENARY LECTURE ABSTRACTS
Plenary session 3: Changing lanscapes of fungal genetics and genomics

Tuesday 5th April
9.30 – 10.00
Gaston Berger

STAJICH Jason (1)
SPATAFORA Joseph (2), GRIGORIEV Igor (3), MARTIN Francis (4), HENRISSAT Bernard (5), 1000 FUNGAL GENOMES PROJECT consortium
(1) University of California-Riverside, Riverside, CA, USA
(2) Oregon State University, Corvallis, OR, USA
(3) Joint Genome Institute, Walnut Creek, CA, USA
(4) INRA, Nancy, France (5) CNRS, Marseille, France

Genome evolution in early diverging fungi from the 1000 fungal genomes perspective

Transitions in morphological complexity, trophism, and lifestyles have occurred throughout the evolution of the fungal kingdom. To examine these changes in the context of genomic changes requires a well-resolved phylogeny of the fungi and a dense sampling of reference genomes. We have made efforts to improve the available diversity of fungal genomes through the 1000 fungal genomes project (1KFG; http://1000.fungalgenomes.org) and other efforts. The first analysis of 350 of these genomes has been use to construct a resolved phylogeny of the fungi with new understanding of the early branching zoosporic (chytrid) and zygomycete fungi. We find support for multiple, early diverging lineages that include zoosporic fungi, two clades of the zygomycetes we have named Zoopagomycota and Mucoromycota which show long standing symbioses with animals and plants respectively. The arbuscular mycorrhizal fungi (Glomeromycota) group with strong support within the Mucoromycota not as separate lineage. Comparison of gene content to reconstruct changes that lead to emergence of complex multicellular forms reveal the likely complex ancestor of the Dikarya fungi. We also examined the multiple evolutionary losses of the flagella inferred from the phylogeny and how that compares to the loss or retention of genes necessary for the flagellar complex and we identify additional genes that show similar retention patterns.

PLENARY LECTURE ABSTRACTS
Plenary session 3: Changing lanscapes of fungal genetics and genomics

Tuesday 5th April
10.00 - 10.30
Gaston Berger

JANBON Guilhem
RNA Biology of Fungal Pathogens Unit, Department of Mycology, Institut Pasteur, Paris, France

Introns in *Cryptococcus neoformans*

Cryptococcus neoformans is a basidiomycetous opportunistic pathogen leaving in the environment responsible for more than 500 000 deaths every years toward to the globe. Our recent work suggests that a fascinating, complex pattern of RNA molecules composes its transcriptome and this fungus is emerging for different aspects as an ideal model to study RNA metabolism in eukaryotes. It is also tempting to hypothesize that this complex RNA metabolism provides a mechanism for this yeast to respond to different environmental cues and to be an efficient pathogen. The most prominent features of this transcriptome are introns. Our recent re-annotation of the *C. neoformans* genome revealed that nearly all the expressed genes contain intron(s). They can be present within the coding sequence but also within UTR regions. We also demonstrated that these introns are essential for gene expression. We also identified a large number of alternative splicing events. However, alternative splicing in this yeast seems to be more a mean to regulate gene expression than to diversify the proteome.

PLENARY LECTURE ABSTRACTS
Plenary session 3: Changing lanscapes of fungal genetics and genomics

Tuesday 5th April
10.30 - 11.00
Gaston Berger

TAYLOR John (1)
ESTRELLA Raissa (1), CATE Jamaie H. D. (1), YU Ka Man Jasmine (1), BAKER Jacob Scott (1), BREM Rachel B. (1), ELLISON Chris E. (1), DONNELLY Marie K. (1)
(1) University of California, Berkeley, Berkeley, USA

Evolution and ecology meet development: Harnessing natural variation to inform studies of molecular function.

Population genomics is changing the landscape of fungal genetics by making it possible to use natural variation to inform research on molecular development. Two successful approaches will be presented, genome wide association studies (GWAS) to find genes associated with traits of interest to the researcher and Reverse Ecology to find genes associated with fungal adaptive traits that may also be traits that interest the researcher. To take advantage of natural variation in genotype and phenotype, mycologists need to collect individuals of their favorite fungus; as few as 24 have been used, but this is a case where more is better, e.g., 100 or more. The next step is to sequence the genomes of each individual and use the polymorphic nucleotides (SNPs) to search for genetically isolated populations within the collection. Any genetically isolated, interbreeding population is a candidate for GWAS, as has been shown in studies that found virulence genes for plant pathogenesis with 24 *Heterobasidion* individuals (Dalman et al. 2013. Plos One 8) and communication genes for germling fusion with 24 Neurospora individuals (Palma-Guerrero et al. 2013. Plos Genetics). If more than one population is found within the collection, the genomes can be compared to find regions of unusual divergence that also show low variation within a population. These regions are likely the result of hybridization and introgression due to natural selection for an adaptive trait. Knowing the function of the genes in these regions can reveal the adaptive trait, as has been hypothesized for virulence in the human pathogenic fungus, *Coccidioides* (Neafsey et al. 2010. Genome Research 20: 938-94), for growth at low temperatures in *Neurospora* (Ellison et al., 2011. PNAS 108: 2831-2836), and for salt tolerance in *Suillus* (Branco et al. 2015. Molecular Ecology 24: 2747-2758), and is being done for thermophily in *Kluyveromyces*. The final step is one that molecular developmental biologists know well, testing the hypotheses about correlation between genotype and trait by disabling genes, as has been done in the case of *Neurospora* (Ellison et al. 2011. PNAS 108: 2831-2836) and is being done for Kluyveromyces. Each of these studies creates a tool in the form of isolates and genomes that any other researcher can use by simply screening the individuals for new traits. The challenge to our community is to curate these collections and genomes for future research.

PLENARY LECTURE ABSTRACTS
Plenary session 4: Evolutionary genomics

Tuesday 5th April
11.30 – 12.00
Gaston Berger

BRANCA Antoine (1)
RODRIGUEZ DE LA VEGA Ricardo (1), ROPARS Jeanne (1), DUMAS Emile (1), DUPONT Joelle (2), LOPEZ VILLAVICENCIO Manuela (2), GOUZY Jerome (3), SALLET Erika (3), GIRAUD Tatiana (4)
(1) ESE, CNRS, Université Paris-Sud, AgroParisTech, Université Paris-Saclay, Orsay, France
(2) ISEB, CNRS, MNHN, UPMC, EPHE, Paris, France
(3) LIPM, INRA,CNRS, Toulouse, France

The adaptive genomic toolbox of *Penicillium* molds: insights from between and within species comparisons

Penicillium is a diverse fungal genus with hundreds of species occurring worldwide in various substrates, from soil to food, and with various lifestyles, from necrotrophic pathogenicity to endophytic mutualism. Studying parallel evolution of related species on the same environment is a powerful approach for understanding the genomic processes of adaptation. Several species of *Penicillium* have been independently domesticated for the ripening of cheese since at least the 18th century. Currently, the two key *Penicillium* species for cheese-making are *P. roqueforti* for blue cheeses and *P. camemberti* for Brie-type cheeses. These distantly related species have been independently selected in a human-made nutrient-rich environment containing numerous bacterial and fungal competitors. First, by comparing the genomes of the *Penicillium* used as starter culture in cheese production with other *Penicillium* genomes, we were able to demonstrate the parallel gains, losses and positive selection on genes involved in the utilization of the cheese nutrients and competition with other microorganisms. Some of the genes have been acquired through lateral transfers and increase fitness in cheese environment. Second, we focused at the intraspecific level, since *P. roqueforti* grows not only in cheese but also in various environments. We could reconstruct the history of *P. roqueforti* domestication, which gives another example of the ease for *Penicillium* species to adapt to new substrates at short evolutionary timescale. Our results show that *Penicillium* molds constitute an excellent model for combining micro- and macroevolutionary approaches to understand the genetics of adaptation.

PLENARY LECTURE ABSTRACTS
Plenary session 4: Evolutionary genomics

Tuesday 5th April
12:00 - 12:30
Gaston Berger

STUKENBROCK Eva (1)
GRANDAUBERT Jonathan (1), DUTHEIL Julien (1)
(1) Max Planck Institute for Evolutionary Biology, Plön, Germany

Genome-wide recombination landscapes and adaptation in fungal plant pathogens

Antagonistic co-evolution between pathogens and their hosts drives rapid adaptive changes in both partners. We aim to understand the mechanisms underlying such rapid adaptation in two closely related fungal plant pathogens *Zymoseptoria tritici* (wheat pathogen) and *Zymoseptoria ardabiliae* (wild grass pathogen). We previously reported that *Z. tritici* has a significantly higher effective population size in spite of strong directional selection pressure imposed to the pathogen in the wheat field. We also showed a strong impact of natural selection on the genome evolution of *Z. tritici*. The efficacy of selection may be increased by a high recombination rate. Indeed we compute very high rates of genome-wide recombination rates in both *Z. tritici* and *Z. ardabiliae* using population genomics approaches. Comparing mean recombination rates of coding and non-coding sequences, we find significantly higher recombination rates in the former implying a central role of recombination in gene evolution. Some recombination hot spots are located in genes, including genes encoding putative effectors, further supporting a central role of recombination in adaptation. To study the evolution of recombination, we correlated recombination maps in the syntenic regions of the *Z. tritici* and *Z. ardabiliae* genomes. We find that recombination rate varies extensively across chromosomes in both species, and that some regions have conserved patterns of recombination between the two species while others show highly different patterns. We conclude that the recombination landscape and hot spot positions evolve rapidly in *Zymoseptoria*. This has strong implications for the tempo and mode of adaptive evolution of these pathogen species.

PLENARY LECTURE ABSTRACTS
Plenary session 4: Evolutionary genomics

Tuesday 5th April
12.30 – 13.00
Gaston Berger

JOHANNESSON Hanna (1)
SVEDBERG Jesper (1), MOLNAR Ruxandra (1), HAMMOND Tom (2)
(1) Department of Organismal Biology, Norbyvagen 18D, Uppsala, Sweden
(2) Illinois State University, Illinois, USA

Genomic conflict as a motor for evolutionary change: insights from the fungal spore killers

Conflicts caused by selfish genetic elements is expected to be a driving force for evolutionary innovation, and hence, of fundamental importance for all aspects of evolution. Empirical data on the topic is largely lacking and needed in order to support or refute this theory. In my research group, we use *Neurospora* as a study system of the evolutionary significance of meiotic drive. In this model system, the meiotic drive element Spore killer is found. In sexual crosses between strains carrying a Spore killer genetic element and strains that are sensitive to Spore killer, half of the spores will die and the surviving spores will all carry the Spore killer element. The Spore killer and its resistance factor have been mapped to an approximately 2 Mbp large region of suppressed recombination on LG3. We have introgressed the Spore killer element, the resistance and sensitive alleles into uniform genetic background, and screened the phenotypic effects of carrying the element. By this approach, we have confirmed that there is both a direct and an indirect cost of carrying the Spore killer haplotype. Furthermore, we have sequenced 90 genomes, representing all known Spore killers as well as sensitive and resistant strains of *N. sitophila* and *N. intermedia* using Illumina HiSeq (to 30X coverage). From seven of these we have also gathered MiSeq (100x) and Pac Bio (50x) data to create high quality, full chromosome assemblies. This data has revealed a complex pattern of inversions, insertions and deletions that may play an essential role in maintaining the region of suppressed recombination and in explaining the birth and death of Spore killers over evolutionary time. Taken in combination, results emerging from this project suggest that meiotic drive has a profound effect of genomic architecture in *Neurospora*.

PLENARY LECTURE ABSTRACTS
Plenary session 5: Epigenetics

Wednesday 6th April
9.30 - 10.00
Gaston Berger

LIU Yi
Dept. of Physiology, University of Texas Southwestern Medical Center, Dallas, USA

Synonymous but not the same: new codes within genetic codons

Codon usage bias has been observed in the genomes of almost all organisms and is thought to result from selection for efficient and accurate translation. Many genes exhibit little codon usage bias. The lack of codon bias for a gene is thought to be due to lack of selection for mRNA translation. The rhythmic expression and the proper function of the *Neurospora* FREQUENCY (FRQ) protein are essential for circadian clock function. *frq* exhibits non-optimal codon usage across its entire open reading frame. Optimization of *frq* codon usage abolishes both overt and molecular circadian rhythms. Codon manipuplation not only affects FRQ expression level but surprisingly, also results in conformational changes in FRQ protein, altered FRQ phosphorylation profile and stability, and impaired functions in the circadian clock. In addition, we discovered genome-wide correlations between codon usage and protein structure motifs in *Neurospora* and other organisms. Finally, we demonstrated that codon usage regulates speed of translation elongation and by doing so, affects co-translational protein folding. Together, these results indicate the existence of new codes within the genetic codons that also specify protein structures.

PLENARY LECTURE ABSTRACTS
Plenary session 5: Epigenetics

Wednesday 6th April
10.00 - 10.30
Gaston Berger

LEWIS Zachary
Biosciences Building, University of Georgia, Athens, USA

Heterochromatin is essential for normal chromatin architecture and genome function.

Repressive chromatin modifications are important for fungal gene regulation and genome stability. In *Neurospora crassa*, two classes of heterochromatin are defined by H3 lysine-9 methylation (H3K9me) and H3 lysine-27 methylation (H3K27me), respectively. The single H3K9 methyltransferase, called DCDC, is required for normal growth and development. DCDC-deficient mutants are hypersensitive to the genotoxic agent methyl methanesulfonate (MMS) and during unperturbed vegetative growth display altered localization of phosphorylated H2A, a marker for the activated DNA damage response. To determine the molecular events that drive genotoxic stress, we isolated genetic suppressors of DCDC-deficient mutants. We found that MMS-sensitivity and growth phenotypes of DCDC-deficient strains are suppressed by mutation of eed or set-7, encoding components of the H3K27 methyltransferase PRC2 (Polycomb repressive complex-2). H3K27 methylation typically associates with clusters of silent genes, but undergoes genome-wide redistribution to constitutive heterochromatin in DCDC - or HP1-deficient mutants. We conclude that disruption of H3K9 methylation triggers global epigenetic reprogramming that drives aberrant gene expression and genotoxic stress. Similar epigenetic reprogramming in mutants lacking H3K9 methylation may drive altered gene expression in other fungi, such as *Aspergillus nidulans*.

PLENARY LECTURE ABSTRACTS
Plenary session 5: Epigenetics

Wednesday 6th April
10.30 - 11.00
Gaston Berger

SAUPE Sven (1)
DASKALOV Asen (6), NESS Frederique (1), DYRKA Witold (2), BIRGIT Habenstein (3), RAIMON Sabaté (4), CLAVÉ Corinne (1), HOFMANN Kay (5), LOQUET Antoine (3)
(1) IBGC CNRS Université de Bordeaux,Bordeaux, France
(2) Wroclaw university of technology, Wroclaw, Poland
(3) IECB CBMN, Pessac, France
(4) Institute of Nanoscience and Nanotechnology, University of Barcelona, Barcelona, Spain
(5) University of Cologne, Cologne, Germany
(6) University of California Berkeley, Berkeley, USA

Prion amyloid folds in NLR signalling in fungi

Nod-like receptors (NLRs) are a conserved class of intracellular receptor that function in innate immunity and establishment of symbiotic interactions both in plants and mammals. We and others have identified fungal NLR homologs in the context of a non-self recognition process common in fungi and termed heterokaryon incompatibility. We find that a subclass of fungal NLRs operate by a mechanism involving amyloid-based signalling in which the receptor activates its cognate downstream effector proteins by templating amyloid folding. These effector proteins are of different types and their activation usually leads to programmed cell death. We will describe several examples of fungal amyloid-based signalling domains drawn from various species (*Podospora anserina, Nectria haematococca, Chaetomium globosum*) and explicit the evolutionary relationship this form of fungal programmed cell death bears with necroptotic cell death described in mammals.

PLENARY LECTURE ABSTRACTS
Plenary session 6: System and synthetic biology

Wednesday 6th April
11.30 - 12.00
Gaston Berger

MEYER Vera
Berlin University of Technology, Institute of Biotechnology, Department Applied and Molecular Microbiology, Germany

Aspergillus in synthetic biology – where is it taking us?

The current epoch of Anthropocene is a new geological epoch of earth's lithosphere due to human activities in the last centuries. Synthetic biology provides a new dimension to the Anthropocene. It could take us in the not too distant future to an age, where the limits to our capabilities to design and reshape biological systems are only limited by our imaginations and our ethical values. The possibilities of synthetic biology are mind-blowing and have the potential to transform medicine, food production, biofuel research, bioplastics industry and nanofabrication of materials. Central themes of synthetic biology include, amongst others, the construction and fine-tuning of chassis genomes, the engineering of transcriptional circuits and metabolic pathways as well as the synthesis of non-natural molecules. Synthetic biology has a strong background in systems biology as understanding of design principles underlying genetic and chemical regulation in natural biological systems is a prerequisite to re-design biochemical systems. Our focus is to pioneer genetic tools and molecular gene switches for the industrial strain *Aspergillus niger* and to explore its capabilities as producer of non-natural products by re-wiring its secondary metabolism. *A. niger* is a cell factory with a long-standing experience in biotech industry as host for citric acid and protein production. With its well-annotated genome, the availability of a genome-wide metabolic network, hundreds of transcriptomic and proteomics data and its genetic amenability, it is a well-suited and versatile model system for fungal synthetic biology. In my talk, I will present our recent approaches which aim to engineer titratable, stable and tightly regulated conditional expression systems (e.g., Tet-On, Tet-Off) and our efforts to genetically trim *A. niger* to become an expression host for the synthesis of natural but heterologous nonribosomal peptides with product yields high enough for commercial exploitation. In addition, I will discuss our strategies to diversify its natural product portfolio by combinatorial biosynthesis for the generation of "new-to-nature" compounds.

PLENARY LECTURE ABSTRACTS
Plenary session 6: System and synthetic biology

Wednesday 6th April
12.00 - 12.30
Gaston Berger

KOSZUL Romain
Spatial regulation of genomes unit, Genomics Department, Pasteur Institute, Paris, France

Redesigning chromosomes to optimize 3D conformation assays

Genomic derivatives of the capture of chromosome conformation assay (3C, Hi-C, Capture-C) are widely applied to decipher the average intra- and inter-chromosomal organization of eukaryotes and prokaryotes. In all chromosome conformation capture based experiments the accuracy with which contacts are detected varies considerably because of the uneven distribution of restriction sites along genomes. We redesigned and reassembled in yeast a 150kb region with regularly spaced restriction sites for various enzymes. Thanks to this design, we enhanced the signal to noise ratio and improved the visibility of the entire region as well as our understanding of Hi-C data, while opening new perspectives to future studies in other organisms. In addition, I will also present our investigation of the 3D structure of synthetic yeast chromosomes assembled by the SC2.0 consortium.

PLENARY LECTURE ABSTRACTS
Plenary session 6: System and synthetic biology

Wednesday 6th April
12.30 – 13.00
Gaston Berger

MORTENSEN Uffe
Department of Systems Biology, Technical University of Denmark, Lyngby, Denmark

CRISPR methods for product discovery and fungal cell factory construction

The rapidly increasing number of fully sequenced fungal genomes constitutes a goldmine of new research possibilities. However, for most newly sequenced species, exploitation of this potential is hampered by low gene targeting frequencies. We have therefore developed a flexible CRISPR/Cas9 toolbox adapted for filamentous fungi to facilitate genome editing. Our toolbox currently contains a set of four AMA1 based vectors with different selections markers, which all contain a *cas9* gene codon optimized for *Aspergillus niger* and a USER cloning cassette for simple insertion of the sequences encoding *sgRNA*. Moreover, we have also developed an sgRNA design software that facilitates identification of *sgRNA* sequences that can target a desired gene in several different species, hence, reducing the plasmid construction workload. To investigate the versatility of our system we have embarked on a project aiming at testing tool parts in the black *Aspergillus* species where several species have not previously been genetically engineered. To address the efficiency of the system and to facilitate future work, we initially introduce mutations in homologs of the pigment gene *albA* and in the marker gene *pyrG*. In species where CRISPR-Cas9 works efficiently, we envision that it will now be possible to rapidly address specific scientific questions by changing or eliminating specific gene functions using our system directly; or by using it to eliminate the NHEJ pathway setting the stage for conventional gene targeting. We will provide examples of this strategy. CRISPR-Cas9 may also be advantageously used for cell factory construction as our versatile system allows genes to inserted and tested for activity in several different species using the same building blocks. In this way the repertoire of strains for production of a given product can be quickly tested and the best host chosen at an early point. For examining pathways in more detail or for metabolic engineering purposes, we are investigating the possibility of editing genes by RNA guided Cas9 activity using oligo-nucleotides as repair template for the repair of the resulting DNA DSBs. We have shown that it is possible to introduce defined point mutations in specific genes using this strategy in a number of genes. Efficiencies and possibilities will be discussed.

CONCURRENT SESSION ABSTRACTS

CONCURRENT SESSION ABSTRACTS
CS1: Cell biology and traffic

Monday 4th April
16:00 - 16:20
Louis Armand Ouest

PENALVA Miguel A (1)
PINAR Mario (1), HERNÁNDEZ-GONZÁLEZ Miguel (1), ARST Herbert N. (1), GARCÍA-TAGUA Victor (1), ZHANG Jun (2), XIANG Xin (2), PANTAZOPOULOU Areti (1)
(1) Centro de Investigaciones Biológicas CSIC, Madrid, Spain
(2) Uniformed Services University of Health Sciences, Bethesda, USA

Golgi exit by maturation of TGN cistern

Exocytic carriers exiting the Golgi towards the Spitzenkörper arise by maturation of TGN (trans-Golgi network) cisternae. The transition between Golgi and post-Golgi identity is determined by the recruitment of *Aspergillus nidulans* RabE (yeast Ypt31, mammalian RAB11) to maturing TGN cisternae, which is in turn mediated by the guanine-nucleotide exchange factor that activates RabE, TRAPPII. *Aspergillus nidulans* hypA encodes Trs120, a key component of TRAPPII essential for exocytosis to occur. Post-Golgi carriers travel towards the Spitzenkörper using microtubule- and actin-dependent motors. In the tip region, carriers shift from microtubules to actin, such that myosin-5 focuses them at the apex. Should this relay fail, carriers are transported basipetally by dynein, plausibly to have another chance to return to the tip region riding on kinesin and successfully engage myosin-5.

CONCURRENT SESSION ABSTRACTS
CS1: Cell biology and traffic

Monday 4th April
16:20 - 16:40
Louis Armand Ouest

TAKESHITA Norio (1)
ISHITSUKA Yuji (2), SAVAGE Natasha (3), BERGS Anna (1), NIENHAUS G. Ulrich (2), FISCHER Reinhard (1)
(1) Karlsruhe Institute of Technology, Department of Microbiology, Karlsruhe, Germany
(2) Karlsruhe Institute of Technology, Institute of Applied Physics, Karlsruhe, Germany
(3) University of Liverpool, Institute of Integrative Biology, Liverpool, UK

Super-resolution microscopy reveals a dynamic picture of cell polarity maintenance during hyphal growth

Polar cell growth, a key cellular mechanism shared among a wide range of species, relies on targeted insertion of new material at specific locations of the plasma membrane. How these cell polarity sites are stably maintained during massive membrane insertion has yet remained elusive. Conventional live-cell optical microscopy fails to visualize polarity site formation in the crowded cell membrane environment due to its limited resolution. Here we have employed advanced live-cell imaging techniques to directly observe localization, assembly and disassembly processes of cell polarity sites with high spatio-temporal resolution in a rapidly growing filamentous fungus, *Aspergillus nidulans*. We show that the membrane-associated polarity site marker TeaR is transported on microtubules along with secretory vesicles and forms a protein cluster at that point of the apical membrane where the plus-end of the microtubule touches. There, a small patch of membrane is added via exocytosis, and the TeaR cluster gets quickly dispersed over the membrane. There is an incessant disassembly and reassembly of polarity sites at the growth zone, and each new polarity site locus is slightly offset from preceding ones. Based on our imaging results and computational modeling, we propose a transient polarity model that explains how cell polarity is stably maintained during highly active directional growth.

CONCURRENT SESSION ABSTRACTS
CS1: Cell biology and traffic

Monday 4th April
16:40 - 17:00
Louis Armand Ouest

WÖSTEN Han
Utrecht University, Utrecht, The Netherlands

Intercompartmental streaming in *Aspergillus*

Hyphae of higher fungi are compartmentalized by porous septa that enable cytosolic streaming. Therefore, it was believed that the mycelium shares a common cytoplasm. However, we have shown that septa of growing hyphae of *Aspergillus* are often closed. For instance, about 50% of the apical septa of *A. niger* are plugged, while they are always closed in older parts of hyphae. Closure of septal pores physically isolates compartments. Yet, experimental evidence indicates that transporters in the plasma membrane lining the septal wall allow for selective inter-compartmental transport under this condition. Not only closed septa but also open septa act as a barrier for inter-compartmental cytoplasmic mixing. The septal pore diameter reduces with increasing septal age and under stress conditions. Modelling shows that the septal pore width is set such that its regulation offers maximal control of compound concentration levels within the compartments. Results obtained so far show that that apical compartments of growing *Aspergillus hyphae* behave unicellularly, while older compartments have a multicellular organization. This suggested that apical growth is supported by subapical compartments. However, we have recently shown that growth speed of apical compartments is not affected when hyphae are dissected in the second compartment. We thus conclude, that apical compartments can be self-providing units. Septal opening or transporter proteins may provide strategies to support of apical compartments under low nutrient conditions or for communication within the mycelium.

CONCURRENT SESSION ABSTRACTS
CS1: Cell biology and traffic

Monday 4th April
17:00 - 17:20
Louis Armand Ouest

PEREZ DE NANCLARES Elixabet (1)
ABAD Ion Luis (1), ESPESO Eduardo A (2), UGALDE Unai O (1), ETXEBESTE Oier (1)
(1) Dept. of Applied Chemistry, Faculty of Chemistry, University of the Basque Country, Donostia, Spain
(2) Dept. of Cellular and Molecular Biology, Centro de Investigaciones Biológicas (CSIC), Madrid, Spain

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

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

CONCURRENT SESSION ABSTRACTS
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Monday 4th April
17:20 - 17:40
Louis Armand Ouest

TANNER Widmar (1)
MALINSKY Jan (2), OPEKAROVA Miroslava (2)
(1) Cell Biology and Plant Biochemistry, University of Regensburg, Regensburg, Germany
(2) Institute of Experimental Medicine, Academy of Sciences, Prague, Czech RepublicC

Plasma membrane microdomains in *S. cerevisiae*. effect of de-energization on plasma membrane properties.

The plasma membrane of *S. cerevisiae* is subdivided into a large number of stable microdomains, hosting different membrane proteins (1). The membrane compartment of Can1 (MCC) has been studied in detail (2-4). It is associated with so-called eisosomes, a cytosolic structural element (5). MCCs are enriched in ergosterol (4) and together with eisosomal components constitute typical membrane invaginations (6). Functionally the MCC plays a role in homeostasis of sphingolipids (7), in the turnover of specific membrane proteins (3), and is involved in stress responses, for example in osmotic and mechanic stresses (8, 9), and glucose deprivation (10). In the latter case, mRNA degrading exo-ribonuclease, Xrn1, associates with eisosomes (10), where it does not degrade mRNA anymore (Vaskovicova et al, submitted). When the membrane potential of yeast cells is decreased by uncouplers, some proteins as well as ergosterol leave MCC and distribute homogenously (4). De-energization dramatically changes the properties of the plasma membrane: the amount of gel-like microdomains, which are sphingolipid enriched, reduces significantly, whereas the overall rigidity of the plasma membrane increases (11, 12). Most likely this is the reason for the highly increased resistance of de-energized cells towards detergents, polyene antimycotics and toxic peptides (12, 13).

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CONCURRENT SESSION ABSTRACTS
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Monday 4th April
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Louis Armand Ouest

ROBERTS Samantha (1)
CHANG Shyr-Shea (2), KUMAR Abhishek (3), OCCHIPINTI Patricia (1), SHROFF Hari (3), ROPER Marcus (2), GLADFELTER Amy (1)
(1) Department of Biological Sciences, Dartmouth, Lebanon, NH, USA
(2) Department of Mathematics and Biomathematics, University of California, Los Angeles, CA, USA
(3) Section on High Resolution Optical Imaging, National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, MD, USA

Clustered nuclei maintain autonomy and nucleo-cytoplasmic ratio control in as syncytium

Nuclei of some multinucleate cells, including the fungus *Ashbya gossypii*, have variable division times despite being bathed in the same cytoplasm, which could buffer variation from noisy gene expression or cytokinesis. How are nuclei sharing one cell autonomous? One possibility is that, within the seemingly continuous cytoplasm, regulators are restricted and the cytoplasmic composition is variable between nuclei. Consistent with this hypothesis, we found that transcripts for all genes examined are enriched close to the nuclear envelope. Additionally, we demonstrated that at any time only subsets of *A. gossypii* nuclei transcribe cyclin genes, and found transcripts are more concentrated near nuclei transcribing their gene. We next analyzed whether transcriptional activity was associated with cell cycle stage of asynchronous nuclei. Only G1 cyclin CLN3 transcription correlated with nuclear stage, and we demonstrated that the number of transcripts located near a nucleus is uncorrelated with cell cycle stage. However, we found that the spatial distribution of some cyclin transcripts changed throughout the cell cycle. It is intriguing that the concentration of these transcripts does not change over time, but their spatial distribution may be essential for cell cycle regulation in this syncytium. In *A. gossypii*, internuclear distance is tightly regulated. We therefore hypothesized that internuclear cytoplasmic regions are required for construction of independent nuclear territories and maintenance of nuclear autonomy. To test this hypothesis, we utilized an *A. gossypii* dynactin mutant, in which nuclei become clustered with limited regions of internuclear cytoplasm. Surprisingly, we found asynchronous division and transcriptional autonomy persists among clustered nuclei, indicating internuclear cytoplasmic buffer zones are nonessential for nuclear autonomy in this syncytium. We found that transcriptional activity correlated with position in a cluster and nuclei can move within a cluster. These data suggest that clustered nuclei dynamically respond to changes in their local environment. Quantitative analysis of mRNA spatial distributions revealed striking similarities between wild-type and mutant cells, despite a higher concentration of mRNA in the vicinity of a cluster. This further supports that the spatial distribution of transcripts plays a more important role in nuclear autonomy than transcript concentration. This work demonstrates that *A. gossypii* are transcriptionally autonomous, that clustered nuclei maintain autonomy, and provides insights into the role of cytoplasmic organization in generation of nuclear autonomy.

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Monday 4th April
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Louis Armand Ouest

MARTIN-URDIROZ Magdalena (1)
EGAN Martin J. (2), OSES-RUIZ Miriam (1), SOANES Darren (1), TALBOT Nicholas J. (1)
(1) School of Biosciences, University of Exeter, Geoffrey Pope Building, Exeter, UK
(2) Department of Cellular and Molecular Medicine, University of California San Diego, La Jolla, USA

Investigating the role of BAR domain proteins during plant infection by *Magnaporthe oryzae*

The filamentous fungus *Magnaporthe oryzae* causes blast disease on a wide variety of grasses, including rice. The infection cycle starts when a spore lands on the surface of a rice leaf where it germinates to form a polarised tube, which swells at its tip to develop a specialised infection cell called an appressorium. This cell switches from isotropic expansion to polarised growth at its base to generate a penetration peg to rupture the leaf cuticle and allow the fungus to colonise plant tissue. However, the mechanism that regulates actin polymerization required for the formation of this protrusion is unknown. We have observed that concomitant with these morphological changes, an asymmetrical distribution of phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) occurs at the plasma membrane, indicating that differences in membrane composition may be involved in appressorium morphogenesis and host invasion. The membrane deforming BAR domain family proteins generally bind to the negatively charged inner surface of the plasma membrane through PI(4,5)P2 and phosphatidylserine. In *M. oryzae*, seventeen BAR domain proteins have been identified with homology to proteins involved in endocytosis, endosomal protein sorting, karyogamy, actin cytoskeleton organization and the activation of the regulator of cell polarity, Cdc42. In this study, we have analysed the association of phosphoinositides, BAR domain proteins and Cdc42 with actin polymerization and penetration hypha formation during plant infection. Progress in understanding the role of BAR domain protein function and PI(4,5)P2 asymmetry will be presented.

CONCURRENT SESSION ABSTRACTS
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Monday 4th April
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Louis Armand Ouest

OLIVEIRA-GARCIA Ely (1)
VALENT Barbara (1)
(1) Kansas State University, Dept of Plant Pathology, Manhattan, Kansas, USA

Understanding plant cell effector uptake in rice-*Magnaporthe oryzae* interactions

Rice blast caused by *Magnaporthe oryzae*, a hemibiotroph and facultative pathogen, is the most destructive disease of rice worldwide. After host penetration, *M. oryzae* establishes a biotrophic interaction. It is assumed that different strategies employed by the fungus to avoid triggering defense responses, including masking of invading hyphae or active suppression of host defense mechanisms, are essential for a biotrophic parasitic lifestyle. During the infection process, *M. oryzae* secretes various effectors, which are hypothesized to be involved in effective host infection. Effectors are classified by their destinations in the interaction court, with apoplastic effectors residing in the extracellular plant compartment and cytoplasmic effectors translocating into the cytoplasm of living plant cells. Notably, cytoplasmic effectors of *M. oryzae* are associated with a specialized interfacial structure, the biotrophic interfacial complex (BIC). To date, little is known about the mechanisms of effector uptake into plant cells during fungal infection. Here we show translocation of the cytoplasmic effectors Bas1, Pwl1 and Pwl2 in vesicles from BICs to rice cytoplasm during biotrophic development. Using fluorescent protein tagging, we found that cytoplasmic effectors Bas1, Pwl1 and Pwl2 are sorted into different vesicles in BICs formed on primary hyphae (PH), revealing new levels of functional complexity for this biotrophic structure. In contrast, most of the vesicles from BICs on mature bulbous hyphae showed colocalization of the cytoplasmic effectors. Whereas BICs on primary hyphae deliver effectors in micro-vesicles, BICs on mature bulbous hypha deliver effectors in macro-vesicles, at times reaching diameter sizes over 3 µm. Furthermore, we demonstrate that Wortmannin, a potent endocytosis inhibitor, induces abnormally shapes and swollen BICs as well as the accumulation of cytoplasmic effectors in the BICs. Moreover, Wortamannin treatment induced the accumulation of the cytoplasmic effectors under all penetration pores, suggesting that effector uptake begins even before host penetration. Based on these results we conclude that cytoplasmic effector translocation is mediated by vesicle formation and transport, and may be characteristic of appressoria as well as biotrophic invasive hyphae. Our results also suggest a potential role of *M. oryzae* effectors for manipulation of the host cell endocytosis process.

CONCURRENT SESSION ABSTRACTS
CS2: Pathogenesis and symbiosis

Monday 4th April
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Gaston Berger

BROWN Alistair (1)
CHILDERS Delma (1), BALLOU Elizabeth (1), RAZIUNAITE Ingrida (1), MOL AVELAR Gabriela (1), BUDGE Susan (1), LENARDON Megan (1), MACCALLUM Donna (1), BROWN Gordon (1), GOW Neil (1),
(1) University of Aberdeen, Aberdeen, UK

Environmental adaptation in *Candida* during colonisation and infection

The major fungal pathogen, *Candida albicans*, can colonise a diverse range of niches in humans such as the GI tract, skin, mucosal surfaces, blood and internal organs. This flexibility is dependent upon robust metabolic and stress adaptation to the local microenvironments in these niches. In particular, *C. albicans* tunes its metabolism to the local carbon sources, which differ between niches. Two aspects of this metabolic adaptation will be discussed. First, carbon regulation in *C. albicans* has diverged from the model yeast, *S. cerevisiae*. In, *S. cerevisiae*, the catabolite inactivation of key central metabolic enzymes promotes the sequential utilisation of sugars then alternative carbon sources. In *C. albicans*, the evolutionary rewiring of ubiquitination targets in central carbon metabolism has relaxed this catabolite inactivation, thereby permitting the simultaneous utilisation of sugars and alternative carbon sources. This enhances the capacity of *C. albicans* to colonise the GI tract and to cause systemic infection. Second, carbon adaptation promotes the virulence of *C. albicans* in additional ways. It influences: (a) the expression of key virulence factors; (b) the susceptibility of *C. albicans* to environmental stresses; (c) antifungal drug resistance; and (d) the visibility of this pathogen to innate immune defences. Therefore, metabolic adaptation is tightly integrated with other cellular processes that influence *Candida*-host interactions and pathogenicity.

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Gaston Berger

KRETSCHMER Matthias *(1)*
CROLL Daniel (2), ROGIC Sanja (3), PAVLIDIS Paul (3), KRONSTAD James (1)
(1) Michael Smith Laboratories, The University of British Columbia, Vancouver/ BC, Canada
(2) Dep. of Environmental Systems Science, ETH Zürich, Zürich, Switzerland
(3) Centre for High-Throughput Biology, The University of British Columbia, Vancouver/ BC, Canada

Modulating host carbohydrate metabolism: A silver bullet for impairing *Ustilago maydis* infection?

The ability of biotrophic fungi to metabolically adapt to the host environment is a critical factor in plant diseases. We are investigating adaptation both in the pathogen and the host, and we previously demonstrated the importance of beta-oxidation in the disease caused by the corn smut *Ustilago maydis*. Here we used RNA-Seq to analyze the transcriptional response of *Zea mays* to infection by *U. maydis*. We identified key changes that reflect metabolic shifts. This analysis highlighted modifications in transcriptional regulation, sucrose/starch, amino acid and secondary metabolism, and chloroplast function during infection. We confirmed the relevance of these changes in virulence assays with selected maize lines with defects in the vegetative to reproductive transition, sucrose/starch metabolism and chloroplast function. Furthermore, a chemical genomic approach was used to impair specific host functions. Overall, these approaches revealed that specific regulatory and metabolic changes that influence the vegetative to reproductive transition and sucrose/starch metabolism contribute to resistance to infection. In contrast, loss of chloroplast function as well as inhibition of amino acid biosynthesis or secondary metabolism increased the disease severity. These functions therefore contribute to susceptibility to infection. Taken together, these studies reveal key aspects of metabolism that are critical for biotrophic adaptation during the maize-*U. maydis* interaction.

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Gaston Berger

JIN Hailing *(1)*
WANG Ming (1), WEIBERG Arne 3 (1), LIN Feng-Mao (2), HUANG Hsien-Da (2)
(1) Department of Plant Pathology and Microbiology, Center for Plant Cell Biology, Institute for Integrative Genome Biology, University of California, Riverside, USA
(2) Institute of Bioinformatics and Systems Biology, National Chiao Tung University, Taiwan

Cross-kingdom RNAi in plant - pathogen interactions

Small RNAs (sRNAs) are a class of short non-coding regulatory RNAs that are present in almost all the eukaryotes and mediate gene silencing in a sequence-specific manner. Studies from my lab and others have shown that plant endogenous sRNAs play a critical role in host immune responses against pathogen attacks. We have also demonstrated that some sRNAs from eukaryotic pathogens, such as *Botrytis cinerea*, the fungal pathogen that causes grey mold disease on more than 200 plant species, could be translocated into host plant cells. These sRNAs act as effector molecules to suppress host immunity genes for successful infection. This finding represented the first example of naturally occurring Cross-Kingdom RNAi during the host - pathogen interactions. Similar phenomenon was recently reported in mammalian system, where gastrointestinal nematodes also deliver sRNAs to mammalian cells and target host genes involved in innate immunity. Thus, Cross-Kingdom RNAi was used as an aggressive virulence mechanism by both plant and animal pathogens and pests. Such sRNA effectors are mainly produced from retrotransposons by *B. cinerea* Dicer-like (Bc-DCL) proteins. We found that expressing sRNAs that target Bc-DCL1 and Bc-DCL2 in *Arabidopsis* or *Solanum lycopersium* (tomato) silences Bc-DCL genes and attenuates fungal pathogenicity and growth. This suggests that sRNAs can move from the host plant to the fungus; thus, sRNA trafficking between *B. cinerea* and its hosts is bidirectional. We demonstrate that *Verticillum dahliae*, a fungal pathogen that causes wilt disease, also uses sRNA effectors for its virulence. Plants expressing sRNAs that target DCLs of *B. cinerea* and *V. dahliae*, show reduced susceptibility to both pathogens. Furthermore, *B. cinerea* could take up external sRNAs and dsRNAs from the environment. Applying Bc-DCL-targeting sRNAs or the double-stranded RNA (dsRNA) precursors on the surface of fruits, vegetables, or flowers significantly inhibits gray mold disease. These results demonstrate an effective RNAi-based strategy for controlling multiple diseases, which could potentially replace toxic fungicides and circumvents the need for transgenic plants.

CONCURRENT SESSION ABSTRACTS
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Monday 4th April
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Gaston Berger

NOTTENSTEINER Mathias (1)
ZECHMANN Bernd (2), HUECKELHOVEN Ralph (1)
(1) Technische Universitaet Muenchen, Muenchen, Germany
(2) University of Graz, Graz, Austria

ROPIP1 of the barley powdery mildew fungus is a retroelement-derived virulence effector targeting barley susceptibility factor RACB

Plants and their pathogens are engaged in a constant battle. Pathogens trigger susceptibility of their host cells by secretion of effector proteins. We identified ROP-Interacting Peptide 1 (ROPIP1) as an effector of a novel type in the interaction of barley (Hordeum vulgare L.) with *Blumeria graminis f.sp. hordei* (*Bgh*), the causal agent of powdery mildew disease. ROPIP1 was found to directly target the host susceptibility factor HvRACB in yeast and in planta. HvRACB is a small monomeric GTPase required for full susceptibility of barley against *Bgh* inter alia by influencing cytoskeleton dynamics. Transient over-expression of GFP-ROPIP1 together with RFP-HvMAGAP1, a microtubule-associated negative regulator of HvRACB activity, caused a significant decrease in cortical microtubule network stability. Both, over-expression and Host-induced gene silencing (HIGS) of ROPIP1 showed its potential to modulate the susceptibility level of barley epidermal cells towards *Bgh*. We hypothesize that presence of ROPIP1 in barley epidermal cells hinders deregulation of HvRACB activity by HvMAGAP1 which in turn might promote microtubule network loosening. Further, we provide evidence of a native ROPIP1 protein which is encoded by an active retroelement of *Bgh*.

CONCURRENT SESSION ABSTRACTS
CS2: Pathogenesis and symbiosis

Monday 4th April
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Gaston Berger

VENEAULT-FOURREY Claire (1)
DAGUERRE Yohann (1), PELLEGRIN Clément (1), ZHANG Feng (1), SCHELLENBERGER Romain (1), KOHLER Annegret (1), MARTIN Francis (1)
(1) Tree-Microbe Interactions, INRA, University of Lorraine, Labex ARBRE, Champenoux, France

Mutualistic ectomycorrhizal fungus: a delicate edge between saprotrophic and biotrophic plant-pathogenic fungi ?

In forest ecosystems, hyphae of ectomycorrhizal (ECM) fungi constitute a significant proportion of soil microbial biomass. Soil-borne hyphae interact with tree roots to form ectomycorrhizal symbiosis, providing N, P nutrients to the tree in return for photosynthetically-derived carbon from their hosts. While colonization of roots by ECM fungi is invasive leading to dramatic morphogenetic changes of roots and cell wall remodelling, weak plant defence responses are triggered. We showed that, similarly with plant-pathogenic microbes, the ectomycorrhizal fungus *Laccaria bicolor* use secreted effector proteins (carbohydrate-active enzyme CAZyme and Mycorrhiza-induced Small Secreted Protein MiSSPs) to establish mutualistic interaction. I will summarize our most recent findings concerning the role of several effectors in the symbiosis development. I will also discuss how the studies of effectors from ectomycorrhizal fungi change our view of the tenuous frontier between pathogenicity and mutualism.

CONCURRENT SESSION ABSTRACTS
CS2: Pathogenesis and symbiosis

Monday 4th April
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Gaston Berger

WAWRA Stephan (1)
FESEL Philipp (1), WIDMER Heidi (1), LESON Lisa (1), LANGEN Gregor (1), ZUCCARO Alga (1)
(1) University of Cologne, Cluster of Excellence on Plant Sciences (CEPLAS), Botanical Institute, Cologne, Germany

The novel fungal specific β-glucan binding lectin FGB1 alters susceptibility to cell wall stress and prevents glucan-triggered immunity in plants

beta-glucans are well-known modulators of the immune system in mammals, but little is known about beta-glucan triggered immunity in plants. We found that the secreted plant-induced protein FGB1 from *Piriformospora indica*, a novel type of beta-glucan binding lectin conserved among fungi, is able to increase the resistance of *P. indica* to cell wall stress and to efficiently suppress beta-glucan triggered immunity in different host plants. Our results highlight the importance of beta-glucan as a fungal MAMP and the necessity of mutualistic and pathogenic fungi to protect beta-glucan polymers from host recognition during colonization of different plants.

CONCURRENT SESSION ABSTRACTS
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Monday 4th April
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Gaston Berger

PAOLETTI Mathieu (1)
LAMACCHIA Marina (1), DYRKA Witold (2), KJELLBERG Matti (3), MATTJUS Peter (3), SHERMAN David (4), DURRENS Pascal (4), SAUPE Sven (1)
(1) CNRS, Bordeaux, France
(2) Wroclaw University of Technology, Wroclaw, Poland
(3) Abo University, Turku, Finland
(4) INRIA, Bordeaux, France

NOD-like receptors (NLR) and non self recognition in fungi : *Podospora* / *Serratia* interactions as a model for fungal innate immune response.

Innate immunity constitutes the first line of defense against pathogens. Eukaryotes rely on NOD-like Receptors (NLR) to initiate a proper innate immune response. NLR display tripartite organizations including a central Nucleotide and Oligomerization Domain (NOD), a C terminal ligand binding domain made of tandem repeat sequences, and a N terminal effecter domain activating a downstream reaction. While plant and animal NLR repertoires are built from a restricted number of architectures based on NB LRR receptors, fungal genomes code an enormous diversity of NLR made of the combinatorial assortment of at least 10 different N terminal domains, 3 NOD central domains and three C terminal repeat domains. The most frequently NLR receptors recognize pathogen indirectly by sensing alterations they provoke on host cell proteins to weaken the immune response, a model known as the guard model. Using the model *Podospora anserina*, we investigate the role of HET-E and HET-c that were identified in the context of vegetative incompatibility (VI, a non self recognition process between genetically different isolates of the same species) in a hetero-specific non self recognition mechanism in response to bacteria in the frame of the guard model. het-e encodes a NLR protein of the HNWD family, while het-c encodes a glycolipid transfer protein. Both genes are submitted to positive selection which can be explained in the context of an evolutionary arms race with a pathogen. This model implies that HET-c protein has a role in *P. anserina*"s response to pathogens, and HET-E senses alterations of HET-c. We have selected two bacterial species, *Serratia fonticola* and *S. marcescens*, that initiate different fungal responses both weakened in absence of HET-c. We investigate the role of HET-c / HET-E in the initiation and development of a response to these bacteria. HET-c is required for proper cell wall organization in response to bacteria. Different HET-c protein variants display variable sensitivity to degradation by a *Serratia* protease known as a virulence factor. These data are consistent with the guard model. We also analyzed the *P. anserina* transcriptomic response to both bacteria in comparison to the VI reaction. While the responses display significant overlap, specificities suggest the existence of a multilayered innate immune response to heterospecific non self in *P. anserina*.

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CS2: Pathogenesis and symbiosis

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18:20 - 18:40
Gaston Berger

KIND Sabine (1)
HINSCH Janine (1), GALUSZKA Petr (2), TUDZYNSKI Paul (1)
(1) Institute of Plant Biology and Biotechnology, Westphalian Wilhelms-University Münster, Muenster, Germany
(2) Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University , Olomouc, Czech Republic

Identification of the first fungal cytokinin biosynthetic de novo pathway and its relevance for virulence

The plant hormones cytokinins (CKs) are well-known to be involved in many aspects of plant development and growth. However, it is becoming more and more evident that CKs are not unique to plants but can be found in a wide range of organisms including plant pathogenic fungi, though here the biosynthetic mechanisms are unknown. We recently identified a de novo CK biosynthesis pathway in the biotrophic ascomycete *Claviceps purpurea*. Noticeably, in this fungus the isopentenyltransferase and the domain necessary for isopentenyladenine production are combined into a single bifunctional enzyme (CpIPT-LOG). In addition, this gene belongs to a small cluster with a CK specific P450 monooxygenase. However, the ∆cpipt-log mutant still contains iP, tZ and cZ CKs and is fully virulent on rye (Hinsch et al., 2015, Environ Microbiol 17: 2935-2951). We further investigated a conserved CK biosynthesis mechanism involving degradation of modified tRNAs. Deletion of the key gene cptRNA-IPT led to the complete loss of cZ-type CKs while iP and tZ formation was again only slightly altered. The double deletion mutant ∆∆cpipt-log/cptRNA-ipt does not produce any CKs proving that the de novo and the tRNA degradation pathways are the sole CK sources in *C. purpurea* with a redundancy for iP and tZ type CK formation. The drastically reduced virulence of this mutant shows the importance of CKs in the successful establishment of *C. purpurea*-rye interaction.

CONCURRENT SESSION ABSTRACTS
CS3: Environmental sensing, stress response

Monday 4th April
16:00 - 16:20
Louis Armand Est

KRONHOLM Ilkka
University of Jyväskylä, Jyväskylä, Finland

Contribution of epigenetic mechanisms to phenotypic plasticity in *Neurospora crassa*

Environment never stays constant throughout an organism's life. Physiological homeostasis must be maintained on the face of changing environments; many developmental transitions must be timed correctly using environmental cues. This phenomenon is called phenotypic plasticity. While we have made some progress in unraveling the genetic basis of phenotypic plasticity, much still remains unknown. The role of epigenetic mechanisms, or chromatin modifications, in particular is poorly known. We used the filamentous fungus *Neurospora crassa* to investigate the contribution of epigenetic mechanisms to phenotypic plasticity. We measured reaction norms of mutants that are deficient in different epigenetic mechanisms, such as DNA methylation, histone methylation, histone deacetylation, or RNA interference. We asked how do epigenetic mechanisms affect the shape of the reaction norms, are the effects specific to certain environments, and which epigenetic mechanisms are involved. We find that epigenetic mechanisms do affect phenotypic plasticity and these effects are mechanism and environment specific. In particular Histone 3 lysine 36 methylation is required for response to high temperatures, and histone 3 lysine 4 trimethylation is required for response to low pH. Temperature response is also affected by a histone demethylase and the RNA interference pathway. Shapes of the reaction norms are affected in several cases. Furthermore, have begun investigating whether transgenerational effects are present in *Neurospora* and whether epigenetics could be a mechanism that mediates those effects.

CONCURRENT SESSION ABSTRACTS
CS3: Environmental sensing, stress response

Monday 4th April
16:20 - 16:40
Louis Armand Est

SCHUMACHER Julia (1)
BRANDHOFF Beate (1), SIMON Adeline (2), COHRS Kim (1), VIAUD Muriel (2)
(1) IBBP, WWU Münster, Münster, Germany
(2) BIOGER, INRA, AgroParisTech, Thiverval-Grignon, France

Transcriptional responses to white light in *Botrytis cinerea* involve the GATA-transcription factors BcWCL1 and BcLTF1 and the MAP kinase BcSAK1

Botrytis cinerea is a necrotrophic plant pathogen that exhibits prominent light responses including the formation of the reproduction structures (photomorphogenesis), secondary metabolites/ pigments, and antioxidant enzymes. A complex regulatory network of photoreceptors, transcription factors (TFs) and chromatin modulates is supposed to initiate, transmit, and fine-tune the responses to different wavelengths of light on the transcriptional level that finally leads to the observable phenotypes. As the formation of the reproduction structures is strictly regulated by light in this fungus - conidia are formed in the light, sclerotia in the dark - the output can be easily monitored. The GATA-type TFs BcLTF1 and BcWCL1 are important regulators as their deletions resulted in conidiation in light and dark («always conidia») (Schumacher et al. 2014; PLoS Genet 10:e1004040, Canessa et al. 2013; PLoS One 8:e84223). Study of gene expression in both deletion mutants by microarray analyses highlighted the role of the TFs in mediating the transcriptional responses of the majority of light-responsive genes including a number of other transcriptional regulators. As the group of light-responsive genes also contained genes that are induced by oxidative and osmotic stress in a BcSAK1-dependent manner (Heller et al. 2012; MPMI 25:802-816), their expression levels were studied in the delta-Bcsak1 mutant. Indeed, light induction does not occur in the mutant background indicating that the MAP kinase cascade is another functional unit of the light regulatory network. More detailed expression analyses of chosen LTF-encoding genes showed that three major transcriptional profiles exist: genes that exhibit maximal expression after 15 min (early), 60 min (moderate) or 120 min (late) of light treatment. Expression levels of the majority of genes decrease after prolonged light exposure (photoadaptation); however, this is not observed for bcltf1. Eight LTFs have been functionally characterized by deletion and overexpression analyses so far: While BcLTF1 has various functions (growth in light, ROS homoeostasis, virulence, differentiation), other LTFs specifically regulate certain developmental stages such as the initiation of conidiophore development or conidiogenesis.

CONCURRENT SESSION ABSTRACTS
CS3: Environmental sensing, stress response

Monday 4th April
16:40 - 17:00
Louis Armand Est

BRAND Alexandra (1)
BEDEKOVIC Tina (1)
(1) University of Aberdeen, Aberdeen, UK

Protein complexes involved in hyphal steering in *Candida albicans*

The fungus, *Candida albicans*, lives as a harmless yeast in most humans but, in immunodeficient patients, it can become pathogenic by forming invasive hyphae that damage tissue and trigger an inflammatory immune response. The ability of growing hyphae to re-orient growth in response to external cues is essential for tissue penetration and depends on the activity of the Ras-like small GTPase, Rsr1/Bud1. In yeast, this protein is locked in position adjacent to bud scars but, in hyphae, Rsr1 is free to direct growth in response to tip-contact. We are investigating how contact is sensed and signalled through Rsr1. Tip contact induced a stable, asymmetric bias of polarity complexes towards the site of contact and this was required for re-orientation in response to obstacles and underlying gaps. Hyphae of the delta-rsr1 mutant, or strains where Rsr1 was GDP- or GTP-locked, were unresponsive to cues because they were unable to maintain this asymmetry. Examination of these strains and mutants lacking the regulators of Rsr1, the GEF Bud5 or the GAP Bud2, indicated that GDP-GTP cycling is important for Rsr1 localisation and hyphal contact-induced responses. The mutants were unable to maintain hyphal growth in the presence of 1 mM calcium, reverting to growth as yeast, suggesting that Rsr1 may be involved in the negative regulation of calcium influx. The failure of the delta-bud5 mutant to exhibit several Rsr1-dependent phenotypes suggested that there may be a second Rsr1 activator in *C. albicans*. Rsr1 appears to be pivotal in transducing external signals and its function is therefore more complicated in *C. albicans* than in the model yeast, *Saccharomyces cerevisiae*.

CONCURRENT SESSION ABSTRACTS
CS3: Environmental sensing, stress response

Monday 4th April
17:00 - 17:20
Louis Armand Est

ZEILINGER Susanne (1)
GRUBER Sabine (1), LICHIUS Alexander (1), RADEBNER Theresa (2), LABAJ Pawel (3), KREIL David (3),
(1) Institute of Microbiology, University of Innsbruck, Innsbruck, Austria
(2) Institute of Chemical Engineering, Vienna, Austria
(3) Chair of Bioinformatics, University of Natural Resources and Life Sciences, Vienna, Austria

Host sensing and attack in the mycoparasite *Trichoderma atroviride*: the 7-TM receptor Gpr1 and its putative interactor, a Sur7-family protein

The application of mycoparasitic fungi being able to antagonize fungal phytopathogens is a promising alternative to hazardous chemical fungicides in plant disease control and species of the fungal genus *Trichoderma* are among the most successful mycoparasites. The direct antagonism of phyto-pathogenic fungi by *Trichoderma* mycoparasites comprises sensing of the prey and chemotropic growth towards it followed by activation of «molecular weapons» such as cell wall-lytic enzymes, secondary metabolites, and infection structures. Consequently, the receptors and signaling pathways involved in sensing and responding to the prey fungus are of special interest. The *Trichoderma atroviride* Gpr1 7-TM receptor is implicated in the recognition of prey-derived signals as *gpr1*-mutants are unable to respond to the presence of a living prey fungus by expressing cell wall-degrading enzymes such as chitinases and proteases and to attach to prey hyphae. Transcriptome profiling revealed a set of genes involved in primary and secondary metabolic processes, stress response, defense and virulence to be targeted by Gpr1 upon prey recognition. Receptor-interacting proteins play important roles in regulating the amount of receptor on the cell surface and in localizing and anchoring the receptor to specific membrane compartments. A membrane-based yeast two-hybrid-based screen for Gpr1 interactors resulted in the identification of several candidates with functions related to protein targeting and sorting as well as plasma membrane and cell wall-associated proteins. Functional characterization of one of the identified interactors, a plasma membrane protein belonging to the fungal-specific Sur7 family, revealed a role in the regulation of conidiation, polar growth, and the mycoparasitic interaction with the prey fungus. Monitoring of fluorescently labelled versions of Sur7 and Gpr1 revealed largely overlapping localization patterns in the plasma membrane, secretory and endocytic vesicles as well as septa indicating co-localization and possible interaction.

CONCURRENT SESSION ABSTRACTS
CS3: Environmental sensing, stress response

Monday 4th April
17:20 - 17:40
Louis Armand Est

DI PIETRO Antonio
Department of Genetics, University of Córdoba, Cordoba, Spain

A stress response MAPK pathway mediates host signal sensing in *Fusarium oxysporum*

Soil-inhabiting fungal pathogens and symbionts use chemical stimuli from roots to locate the host plant. We found that directed hyphal growth of the vascular wilt fungus *Fusarium oxysporum* towards tomato roots is triggered by the catalytic activity of secreted class III peroxidases, a family of haem-containing enzymes present in all land plants. Unexpectedly, chemotropic sensing of peroxidase-generated signals requires conserved elements of the fungal cell wall integrity MAPK cascade, as well as the two seven-pass transmembrane proteins Ste2 and Ste3 which are functional homologues of the *Saccharomyces cerevisiae* sex pheromone receptors. In addition, the ROS-generating enzyme NADPH oxidase also contributes to this process. Our goal is to understand how conserved transmembrane receptors and cell wall stress signalling components cooperate in the directional response towards distinct types of chemoattractants such as peptide pheromones and plant peroxidases.

CONCURRENT SESSION ABSTRACTS
CS3: Environmental sensing, stress response

Monday 4th April
17:40 - 18:00
Louis Armand Est

SCHUMANN Marcel René (1)
OOSTLANDER Anne (1), WISSING Josef (2), JÄNSCH Lothar (2), FLEIßNER André (1)
(1) Institut für Genetik, Technische Universität Braunschweig, Braunschweig, Germany
(2) Zelluläre Proteomforschung, Helmholtz Zentrum für Infektionsforschung, Braunschweig, Germany

SIP-1 is essential for anastomosis formation in *Neurospora crassa*, probably by promoting cell fusion competence.

Cell fusion is an essential process for the growth and development of eukaryotic organism. In the ascomycete fungus *Neurospora crassa*, germinating conidia undergo chemotropic interaction and fuse to form a supracellular network, which further develops into the mycelial colony. Fusing germlings employ an unusual mode of communication. In a kind of «cell dialog», the two fusion partners coordinately switch between two physiological stages probably related to signal sending and receiving. On the molecular level, this unusual cellular behavior involves the alternating recruitment of the SO protein and the MAK-2 MAP kinase module to the germling tips. By co-immunoprecipitation and mass spectrometry, we identified SIP-1 as a new interaction partner of the SO protein. A deletion of the sip-1 gene results in a delta-so-like phenotype, including the inability to undergo chemotropic interactions and subsequent fusion. Live-cell imaging revealed that a GFP-SIP-1 construct is recruited to the tips of interacting germlings in the same oscillating fashion as SO. Surprisingly, in contrast to SO, SIP-1 also accumulates in the same oscillatory manner at the membrane of isolated, non-interacting germlings. The first membrane recruitment of SIP-1 is already observed in ungerminated spores at forming polarization side. This unexpected finding suggests that already individual germlings switch between two physiological stages while growing. These dynamics then become coordinated during cell-cell interactions. We hypothesize that SIP-1 is involved in gaining fusion competence during spore germination. Future characterization of SIP-1 functions will therefore further our understanding of fungal cell communication and the unique «cell dialog» mechanism.

CONCURRENT SESSION ABSTRACTS
CS3: Environmental sensing, stress response

Monday 4th April
18:00 - 18:20
Louis Armand Est

KÜNZLER Markus (1)
SCHMIEDER Stefanie S. (1), STANLEY Claire E. (2), VAN SWAAY Dirk (2), DEMELLO Andrew (2), AEBI Markus (1),
(1) Institute of Microbiology, Department of Biology, ETH Zürich, Zürich, Switzerland
(2) Institute for Chemical and Bioengineering, Department of Chemistry and Applied Biosciences, ETH Zürich, Zürich, Switzerland

The defense response of *Coprinopsis cinerea* against fungivorous nematodes reveals differentiation and communication among vegetative hyphae

Vegetative mycelia of multicellular fungi are syncytial networks of interconnected hyphae resulting from hyphal tip growth, branching and anastomosis. There is considerable knowledge about the functions of fungal mycelia as an entity but comparably little knowledge about functions at the level of single hyphae. As an example, fungal mycelia are known to be able to respond to abiotic or biotic stresses, such as a lack of nutrients or an attack by fungivores. However, such stresses often affect only part of the mycelium and little is known about the spatiotemporal regulation of the respective responses within the mycelial network. We used a tailor-made microfluidics device in combination with a fluorescent reporter gene to study the spatio-temporal regulation of defense effector gene expression in the basidiomycete *Coprinopsis cinerea* in response to fungivorous nematodes. Our results reveal an initial strong upregulation of defense effector gene expression in hyphal compartments that are under nematode attack. This response is then propagated in the mycelium in both acropetal and basipetal directions via specific hyphae. This propagation cannot be explained by the distribution of the fluorescent protein by cytoplasmic streaming. Thus, our system reveals a previously unrecognized type of hyphal differentiation and communication in mycelia of multicellular fungi. We are aiming to identify the signals and underlying signaling cascades responsible for the elicitation and propagation of the anti-nematode defense response in the mycelium of *C. cinerea*.

CONCURRENT SESSION ABSTRACTS
CS3: Environmental sensing, stress response

Monday 4th April
18:20 - 18:40
Louis Armand Est

BORMANN Joerg (1)
HEINZE Cornelia (1), MENTGES Michael (1), BROCKMANN Anke (1), ALDER Arne (1), BLUM Christine (1), HARTUNG Josephine (1), GLÖCKNER Annemarie (1), SCHÄFER Wilhelm (1)
(1) University of Hamburg, Biocenter Klein Flottbek, Department of Molecular Phytopathology, Hamburg, Germany

A novel virus response gene determines fitness in the cereal pathogen *Fusarium graminearum*

Virus replication in fungi, in general, either remains symptomless or results in an impaired fungal physiology. The underlying molecular cues are unknown. The mycovirus FgV-ch9 infects *Fusarium graminearum*, a devastating fungal pathogen of cereals with a world-wide distribution. Due to FgV-ch9 viral infection all aspects of the fungal life cycle are impaired: sexual and asexual propagation, vegetative growth, and virulence. Heterologous expression of a single FgV-ch9 structural protein in the fungus phenocopies virus infection. We identified a potentially mRNA-binding protein as the central facilitator of fungal response to virus infection and named it fungal immune response gene 1 (*FIR1*). Its transcription level is strongly reduced in the presence of the virus or the viral structural protein and deletion of *FIR1* causes phenotypes identical to virus infection. Constitutive overexpression of *FIR1* over-rules the devastating effects of viral infection leading to a symptomless replication of the virus. We conclude that this virus-fungus interaction follows a gene-for-gene interaction: *FIR1* expression is downregulated by the virus infection and, thereby, impairs all aspects of the fungal life cycle. This is the first description of a fungal immune response which counteracts virus propagation.

CONCURRENT SESSION ABSTRACTS
CS4: Regulatory networks

Tuesday 5th April
16:00 - 16:20
Louis Armand Ouest

BAHN Yong-Sun
(Department of Biotechnology, Center for Fungal Pathogenesis, Yonsei University, SEOUL, South Korea

Systematic Functional Profiling of Pathobiological Regulatory Networks in a Global Human Meningitis Fungal Pathogen

Cryptococcus neoformans causes life-threatening meningoencephalitis in humans, but the treatment of cryptococcosis remains challenging. To develop novel therapeutic targets and approaches, signaling cascades goveming pathogenicity of *C. neoformans* have been extensively studied but the underlying pathobiological regulatory circuits remain elusive. In this study, we constructed a high-quality library of more than 550 signature-tagged gene-deletion strains through homologous recombination methods for 155 putative transcription factor and 114 kinase genes and examined their in vitro and in vivo phenotypic traits under 32 distinct growth conditions. This high- coverage functional phenome analysis uncovered myriad of novel transcription factors and kinases, which play critical roles in growth, differentiation, stress responses, antifungal drug resistance, and virulence. Large-scale virulence and infectivity assays in insect and mouse host models identified 45 transcription factors and 50 kinases that are critical for pathogenicity. These pathogenicity-related transcription factors and kinases are involved in the following biological functions: growth and the cell cycle, nutrient metabolism, stress response and adaptation, cell signalling, cell polarity and morphology, vacuole trafficking, tRNA modification, and previously unknown functions. The genotypic and phenotypic data for each transcription factor and kinase are all publicly available in the *C. neoformans* transcription factor phenome database (http://tf.cryptococcus.org) and kinase phenome database (http://kinase.cryptococcus.org), respectively. In conclusion, our phenome-based functional analyses of the *C. neoformans* transcription factor and kinase mutant libraries provide key insights into regulatory networks of basidiomycetous fungi as well as an ubiquitous human fungal pathogen.

CONCURRENT SESSION ABSTRACTS
CS4: Regulatory networks

Tuesday 5th April
16:20 - 16:40
Louis Armand Ouest

SELLAM Adnane (1)
CHAILLOT Julien (1), MALLICK Jaideep (2), TEBBJI Faiza (1), TYERS Mike (2)
(1) Infectious Diseases Research Centre (CRI), Université Laval, Quebec, Canada; CHU de Québec Research Center (CHUQ), Université Laval, Quebec, Canada
(2) Institute for Research in Immunology and Cancer (IRIC), Université de Montréal, Montreal, Canada

Systematic identification of biological circuits that couple cell growth and division in the opportunistic yeast *Candida albicans*

The basis for commitment to cell division in late G1 phase, called Start in yeast and the Restriction Point in metazoans, is a critical but still poorly understood aspect of eukaryotic cell proliferation. All eukaryotic cells must grow to a critical cell size before commitment to division occurs. This size threshold couples cell growth to division and thereby establishes long-term size homeostasis. A comprehensive collection of *C. albicans* homozygous and heterozygous mutants (representing more than 60% and 96 % of the *C. albicans* ORFeome, respectively) was screened for cell size defect using a particle Z2-Coulter Counter sizer. This work establishes the first systematic characterization of the mechanisms underlying regulation of growth and division in a pathogenic fungus. We identified 195 mutants that exhibited a size defect compared to their parental strain including 104 small size and 91 large size mutants. Cross-species analysis of the C. albicans size phenome with those of the model yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* underlines a considerable degree of rewiring and evolutionary plasticity. Genetic epistasis and suppressive-dosage genetic interactions were used to order the cell size regulatory network in *C. albicans*. This comprehensive analysis revealed a complex network of novel regulator of Start and cell size. The most potent were the transcription factors Ahr1, Sfp1, Dot6 and the AGC Sch9 and the HOG MAPK kinase pathway. Additionally, we have used numerous genome-scale approaches including transcriptional profiling (Microarray), genome-wide occupancy mapping (ChIP-chip) and protein complex purification (TAP-affinity purification) to investigate the contribution of each regulator in coordinating growth and division in *C. albicans*. For instance, we found that the zinc finger transcription factor Ahr1 act as a negative regulator of Start in *C. albicans* by modulating protein production and amino acid metabolism. Our genetic epistasis experiments demonstrated that Ahr1 is an effector of the kinase Sch9 and that the two proteins physically interact. We also demonstrated that the p38/HOG MAPK pathway is a critical regulator of Start and acts as inhibitor of the G1-S transcriptional regulator complex SBF (Swi4-Swi6). Furthermore, we found that the HOG pathway controls ribosome biogenesis genes through the master transcriptional regulator, Sfp1. Other regulators of size homeostasis will be discussed and a comprehensive genetic connectivity with cell cycle and growth regulators will be shown. From an evolutionary perspective, our study also provided a framework to compare and understand how regulatory pathways that couple cell growth to cell division evolve especially in a context of a pathogenic fungus compared to saprophytic fungi.

CONCURRENT SESSION ABSTRACTS
CS4: Regulatory networks

Tuesday 5th April
16:40 - 17:00
Louis Armand Ouest

XU Jin-Rong (1)
HUIQUAN Liu (2)
(1) Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, USA
(2) Purdue-NWAFU Joint Research Center, State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, China

Genome-wide A-to-I RNA editing occurs specifically during sexual reproduction independent of ADAR enzymes

Yeasts and filamentous fungi do not have Adenosine Deaminase Acting on RNA (ADAR) orthologs and are believed to lack A-to-I RNA editing, which is the most prevalent editing of mRNA in animals. However, during the study of the PUK1 pseudo-kinase gene important for sexual reproduction in *Fusarium graminearum*, we found that two tandem stop codons UG^{1831}GUG^{1834} in its kinase domain were changed to UA^{1831}GUA^{1834}G by RNA editing in perithecia. To confirm A-to-I editing of PUK1 transcripts, strand-specific RNA-seq data were generated with RNA isolated from conidia, hyphae, and perithecia. PUK1 was almost specifically expressed in perithecia and 90% of its transcripts were edited to UG^{1831}GUG^{1834}G. Genome-wide analysis identified 26,056 perithecium-specific A-to-I editing sites. Unlike those in animals, 70.5% of A-to-I editing sites in *F. graminearum* occur in coding regions and over two-thirds of them result in amino acid changes, including editing of 69 PUK1-like pseudogenes with stop codons in ORFs. PUK1 orthologs and other pseudogenes also displayed stage-specific expression and editing in *Neurospora crassa* and *F. verticillioides*. Furthermore, *F. graminearum* differs from animals in the sequence-preference and structure-selectivity of A-to-I editing sites. Whereas As embedded in RNA stems are targeted by ADARs, RNA editing in *F. graminearum* preferentially targets As in hairpin loops, which is similar to the anticodon loop of tRNA targeted by Adenosine Deaminases Acting on tRNA (ADATs). Besides PUK1, a few genes with stage-specific editing events were functionally characterized for their functions in ascosporogenesis or ascospore release. Overall, our results showed that A-to-I editing occurs specifically during sexual reproduction and mainly in the coding regions in filamentous ascomycetes, involving adenosine deamination mechanisms distinct from metazoan ADARs.

CONCURRENT SESSION ABSTRACTS
CS4: Regulatory networks

Tuesday 5th April
17:00 - 17:20
Louis Armand Ouest

BRUNNER Michael (1)
CESBRON Francois (1), OEHLER Michael (1), HA Nati (1), SANCAR Gencer (1), LI Congxin (2), HÖFER Thomas (2)
(1) Heidelberg University Biochemistry Center, Heidelberg, Germany
(2) Heidelberg University Bioquant, Heidelberg, Germany

Dynamics of light-induced transcription in *Neurospora*

Genes are often transcribed in random bursts. We analyzed induction of frequency (frq) and vivid (vvd) transcription by the light-activatable White Collar Complex (WCC) of *Neurospora* to characterize and quantify transcriptional bursts. In the dark frq and vvd transcripts are present in < 0.5 copies per gene. Activation of WCC by a light pulse triggers a rather synchronized wave of transcription from the frq and vvd promoters, which adapts to dark levels after ~ 30 min. frq and vvd RNA accumulate transiently to 4 and 60 copies per gene, respectively. The light-induced transcription burst of the frq promoter is followed by an extended period of ~1 h during which the promoter is refractory towards restimulation. When challenged by a second light pulse, the newly activated WCC binds to refractory frq promoters and has the potential to recruit RNA polymerase II. However, accumulation of Pol II and phosphorylation of its C-terminal domain repeats at serine 5 is impaired. Quantitative analysis of vvd transcription and mathematical modeling suggest that the vvd promoter also exhibits transcriptional bursting and subsequent refractoriness. The data indicate that refractory promoters carry a physical (negative) memory of their previous history.

CONCURRENT SESSION ABSTRACTS
CS4: Regulatory networks

Tuesday 5th April
17:20 - 17:40
Louis Armand Ouest

FUDAL Isabelle (1)
SOYER Jessica (1), EL GHALID Mennat (1), GRANDAUBERT Jonathan (1), BALESDENT Marie-Hélène (1), CONNOLLY Lanelle R. (2), FREITAG Michael (2), ROUXEL Thierry (1)
(1) UMR BIOGER, INRA / AgroParisTech, Thiverval-Grignon, France
(2) Department of Biochemistry and Biophysics, Oregon State University, Corvallis, USA

Transcriptional and chromatin-based control of effector gene expression in the plant pathogenic fungus *Leptosphaeria maculans*

Plant pathogens secrete an arsenal of small secreted proteins (SSPs) acting as effectors that modulate host immunity to facilitate infection. In fungi, SSP-encoding genes are often located in particular genomic environments and show waves of concerted expression during plant infection. To date, little is known about the regulation of their expression. *Leptosphaeria maculans*, a fungus responsible for stem canker of oilseed rape, has a bipartite genome structure alternating gene-rich and transposable element (TE)-rich regions. TE-rich regions, which encompass one third of the genome, 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 in planta). On these bases, we investigated the determinism of the concerted effector gene expression testing two hypotheses: (i) are one or several common regulators involved in the control of the concerted expression of effector genes? and / or (ii) are TE-rich regions targets of reversible chromatin modifications that affect the regulation of effector gene expression? To identify putative regulators of effector gene expression, we established the repertoire of TFs of *L. maculans* and identified TFs only found in *L. maculans* genome or specifically induced during infection. We performed functional analyses on several TFs and showed that StuA plays a major role in infection and expression of effector genes in *L. maculans*. We also investigated the involvement of one histone modification, histone H3 lysine 9 tri-methylation (H3K9me3) in chromatin-based regulation of concerted effector gene expression. For this purpose, we silenced expression of two key players in heterochromatin assembly and maintenance, HP1 and Kmt1, by RNAi. Whole genome oligoarrays performed on silenced-HP1 and silenced-Kmt1 transformants revealed an over-expression of SSP-encoding genes in TE-rich regions during in vitro growth. That increase of expression was associated with a reduction of H3K9 tri-methylation at two SSP-encoding gene loci. These data strongly suggest that a chromatin-based control, mediated by HP1 and KMT1, represses the expression of at least some of the effector genes located in TE-rich regions during growth in axenic culture. Our hypothesis is that changes of lifestyle and a switch toward pathogenesis relieve chromatin-mediated repression, allowing a concerted expression of effector genes mediated by one / or several TF(s).

CONCURRENT SESSION ABSTRACTS
CS4: Regulatory networks

Tuesday 5th April
17:40 - 18:00
Louis Armand Ouest

WIRTH Sophia (1)
BROSKA Selina (1), AHRENS Lisa-Marija (1), KRAUSE Katrin (1), KUNERT Maritta (2), BOLAND Wilhelm (2), KOTHE Erika (1)
(1) Institute of Microbiology – Microbial Communication, Friedrich Schiller University, Jena, Germany
(2) Department of Bioorganic Chemistry, Max Planck Institute for Chemical Ecology, Jena, Germany

Sexual development affects volatile production of *Schizophyllum commune*

Understanding signal transduction pathways by heterotrimeric guanine-nucleotide binding protein (G-protein) signaling is critical for pheromone response in the basidomycete *Schizophyllum commune*. Regulators of G-protein signaling (RGS) are involved in the modulation of heterotrimetric G-protein signaling cascades and control mycelia growth, hydrophobicity and sexual development. G-proteins might be also an important control point for differential expression of fungal secondary metabolites. In *S. commune* a spontaneous mutations occurring in the RGS gene *thn1* is caused by transposon insertions. These mutants show a partial defect in mating, abnormal clamp formation and an absence of fruiting body development. The aerial mycelia formation is reduced and mutants show an easily wettable phenotype, which indicate that Thn1 regulates surface hydrophobicity. Deletion of *thn1* has a similar effect on vegetative growth, but the delta-thn1 deletion mutant was found to mate unilateral, suggesting the regulation of pheromone signaling by *thn1*. This is reflected in the volatilome. The chemical composition of volatiles was investigated using solid phase microextraction coupled with GC-MS. The wild-type was found to produce mainly esters, whereas transposon mutants and *thn1* deletion mutant emit a mixture of different sesquiterpenes, including Y-bisabolol as the main component. These findings reveal that synthesis of volatile organic compounds is controlled by Thn1. Sesquiterpenes have diverse biological functions, e.g. as autoinducers, in attraction of pollinators or as defense compounds. In bioassays, volatiles of sesquiterpene producing *S. commune* mutants inhibit the growth of various fungi. It was shown that the sesquiterpenes A-bisabolol and bisabolene contribute to the observed growth inhibition. In the genome of *S. commune* we identified 3 genes encoding terpene synthase-like enzymes. They are supposed to be organized in gene clusters with transporters and substrate modifying enzymes. Also transcriptome analysis indicates a regulation of genes involved in the synthesis of sesquiterpenes and suggests a genetic connection between pheromone signaling and secondary metabolism.

CONCURRENT SESSION ABSTRACTS
CS4: Regulatory networks

Tuesday 5th April
18:00 - 18:20
Louis Armand Ouest

BENZ J. Philipp
TU München - Wood Bioprocesses, Freising, Germany

Unraveling polysaccharide degradation signaling networks in *Neurospora crassa*

Due to their active role in biomass mineralization, fungi are an indispensable part of the global carbon cycling. In particular filamentous fungi are of great economic importance as sources of industrial enzymes such as for polysaccharide hydrolysis, but also as a cause for food spoilage and soft-rot decay of construction wood. However, both the rational engineering of filamentous fungi for improved plant cell wall deconstruction, as well as the development of novel wood-protection mechanisms, is hampered by incomplete knowledge of the underlying regulatory and metabolic networks. The filamentous ascomycete *Neurospora crassa* is ideally suited for such analysis since it is not only a well-known model system for eukaryotic cell biology and genetics, but also shows robust growth on lignocellulosic material. In the present work, we studied the interconnection between cellulose and mannan regulatory networks and utilization pathways and found that both signaling pathways compete with each other. Intriguingly, competition is taking place both at the level of inducer uptake and intracellularly. The obtained knowledge provide insights on the transcriptional regulation of cellulase genes allowing engineering of fungal strains for increased production of cellulases. Moreover, it also open up ways to identify antifungal targetsthat could lead to novel, and potentially less toxic, wood protection methods.

CONCURRENT SESSION ABSTRACTS
CS4: Regulatory networks

Tuesday 5th April
18:20 - 18:40
Louis Armand Ouest

PAKULA Tiina (1)
NGUYEN Elizabeth V. (2), IMANISHI Susumu Y. (2), HAAPANIEMI Pekka (2), AVINASH Yadav (2), SALOHEIMO Markku (1), CORTHALS Garry L. (2)
(1) VTT Technical Research Centre of Finland Ltd, P.O. Box 1000, FI-02044, VTT/Espoo, Finland
(2) Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, FI-205210, Turku, Finland

Quantitative site-specific phosphoproteomics of *Trichoderma reesei* signaling pathways upon induction of hydrolytic enzyme production

The filamentous fungus *Trichoderma reesei* is widely used for industrial production of secreted enzymes, including carbohydrate active enzymes, such as cellulases and hemicellulases. Production of many of the enzymes by *T. reesei* is influenced by the carbon source available as well as by a variety of environmental and physiological factors. We have applied phosphoproteome profiling to get information on the signaling pathways related to the carbon source dependent regulation of the hydrolase genes. For the analysis, *T. reesei* was first cultivated in the presence of a carbon source that is not considered to affect the expression of genes encoding cellulases or hemicellulases markedly, and then exposed either to sophorose or spent grain extract to induce cellulase and hemicellulase gene expression. In order to detect early responses related to carbon source sensing and resulting in the onset of induction, proteome-wide phosphorylation was investigated at the time points 0, 2, 5, and 10 minutes after addition of the inducing carbon sources. Specific changes at phosphorylation sites were investigated using a MS-based framework. The workflow involved sequential trypsin digestion, TiO2 enrichment, and MS analysis using a Q Exactive mass spectrometer. 1721 phosphorylation sites were identified and quantified. Investigation of the data revealed a complex signaling network activated upon induction involving components related to light-mediated cellulase induction, osmoregulation, and carbon sensing, as well as glycolysis. Differential phosphorylation of factors related to carbon storage, intracellular trafficking, cytoskeleton and cellulase gene regulation were also observed.

CONCURRENT SESSION ABSTRACTS
CS5: Applied genomics and biotechnology

Tuesday 5th April
16:00 - 16:20
Gaston Berger

MASTER Emma
Univeristy of Toronto, Toronto, Canada

New approaches to uncovering function from fungal genomes

The 1000 Fungal Genomes project is now underway, where initial genomic analyses will assess the occurrence of genes known to encode lignocellulose-active enzymes. However, transformative breakthroughs will depend on our ability to discover entirely new lignocellulotytic activities among by the typically 30-40% of genes in genomes that encode proteins with yet unknown function. Existing methods to uncover new lignocellulotytic enzyme function usually monitor solubilized products from models as well as real lignocellulose materials. While these methods benefit from high-throughput platforms, they can miss novel enzyme activities that modify insoluble lignocellulose surfaces or impact fibre accessibility. This presentation will describe three new methods that we have established to detect and quantify enzyme action on lignocellulose, including 1) methods in spectromicroscopy to characterize lignocellulose residues after protein treatment, 2) production of lignocellulose models for QCM-D and multi-well plate screens, and 3) a simple procedure to chemically label insoluble forms of oxidized cellulose and hemicellulose. In addition to providing an overview of each method, examples of new insights gained through their application will be given.

CONCURRENT SESSION ABSTRACTS
CS5: Applied genomics and biotechnology

Tuesday 5th April
16:20 - 16:40
Gaston Berger

COX Russell
Leibniz Universität Hannover, Hannover, Germany

Heterologous expression of complete fungal biosynthetic pathways

Many solutions have been proposed to solve the problem of fungal cryptic secondary metabolism clusters. These include over-expression of pathway specific regulators, 'global' secondary metabolism regulators, epigenetic modifications, co-fermentation with microbes and a lot of others, but none is systematic or universally applicable. The presentation will focus on the application of rapid gene cluster assembly in yeast and heterologous expression in *Aspergillus oryzae* to reveal the precise chemical steps of biosynthetic pathways, as well as the ability to reprogramme pathways for the production of new compounds.

CONCURRENT SESSION ABSTRACTS
CS5: Applied genomics and biotechnology

Tuesday 5th April
16:40 - 17:00
Gaston Berger

HENRISSAT Bernard
Centre National de la Recherche Scientifique (CNRS), Marseille, France

Carbohydrate-active enzymes in fungal genomes

Carbohydrates are crucial for most organisms as carbon sources or as signaling molecules, but also for cell wall synthesis, host pathogen interactions, energy storage. We term carbohydrate-active enzymes (CAZymes) the enzymes that assemble and breakdown glycoconjugates, oligoaccharides and carbohydrate polymers. As such CAZymes find widespread applications in the food and feed, textile, medical and biotechnology sectors. Unlike most other classes of enzymes which carry limited informative power, the peculiarities of carbohydrate-active enzymes -and of their substrates- turn these enzymes into extremely powerful probes to examine and explain the lifestyle of living organisms. Over the last few years we have explored the CAZyme content of hundreds of fungal genomes in an attempt to understand how evolution shapes the CAZyme profile of fungi and to discover novel enzymes for potential applications.

Acknowledgments: My current research is supported by grants from ANR, IDEX Aix-Marseille, the European Commission (ERA-NET FP7), the Novo-Nordisk Foundation and the European Research Council.

CONCURRENT SESSION ABSTRACTS
CS5: Applied genomics and biotechnology

Tuesday 5th April
17:00 - 17:20
Gaston Berger

ALFARO Manuel (1)
CASTANERA Raúl (1), OGUIZA Jose Antonio (1), RAMÍREZ Lucía (1), PISABARRO Antonio G. (1)
(1) Universidad Pública de Navarra, Pamplona, Spain

Comparative, transcriptional and proteomic analysis of the secretome in the lignocellulose degrading basidiomycete *Pleurotus ostreatus*

Fungi interact with their environment by means of secreted proteins to obtain nutrients, elicit responses and modify their surroundings. Consequently, lifestyle influences the set of fungal secreted proteins [1]. To test this hypothesis, we performed a combined bioinformatics, transcriptomics and proteomics study of the secretome of the basidiomycete *Pleurotus ostreatus* and explored the conservation of these secreted proteins across the Basidiomycota. We identified bioinformatically the set of secretable proteins in two monokaryotic strains (haplotypes) of the white-rot basidiomycete *P. ostreatus* (PC9 and PC15). Then, we performed two RNA-seq analyses to study the relationship between the functional profile of the predicted secretome and the expression level of each group. Finally, we used the set of proteins secreted by P. ostreatus as a query to search for similar proteins in the fungal genomes released in JGI Mycocosm [2]. 538 and 554 protein models were predicted to be secreted (4.41% and 4.77% of PC9 and PC15 gene models, respectively). The functional annotation of these proteins revealed the unknown (37.2%), glycosyl hydrolases (26.5%) and red-ox enzymes (11.54%) as the main functional groups, in a similar distribution for the two strains. The expression level of these groups further enhances the relevance of the unknown group and was significantly different in the two strains (revealing different responses to the same environment). Furthermore, the presence of similar proteins to *P. ostreatus* secreted proteins in other basidiomycetes was used to cluster them into groups coherent with their particular lifestyles rather than with their corresponding phylogenetic positions. Finally, the use of a shot gun proteomics technique to deepen in the analysis of the proteins involved, in addition to the analysis of the computationally predicted secretome, have enabled us to compare the enzymatic profile between the monokaryons PC9 and PC15 and the dikaryon N001 and provide valuable insight into how white rot fungi degrades lignocellulosic biomass.

1. Alfaro M, Oguiza JA, Ramírez L, Pisabarro AG: Comparative analysis of secretomes in basidiomycete fungi. J Proteomics 2014, 102:28-43.
2. Grigoriev I V, Nikitin R, Haridas S, Kuo A, Ohm R, et al. MycoCosm portal: gearing up for 1000 fungal genomes. Nucleic Acids Res 2014; 42:699-704.

CONCURRENT SESSION ABSTRACTS
CS5: Applied genomics and biotechnology

Tuesday 5th April
17:20 - 17:40
Gaston Berger

DRUZHININA Irina (1)
ATANASOVA Lea (1), CHENTHAMARA Komal (1), GRUJIC Marica (1), HENRISSAT Bernard (2), ZHANG Jian (4), SHEN Qirong (4), GRIGORIEV Igor (3), KUBICEK Christian (1)
(1) Research Area Biotechnology and Microbiology, Institute of Chemical Engineering, TU Wien, Vienna, Austria
(2) CNRS & Aix-Marseille Université, Marseille, France
(3) Joined Genome Institute, Walnut Creeks, USA
(4) Nanjing Agricultural University, Nanjing, China

Horizontal gene transfers drove the mycoparasite *Trichoderma* to adapt to saprotrophy and cellulase production

A plethora of high cellulases and hemicellulase producing mutant strains of the fungus *Trichoderma reesei* (Hypocreales, Ascomycota) is now used as the major source of such enzymes production for biorefinery platforms and in diversity of other biotechnological industries. Several other *Trichoderma spp.* are being developed as excellent producers of these and other enzymes. Our phylogenomic analysis of Hypocreales fungi based on 100 neutrally evolving orthologous proteins revealed that the genus *Trichoderma* evolved from an entomopathogenic ancestor to become a versatile mycotroph and an efficient environmental opportunist capable of saprotrophic nutrition on decaying plant biomass and soil. Since the carnivorous ancestors have only a very small repertoire of plant cell wall degrading enzymes (CWDE), the origin of such enzymes in *Trichoderma* is unclear. To investigate this, we mined the genomes inventory of nine species of *Trichoderma* (*T. reesei, T. parareesei, T. longibrachiatum, T. citrinoviride, T. harzianum, T. guizhouense, T. virens, T. atroviride and T. asperellum*) for CWDEs, classified them in glycosyl hydrolase (GH) categories and assessed the evolution of each GH family. We found that the resulting 31 GH families comprised 83 CWDEs, of which more than two third were obtained by independent horizontal gene transfers (HGT) from other fungi, in majority Eurotiales such as *Penicillium*. Approximately half of these transfers occurred already in an early ancestor species, whereas the other half was obtained by specific species from *Trichoderma* clades or even by a single species. The genes appeared to have been obtained individually rather than as clusters because there was no synteny in these loci between *Trichoderma* and donor organisms. We also show that *Trichoderma* is capable to parasitize the majority of these donor fungi, and conclude that *Trichoderma*s superior saprotrophic abilities were powered by the gain of the CWDEs by HGT from host fungi that it could parasitize.

CONCURRENT SESSION ABSTRACTS
CS5: Applied genomics and biotechnology

Tuesday 5th April
17:40 - 18:00
Gaston Berger

VESTH Tammi (1)

NYBO Jane L. (1), THEOBALD Sebastian (1), DE VRIES Ronald P. (4), GRIGORIEV Igor V. (3), BAKER Scott E. (2), ANDERSEN Mikael R. (1)
(1) Department of Systems Biology, Technical University of Denmark, Lyngby, Denmark
(2) Joint Bioenergy Institute, Berkeley, CA, USA, Berkeley, CA, USA
(3) Joint Genome Institute, Walnut Creek, CA, USA, Walnut Creek, CA, USA
(4) Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands, Utrecht, The Netherlands

aspMine - online comparative analysis of species from the *Aspergillus* genus

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

CONCURRENT SESSION ABSTRACTS
CS5: Applied genomics and biotechnology

Tuesday 5th April
18:00 - 18:20
Gaston Berger

SHELEST Ekaterina (1)
WOLF Thomas (1), NATH Neetika (1), SHELEST Vladimir (1)
(1) Leibniz Institute for Natural Product Research and Infection Biology, Hans Knoell Institute (HKI), Jena, Germany

CASSIS, a method for promoter-based prediction of secondary metabolite gene clusters in eukaryotic genomes

Secondary metabolites (SM) are structurally diverse natural products of high pharmaceutical importance. Genes involved in their biosynthesis are often organized in clusters, i. e., are co-localized and co-expressed. Although some clusters in selected species are already well investigated, regulatory and biochemical details for the great majority of them remain unknown. In silico cluster prediction in eukaryotic genomes remains problematic mainly due to the high variability of the clusters content and the lack of other distinguishing sequence features. Tools to predict anchor genes and gene clusters in silico already exist but they have a rather low specificity and are often based on prior knowledge from known clusters, which makes problematic the discovery of novel features. We present CASSIS, a method for SM cluster prediction in eukaryotic genomes, and SMIPS, a tool for genome-wide detection of SM key enzymes («anchor» genes): polyketide synthases, non-ribosomal peptide synthetases, and dimethylallyl tryptophan synthases. Unlike tools based on protein similarity, CASSIS exploits the idea of co-regulation of the cluster genes, which assumes the existence of common regulatory patterns in the cluster promoters. The method searches for «islands» of enriched cluster-specific motifs in the vicinity of anchor genes. A series of cross-validation experiments showed high sensitivity and specificity of the method. Moreover, the specificity of CASSIS is superior to prominent similarity-base methods such as antiSMASH. SMIPS is a small tool based on functional protein domain assignments provided by the InterPro database. SMIPS searches the InterProScan output for typical SM anchor gene domains, than analyses and depicts the protein domain arrangement of various anchor gene types. We will present the results of the CASSIS application to prediction of the SM clusters in various asco- and basidiomycete species. The information about the motifs shared by the cluster genes has its own value, as it can be used for the further research of regulatory patterns, evolution and mechanisms of cluster regulation. As an example of such further research, we will show the results of comparative clusters motif study of 20 Aspergillus species. User-friendly online versions of both SMIPS and CASSIS (the «CASSIS suite») are available at https://sbi.hkijena.de/cassis. The suite also provides a comfortable workflow to run CASSIS on the results of SMIPS.

CONCURRENT SESSION ABSTRACTS
CS5: Applied genomics and biotechnology

Tuesday 5th April
18:20 - 18:40
Gaston Berger

VAN PEIJ Noël (1)
VERWAAL Rene (1), KROES Wouter (1), ARENDSEN Yvonne (1), VONDERVOORT Peter (1), PEL Herman (1)
(1) DSM Biotechnology Center, Delft, The Netherlands

Industrial strain construction: Improving the toolbox for faster and more efficient strain engineering

For the industrial production of enzymes at DSM, we use fungal microorganisms such as *Aspergillus niger* and *Kluyveromyces lactis*. These hosts have a long history of safe use. For a new enzyme product, a new production host needs to be developed to produce the protein of interest. The field of molecular and engineering biology is developing rapidly. More tools become available to design strains in a rationalized way, build them in a faster way and/or increase the throughput of testing strains. A number of tools developed will be described such as advanced transformation and cloning methods and a Cre-Lox based method for marker removal. We will present an overview of recently developed tools and how they can be used in improved, rationalized and faster strain construction with a higher success rate.

CONCURRENT SESSION ABSTRACTS
CS6: Ecological and population genomics

Tuesday 5th April
16:00 - 16:20
Louis Armand Est

GLADIEUX Pierre (1)
RAVEL Sébastien (2), RIEUX Adrien (3), FOURNIER Elisabeth (1), THARREAU Didier (2)
(1) INRA, UMR BGPI, Montpellier, France
(2) CIRAD, UMR BGPI, Montpellier, France
(3) CIRAD, UMR PVBMT, Saint Pierre de la Réunion, France

Population genomics of endemic and pandemic lineages of the rice blast fungus

Population genomic structure represents a key observable feature of pathogen lineage emergence, divergence and spread, and population genetic models fitted to genome-wide data can be used as a baseline against which to identify the genomic features and molecular mechanisms involved in pathogen adaptation or reproductive isolation. The rice blast fungus *Magnaporthe oryzae* is the most damaging rice pathogen, and a textbook example of widely distributed, rapidly adapting pathogen, despite limited genetic diversity. The aim of our study was to elucidate the factors and evolutionary changes underlying the emergence, diversification and spread of *M. oryzae*. Analyses of population structure based on single-read Illumina resequencing of 48 isolates identified four pandemic lineages of which three were highly clonal, and one recombining, as previously reported using microsatellite multilocus typing. We also found two lineages endemic to China, represented by only a few individuals in our dataset. Because recombination is limited in this system, we could use a phylogenetic approach to date the emergence and global dispersal of *M. oryzae*. The sequenced isolates were collected between 1973 and 2009, which allowed us to calibrate tree nodes using dated tips. Our analysis provided an estimate of ~10.000 years before present for the split between the population infecting rice from those infecting *Setaria* millet, corresponding to the oldest archaeological evidence for human exploitation of rice. We also found that the six lineages of *M. oryzae* diverged almost simultaneously ~2.000 years ago, which might correspond to the initial spread of rice cultivation outside Asia. Phylogenomic analyses revealed discordant genealogies among chromosomes, suggesting incomplete lineage sorting associated with rapid diversification. Analyses of the distribution of lineage-specific variants revealed possible exchanges of genomic fragments among clonal lineages, suggesting that hyphal fusion and genetic exchange between mycelia may play a role in increasing the genome diversity of asexual rice blast populations. Our work provides a population-level genomic framework for defining molecular markers to assist in the control of rice blast and for investigating the molecular underpinnings of phenotypic and fitness differences between divergent lineages.

CONCURRENT SESSION ABSTRACTS
CS6: Ecological and population genomics

Tuesday 5th April
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Louis Armand Est

CORRADI Nicolas (1)
ROPARS Jeanne (1), SĘDZIELEWSKA-TORO Kinga (2), NOEL Jessica (1), KRUGER Manuela (1), BRACHMANN Andreas (2)
(1) University of Ottawa, Ottawa, Canada (2) LMU Munich, Martinsried, Germany

Genome and Mating-type organization in a model arbuscular mycorrhizal fungus

Sexual reproduction is ubiquitous among eukaryotes, while fully asexual lineages are extremely rare. Prominent among these putative asexual lineages are the arbuscular mycorrhizal fungi (AMF), an ancient group of plant symbionts with a cytoplasm containing hundreds of co-existing nuclei. The existence of divergence among nuclei was first proposed to drive the evolutionary success of AMF in the absence of sex. However, this hypothesis has been contradicted by recent genome analyses that failed to find significant genetic diversity within these organisms. Here, we set to resolve issues surrounding the genome organization and sexual potential of AMF by exploring the genomes of five isolates of Rhizophagus irregularis, a model AMF. We find that genetic diversity in this species varies among isolates and is structured in a mono- dikaryon-like manner usually linked with the existence of a sexual life cycle. We also identify a putative AMF mating-type (MAT) locus, containing two genes with structural and evolutionary similarities with the MAT locus of some Dikarya. Our analyses suggest that this MAT locus may be bipolar, multi-allelic and that AMF could be heterothallic. These findings provide long awaited insights into the genome organization of these ubiquitous plant symbionts, and are of high relevance for understanding the evolution of sex in fungi and other eukaryotes.

CONCURRENT SESSION ABSTRACTS
CS6: Ecological and population genomics

Tuesday 5th April
16:40 - 17:00
Louis Armand Est

GIRAUD Tatiana (1)
BADOUIN Hélène (1), GLADIEUX Pierre (2), GOUZY Jérôme (3), SIGUENZA Sophie (3), AGUILETA Gabriela (1), SNIRC Alodie (1), LE PRIEUR Stéphanie (1), BRANCA Antoine (1)
(1) Ecologie, Systematique et Evolution, Universite Paris Sud, CNRS, AgroParisTEch, Orsay, France
(2) INRA, UMR BGPI, Montpellier, France
(3) INRA Toulouse, Toulouse, France

Species: Microbotryum

Identifying candidate effectors using population genomics and by detecting selective sweeps in the plant pathogenic anther-smut fungi

Unravelling the genomic processes underlying adaptation are important goals in biology. There is still little data to evaluate these questions beyond a few model species. Here, we sequenced 53 genomes of two *Microbotryum* anther-smut fungi. We found no traces of introgression along the genomes of these two sympatric sister species despite lack of premating isolation, indicating strong selection against hybrids. Polymorphism was negatively correlated with levels of linkage disequilibrium along the genome, as expected in cases of recurrent selective sweep or background selection. Within each species we identified several genomic regions with footprints of selective sweeps, some including genes up-regulated in planta and whose putative functions make them good candidates for being effectors. The selective sweeps appeared scattered along genomes and in different locations in the two species. The genes upregulated in planta accumulated more non-synonymous substitutions than other genes, supporting the view that many of them are likely effectors. Our findings may thus finally provide clues on the genes involved in plant - pathogen interaction, that have remained elusive in this otherwise well-studied system. This study shows that selective sweeps are widespread in natural plant pathogens, contributing to a broader picture of the occurrence and frequency of selection in natural populations and illustrating the usefulness of genome scans for detecting adaptive events.

CONCURRENT SESSION ABSTRACTS
CS6: Ecological and population genomics

Tuesday 5th April
17:00 - 17:20
Louis Armand Est

BRANCO Sara (1)
TAYLOR John, W. (2), BRUNS Thomas, D. (2)
(1) Ecologie, Systematique et Evolution, Université Paris Sud, CNRS, AgroParisTech, Orsay, France
(2) University of California, Berkeley, USA

Continental-level population differentiation and environmental adaptation in *Suillus brevipes*

Little is currently known about the drivers of fungal population differentiation and subsequent divergence of species, particularly in symbiotic fungi. Here we investigate the population structure and environmental adaptation in *Suillus brevipes*, a wind-dispersed ectomycorrhizal fungus associated with pine trees. We re-sequenced the whole genomes of 55 individuals from pine forests from across North America, including two sites in Alberta (Canada), coastal and montane California, Colorado, Minnesota and Wyoming. We detected high levels of genetic variation within the species and a complex population structure. *S. brevipes* populations were highly differentiated. However Alberta and Wyoming populations encompassed very high genetic variation and along with Colorado population appear to form a genetic continuum across the Rocky Mountains. Interestingly, we found evidence of admixture between the two Alberta populations. An in-depth analysis of the Californian populations revealed no gene flow and highly differentiated genomic regions, most notably a region containing a gene homologous to a membrane Na+/H+ exchanger known for enhancing salt tolerance in plants and yeast. These results are consistent with a very recent split between the Californian montane and coastal *S. brevipes* populations, with few small genomic regions under positive selection and a pattern of dispersal and/or establishment limitation.

CONCURRENT SESSION ABSTRACTS
CS6: Ecological and population genomics

Tuesday 5th April
17:20 - 17:40
Louis Armand Est

LEMAIRE Christophe
IHRS, Université d'Angers-INRA-ACO UMR 1345, Angers, France

Did domestication of apple tree promoted speciation of its fungal pathogen, *Venturia inaequalis*?

Domestication is a process by which an organism adapts to new environments created by human (1). By recurrent reproduction and selection cycles, cultivated organisms have experienced dramatic phenotypic and genotypic changes in short times (from hundreds to thousands generations). While domestication of many plants and animals is well studied, the impact of such rapid evolution on the pathogens from wild to cultivated hosts remains largely unknown. Indeed, does the rapid evolution of host drive the evolution of pathogen toward another adaptive optimum? Under this so-called host-tracking scenario one expect dramatically highest rates of genetic sweeps in the population infecting domestic hosts than in ones found in non-agricultural environments, reflecting the mirroring adaptive process in pathogens. However, as domesticated species have often expanded their range far from their wild ancestors, divergence followed by secondary contacts may also occur and make difficult the identification of genes really involved in adaptation to agro-ecosystems (i.e. by genome scans). Here we present a population genomics study of the impact of domestication of apple tree on the agent of the apple scab, the ascomycete *Venturia inaequalis*. Apple scab, the main disease of apple trees, was present on wild ancestors of apple trees (*Malus sieversii*) before domestication (i.e 7000 years ago) in Central Asia (2). Previous studies showed that populations of *Venturia inaequalis* sampled of moutain forests of Kazakhstan were both genetically and phenotipically differents from those sampled in plains on both wild and domestic apple trees (Malus x domestica) (2,3). We re-sequenced the genomes of 40 kazakh strains : 20 sampled in non-agricultural moutain regions and 20 from anthropized environments in plains. Using a reference genome, we mapped the polymorphisms on about 10,500 coding sequences. The scenario of recent (less than 100 years) secondary contact with heterogeneous gene flow among loci -reflecting genetic barriers of reproductive isolation- was found to be the best (4). Both genomic and geographic cline analyses were performed at the 181 most diagnostic non-synonymous SNPs using samples located along a gradient between wild and agricultural habitats. Our results show that it is possible to disentangle genetic barriers to gene flow caused by local adaptations from incompatibilities (hybrid depression) revealed by secondary contacts. These approaches revealed that i) a low level of introgression at these loci, despite sympatry between the two types of strains and ii) that direction of introgression is biased toward the wild type, indicating an expansion of agricultural strains into the wild populations.

1- Glemin & Bataillon, New Phytologist 2009
2- Gladieux et al., Plos One 2008
3- Lê Van et al., Evol. Applications 2012
4- Roux et al., Mol. Biol. Evol. 2012

CONCURRENT SESSION ABSTRACTS
CS6: Ecological and population genomics

Tuesday 5th April
17:40 - 18:00
Louis Armand Est

HANN-SODEN Christopher (1)
MONTOYA Liliam (1), LIACHKO Ivan (2), SULLIVAN Shawn (3), TAYLOR John (1)
(1) University of California, Berkeley, CA, USA
(2) University of Washington, Seattle, WA, USA
(3) Phase Genomics Inc., Seattle, WA, USA

Sympatric Speciation by an Evolutionary Ratchet

Transitions to self-mating compatibility can drive speciation by reducing recombination between populations. Within the genus *Neurospora*, the majority of known species are self-compatible, and selfing has evolved approximately nine separate times. We hypothesize that rapid and irreversible transitions to selfing lifestyles are driving diversification within *Neurospora*. While previous studies have shown that selfing lineages accumulate point mutations at a higher rate, the consequences of selfing on genomic rearrangements have not been studied due to historical limitations on the ability to infer synteny. By using the latest next generation sequencing technologies to produce nearly complete genomes of self-compatible and self-incompatible *Neurospora* species, we are able to quantify the rate of inversions along selfing and non-selfing lineages. We find that selfing lineages have a higher rate of inversion events. Furthermore, we observe abundant translocations leading to a lack of syteny between *Neurospora* species, in contrast to the mesosynteny observed between *N. crassa* and it's sister group, *Podospora*. In addition to the previously documented relaxation of selection on point mutations, our findings indicate that selfing leads to a relaxation of selection on genome architecture, with potentially more profound and rapid effects on population structure. We hypothesize a positive feedback cycle between selfing and rearrangements that drives divergence between nascent self-compatible strains and their parent populations.

CONCURRENT SESSION ABSTRACTS
CS6: Ecological and population genomics

Tuesday 5th April
18:00 - 18:20
Louis Armand Est

GABALDON Toni
Centre for Genomic Regulation, Barcelona, Spain

Impacts of genomic hybridization in fungi

Hybridization creates opportunities for adaptive evolution and can cause immediate speciation, but also creates genomic challenges to overcome. Indeed, hybridization results in the combination of two diverged genomes, which are subsequently shaped by processes of recombination, deletion, and other genomic rearrangements. Genomics have recently paved the way to investigate the stochastic and adaptive processes that follow genomic hybridization. As compared to metazoans or plants, fungi have lower prezygotic barriers and can reproduce clonally for long periods of time, thus hybridization is thought to have a large impact in the evolution of this clade. Consistently, the presence of hybrids in fungi have been extensively documented and an increasing number of cases are being described in the literature. However we still lack a full understanding of how common and relevant has been this process in mediating the origin of new lineages and adaptation to new niches in fungi. Here, I will provide an overview of recent results from my group that showcase the study of ancient and recent hybridization events in fungi, and the follow up of the genomic aftermath of these hybridizations. Examples covered include the origin of the yeast whole genome duplication in *Saccharomyces* lineage, and the emergence of novel pathogenic lineages in the *Candida parapsilosis* clade.

CONCURRENT SESSION ABSTRACTS
CS6: Ecological and population genomics

Tuesday 5th April
18:20 - 18:40
Louis Armand Est

VILGALYS Rytas (1)
LIAO Hui Ling (1), BONITO Gregory (2), HAMEED Khalid (1), GRIGORIEV Igor (4), SCHADT Christopher (3), LABBE Jessy (3), TUSKAN Gerald (3), TSCHAPLINSKI Timothy (3), MARTIN Francis (5)
(1) Duke University, Durham, USA
(2) Michigan State University, East Lansing, MI, USA
(3) Oak Ridge National Laboratories, Oak Ridge, TN, USA
(4) Joint Genome Institute, Walnut Creek, CA, USA
(5) INRA, IAM, Nancy, France

Metagenomic study of plant-fungal interactions between *Populus trichocarpa* with its rhizosphere fungi

A highly diverse community of fungi occurs within roots of forest trees that includes beneficial (mycorrhizal) mutualists, saprophytes, pathogens, and other guilds whose function is unknown. Several species of endophytic fungi have been found to have a growth-promoting effect on *Populus* and other plant hosts, though the molecular bases of these effects still are not fully understood. Genome sequencing and targeted metatranscriptome experiments are being undertaken for a key set of *Populus* root-associated fungi to aid in the understanding of mechanisms involved in plant-fungal interactions. We interrogated the ecological function of two fungal species *Mortierella elongata* PMI93 and *Ilyonectria europeae* PMI82 using metagenomic approaches including RNAseq, metabolomics, and proteomics. *P. trichocarpa* plants were grown from cuttings and inoculated using forest soils (soil bioassay) and also individually inoculated with either PMI93 or PMI82. After 3 months growth, comparative metatranscriptomics reveal that both PMI93 and PMI82 are both most active in the rhizosphere (rhizophytic activites) versus the root systems (endophytic activities). Gene profiles of *P. trichocarpa* roots showed significant changes in response to inoculation with *M. elongata* PMI93 but not to *Ilyonectri*a PMI82. Inoculation with *M. elongata* was found to alter plant-environmental interaction and cell signaling, altering the predominant plant genes for nutrient uptake, extracellular secreted proteins and plasma membrane receptor activities, receptor/kinase transduction followed by transcriptional regulation. Up regulation of genes for specific nutrient hydrolysis/uptake and growth promotion, and down regulation of genes for LRR-receptors/kinases, strongly suggest that *M. elongata* manipulates plant defense to equilibrate the energy resource for plant growth. The functional activity of *Mortierella* and *Ilyonectria* in the root and soil systems will also be discussed.

CONCURRENT SESSION ABSTRACTS
CS7: Metabolism and physiology

Wednesday 6th April
16:00 - 16:20
Gaston Berger

WILSON Richard
University of Nebraska, Lincoln, USA

Nutrient sensing and metabolic responses governing rice cell invasion and proliferation by the blast fungus *Magnaporthe oryzae*

Blast, mediated by the ascomycete *Magnaporthe oryzae*, is the most serious disease of cultivated rice and a global food security threat that annually results in a 10-30 % worldwide rice yield losses. *M. oryzae* is also emerging as a major pathogen of wheat. During rice infection, dome-shaped, pressurized fungal cells called appressoria are formed from germinating spores on the nutrient-free surface of the rice leaf in order to access the underlying epidermal cells. Fungal growth in living rice cells, called biotrophy, then occurs for the first days of infection without activating the robust plant defenses that normally work to keep the host plant disease-free. Despite the fundamental importance of this biotrophic growth stage to crop health, little is known about how plant defense suppression and fungal growth is integrated during host cell colonization, or what signaling and metabolic changes occur when the fungus undergoes a transition from appressorialife style on the host surface to an invasive hyphal growth in rice cells. We have recently described how glucose metabolism, sirtuin- and Tps1-mediated antioxidation, and activated TOR signaling facilitate growth in rice cells, while inactivated TOR is critical during appressorium formation. Here, we will discuss our current work on understanding the molecular pathways and metabolic strategies underpinning disease progression by the blast fungus. Our results define key and exploitable differences in the metabolic requirements of *M. oryzae* compared to host rice cells that could be applicable to a wide-range of important fungi with similar lifestyles.

CONCURRENT SESSION ABSTRACTS
CS7: Metabolism and physiology

Wednesday 6th April
16:20 - 16:40
Gaston Berger

LASTOVETSKY Olga *(1)*
GASPAR Maria L. (2), HENRY Susan (2), PAWLOWSKA Teresa (3)
(1) Graduate Field of Microbiology, Cornell University, Ithaca, NY, USA
(2) Department of Molecular Biology and Genetic, s Cornell University, Ithaca, NY, USA
(3) School of Integrative Plant Science, Cornell University, Ithaca, NY, USA

Central roles of lipid metabolism in an intimate fungal-bacterial interaction

Fungal-bacterial interactions are abundant in nature and many of them are recognized as important in human health, agriculture and food production. Despite their abundance and economic impact, very little is known about the underlying molecular mechanism(s) of such interactions. We employed the association between the fungus *Rhizopus microsporus* and its bacterial endosymbiont *Burkholderia rhizoxinica* as a model for the study of molecular mechanisms governing fungal-bacterial interactions. Through the use of RNA-seq, we identified a set of bacterial and fungal genes important for the establishment of this symbiosis. Analysis of fungal transcriptomes revealed the overexpression of many genes involved in lipid biosynthesis and metabolism. Based on the transcriptomic data we were able to reconstruct the lipid-related pathways that were activated in the host fungi during the early stages of symbiosis establishment. It appeared that both de novo phospholipid biosynthesis and the breakdown of triacylglycerol (TAG) were initiated upon physical interaction with endosymbionts. Phosphatidic acid (PA) is a central intermediate in both of these pathways and is known to possess important signaling function in eukaryotes. PA can be made de novo from fatty acids and glycerol 3-phosphate or through the activity of diacylglycerol kinase (DAGK) or phospholipase D (PLD) enzymes. We identified two DAGK and two *PLD* genes overexpressed during the fungal-bacterial interaction and thus hypothesized that PA plays an important role in the fungal-bacterial symbiosis establishment. Using chemical inhibitors, we determined that it was the specific activity of DAGK, but not PLD, that was required for the compatible interaction between host fungi and their endosymbionts. DAGK catalyzes the phosphorylation of diacylglycerol (DAG) to PA. Because DAG is derived from the breakdown of TAG, we searched for differences in lipid droplet morphology between symbiont-free (cured using antibiotics) and symbiont-containing fungi. We found no difference in lipid droplet morphology between symbiont-containing and symbiont-free fungi, therefore the importance of DAGK activity in symbiosis establishment is instead likely tied to the production of the signaling molecule PA. Together our results highlight lipid metabolism at the center of fungal-bacterial symbiosis establishment and support the importance of DAGKs in mediating this compatible interaction.

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Wednesday 6th April
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Gaston Berger

DIALLINAS George
Faculty of Biology, University of Athens, Athens, Greece

Genetics and the structure of the UapA transporter of *Aspergillus nidulans* reveal the functional importance of gating elements and homodimerization

The uric acid-xanthine/H+ symporter UapA of *Aspergillus nidulans* is one of the most extensively studied eukaryotic transporters at the level of regulation of expression, membrane trafficking, endocytic turnover and mostly structure-function relationships concerning substrate specificity (Diallinas, Front Pharmacol. 2014 5:207). Genetic modifications of UapA through random or rationally designed mutations that enlarge its substrate specificity, allow transport of other purines and purine analogues. Interestingly, most of these mutations map outside the presumed substrate binding site and were thus proposed to define putative channel-like gates. The precise molecular basis of how gating elements and in particular Arg481, located distant from the substrate translocation trajectory, function, remained unclear. Here I will present present the crystal structure, at 3.6 Å, of a genetically stabilized version of UapA in complex with xanthine, which fully rationalizes the genetic data and lead to a better understanding of transport function via a substrate elevator mechanism. Most importantly, the UapA structure, together with functional assays, supports, for the first time, that dimerization is critical for the proper functioning of a solute transporter.

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Gaston Berger

FEKETE Erzsebet (1)
ÁG Norbert (1), FLIPPHI Michel (1), KARAFFA Levente (1), CERQUEIRA Gustavo (2), SCAZZOCCHIO Claudio (3)
(1) Department of Biochemical Engineering, University of Debrecen, Debrecen, Hungary
(2) Institute of Harvard and MIT, Cambridge, Massachusetts, USA
(3) Imperial College, London, UK and Institut de Biologie Intégrative de la Cellule (I2BC), Orsay, France

Spliceosomal twin introns: their evolution and possible relevance to post-transcriptional regulation

Spliceosomal twin introns («stwintrons») are unconventional intervening sequences in nuclear transcripts where a standard «internal» U2 intron interrupts a canonical splicing motif of a second, «external» U2 intron. The generation of a full-length ORF thus necessitates two successive U2 splicing reactions. The stwintron hypothesis predicts in each case a specific splicing intermediate. We have demonstrated experimentally the proposed intermediates and thus the presence of internal introns in donor and/or acceptor sequences of external introns for transcripts of *Fusarium verticillioides*, *Trichoderma reesei* (Sordariomycetes), *Helminthosporium solani* (Dothideomycetes) and *Aspergillus nidulans* (Eurotiomycetes). In one instance, we have shown that the excision of an internal intron from a split external intron donor and an alternative splicing pathway, which yields a nonsensical RNA are mutually exclusive alternatives. This may be at the root of a post-transcriptional regulatory mechanism, akin to intron retention where the resulting faulty RNA would be degraded by nonsense-mediated mRNA decay. In two examples of experimentally confirmed stwintrons, which position is conserved in entire classes of Pezizomycotina, the cognate phylogenies suggest that intron/exon structure displays a remarkable evolutionary plasticity, where either the internal intron or indeed the whole stwintron can be gained or lost within related clades.

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Gaston Berger

BRAKHAGE Axel (1,2)
(1) Department Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany
(2) Institute for Microbiology, Friedrich Schiller University of Jena, Jena, Germany

Bacterial reprogramming of fungi by epigenetic manipulation leads to activation of silent gene clusters

The dramatic increase of multi-resistant bacteria has triggered an intense search for new antibiotics. Most of these compounds are low-molecular-weight natural products, which are important for intercellular communication. Genome mining efforts indicate that the capability of fungi to produce natural products has been substantially underestimated. Many of their biosynthesis gene clusters are silent under standard cultivation conditions (1). By genetic engineering, we could activate such silent gene clusters, which led to the production of novel compounds (2,3). Furthermore, we have discovered that communication between microorganisms represents a physiological trigger for activation of such silent fungal gene clusters (4). The physical interaction of the fungus *Aspergillus nidulans* with the soil-dwelling bacterium *Streptomyces rapamycinicus*, led to the selective activation of silent gene clusters (4). This reprogramming of the fungus by the bacterium requires the histone acetylase GcnE of *A. nidulans*, which is part of the Saga/Ada complex. GcnE was shown to specifically increase the K14 and K9 acetylation of histone 3 associated with genes belonging to the orsellinic acid biosynthesis gene clusters after co-incubation with *S. rapamycinicus* (5, 6). Knowledge of these regulatory interactions will pave the way to a novel avenue to drug discovery through targeted activation of silent gene clusters.

(1) Brakhage (2013) Nature Rev Microbiol
(2) Scherlach et al. (2011) Angew Chem Int Ed
(3) Macheleidt et al. (2015) Mol Microbiol
(4) Schroeckh et al. (2009) PNAS
(5) Nützmann et al. (2011) PNAS
(6) Nützmann et al. (2013) Appl Environ Microb

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STRAUSS Joseph (1)
GACEK-MATTHEWS Agnieszka (1), BERGER Harald (1), SASAKI Takahiko (2), WITTSTEIN Kathrin (3), GRUBER Clemens (1), LEWIS Zack (2)
(1) Fungal Genetics and Genomics Unit, Department of Applied Genetics, BOKU University, Vienna, Austria
(2) Department of Microbiology, University of Georgia, Athens, GA, USA
(3) Helmholtz Centre for Infection Research, Department Microbial Drugs, Braunschweig, Germany

KdmB, an *Aspergillus* histone H3K4 demethylase, is a genome-wide chromatin regulator and activator of secondary metabolism

Histone posttranslational modifications (HPTMs) are involved in chromatin-based regulation of fungal secondary metabolite biosynthesis (SMB) in which the corresponding genes, usually physically linked in co-regulated clusters, are silenced under conditions of active growth (primary metabolism) and get activated when environmental conditions are suitable and nutrients are depleted (secondary metabolism). The exact molecular mechanisms of the silencing and activation cycles by these epigenetic events, however, are not fully understood. Here we show by a combined approach of quantitative mass spectrometry (LC-MS/MS), genome-wide chromatin immunoprecipitation (Chip-seq) and transcriptional network analysis (RNA-seq) that the core regions of silent *A. nidulans* SMB clusters generally carry low levels of all tested chromatin modifications and that heterochromatic marks form distinguished peaks flanking most of these SM clusters. Activating histone marks such as trimethylation of lysines K4 and K36 or acetylation of K9 and K14 in histone H3 are established upon activation of the clusters. We investigated the role of a Jarid1 family H3K4 demethylase with a Jumonji-domain, termed KdmB, in this silencing process and the influence of H3K4 trimethylation dynamics on transcription of these genes during primary and secondary metabolism growth conditions. We found that KdmB is an active demethylase *in vitro* and *in vivo* that is necessary for the removal of activating marks and transcriptional repression of around 1750 genes under both conditions. Surprisingly, KdmB also mediates transcriptional activation of around 1850 genes directly or indirectly. Under the control of this coactivator, we found a striking enrichment in genes involved in secondary metabolite biosynthesis. Taken together, this study extends our general knowledge about Jumonji-domain containing histone demethylases and about the role of H3K4me3 and chromatin-based regulation of SMB cluster gene silencing and activation in fungi.

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JANEVSKA Slavica (1)
STUDT Lena (1), NIEHAUS Eva-Maria (1), BURKHARDT Immo (2), ARNDT Birgit (3), SIEBER Christian M.k. (4), HUMPF Hans-Ulrich (3), DICKSCHAT Jeroen S. (2), TUDZYNSKI Bettina (1)
(1) Institute of Plant Biology and Biotechnology, University of Münster, Münster, Germany
(2) Kekulé Institute of Organic Chemistry and Biochemistry, University of Bonn, Bonn, Germany
(3) Institute of Food Chemistry, University of Münster, Münster, Germany
(4) Lawrence Berkeley National Lab, DOE Joint Genome Institute, Walnut Creek/CA, USA

Two separate key enzymes and two pathway-specific transcription factors are involved in fusaric acid biosynthesis in *Fusarium fujikuroi*

It has been known for decades that the rice pathogen *Fusarium fujikuroi* and closely related *Fusarium* spp. produce the harmful mycotoxin fusaric acid (FSA) as well as two derived compounds 9,10-dehydrofusaric acid and 10-hydroxyfusaric acid (fusarinolic acid). Recently, we characterized a small gene cluster of five co-regulated genes, FUB1-FUB5, all involved in FSA biosynthesis. Among these genes, *FUB1* encodes a key enzyme, a polyketide synthase (PKS) [1]. However, the biosynthetic steps leading to FSA as well as the origin of the nitrogen atom, that is incorporated into the polyketide backbone, remained unknown. Through overexpression of *FfSGE1*, encoding a global regulator of secondary metabolism in *F. fujikuroi* [2], we were able to identify seven additional FSA biosynthesis genes, *FUB6-FUB12*, that belong to a new cluster separated from *FUB1-FUB5* cluster by three unrelated genes. While the previously published FSA gene cluster did not harbor a pathway-specific transcription factor (TF) gene, the extended cluster encodes two fungal-specific Zn(II)2Cys6 TFs. Cluster gene expression and product formation was shown to be completely dependent on one of these TFs, Fub10. In contrast, the second TF, Fub12, regulates the conversion of FSA into its two derivatives, dehydrofusaric acid and fusarinolic acid. This derivatization is mediated by FSA cluster-independent P450 monooxygenases and serves as a FSA detoxification mechanism for the fungus. In addition, the cluster-specific major facilitator superfamily transporter Fub11 was shown to be essential for survival at high FSA levels, representing a second mode of fungal self-protection against FSA. Concerning the biosynthesis of FSA, the second half of the gene cluster encodes a non-canonical non-ribosomal peptide synthetase (NRPS), Fub8, which acts as second key enzyme besides the PKS Fub1. Finally, a combination of bioinformatics, molecular and chemical approaches enabled the reconstitution of a putative FSA biosynthetic pathway. We suggest that the NRPS Fub8 activates an aspartate amino acid precursor, while the PKS Fub1 provides the triketide trans-2-hexenal as underlined by feeding experiments. Thus, the interplay of two separate key enzymes in FSA biosynthesis represents a unique way to produce a fungal PKS-NRPS hybrid compound.

[1] E.M. Niehaus et al. (2014) Appl. Microbiol. Biotechnol., 98, 1749-1762.
[2] C.B. Michielse et al. (2015) Environ. Microbiol., 17, 2690-2708.

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PORQUIER Antoine *(1) (2)*
MORGANT Guillaume (1), DALMAIS Bérengère (1), SIMON Adeline (1), VIAUD Muriel (1)
(1) UMR BIOGER, INRA, AgroParisTech, Université Paris-Saclay, Thiverval Grignon, France
(2) Université Paris Sud, 15 avenue Georges Clémenceau, Orsay, France

Specific regulation of Botrydial and Botcinic acid gene clusters in *Botrytis cinerea*

Botrytis cinerea is responsible for the gray mold disease on more than 200 plant species. Among the virulence factors identified in this Ascomycete are two non-host specific toxins (and their derivatives): botrydial (BOT, a sesquiterpene) and botcinic acid (BOA, a polyketide). The genes responsible for their biosynthesis are clustered in AT-rich subtelomeric regions of chromosome 1 (*BOA* genes) and chromosome 12 (*BOT* genes). Previous studies pointed out their co-regulation by conserved signalling pathways as well as by global transcription factors (TFs) like the calcineurin-dependent BcCrz1. A strong link between secondary metabolism gene expression and light-dependent development has been showed in *B. cinerea*, notably by the characterization of Velvet complex members BcVel1 and BcLae1. Global regulation data are available for *BcBOT* and *BcBOA* genes, but their direct regulators have not been characterized so far. A TF encoding gene present within a secondary metabolism cluster usually specifically regulates genes of this cluster. Amongst *BcBOA* genes, *BcBOA13* is predicted to encode a Zn(II)2Cys6 TF. In addition, a new version of *B. cinerea* genome annotation allowed adding new putative *BcBOT* genes to this cluster, including a gene predicted to encode a Zn(II)2Cys6 TF (*BcBOT6*). In our study, we functionally characterized *BcBOT6* and *BcBOA13*. Our hypothesis is that these TFs are specific regulators of their respective cluster. The data accumulated so far through the generation of deletion mutants and the analysis of gene expression by RT-qPCR support this hypothesis. In order to investigate the putative direct interaction between BcBot6 and BcBoa13 with promoters of genes from *BcBOT* and *BcBOA* culsters, a Yeast One Hybrid strategy was carried out. We searched for regulators of *BcBOT6* and *BcBOA13* to make the link between these TFs and known regulatory pathways. In parallel, we tested the role of epigenetic modifications in the transcriptional control of these clusterss. We generated deletion mutants of the orthologs of chromatin modifiers known to affect secondary metabolism gens expression in fungi (Hp1, Dim-5 and Kmt6). The expression profiles of *BcBOT* and *BcBOA* genes will be assessed in these mutants. Altogether, these results give a more precise view of the regulatory network controlling the expression of Bc*BOT* and Bc*BOA* gene clusters in *B. cinerea*.

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Louis Armand Est

RUYTINX Joske (1)
CONINX Laura (1), NGUYEN Hoai (1), MARTINOVA Veronika (1), COLPAERT Jan (1)
(1) Hasselt University, Centre for Environmental Sciences, Diepenbeek, Belgium

Mechanisms of metal homeostasis and adaptation in the ectomycorrhizal fungus, *Suillus luteus*

By natural selection, the ectomycorrhizal fungus *Suillus luteus* has evolved to metal tolerant ecotypes on severely metal contaminated sites. These metal tolerant ecotypes are very effective in protecting their host plant from metal toxicity. It is believed that adaptive metal tolerance is due to a modification in general mechanisms of cellular metal homeostasis. Membrane transporters are key players in the control of cellular metal concentrations and homeostasis. By comparative genetics, we identified several putative metal transporters in *S. luteus*. For some of them, their cellular localization and putative role in metal homeostasis was confirmed by heterologous expression in yeast. Gene expression in response to different concentrations of essential trace metals Zn and Cu and, non-essential Cd was determined in metal sensitive and tolerant *S. luteus* ecotypes. An ER localized P-type ATPase, SlHMA1 is highly expressed in response to elevated Cu and Cd concentrations. A plasma-membrane localized ZIP importer, SlZRT1, is highly expressed in response to limited Zn concentrations. Furthermore, Zn tolerant *S. luteus* ecotypes differ for the expression of the CDF-family transporter encoding gene, *SlZnT2*. Indeed, *SlZnT2* is constitutively highly expressed in Zn tolerant ecotypes whereas in Zn sensitive ecotypes its expression is very low. The high *SlZnT2* expression seems to be partly associated with an increase in gene number, and with differences in cis-regulation. Indeed, most of the Zn tolerant ecotypes have more than one copy of the *SlZnT2* gene, while sensitive ecotypes always have a single copy of this gene. We could distinguish three different genotypes for its promotor sequence. One of these genotypes was exclusively found in tolerant ecotypes. Since, most Zn tolerant ecotypes of *S. luteus* are Zn excluders, further work is needed to show how SlZnT2 contributes to cellular Zn export and host plant protection.

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Louis Armand Est

DAGHINO Stefania (1)
MURAT Claude (2), DE MITA Stéphane (2), MARTINO Elena (1), MARTIN Francis (2), PEROTTO Silvia (1)
(1) Department of Life Sciences and Systems Biology, University of Torino, Torino, Italy
(2) Tree Microbe Interaction, INRA, Lorraine University, Champenoux, France

Phenotypic and genomic adaptation of the ericoid fungus *Oidiodendron maius* to heavy metals

Oidiodendron maius is a symbiotic Ascomycete that associates with Ericaceae to form ericoid mycorrhiza (ERM). ERM greatly increases metal tolerance of the host plant in soils enriched in heavy metals, a property likely due to the fungal partner. Previous experiments on heavy-metal tolerant and sensitive isolates of *O. maius* suggested, for a single locus, a higher genetic diversity in fungal isolates from heavily polluted sites than sensitive isolates (Vallino et al. 2011 FEMS Microbiol Ecol 75: 321). The aim of our studies was to further explore DNA polymorphisms in the genome of 18 heavy-metal tolerant and sensitive isolates of *O. maius*. When tested *in vitro* for their growth in presence of different metals (Cd, Cr, Ni and Zn), we found a significant phenotypic diversity in these *O. maius* isolates, eight being sensitive and ten tolerant on at least one metal tested. The genome of these 18 *O. maius* isolates was sequenced with Illumina technology and the genomic reads were aligned onto the available reference genome of a metal tolerant *O. maius* isolate (*O. maius* Zn, Kohler et al. 2015 Nature Genetics 47: 410). Alignment with stringent parameters led to an average coverage of 62%. When the 18 genomes were compared to the reference, a total number of 1.874.886 Single Nucleotide Polymorphism (SNPs) were identified. A neighbor-joining tree built using these SNPs showed that two heavy-metal tolerant isolates were very similar to the reference O. maius Zn isolate, whereas the other isolates separated into two other groups, tolerant and sensitive. Phylogenetic analyses of two genes known to be involved in the heavy metal response of *O. maius Zn* (OmSOD1 and OmZnt1) produced a clustering similar to the one obtained with all the SNPs. Of the total SNPs, 51% were distributed in the coding sequences and, among them, 36% were non-synonymous. In order to further investigate the possible occurrence of genomic adaptation to metal pollution in *O. maius*, we calculated a few diversity indices. The Tajima's D identified 12 genomic windows and 137 genes under positive selection (D2). Fst values revealed 7.002 genes with Fst >0.25, including four genes already known to be involved in the response of *O. maius* Zn to metals (i.e. *OmZnT1, OmFCR1, OmFET* and *OmMLH3*). These preliminary results, that compare phenotypic and genomic diversity of *O. maius* isolates, suggest genomic adaptation of heavy-metal tolerant ERM fungi.

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MOREL-ROUHIER Mélanie (1)
SORMANI Rodnay (1), MATHIEU Yann (1), MEUX Edgar (1), ANAK NGADIN Andrew (1), THUILLIER Anne (1), RORET Thomas (2), SAIAG Fanny (1), DIDIERJEAN Claude (2), GELHAYE Eric (1)
(1) Lorraine University, INRA, Nancy, France
(2) CRM2, Lorraine University, Nancy, France

Evolution of detoxification systems in lignolytic fungi

Wood decaying fungi have developed unique features allowing them to use complex carbon sources. The adaptation of these organisms to their life style is due at least in part of the existence of extracellular degrading systems. Besides these extensively studied extracellular systems of degradation, wood decaying (and more globally complex organic matter degrading) fungi possess also extended detoxification systems in comparison with other fungi belonging to other trophic types, allowing them to cope with the myriad of toxic molecules released during wood degradation. A comparative genomic approach revealed indeed the presence of an extended multigenic family encoding glutathione transferases (GSTs) in the genomes of wood decayers. This extension concerns at least four classes of GSTs (omega, FuA, Ure2p and GTT), depending on the species. During the last few years, we have focused our research on the biochemical and structural characterization of various GSTs from *Phanerochaete chrysosporium*. These studies have revealed that the activity of these enzymes could be split off into two functional groups with opposite catalyzing reactions, namely: glutathionylation and deglutathionylation. Moreover, they are able to interact with molecules present in wood from different tree species, such as flavonoids, terpenes or resulting from the wood biodegradation. Some of these fungal GSTs are also structurally related to the bacterial GSTs involved in lignin degradation. In these bacterial systems, some GSTs exhibit an activity of Y-O-4 ether cleavage through a glutathionylation activity, whereas other isoforms are involved in the recycling of glutathione through an activity of deglutathionylation. It is thus obvious that extracellular wood degradation and intracellular detoxification are closely linked systems enabling lignolytic fungi to adapt and survive to their hostile environment.

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Louis Armand Est

HAWKINS Nichola (1)
FRAAIJE Bart (1)
(1) Biological Chemistry and Crop Protection, Rothamsted Research, RRES, Harpenden, UK

Mapping the adaptive landscape of azole resistance in *Zymoseptoria tritici*

Resistance to single site inhibitor fungicides often evolves due to single point mutations in the target site encoding gene, such as those resulting in the amino acid substitutions G143A in cytochrome b for the QoIs, or E198A in beta-tubulin for the MBCs. However, in the case of the azole fungicides, individual point mutations in the target site encoding gene *CYP51* confer only quantitative reductions in azole sensitivity, with multiple mutations accumulating to confer higher levels of resistance. The accumulation of resistance through multiple mutations is often referred to as additive. However, there is evidence of extensive epistasis between *CYP51* mutations, in both fungicide resistance and enzyme function. This produces a rugged adaptive landscape, making evolutionary pathways under azole selection less predictable. Furthermore, cross-resistance between different fungicides within the azole class is incomplete, meaning different genotypes are likely to be selected by different fungicides, and so selection pressures change over time as different azoles are used in the field. In the wheat pathogen *Zymoseptoria tritici*, over 30 *CYP51* mutations have been reported, in over 70 non-synonymous haplotypes with up to eight amino acid changes each, in addition to efflux pump and target-site over-expression in some isolates. Functional genetic tools, including heterologous expression and gene replacement, enable the impact of specific mutations, alone and in combination, to be investigated. Experimental evolution provides the possibility to test the repeatability of specific pathways through the adaptive landscape, and whether different trajectories may have been seen under different scenarios of azole use.

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Louis Armand Est

FILLINGER Sabine (1)
OMRANE Selim (1), AUDÉON Colette (1), IGNACE Amandine (1), DUPLAIX Clémentine (1), AOUINI Lamia (2), KEMA Gert (2), WALKER Anne-Sophie (1)
(1) BIOGER, INRA, AgroParisTech, Thiverval-Grignon, France
(2) PRI, Wageningen University, Wageningen, The Netherlands

Several mutations of *Zymoseptoria tritici* field strains lead to *MFS1* overexpression and multi-drug-resistance (MDR)

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*. Plant pathogenic fungi are also concerned by this phenomenon. MDR strains were detected in septoria leaf blotch (*Zymoseptoria tritici*) field populations since 2008. These strains are cross-resistant to fungicides with different modes of action due to active fungicide efflux. In a previous study, we identified the *MFS1* gene overexpressed in all tested MDR field strains (1). This gene encodes a major facilitator membrane transporter whose inactivation abolished the MDR phenotype in two resistant isolates (MDR6 and MDR7). To identify the mutation(s) responsible for MDR phenotype we applied bulk-progeny sequencing to crosses involving MDR6 and MDR7 strains. This analysis enabled us to identify a 519 bp insert in the *MFS1* promoter in both strains. The insert, a reminiscence of a recent retrotransposition event, is responsible for *MFS1* overexpression and the MDR phenotype. Genotyping of various field strains revealed that at least one additional mutation is responsible for the MDR phenotype.

1- Omrane et al., (2015), Env. Microbiol., 17: 2805-2823.

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Louis Armand Est

DUBEY Mukesh (1)
BRANDSTRÖM DURLING Mikael (1), KOSAWANG Chatchai (2), COLLINGE David (2), JENSEN Dan Funck (1), KARLSSON Magnus (1)
(1) Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden (2) Department of Plant and Environmental Sciences and Copenhagen Plant Science Centre, University of Copenhagen, Copenhagen, Denmak

ABC transporters are important for xenobiotic tolerance and biocontrol traits in the fungus *Clonostachys rosea*

The mycoparasitic fungus *Clonostachys rosea* is an efficient biological control agent (BCA) under field conditions for a variety of plant diseases on agricultural crops. In addition, *C. rosea* can tolerate diverse groups of fungicides when exposed to doses similar to those recommended for controlling plant pathogenic fungi. Tolerance of *C. rosea* to fungicides can be useful for developing strategies for applications of *C. rosea* together with low doses of fungicides in integrated pest management programs. We sequenced the genome of *C. rosea* strain IK726, and transcriptomes from *C. rosea* interacting with *Botrytis cinerea* and *Fusarium graminearum*, and during exposure to certain mycotoxins. Comparative genomics revealed a significant (P0.05) increase in the number of ABC-transporters, polyketide synthases, cytochrome P450 monooxygenases, pectin lyases and GMC oxidoreductases encoding genes compared with other filamentous ascomycetes, including BCAs *Trichoderma atroviride* and *T. virens*. Interestingly, the increase in ABC-transporter gene number in *C. rosea* was associated with the phylogenetic subgroup B (multidrug resistance) and subgroup G (pleiotropic drug resistance). Transcriptome data from *C. rosea* interacting with *B. cinerea* and *F. graminearum* showed that 68% of all induced (P0.05) genes encoded membrane transporters including ABC- and major facilitator superfamily (MFS) transporters. Expression of certain group B and group G ABC transporter genes was induced in *C. rosea* during exposure to the *Fusarium* mycotoxin zearalenone (ZEA), fungicides or metabolites from the biocontrol bacterium *Pseudomonas chlororaphis*. Deletion of two highly induced subgroup G ABC transporter gene *ABC-G5* and *ABC-G29* resulted in mutants that had a reduced ability to protect barley seedlings from *F. graminearium* rot disease in growth chamber tests. In additions, *ABC-G5* deletion strains were more sensitive to ZEA, and fungicides iprodione and mefenoxam. In contrast with gene expression data, *ABC-G29* deletion strains did not show differences in sensitivity to ZEA or fungicides, but were more sensitive to H2O2 compared to wildtype *C. rosea*. This contradiction may be related to the overlap in substrate specificity between ABC-G29 and other C. rosea ABC transporters, since C. rosea genome has 86 ABC transporters that may complement the function of ABC-G29. In summary, our results suggest that ABC transporters are important for xenobiotic tolerance and biocontrol traits in *C. rosea*.

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18:00 - 18:20
Louis Armand Est

PIANALTO Kaila (1)
ALSPAUGH Andrew (2)
(1) Department of Molecular Genetics and Microbiology, Duke University, Durham, North Carolina, USA
(2) Department of Medicine, Duke University, Durham, North Carolina, USA

Cell-Wall-Mediated Mechanisms of Echinocandin Resistance in *Cryptococcus neoformans*

The fungal cell wall, a structure crucial for fungal viability and pathogenesis, is an ideal target for antifungal therapeutics. Echinocandins represent a class of antifungal agents that inhibit beta-1,3-glucan synthase, an essential enzyme for cell wall biosynthesis. One member of this drug family, caspofungin, is highly effective at inhibiting beta-1,3-glucan synthase activity at the protein level in *Cryptococcus neoformans*. However, it is ineffective at inhibiting growth of *C. neoformans* in culture and *in vivo* at clinically relevant concentrations. The mechanism behind this resistance is not well characterized. We have performed a forward genetics screen to identify gene products and cellular processes of *C. neoformans* involved in caspofungin resistance. So far, a total of 3,880 targeted deletion strains were screened for caspofungin sensitivity. Of these, 53 strains show an increase caspofungin sensitivity, equal to or greater than a known hyper-sensitive mutant (calcineurin mutant). One mutant, *pfa4*Δ, carrying a deletion of an S-palmitoyltransferase, displayed a drug sensitivity slightly greater than the calcineurin mutant. The Pfa4 enzyme is crucial for the proper localization of membrane proteins, such as the chitin synthase Chs3, to the cell surface. The *chs3*Δ mutant is also hypersensitive to caspofungin, indicating that the levels of chitin, and likley chitosan, produced by this enzyme may be crucial for *C. neoformans* tolerance to caspofungin. Ras1, a target of Pfa4, plays a role in cell cycle control, morphogenesis, and mating. A *ras1*Δ mutant is also highly sensitive to caspofungin compared to wild-type. To elucidate the role of this palmitoyltransferase in caspofungin resistance , we will assess the roles of its substrates in echinocandin resistance as well as cell wall biosynthesis and cell integrity. These studies will lead to better understanding of the mechanism of caspofungin resistance in *C. neoformans* and may define targets for combinatorial therapeutics acting synergistically with echinocandins.

CONCURRENT SESSION ABSTRACTS
CS8: Adaptation to xenobiotics

Wednesday 6th April
18:20 - 18:40
Louis Armand Est

SCALLIET Gabriel (1)
MOSBACH Andreas (1), WIDDISON Stephanie (2), FARMER Andrew (3), EDEL Dominique (1), DIETRICH Bob (4), CORRAN Andy (2)
(1) Syngenta Crop Protection Münchwilen AG, Stein, Switzerland
(2) Syngenta Jealotts Hill Int. Research Centre, Bracknell, UK
(3) National Center for Genome Resources, Santa Fe, USA
(4) Syngenta Biotechnology Inc., Research Triangle Park, USA

Resistance Mechanisms to Anilinopyrimidines Fungicides in *Botrytis cinerea*

Anilinopyrimidines (AP) fungicides (cyprodinil, mepanipyrin, pyrimethanil) were first introduced in Europe more than 20 years ago. These fungicides still constitute an integral part of the plant protection program in various crops worldwide such as grapes, apple and cereals. Anilinopyrimidines fungicides display good efficacy for the control of a range of Ascomycetes and Adelomycetes pathogens such as *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Venturia inequalis, Pyrenophora teres* and *Rynchosporium secalis*. In most cases resistant field isolates have been reported at a low to moderate frequency, but the underlying resistance mechanisms were not deciphered yet. We report the discovery of a wide range of AP resistance mechanisms in *Botrytis cinerea* using two complementary approaches. Firstly, mutants resistant to AP were obtained using *in vitro* laboratory screens and candidate genes mutated specifically in resistant mutants were identified by full genome re-sequencing. The role in resistance of these candidate genes was tested using reverse genetics. Secondly, mapping populations were obtained from crosses between resistant and sensitive field isolates, allowing the fine mapping of loci involved in resistance. The role in resistance of the candidate genes located at resistance loci, was tested using reverse genetics. In total, our work led to the identification of mutations affecting ten different genes which are, for the majority of them, involved in mitochondrial maintenance functions. Amongst these mutations, two resistance mechanisms account for all cases of field resistance. Complementary OMICS, chemical interactions and chemical genetics studies enabled us to uncover the key target of these class of antifungals as major signaling cascades involved in the inhibition of cell cycle progression.

CONCURRENT SESSION ABSTRACTS
CS9: New tools for fungal biology

Wednesday 6th April
16:00 - 16:20
Louis Armand Ouest

SEIDL Michael (1)
FAINO Luigi (1), THOMMA Bart P. H. J. (1)
(1) Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands

Mind the gap: Lessons from completed fungal genomes

The advent of next-generation sequencing (NGS) technologies has been a catalyst that facilitated studies into the biology of diverse species from all kingdoms of life. Until today, hundreds of fungal genomes have been sequenced, but the vast majority yielded highly fragmented genome assemblies that lack contiguous genomic regions. I will discuss multiple important reasons to produce complete, gapless fungal genome assemblies. Using two strains of the fungal pathogen *Verticillium dahliae* as an example, I will outline our genome sequencing and assembly strategy that, based on long-read sequencing technology and optical mapping, yielded complete, gapless, telomere-to-telomere genome assemblies. By focusing on highly abundant transposable elements that were previously not or only poorly assembled, we reveal active and passive roles of transposable elements in the genome evolution and in the evolution of fungal aggressiveness of *V. dahliae*. Notably, the outlined genome assembly approach is not restricted to *V. dahliae*. We recently embarked on multiple sequencing initiatives that utilize the power of long-read sequencing technologies to produce (near) complete genome assemblies of plant pathogens from distinct fungal genera with different genome characteristics. Arguably, complete fungal genome assemblies will become the new standard as they are imperative to disclose insights into genome organization, function and evolution in unprecedented detail.

CONCURRENT SESSION ABSTRACTS
CS9: New tools for fungal biology

Wednesday 6th April
16:20 - 16:40
Louis Armand Ouest

VOIGT Christian (1)
GLÖCKNER Annemarie (1), EGGERT Dennis (2), REIMER Rudolph (2)
(1) Phytopathology and Biochemistry, Biocenter Klein Flottbek, University of Hamburg, Hamburg, Germany
(2) Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany

3D-Visualization of the Fungal Cell Wall through Super-Resolution Microscopy

The fungal cell wall is generally composed of the long-chain polysaccharides (1,3)-beta-glucan with additional (1,6)-beta-linkages and (1,4)-beta-linked N-acetylglucosamine, known as chitin, as well as the structural proteins mannans, which contain the saccharide mannose. Whereas chitin forms the inner layer of the cell wall that is attached to the plasma membrane, (1,3)-beta-glucan is directed to the outside of the cell wall and covered by mannans. The application of recently developed super-resolution microscopy techniques now allows for the first time, visualizing the cell wall 3D network formation and interaction of these three major fungal cell wall components in live cell imaging on a nanoscale level. Interestingly, the conventional fungal cell wall architecture was modified in *Fusarium graminearum* mutants carrying a deletion of the serine/threonine kinase gene *PK1*. Apart from a reduced growth rate, disturbed polar growth, and a strongly reduced virulence on wheat spikes, the most striking phenotype of *PK1* deletion mutants, was their aberrant hyphal morphology. During vegetative growth of *PK1* deletion mutants, single cells at the growing tip of an hypha tended to have a bulb-like shape. By applying super-resolution microscopy on fluorescent-labeled cell walls polysaccharides of these bulb-like cells, we unexpectedly uncovered a unique cell wall with a two-layer architecture corresponding to a chitin/(1,3)-beta-glucan/chitin/(1,3)-beta-glucan two layer arrangement.

CONCURRENT SESSION ABSTRACTS
CS9: New tools for fungal biology

Wednesday 6th April
16:40 - 17:00
Louis Armand Ouest

O'MALLEY Michelle (1)
SOLOMON Kevin (1), HAITJEMA Charles (1), HENSKE John (1), GILMORE Sean (1)
(1) UC-Santa Barbara, Santa Barbara, USA

Deciphering the biomass-degrading behavior of the anaerobic gut fungi from Neocallimastigales

Renewable chemicals derived from plant biomass (mainly composed of cellulose and lignin) are attractive alternatives to those made from petroleum. To produce chemicals from biomass, enzymes are used to break down cellulose into simple sugars, which are later fermented into value-added products. However, since cellulose is tightly bound within a network of crystalline cellulosic fibers and lignin, existing biomass degrading enzymes are not very efficient. To develop new technologies to break down plant material into sugar, much can be learned by studying how microbes digest lignocellulose in biomass-rich environments, such as the digestive tract of large herbivores. Anaerobic fungi (Neocallimastigales) are native to the gut and rumen of these animals, where they have evolved powerful enzymes to degrade plant biomass. Our goal is to develop new experimental tools to engineer anaerobic fungi and anaerobic microbial consortia for lignocellulose breakdown and chemical production. To accomplish this goal, we isolated a panel of anaerobic fungi and associated microbes from different herbivores and screened for their ability to degrade several types of lignin-rich grasses and agricultural waste. By focusing on model anaerobic fungi from the *Piromyces*, *Neocallimastix*, and *Anaeromyces* genera, we have employed next-generation sequencing to discover thousands of new genes, revealing hundreds of novel biomass-degrading enzymes. Additionally, we have characterized key regulatory patterns for these enzymes, which depend on the environment of the fungus. Using this information, we are developing new genetic engineering strategies to manipulate gut fungi at the molecular level, along with "bottom-up" strategies to synthesize microbial consortia for compartmentalized breakdown and bioproduction.

CONCURRENT SESSION ABSTRACTS
CS9: New tools for fungal biology

Wednesday 6th April
17:00 - 17:20
Louis Armand Ouest

SCHILLING Jonathan (1)
ZHANG Jiwei (1), PRESLEY Gerald (1), HAMMEL Kenneth (2), MENKE Jon (1), HU Dehong (3), RYU Jae San (2), ORR Galya (3), FIGUEROA Melania (1)
(1) University of Minnesota, Saint Paul, Minnesota, USA
(2) USDA Forest Products Laboratory, Madison, Wisconsin, USA
(3) Pacific Northwest National Laboratory, Richland, Washington, USA

Space-for-time designs to resolve gene regulation and feedback as fungi degrade wood.

Fungi differ in their metabolic strategies for unlocking carbohydrates from wood. Some fungi (white rot type) oxidatively degrade lignin via peroxidases (PODs) prior to deploying typical glycosyl hydrolases (GHs) to saccharify polysaccharides. Other fungi (brown rot type) employ oxidative mechanisms initially that are less substrate-selective, ultimately resulting in lignin modifications without extensive lignin removal. These brown rot fungi are polyphyletic, and most lineages are characterized by gene losses (e.g. cellobiohydrolases; CBHs) yet faster decay rates. These mechanisms, as currently theorized, depend on temporal sequences of reactions, many non-compatible in discrete space. Defining these mechanisms has been challenging, in part due to the need to work *in planta* with fungi growing in solid wood. We are using wood wafers cut at a specific grain angle to develop fungal mycelial fronts as they grow into the wood matrix. This creates a space-for-time surrogate. Focusing on the brown rot fungus *Postia placenta*, these directional systems have improved resolution of key initial events over those observed using whole-block time series. Specifically, we have observed via qPCR and RNAseq an upregulation of oxidoreductases in the first 5 mm of *P. placenta's* hyphal front, prior to GH upregulation. This represents a window of less than 48 hours, after which GHs are induced by cellobiose. Gene upregulation is matched, spatially, by enzyme activities (e.g. endoglucanases) and measured proteins, and we can also overlay structural and non-structural carbohydrates to create a map of induction/response relationships. This simple set-up has a powerful potential to reduce noise in data generated from global analytical tools used for transcriptomics, proteomics, and metabolomics. This 'winnowing' approach to train powerful omics tools with spatial (thus temporal) resolution will help efforts to distill the metabolic steps that matter as fungi degrade wood.

CONCURRENT SESSION ABSTRACTS
CS9: New tools for fungal biology

Wednesday 6th April
17:20 - 17:40
Louis Armand Ouest

SCHUETZE Tabea (1)
MEYER Vera (1)
(1) TU Berlin, Berlin, Germany

Use of polycistronic gene expression to produce secondary metabolites in *Aspergillus niger*

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

CONCURRENT SESSION ABSTRACTS
CS9: New tools for fungal biology

Wednesday 6th April
17:40 - 18:00
Louis Armand Ouest

CARERE Jason
COLGRAVE Michelle (1), STILLER Jiri (1), LIU Chunji (1), MANNERS John (2), KAZAN Kemal (1), GARDINER Donald (1)
(1) Commonwealth Scientific and Industrial Research Organization (CSIRO), Brisbane, Australia
(2) Commonwealth Scientific and Industrial Research Organization (CSIRO), Canberra, Australia

Discovery of *in planta* targets of pathogen enzymes

Plants produce a variety of secondary metabolites to defend themselves from pathogen attack. Pathogens often overcome these defences by utilizing enzymes which degrade or modify plant defence compounds. However, in most cases, plant defence compounds targeted by pathogen enzymes remain enigmatic and elucidating this information would facilitate the modification of pathogen targets to provide increased disease resistance. We developed an LC-MS-based method to identify plant defence compounds targeted by pathogen enzymes. To validate this method, we used two *Fusarium* enzymes known to degrade distinct plant defence compounds in two different plant species. Of these two enzymes, the Fdb1 lactamase produced by the wheat crown rot pathogen *F. pseudograminearum* is known to degrade the wheat defence compound MBOA while the Tom1 tomatinase (a glycosyl hydrolase) produced by the tomato wilt pathogen *F. oxysporum* f. sp. lycopersici is known to degrade the tomato defence compounds tomatine. Fdb1 and Tom1 were expressed in *E. coli*, purified and applied to wheat and tomato extracts, respectively. Treated and mock-treated extracts were subjected to LC-MS to comparatively analyse the relative abundance of compounds with and without enzyme treatment. As expected, after treating the wheat samples with Fdb1, MBOA was no longer detectable while the abundance of 2-AMPO, the MBOA degradation product, increased nearly 40-fold. Similarly, the abundance of tomatine was drastically reduced after the tomato extract was treated with Tom1 while the amount of tomatidine, the product of tomatine cleavage, was increased nearly 1000-fold. In addition to these expected products, a suite of potential substrates and/or products were identified after enzyme treatment of plant extracts, demonstrating the potential utility of the method presented here in discovering in planta targets/substrates of any pathogen enzyme and also potential novel defence compounds in plants. We expect this information can aid in our understanding of plant-pathogen interactions and lead to the development of new plant protection strategies.

CONCURRENT SESSION ABSTRACTS
CS9: New tools for fungal biology

Wednesday 6th April
18:00 - 18:20
Louis Armand Ouest

LARRONDO Luis (1)
HEVIA Montserrat (1), HEVIA Constanza (1), GALLEGOS Andrés (1), SALINAS Francisco (1), ROJAS Vicente (1), DELGADO Verónica (1), CANESSA Paulo (1)
(1) Millennium Nucleus for Fungal Integrative and Synthetic Biology, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile

Synthetic Biology of Fungal Systems: optogenetic tools to manipulate gene expression for scientific and artistic purposes

Light is a strong environmental cue that informs organisms about their whereabouts, and can reprogram gene expression allowing a better adaptation to the surrounding environment. The fungus *Neurospora crassa* has been one of the main models for the study of photobiology, providing great insights on how microorganisms perceive and respond to light. This ascomycete responds specifically to blue-light (but not to other wavelengths) through a transcriptional heterocomplex named White Collar Complex (WCC). One of its components, WC-1, possesses a LOV (Light Oxygen Voltage) domain capable of detecting blue light, which promotes a conformational change that leads to a light-dependent dimerization resulting in strong transcriptional activation of target genes. Thus, the expression of WCC-target genes is rapidly and precisely controlled in a dose-dependent manner when light is present. In order to design and improve optogenetic switches that can be utilized in other organisms as orthogonal controllers, we have been exploring the dynamics of light responses in this organism. Through the development of simple synthetic switches we have successfully implemented a blue-light responding transcriptional system in *Saccharomyces cerevisiae*. Therefore, now in yeast (which naturally does not respond to light), we can efficiently and orthogonally induce gene expression. Most importantly, in order to better identify the kinetics of light-responses in *Neurospora*, we have explored the sensitivity and spatial resolution of this system. In doing so, we have been able to genetically program images in this organism. Thus, we can project a photograph on a surface covered with *Neurospora* carrying a real-time reporter under the control of a light responsive promoter and obtain back a response that mimics the pattern of the original image. Thus, we have established a live canvas in which images can be genetically interpreted and reconstituted with real-time dynamics.

MN-FISB120043, FONDECYT 1131030.

CONCURRENT SESSION ABSTRACTS
CS9: New tools for fungal biology

Wednesday 6th April
18:20 - 18:40
Louis Armand Ouest

LEGRAND Mélanie
FERI Adeline (1), LOLL-KRIPPLEBER Raphaël (1), MAUFRAIS Corinne (2), D'ENFERT Christophe (1)
(1) Institut Pasteur & INRA USC2019, Paris, France
(2) Institut Pasteur, Paris , France

Candida albicans, a model organism for studying Loss-Of-Heterozygosity events in eukaryotes

Candida albicans is responsible for the majority of life-threatening nosocomial fungal infections and is also the most frequently isolated fungal commensal of humans. Genomics studies in *C. albicans* have highlighted various degrees of tolerance to genome plasticity. In different instances, the observation of genome rearrangements has been correlated with adaptation of the cells to various stress both in vivo and in vitro. In particular, loss-of-heterozygosity (LOH) events, aneuploidies and isochromosome formation have been shown to contribute to increasing the resistance to azole antifungals. While LOH events are pervasive across the *C. albicans* population, few hot spots of LOH have been identified, suggesting that most LOH events result from independent double-strand breaks (DSB) experienced by individual isolates, as a consequence of normal metabolic activities or environmental insults such as exposure to antifungals or host attack. In order to further explore the mechanisms that regulate LOH in *C. albicans*, we have established (i) a novel method combining an artificial heterozygous locus harboring the BFP and GFP fluorescent markers and flow cytometry to detect LOH events at the single cell level and (ii) a DSB-inducible system based on the inducible expression of the *Saccharomyces cerevisiae* I-SceI endonuclease. Using collections of overexpression plasmids, we show that this LOH-reporter system can be used to identify genes whose overexpression triggers genome instability, specifically through LOH events. These include genes encoding components and regulators of the anaphase promoting complex, components of the recombination machinery and microtubule-interacting proteins. Because DNA DSBs have been shown to be very potent initiators of recombination in yeast and other organisms, we also investigated the molecular mechanisms involved in the repair of a targeted DNA DSB in a true heterozygous diploid organism. Our data suggested that most of the I-SceI-induced DNA DSBs are repaired by gene conversion in *C. albicans*. Interestingly, the precise characterization of the progenies that arose from a targeted DSB allowed us to identify recessive lethal and deleterious alleles at the *GPI16* and *MRF1* loci, respectively. Our data suggest that *C. albicans* is a valid model to study mechanisms involved in eukaryotic genome integrity.

POSTER SESSION ABSTRACTS

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M01

Monday 4th April
14:00 - 16:00

PAPAIOANNOU Ioannis *(1), VAGGALIS Vasilis (1), TYPAS Milton (1)*
(1) Department of Genetics & Biotechnology, Faculty of Biology, University of Athens, Athens, Greece

Exploring the molecular genetics of heterokaryon incompatibility in the asexual ascomycete *Verticillium dahliae*

Heterokaryon incompatibility (HI) is the inability of fungal strains to fuse for the establishment of viable vegetative heterokaryons. A series of HI (het) loci control this nonself recognition mechanism, which leads to the rejection of incompatible heterokaryons. Some of these genes have been molecularly analyzed mainly in the sexual ascomycetes *Neurospora crassa*, *Podospora anserina* and *Cryphonectria parasitica*. Although HI is predicted to play an important role in the population genetics of asexual fungi, which can exchange genetic material only through heterokaryosis and the parasexual cycle, its molecular basis in these fungi remains unknown. Here we report the results of our investigation of HI in the asexual plant-pathogenic ascomycete *Verticillium dahliae*. Functional analysis of *V. dahliae* signaling pathways revealed that both MAP kinase Vmk1 and NADPH oxidase NoxA are essential for hyphal and conidial anastomosis, which is the first step towards heterokaryon formation. Through the combination of experimental and *in silico* approaches, clear-cut homologues of the majority of characterized het genes were identified in the genomes of *V. dahliae* and related species, although they lacked the close linkage that is typical of some of their homologous het genes in the model fungi. The homologues of *N. crassa* het-c, mat and un-24, and *P. anserina* het-c genes were selected for population and functional analyses in *V. dahliae*. All of them were characterized by very low levels of polymorphism in a diverse collection of *V. dahliae* strains, and their deletion (knock-out) mutants failed to exhibit any alterations in their compatibility behaviour. These results indicate that the het homologues tested are not directly involved in HI control in *V. dahliae* and confirm that these het genes do not play a universal role in the control of HI in different fungi. When Hidden Markov Models of HI-related protein domains were used in a genomic analysis, 35 HET domain and 27 NACHT domain-containing genes were detected in the genome of *V. dahliae*. Through a population genomics approach, 4 of these genes (VdHET1-4) were found to be polymorphic between *V. dahliae* strains, and they were targeted for functional characterization, which has produced indications of putative participation in HI control. This study has contributed to a better understanding of HI in the asexual fungus *V. dahliae* and provides new insights into HI control in asexual fungi.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M02

Monday 4th April
14:00 - 16:00

KLUGE Janina *(1), LUTOMSKI Miriam (1), KÜCK Ulrich (1)*
(1) Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-University, Bochum, Germany

Components of the bud site selection system and the septation inititation network affect arthrospore formation in *Acremonium chrysogenum*

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

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M03

Monday 4th April
14:00 - 16:00

PFISTER Carole *(1), FORGES Marine (1), COURTY Pierre Emmanuel (2), LEBORGNE CASTEL Nathalie (1), WIPF Daniel (1)*
(1) Agroécologie, INRA, Agrosup, CNRS, Université de Bourgogne, , Dijon, France
(2) University of Fribourg, Department of Biology, Fribourg, Switzerland

Membrane dynamics of sugar transports in plant-microbe interactions

Plants can influence populations of mutualistic and pathogenic microorganisms present in their rhizosphere through exudation of sugars, a carbon source crucial for their growth and development. Beside their nutritional role, sugars could act as signaling molecules in plant-microorganism interactions. Mycorrhizal symbiosis is a mutualistic association in which the plant receives mineral nutrients (phosphate, nitrogen...) by the fungal partner, which in return receives sugars. In a pathogenic association, the microorganism diverts sugars provided by the plant without any compensation. Microorganisms are thus able to manipulate the host to modify fluxes of sugar(s). Despite the identification of sugar transport proteins at biotrophic interfaces, molecular and cellular mechanisms by which microorganisms operate the distribution of sugars produced by plants are still poorly understood. In this context, we aim to characterize plant membrane dynamics related to the transport of sugars in mutualistic and pathogenic interactions. The project is first conducted on *Nicotiana tabacum* cell suspension to analyze sugar flows (sucrose and glucose) through the plasma membrane of WT and endocytosis affected mutant cells subjected to various microbial molecules (e.g. defense elicitors, mycorrhizal factors). Secondly, tobacco sugar transporters are identified by screening gene and protein databases, and characterized by expression analysis to select leading candidates. Finally, selected carriers will be localized at the cellular level and their dynamic into the membrane system will be followed in response to mutualistic and pathogenic microorganisms or microbial molecules.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M04

Monday 4th April
14:00 - 16:00

YARDEN Oded (1), *SHOMIN Hila (1)*
(1) The Hebrew University of Jerusalem, Rehovot, Israel

Protein phosphatase 2A, a heterotrimeric protein involved in regulation of hyphal elongation in *Neurospora crassa*, interacts with the NDR kinase COT1

Inactivation of COT1 or alteration of its phosphorylation status affects hyphal elongation and branching in *N. crassa*. PP2A is a Ser/Thr phosphatase that plays a role in regulation of growth and development in *N. crassa* and other fungi. RGB-1 and B56 are two of the interchangeable regulatory subunits of PP2A. Inactivation of *rgb*-1 or *b56* impairs hyphal growth, branching and conidiation. We have determined that the catalytic subunit, RGB-1 and B56 can physically interact with COT1. RGB1::GFP localizes to hyphal tips and along the plasma membrane, in a manner similar to the cellular distribution of COT1. B56::GFP is localized at hyphal tips and septa. The presence of COT1 alleles mimicking constitutive phosphorylation of residues known to be phosphorylated [cot-1(S189E), cot-1(S417E) or cot-1(T589E)] suppressed the slow growth phenotype of rgb-1 RIP mutant, indicating a functional link between the two gene products, on the basis of COT1 phosphorylation state. In contrast, none of the phosphomimetic mutants suppressed the slow growth of the *b56* subunit mutant, suggesting that this subunit is involved in indirect regulation of COT1 phosphorylation. In the presence of sublethal concentrations of the PP2A inhibitor cantharidin, COT1 is hyperphosphorylated in a manner which is similar to that observed in an *rgb-1* RIP mutant. We conclude that PP2A is involved in regulation of COT1 phosphorylation state and that the regulatory subunits have distinct functions in regulation of growth and development in *N. crassa*.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M05

Monday 4th April
14:00 - 16:00

HEROLD Inbal *(1), YARDEN Oded (1)*
(1) The Hebrew University of Jerusalem, Rehovot, Israel

GUL-1 affects RNA abundance of genes encoding cell wall remodeling proteins in *Neurospora crassa*

Structure and maintenance of the cell wall are determined, in part, by balanced function of the cell wall biosynthesis and degradation. We hypothesized that the abnormally-thick cell walls and septa of the *N. crassa* NDR kinase *cot-1* mutant may be a result of altered expression of the cell wall remodeling machinery. Inactivation of *gul-1* (a homologue of the yeast *Ssd1* RNA-binding protein involved in translational regulation of cell wall remodeling proteins) partially suppresses the *cot-1* phenotype, accompanied by improved characteristics of the cell wall and septa. A 40% increase in chitin content in the cell wall of *cot-1* (when compared to the wild type) was almost completely abolished in the gul-1/cot-1 double mutant. Furthermore, the *gul-1* mutant was also found to be almost 2-fold more sensitive to chitin synthase inhibitors, when compared to the wild type. We have determined that *gul-1* is involved in regulation of the expression of cell wall remodeling genes such as glucan synthase, chitin synthases and a chitinase in a manner which is at least partially independent of the classic cell wall integrity pathway. Overall, expression of at least 25 genes involved in cell-wall remodeling was GUL-1-dependent. Moreover, based on GO analysis, GUL-1 was also found to regulate additional pathways such as transmembrane transport (34 genes) and amino acid metabolism (8 genes).We conclude that GUL1 is a regulator of cell wall remodeling and that the suppressive effect on *cot-1* is conferred via this capacity.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M06

Monday 4th April
14:00 - 16:00

AHARONI Liran *(1), ZIV Carmit (1), CHEN She (2), YARDEN Oded (1)*
(1) The Hebrew University of Jerusalem, Rehovot, Israel
(2) The National Institute of Biological Sciences, Beijing, China

Tyr117 and Tyr119 are required for proper MOB2A function and interaction with the NDR kinase COT1 in *Neurospora crassa*

MOB (MPS-1 binding) proteins act as activating subunits which are required for NDR kinase function. Phosphorylation has been suggested to play a role in the regulation of MOB function. In *N. crassa*, MOB2A and MOB2B have been shown to have overlapping functions. Both MOB2 proteins physically and genetically interact with COT1, a Ser/Thr kinase that is involved in the regulation of hyphal polarity and branching. Tyr117 and Tyr119 of MOB2A which can potentially undergo phosphorylation, were altered by site directed mutagenesis to produce mutants harboring two Phe or Glu residues (mimicking the putative unphosphorylated or constantly phosphorylated MOB2A forms, respectively). Either alteration resulted in a significant reduction in conidia formation, in a manner similar to that observed in the *Δmob-2a* mutant. However, *mob-2a*(Y117F,Y119F) as well as *mob-2a*(Y117E,Y119E) exhibited only a minor, although significant, reduction (10%) in growth rate and distance between branches, when compared to the wild type. *mob-2a*(Y117F,Y119F) also exhibited dense hyphal growth. These results indicate that Tyr117 and Tyr119 are important for MOB2A function. Even though Tyr117 and Tyr119 do not reside within the predicted MOB2A-COT1 interaction sites and are not predicted to be physically associated with the NDR kinase (based on the yeast model), altering these residues also affected the physical interaction between MOB2A and COT1, as determined by yeast two hybrid analyses. We conclude that in addition to its function in regulation of hyphal elongation, MOB2A is also required for conidiation in a manner which is dependent on these residues.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M07

Monday 4th April
14:00 - 16:00

SERRANO Antonio *(1), ILLGEN Julia (1), THIEME Nils (1), BRANDT Ulrike (1), LICHIUS Alexander (2), READ Nick (2), FLEIßNER André (1)*
(1) Technische Universität Braunschweig, Institut für Genetik, Braunschweig, Germany
(2) Institute of Inflammation and Repair, University of Manchester, Manchester, UK

Subcellular localization influences the activity of the MAP Kinase MAK-2 during cell fusion in *Neurospora crassa*

Cell-cell fusion is essential for the development of most eukaryotic organisms. However, the molecular basis of this process is only poorly understood. In the ascomycete fungus *Neurospora crassa*, fusion occurs between germinating vegetative spores as well as between hyphae of the inner part of the colony. Analysis of fusion mutants revealed an unusual mode of communication, in which two fusion partners coordinately alternate between two physiological stages. This peculiar behavior includes the alternating membrane recruitment of the SO protein and MAK-2, a MAP kinase homologous to fus3p of yeast. To analyze the relationship of the localization and activity of MAK-2 during switching, we permanently tethered the kinase to the plasma membrane by using a CAAX motif. This mislocalization resulted in a fusion-defective phenotype in the *Δmak-2* background, indicating that the dynamic of MAK-2 is essential for its function. Western blot analyses revealed a strong hyper-activation of MAK-2 by its upstream MAP Kinase (MEK-2/MIK-2). In addition, SO is not properly recruited in these strains during the communication process. To understand the hyper-phosphorylation, we tested it in different mutants related with cell fusion. Here, we analyzed components of the reactive oxygen species (ROS) generating system, which is essential for the cell fusion in filamentous fungi, some components of the polarisome and a possible cross-talk between MAP Kinases. These experiments revealed that the phosphorylation of the membrane tethered MAK-2 is still present in the NADPH oxidase mutants, but disappear in mutants lacking the polarity factor BEM-1 and the SO protein. Furthermore, by using this tool, we placed these factors either upstream or downstream of the MAK-2 phosphorylation cascade. In addition, we tested the role of phosphorylation for the spatial dynamics of MAK-2 by mutating both phosphorylation sites. We found that phosphorylation is essential for cell communication in *Δmak-2* background. Interestingly, however, these mutated versions were still recruited to the plasma membrane in a wild-type background, indicating that phosphorylation is not essential for the subcellular translocation in the presence of wild-type MAK-2. In future studies we will identify the link between MAP kinase signaling, polarity and the ROS generating system.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M08

Monday 4th April
14:00 - 16:00

WERNER Antonia *(1), HERZOG Britta (1), PÖGGELER Stefanie (1)*
(1) Georg-August University Göttingen, Göttingen, Germany

Role of the autophagy-related gene Sm*atg12* in fruiting-body development of the filamentous ascomycete *Sordaria macrospora*

In filamentous fungi, autophagy functions as a catabolic mechanism to overcome starvation conditions and to control diverse developmental processes under normal nutritional conditions. Autophagy involves the formation of double-membrane autophagosomes engulfing cellular components for the degradation in the vacuole. Two ubiquitin-like conjugation systems are essential for the expansion of the autophagosomal membrane. We recently showed that in the homothallic ascomycete *Sordaria macrospora*, autophagy related genes encoding components of the conjugation systems are required for fruiting body development or are essential for viability. Here, we cloned and characterized the *S. macrospora* (Sm) *atg12* gene, encoding a ubiquitin-fold protein. To examine its role in detail, we replaced it with a hygromycin resistance cassette and generated a homokaryotic ΔSmatg12 strain, which displayed a decreased vegetative growth rate and was unable to form fruiting bodies. GFP-labeled SmATG12 was detected in punctured intracellular structures that co-localized with some of the DsRED-SmATG8 intracellular structuress. We also showed that lipidation of SmATG8 and proper autophagosome formation depends on SmATG12.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M09

Monday 4th April
14:00 - 16:00

PANTAZOPOULOU Areti *(1), ARST Herbert N (1), HERNANDEZ-GONZALEZ Miguel (1), PEÑALVA Miguel A (1)*
(1) Centro de Investigaciones Biológicas-CSIC, Madrid, Spain

Bypassing the requirement for asymmetric distribution of Arf1 regulators across the Golgi: “Unconventional” secretion in *Aspergillus nidulans*

Following activation by cognate guanine nucleotide exchange factors (GEFs), Arf1 is stabilized on Golgi membranes, where it recruits specialized effectors. For example, at the early / cis Golgi, Arf1 recruits the COPI coat, necessary for intra-Golgi and Golgi-to-ER transport, while at the late Golgi / TGN Arf1 recruits clathrin and its adaptors, mediating sorting of cargo to the endo-lysosomal system. The molecular basis of Arf1 specialization within the Golgi is uncertain. Arf1 is activated by two Golgi GEFs: a GBF/Gea-subfamily member acting at the early Golgi (*A. nidulans* GeaA) and a BIG/Sec7-subfamily member acting at the late Golgi (*A. nidulans* HypB). Both are essential. Which are their non-overlapping functions and how each is recruited to a specific Golgi sub-compartment is unclear. We have isolated *geaA1* as a suppressor mutation that rescues the null *ΔhypB* mutant. The mutation maps in the gene encoding GeaA. GeaA1 impairs growth, suggesting that the ability to bypass HypB is acquired at the expense of GeaA-specific roles. GeaA1 alters a previously unnoticed GBF/Gea-specific tripeptide motif in GeaA and results in a remarkable redistribution of the protein from the Golgi to the apical plasma membrane. How the secretory pathway is organized and whether the Golgi is bypassed or re-organized in hyphae growing with a single Arf1 GEF are questions that we are pursuing, intending to understand why two Golgi Arf1 GEFs have been conserved through the evolution of eukaryotes.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M10

Monday 4th April
14:00 - 16:00

BOENISCH Marike *(1), BROZ Karen (2), KISTLER H. Corby (2)*
(1) University of Minnesota, Department of Agronomy and Plant Genetics, Saint Paul, Minnesota , USA
(2) USDA ARS, Cereal Disease Laboratory, Saint Paul, Minnesota, USA

Reorganization of the endoplasmic reticulum of *Fusarium graminearum* during trichothecene mycotoxin induction

The ascomycete fungus *Fusarium graminearum* causes disease on wheat and barley and contaminates grains with trichothecene mycotoxins making them unfit for human consumption. While genetic regulation of the trichothecene gene cluster is well studied, cellular organization of trichothecene biosynthetic enzymes in the fungal cell and subcellular changes during toxigenesis are not well understood. By using fluorescence tagged proteins and the fluorescent live cell dye ER-Tracker Blue-White DPX, we discovered that three enzymes, catalyzing early and late steps in trichothecene biosynthesis, hydroxymethylglutaryl CoA reductase (Hmr1p), trichodiene oxygenase (Tri4p), and calonectrin oxygenase (Tri1p), co-localize with each other under toxin inducing conditions *in vitro* and *in planta* at the endoplasmic reticulum (ER). Recently, we discovered that the Tri14 protein encoded by a gene of the trichothecene gene cluster is also localized at the ER and co-localizes with Tri4p during toxin production. Applying super resolution microscopy, we determined that the ER organization shifts from being highly reticulate under non-toxin inducing conditions, to being tubular and exhibiting pronounced perinuclear ER upon toxin induction. The reorganization of the ER upon toxin induction was further confirmed by observing similar fluorescence patterns with the native ER resident protein Sec22p of *F. graminearum* tagged with GFP (green fluorescent protein), as well as with GFP containing the ER-retrieval sequence HDEL. High pressure freezing and freeze substitution for TEM (transmission electron microscopy) revealed the ultrastructure of ER membranes in toxin producing and non-toxin producing cells. Different types of proliferations of the smooth ER, including lamellar stacks of perinuclear ER membranes («karmellae») as well as lamellar stacks of peripheral ER membranes («stripes») and concentric stacks («whorls») were observed in cells grown under toxin producing conditions. Visualizing nuclei of a histone H4p::GFP, Tri4p::RFP (red fluorescent protein) doubly tagged strain under toxin induction supported the hypothesis that trichothecene biosynthesis is localized at both smooth perinuclear ER and peripheral ER. As a consequence, trichothecene biosynthesis seems to be localized at particular regions of the ER. Subcellular changes which occur during trichothecene production *in vitro* and *in planta* might facilitate toxin biosynthesis and self-protection of the fungus.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M11

Monday 4th April
14:00 - 16:00

OSES-RUIZ Miriam *(1), SAKULKOO Wasin (1), LITTLEJOHN George R. (1), MARTIN-URDIROZ Magdalena (1), SOANES Darren M. (1), KERSHAW Michael (1), TALBOT Nicholas J. (1)*
(1) School of Biosciences, University of Exeter, United Kingdom, Exeter, UK

An S-phase checkpoint and associated transcriptional re-programming regulates septin-dependent host invasion by *Magnaporthe oryzae*

To cause rice blast disease, the fungus *Magnaporthe oryzae* develops a specialised infection structure called an appressorium. Appressorium development requires genetic control of cellular differentiation, first from anisotropic to isotropic cellular expansion to generate the dome shaped appressorium, and second, from isotropic to anisotropic growth to translate turgor pressure into vertical mechanical force, which requires septin-dependent re-orientation of the F-actin cytoskeleton. Here, we show that the geometrical changes and physical forces associated with appressorium development are tightly linked to cell cycle control. We report that the polarization switches are tightly controlled by S-phase checkpoints operating through different mechanisms. We present an integrated model in which we show that the switch from anisotropic to isotropic growth, leading to initial development of an appressorium, is tightly controlled by a G1 to S-phase cell cycle transition mediated through the DNA damage response. We also show that appressorium-mediated plant penetration and cytoskeletal re-polarisation is regulated by an S-phase checkpoint linked to turgor control. We go on to use global transcriptional RNA-seq to define the transcriptional signature associated with appressorium development, using comparative transcriptome analysis to define a transcriptional network associated to appressorium development, involving a hierarchical network of 63 transcription factors that regulate appressorium development and function in *M. oryzae*.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M12

Monday 4th April
14:00 - 16:00

MÖLLER Mareike *(1), SOYER Jessica (2), SCHOTANUS Klaas (1), FREITAG Michael (3), STUKENBROCK Eva (1)*
(1) Environmental Genomics, Christian-Albrechts University, Kiel; Max Planck Institute for Evolutionary Biology, Kiel/Plön, Germany
(2) INRA-AgroParisTech BIOGER, Thiverval-Grignon, France
(3) Department of Biochemistry and Biophysics, Center for Genome Research and Biocomputing, Oregon State University, Corvallis, USA

The role of histone-methyltransferases KMT1 and KMT6 in chromatin organization and gene regulation in *Zymoseptoria tritici*

Zymoseptoria tritici is a plant pathogenic fungus specialized to infect wheat (*Triticum aestivum*). The genome of the sequenced reference *Z. tritici* isolate consists of 21 chromosomes of which eight are accessory chromosomes. These chromosomes are highly instable during meiosis, transcriptionally repressed and show enrichment in repetitive elements and heterochromatic histone marks. The methylation of specific histone tails and the resulting changes in chromatin structure has been shown to play a crucial role in the regulation of secondary metabolism and pathogenicity-associated gene expression in filamentous fungi. To elucidate the role of histone modifications on transcriptional regulation and pathogenicity in *Z. tritici*, we created deletion mutants of the methyltransferases KMT6 and KMT1 that are responsible for the methylation of histone 3 at H3K27me3 and H3K9me3, respectively. We combined genetic and phenotypic analyses to follow the impact of these deletions *in vitro* and during the infection of the host plant. We used ChIPseq and RNAseq to compare changes in chromatin structure and the resulting differences in gene expression between mutants and wild type strains. We observed dramatic chromatin and genome rearrangements reflected in severe phenotypical changes in the *Δkmt1* mutants. The *Δkmt6* mutants however showed little differences to wild type under normal growth conditions *in vitro* and *in planta*, but significant alterations in secondary metabolite production under stress conditions. Based on these results we conclude a strong impact of H3K9me3 in chromatin organization and normal growth, and an important role of H3K27me3in gene regulation and the production of secondary metabolites in *Z. tritici*.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M13

Monday 4th April
14:00 - 16:00

TZELEPIS Georgios *(1), HOSOMI Akira (2), HOSSAIN Tanim (2), HIRAYAMA Hiroto (2), DUBEY Mukesh (1), FUNCK JENSEN Dan (1), SUZUKI Tadashi (2), KARLSSON Magnus (1)*
(1) Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden (2) Glycometabolome Team, Systems Glycobiology Research Group, RIKEN-Max Planck Joint Research Center, Wako Saitama, Japan

The role of PNGases and ENGases in the endoplasmic reticulum associated degradation process of misfolded glycoproteins in ascomycete fungi

N-glycosylation is an important post-translational modification of proteins, which mainly occurs in the endoplasmic reticulum (ER). Misfolded glycoproteins are degraded by a cellular system called the ER-associated degradation process (ERAD), which involves deglycosylation by peptide:N-glycanase (PNGase) and endo -N-acetylglucosamidase (ENGase). A survey of fungal and oomycete genomes revealed high variability in the number and distribution of PNGases and ENGases. The current work involves a comparative study of the role of PNGases and ENGases between *Neurospora crassa* and *Trichoderma atroviride*. Both species contained an enzymatically inactivated PNGase ortholog (png-1/png1) and two putative ENGase orthologs: gh18-10/Eng18B with a predicted cytosolic localization, and gh18-11/Eng18A that were predicted to be secreted. *N. crassa* carried an additional, putative acidic-type PNGase. By heterologous expression in *Saccharomyces cerevisiae* and Western blot analyses, gh18-10, Eng18A and Eng18B, but not gh18-11 nor the acidic-type PNGase, were shown to be active deglycosylating enzymes. We therefore hypothesized that gh18-10 of *N. crassa* and Eng18A or Eng18B of *T. atroviride* solely acts as the deglycosylating enzyme in the ERAD-pathway. By using an in vivo RTL assay based on *S. cerevisiae*, we showed that the orthologs gh18-10 and Eng18B, but not gh18-11, Eng18A nor the acidic-type PNGase, were able to restore the ERAD-pathway function in *S. cerevisiae*. Deletion of the gh18-10/Eng18B genes resulted in significantly slower growth rate, reduced secretion but higher resistance to abiotic stress compared to wild type in both species, suggesting a conserved function. HPLC analyses showed that the *T. atroviride* Δ*Eng18B* mutant was unable to produce cytosolic free oligosaccharides, which shows that Eng18B is the only deglycosylating enzyme that acts on intracellular glycoproteins in *T. atroviride*. The situation was very different in *N. crassa* where free oligosaccharides were detected even in the Δ*gh18-10* background. In summary, our data indicate that distinct deglycosylation pathways may function in different ascomycete species, which in turn differ from the situation found in *S. cerevisiae*, plants and animals. Therefore, the generated data provides important information on the evolution and function of the ERAD-pathway in different eucaryotes that may help us to understand the basis of important diseases caused by deglycosylation defects.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M14

Monday 4th April
14:00 - 16:00

HERZOG Robert *(1), SOLOVYEVA Irina (1), GUPTA Deepak K. (2), SHARMA Rahul (2), RÜHL Martin (3), THINES Marco (2), HENNICKE Florian (1)*
(1) Junior Research Group Genetics and Genomics of Fungi, Senckenberg Gesellschaft für Naturforschung, Frankfurt am Main, Germany
(2) Goethe-University Frankfurt/Senckenberg Biodiversity and Climate Research Centre (BiK-F), Frankfurt am Main, Germany
(3) Justus-Liebig-University Giessen, Institute of Food Chemistry and Food Biotechnology, Giessen, Germany

Agrocybe aegerita as an emerging modern model basidiomycete for edible mushroom formation

The increase of yield and quality in edible mushroom production is one of the goals of model basidiomycete-based research. Currently, modern studies on mushroom formation including molecular genetics approaches are mainly undertaken in a few well-established model basidiomycetes due to certain upsides they exhibit. However, all of them also display major individual downsides such as not being suited for edible mushroom production. Starting from this, the commercially grown choice edible mushroom *Agrocybe aegerita*, which has been studied as a model basidiomycete before, is proposed to become a modern model organism for possessing most of the individual upsides of established model mushrooms allowing for molecular genetics approaches. As a first step, a set of standard strains of *A. aegerita* was selected including the dikaryon AAE-3 and a set of AAE-3-derived sibling monokaryons. Each sibling monokaryon is mating compatible with at least one other sibling strain and each of them features a distinct degree of monokaryotic fruiting. The selected strain set exhibits both basidiomycete and other highly beneficial properties. This includes dikaryotic hyphae with clamps, dikaryotic fruiting body formation completed after 21 days, monokaryotic oidiation and a complete array of monokaryotic fruiting degrees on agar media. Taken together with the suitability of *A. aegerita* for molecular genetics and the whole-genome of the dikaryon AAE-3 sequenced and in silico annotated, *A. aegerita* seems to be a very good candidate to become a modern model basidiomycete for mushroom formation.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M15

Monday 4th April
14:00 - 16:00

KOLLÁTH-LEISS Krisztina *(1), SARDAR Puspendu (1), KEMPKEN Frank (1)*
(1) Christian-Albrechts-University, Institute of Botany, Olshausenstr. 40, 24098 Kiel, Germany, Kiel, Germany

The BEM46 protein in *N. crassa*: Eisosomal localization and its connection to tryptophan and auxin biosynthesis

The BEM46 proteins are evolutionary conserved in eukaryotes [1]. Although their function remains elusive, some studies indicated a role in processes associated with cell polarity. We previously reported that BEM46 in *N. crassa* is localized to the perinuclear ER and it is part of the fungal eisosome [2-3], as it co-localizes with PilA, which is an integral part of the fungal eisosome. In addition we showed that the tryptophan transporter MTR is localized in the eisosome. Over-expression of bem46 strains, RNAi mediated knock-down strains and knock-out deletion mutants show impaired germination of ascospores with reduced germination rate and non-directed germination tubes [2]. The BEM46 protein interacts with the anthranylate-synthase (trp-1) and may influence the auxin biosynthesis of the fungus [2], as the gene expression of several genes encoding auxin biosynthesis enzymes and the tryptophan transporter gene mtr differs strongly in bem46 over-expressing and down-regulated strains. Currently our investigations are focused on the complete description of the auxin biosynthesis gene network of *N. crassa* using bioinformatical tools and by investigating double and triple knock-out strains for their abilitiy to synthesize auxin. In addition new components of the fungal eisosome have been identified with a special focus on amino acid transporter and permeases.

[1] Kumar A, Kollath-Leiß K and Kempken F (2013) Biochem Biophys Res Comm 438:526-532
[2] Mercker M, Kollath-Leiß K, Allgaier S, Weiland N and Kempken F (2009) Curr Genet 55:151-161
[3] Kollat-Leiß K, Bönniger C, Sardar P and Kempken F (2013) Euk Cell 13:1051-1063

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M16

Monday 4th April
14:00 - 16:00

POHLMANN Thomas *(1), BAUMANN Sebastian (2), HAAG Carl (1), ALBRECHT Mario (3), FELDBRÜGGE Michael (1)*
(1) Institute for Microbiology, Heinrich Heine University, Düsseldorf, Germany
(2) Cell and Developmental Biology, Centre for Genomic Regulation, Universitat Pompeu Fabra, Barcelona, Spain
(3) Institute for Knowledge Discovery, Graz University of Technology, Graz, Austria

The FYVE domain containing protein Upa1 links mRNA transport to endosome trafficking

A growing number of studies support the idea of a close connection between mRNA transport and membrane trafficking. In the phytopathogenic fungus *Ustilago maydis*, the key RNA-binding protein Rrm4 is co-transported with Rab5a-positive endosomes. This process confers the long-distant transport of mRNPs through the fungal hyphae. Further studies suggest that this transport process is coupled with local translation of the target mRNAs on these organelles. This mechanism is thought to facilitate the correct assembly of higher order protein structures, such as septin oligomers. Nevertheless, it remained unclear how the Rrm4-dependent mRNPs are connected to the endosomal membranes. Here we identify the endosomal protein Upa1 as a link between mRNP and membrane trafficking in *U. maydis*. The protein contains a FYVE domain for association with the endosomal lipids and two novel PAM2-like motifs which mediate the interaction with the MLLE domains of Rrm4. The loss of Upa1 results in defective mRNA transport but does not affect endosomal shuttling. In summary, the FYVE domain containing protein Upa1 is the first endosomal protein which specifically regulates mRNA transport.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M17

Monday 4th April
14:00 - 16:00

MOTA Suellen Finamor *(1), SOUZA Elaine Aparecida (2), FLEISSNER Andre (1), ROCA Maria Gabriela (1)*
(1) Institute für Genetik, Techinische Universität Braunschweig, Braunschweig, Germany
(2) Genetics and Plant Breeding, Universidade Federal de Lavras, Lavras, Brasil

Conidial anastomosis tubes- study in two different *Colletotrichum* species isolated from the same plant lesions

Conidial anastomosis tubes (CATs) are specialized hyphal structures. They emerge as protuberances from conidia before or during germination, depending on the species. Fusions of CATs permits the cytoplasm of two or more conidia to communicate resulting in the transfer of organelles such as nuclei and mitochondria. A novel *Glomerella* species (not closely related to *C. lindemuthanium*) was isolated from anthracnose lesions in the common bean plant, from Brazilian fields. In the same anthracnose lesions, *Colletotrichum lindemuthanium* (teleomorph *Glomerella cingulata f. sp. phaseoli*) was identified, which is the expected pathogen to cause this plant disease. The new lesion cohabitant, *Glomerella*, was isolated from ascospores, but does not sexually reproduce in the laboratory. Exposure of bean plants to these isolates alone, does not result in symptoms on the plant. In our study, we confirm that both species are uninucleated. Interestingly, interspecies CAT fusion occurs frequently between conidia of these isolates, however these interactions result in vegetative incompatibility. Currently, we are investigating the molecular basis and the consequences of these unusual interspecies interactions. One of our achieved objectives was to identify the soft gene (so) in both species, and to compare the sequences with other related species. The SO protein has been reported to be involved in *N. crassa* cell-cell communication processes, mediating CAT and hyphal fusion. We will investigate the role and function of SO in *Glomerella*, to describe the dynamic of events during intra- and interspecies CATs fusion. Finally we will investigate the genetic and phenotypic consequences of these fusions. The results of these studies will further our understanding of the causes of genetic and phenotypical variation of *Glomerella* species observed in Brazilian fields. In the long term these findings might assist the genetic and plant breeding programs against this disease.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M18

Monday 4th April
14:00 - 16:00

TAVARES Sílvia *(2), PIRES Ana Sofia (2), LOUREIRO Andreia (2), AZINHEIRA Helena (2), RAMOS Ana Paula (1), GARDNER Rui (3), ABRANCHES Rita (4), SILVA Maria Do Céu (1), LOUREIRO João (5), TALHINHAS Pedro (1)*
(1) LEAF, Instituto Superior de Agronomia, Lisboa, Portugal
(2) CIFC, Instituto Superior de Agronomia, Oeiras, PORTUGAL
(3) Instituto Gulbenkian de Ciência, Oeiras, Portugal (4) Instituto de Tecnologia Química e Biológica António Xavier, Oeiras, Portugal
(5) CFE, Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Coimbra, Portugal

Cytogenomic analyses show expanded genomes in the Pucciniales (rusts fungi) and reveal nuclear content variation along their life cycles

Rust fungi (Basidiomycota, Pucciniales) are biotrophic plant pathogens with complex life cycles (up to five spore types). The urediniosporic infection cycle is frequently the most important in disease dissemination as the only stage capable of repeating itself. The cell nuclear content of rust fungi is thought to follow that of other Basidiomycota, with haploid nuclei throughout the life cycle, only becoming diploid upon karyogamy in telia and immediately returning to the haploid state as meiosis takes place leading to the formation of basidiospores. Recently, using genome size quantification techniques, the presence of 1C, 2C and a low proportion of 4C nuclei was detected in different stages of the urediniosporic cycle of several rust fungi. These results suggest the presence of diploid nuclei that supposedly only occur in teliospores, and compatible with the occurrence of karyogamy and meiosis prior to urediniospore formation, although endopolyplody or other parasexuality phenomena cannot be ruled out. This unexpected phenomenon seems to be transversal to the Pucciniales. Moreover, the estimation of genome size of 60 rust species sets the average Pucciniales genome at 380 Mbp, ranging from 70 to 2489 Mbp (the average fungal genome is 44 Mbp), with no apparent phylogenetic structuration. Such genome size variations may be due to polyploidy phenomena, and may be linked to the nuclear content variation along rust life cycles.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M19

Monday 4th April
14:00 - 16:00

ILLANA Adriana *(1), MARCONI Marco (1), RODRÍGUEZ-ROMERO Julio (1), XU Ping (2), DALMAY Tamas (2), WILKINSON Mark (1), SESMA Ane (1)*
(1) Centro de Biotecnología y Genómica de Plantas (CBGP), Universidad Politécnica de Madrid, Madrid, Spain
(2) School of Biological Sciences, University of East Anglia, Norwich, UK

The karyopherin Exp5/Msn5 is required for transport of small RNAs in *Magnaporthe oryzae*

The rice blast fungus *Magnaporthe oryzae* is an important plant pathogenic ascomycetous fungi. *M. oryzae* Exp5 is the orthologue of the karyopherin exportin-5 from humans and Msn5p from *Saccharomyces cerevisiae*. Karyopherins are involved in the translocation of proteins and/or RNAs, between the nucleus and the cytoplasm. The human EXP5 is also required for maturation of pre-miRNA precursors, and yeast Msn5 can bind small RNAs. The *M. oryzae Δexp5* mutant is strongly impaired in disease symptoms formation, suggesting that RNAs or proteins translocated by *M. oryzae* Exp5 play an important role in infection. We are using different approaches to identify cargo proteins and/or RNAs transported by Exp5. Immunoprecipitation experiments allowed us to identify proteins potentially translocated by Exp5, including several t-RNA synthetases, seven subunits of the 26S proteasome and components of signal transduction pathways such as kinases and small GTPases. To confirm any link between Exp5 and small RNA metabolism in *M. oryzae*, we followed two different approaches. First, we sequenced small RNA (smRNA) libraries derived from wild-type and *Δexp5* mutants. Most of genomic smRNA loci identified in the wild type strain are located in transposable elements, followed by non-coding RNAs and intergenic regions. *M. oryzae* has about 20 different types of transposon elements with very diverse copy number. Our results indicated that *Δexp5* is potentially required for the transport of a specific class of retrotransposons RNAs. Second, we have developed a cellular fractionation protocol which allows us to isolate smRNAs from total, nuclear and cytoplasmic fractions using wild-type (WT) and *Δexp5* mutants. Northern hybridization have been carried out with the probes snRNA U6 and t-RNA-met, that are specific RNA markers for nuclear and cytoplasmic fractions, respectively. Additional probes identified from the sequencing of smRNAs have been used to compare the RNA profile patterns in different subcellular compartments between WT and *Δexp5* strains. Our results suggest an involvement of Exp5 in the transport of specific classes of smRNAs between the nucleus and the cytoplasm of *M. oryzae*.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M20

Monday 4th April
14:00 - 16:00

ICHINOSE Sakurako *(1), TANAKA Mizuki (1), SHINTANI Takahiro (1), GOMI Katsuya (1)*
(1) Tohoku university, Sendai, Japan

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

Aspergillus oryzae produces large amounts of amylolytic enzymes in the presence of malto-oligosaccharides. In the presence of glucose, however, their production is repressed by carbon catabolite repression. In filamentous fungi, it has been proposed that glucose repression is regulated by the transcription factor CreA and the ubiquitin processing protease CreB. We generated creA and creB gene deletion mutants in *A. oryzae*, and observed that the wild-type (WT) and *ΔcreB* mutants grew as compact hyphal pellets, whereas the *ΔcreA* strain grew as pellets of smaller sizes or with a pulpy-like morphology in submerged culture [1]. In this study, we examined the effect of creA and creB deletions on cell wall components to elucidate the cause of *ΔcreA* morphological changes during submerged culture. Cell wall components of each mutant were fractionated by alkali treatment and carbohydrate composition of each fraction was determined by high-performance anion-exchange chromatography (HPAC). The alpha-1,3-glucan cell wall content was higher in *ΔcreA* than in WT. Furthermore, the major alpha-1,3-glucan synthase gene *agsB* and the putative alpha-amylase gene *amyD,* which is proposed to be involved in alpha-1,3-glucan biosynthesis, have higher expression levels in *ΔcreA* than in WT or in *ΔcreB*. In contrast, alpha-1,3-glucan cell wall content decreased in *ΔcreB* compared to WT, even though the morphology of *ΔcreB* was similar to WT. These results suggest that CreA and CreB regulates the biosynthesis of alpha-1,3-glucans. In addition, the morphology changes associated with *creA* gene deletion is likley caused by an increase in alpha-1,3-glucans content of the cell wall.

[1] Ichinose et al (2014) Appl. Microbiol. Biotechnol.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M21

Monday 4th April
14:00 - 16:00

BARTNICKI-GARCIA Salomon *(1), LARA-ROJAS Fernando (1), MOURIÑO-PÉREZ Rosa R. (1)*
(1) CICESE- Ensenada Center for Scientific Research & Higher Education, Ensenada, Baja California, Mexico

Myosin-1 is an essential component of the endocytic collar of hyphae of *Neurospora crassa*

The subapical endocytic collar is a prominent feature of hyphae of *Neurospora crassa*. It comprises a dynamic collection of actin patches associated with a number of proteins required for endocytosis, namely, ARP-2/3 complex, fimbrin, and coronin. We have found that myosin-1 is another key component of this endocytic collar. A *myo-1* sequence was identified in the genome of *N. crassa* and used it to generate a strain with a myo-1:GFP allele under the ccg1 promoter. Examination of living hyphae by confocal microscopy, revealed myosin-1:GFP located mainly as a dynamic collection of small patches arranged in collar-like fashion in the hyphal subapex. Dual tagging showed myosin-1:GFP partially colocalized with two other endocytic proteins, fimbrin and coronin. Myosin-1 also participated in septum formation. By recovering a viable strain, albeit severely inhibited in growth and sporulation, after deletion of *myo-1*, it was possible to investigate the phenotypic consequences of the elimination of MYO-1. Deletion of *myo-1* caused a severe reduction in growth rate (95%), near absence of aerial mycelium and no conidiation. A reduced uptake of the lipophilic dye FM4-64 indicated a deficiency in endocytosis of the *Δmyo-1* mutant. Hyphae were produced by the *Δmyo-1* mutant, but their morphogenesis was severely affected. Hyphal morphology was distorted displaying irregular periods of isotropic and polarized growth. The morphological alterations were accompanied, and presumably caused, by a disruption in the organization and dynamics of a myosin-deprived actin cytoskeleton which compromised the stability and function of the Spitzenkörper as a vesicle supply center.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M22

Monday 4th April
14:00 - 16:00

FIEDLER Markus *(1), MEYER Vera (1)*
(1) Institute of Biotechnology, Department Applied and Molecular Microbiology, Berlin University of Technology, Berlin, Germany

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

Comprehensive understanding on how fungal growth and branching are intertwined with the secretory flux of proteins towards the hyphal tip is still missing. To gain deeper insights into these processes, we established a fluorescently labelled v-SNARE reporter strain (GFP:SncA) in *Aspergillus niger* [1]. This strain was used as a background to replace the *A. niger glaA* gene coding for glucoamylase, which is the most abundant secretory protein of *A. niger*, with a *glaA* gene under control of the metabolism-independent Tet-on promoter. This *glaA* modification was also performed in a GFP:SncA reporter strain deleted for r*acA*. RacA is a Rho GTPase GTPase which is key for polarity establishment and maintenance of *A. niger* hyphae and its deletion is known to provoke an hyperbranching phenotype [1]. The availability of both strains enable us to study the link between cargo load, amount of secretory vesicles and number of hyphal tips.

[1] Kwon et al (2013) Plos One 8:7e68946

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M23

Monday 4th April
14:00 - 16:00

FIEDLER Markus *(1), KUBISCH Christin (1), MEYER Vera (1)*
(1) Institute of Biotechnology, Department Applied and Molecular Microbiology, Berlin University of Technology, Berlin, Germany

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

A comprehensive understanding of the secretory pathway of filamentous fungi and its interplay with polar growth and cell wall integrity is still lacking in industrial fungi such as *Aspergillus niger*. GTP-binding proteins such as the members of the Arf family, are major switches controlling coated vesicle formation during intracellular trafficking in eukaryotes. The function of all six Arf family proteins have been studied in *S. cerevisiae*, whereas none of the seven predicted ones have been examined in *A. niger* so far. In the current study, we have investigated the function of ArfA, the orthologue of the *S. cerevisiae* ARF1 and ARF2 genes in *A. niger*, as the encoding gene is specifically up-regulated in *A. niger* under conditions that lead to high secretion of glucoamylase [1]. Arf1 and Arf2 have multiple roles within the secretory pathway of *S. cerevisiae* and the closet human orthologue was recently shown to be a key factor ensuring the hypersecretion phenotype of neuroendocrine tumor cells [2]. The GTP-binding protein ArfA seems to be an essential protein for *A. niger* as deletion of the arfA gene (An08g03690) turned out to be lethal. We thus generated a conditional *arfA* mutant with the help of the Tet-On expression system [3] in a fluorescently labelled v-SNARE background strain (GFP:SncA) [4]. This approach allowed us to follow the effect of arfA downregulation and over-expression on post-Golgi cargo distribution (v-SNARE) and glucoamylase secretion in a single isolate. We could also show that ArfA is able to complement the lethal phenotype of a *S. cerevisiae arf1 arf2* double null mutant.

[1] Jørgensen et al. (2009) BMC Genomics, 10:44
[2] Münzberg et al. (2015) Journal Cell Mol Med. 19(5): 948–959
[3] Meyer et al. (2007) Melanoma Res. 17(2):109-16.
[4] Kwon et al (2013) Plos One 8:7e68946

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M24

Monday 4th April
14:00 - 16:00

MIYAZAWA Ken *(2), YOSHIMI Akira (1), ZHANG Silai (2), SANO Motoaki (3), GOMI Katsuya (2), HASEGAWA Fumihiko (1), ABE Keietsu (1)*
(1) NICHe, Tohoku Univ., Sendai, Japan
(2) Grad. Sch. Agric. Sci., Tohoku Univ, Sendai, Japan
(3) Kanazawa Institute of Technology, Nonoichi, Japan

Growth characteristics and protein productivity of *Aspergillus oryzae* quintuple disruption mutants of alpha-1,3-glucan related genes.

Because of the hyphal pellet formation, the difficulty of high-density cultivation has become a problem in the production of biomaterials by filamentous fungi. We previously analyzed the function of two alpha-1,3-glucan (AG) synthase genes, *agsA* and *agsB*, in the model fungus *Aspergillus nidulans*. The *agsA* disruption mutant has similar morphological phenotypes as wild-type. In contrast, the *agsB* disruption mutant differed from WT in forming dispersed hyphal cells under liquid culture conditions, regardless of the *agsA* genetic background. The mycelial dry weight of the *agsB* disruption strain cultured in liquid medium was increased compared to wild-type, suggesting that the dispersed hyphal cells observed in *agsB* disruption mutant are applicable to high-density cultivation to achieve higher-productivity of biomaterials. In the present study, we carried out a functional analysis of AG-related genes in the koji mold *A. oryzae*, which is an important filamentous fungus used in the traditional Japanese fermentation industry. *Aspergillus oryzae* possesses three AG synthase genes, *agsA, agsB,* and *agsC*. In addition, a conserved gene cluster, which consists of *agsB* and two amylase genes, *agtA* and *amyG*, are found in *A. oryzae* genome. We constructed the disruption mutants of these AG-related genes in *A. oryzae*: triple-disruption mutant of AG synthase genes *(*Δ*agsA,* Δ*agsB,* Δ*agsC*) and quintuple-disruption mutant of AG-related genes *(*Δ*agsA,* Δ*agsB,* Δ*agsC,* Δ*agtA,* Δ*amyG*). Phenotypic analysis revealed that both the triple- and quintuple-disruption mutant formed smaller hyphal pellets compared to wild-type. We also constructed the expression mutants of the cutinase, *CutL1*, as a model secreted protein in the triple- and quintuple-disruption AG-related genes mutants, and the protein productivity in CutL1 production was assessed. The mycelial dry weight cultured in liquid medium and the protein productivity of both triple- and quintuple-disruption mutants were increased compared to wild-type. Taken together, our data suggest that the small hyphal pellets of AG-related genes disruption mutants are suitable for improving protein production in *A. oryzae*, but that *A. oryzae* has additional components related to hyphal aggregation in its cell wall.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M25

Monday 4th April
14:00 - 16:00

TAKANO-ROJAS Harumi *(1), CASTILLO-CANIZÁLES Jorge Luis (1), ZICKLER Denise (2), PERAZA REYES Leonardo (1)*
(1) Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Mexico City, Mexico
(2) Institute for Integrative Biology of the Cell, CEA, CNRS, Univ Paris-Sud, Orsay, France

Peroxisome dynamics during the life cycle of *Podospora anserina*

Peroxisomes are highly versatile and dynamic organelles required for the development of most eukaryotic organisms. In the fungus *Podospora anserina*, peroxisomes play important roles during both vegetative and sexual development, involving a high regulation of the protein machinery that drives peroxisome biogenesis. Here we demonstrate firstly that peroxisome dynamics is highly regulated during the vegetative cycle of this fungus. We show that peroxisomes of vegetative cells are highly mobile and a number of them are engaged in fast long-distance movements towards and backwards from the extending hyphal apex. Moreover, peroxisomes undergo changes in morphology, size and number in response to metabolic and environmental cues. These changes include: (i) Dramatic increase in peroxisome number when growing in substrates that require peroxisome metabolism such as fatty acids and this increase involves the peroxisome/mitochondrion fission factor FIS1; (ii) Elongation of the peroxisome shape in response to stress conditions like oxidative stress. Secondly, we show that peroxisomes are structurally different in vegetative and sexual cells. First, peroxisomes change in number and distribution through the sexual cycle. These changes include: (i) concentration of peroxisomes at the ascus (meiocyte) apex after differentiation from the dikaryotic crozier cell; (ii) peroxisome proliferation during ascus growth and, (iii) a further increase during ascospore differentiation. Second, peroxisome morphology changes from punctuate at meiosis I to elongated from second meiotic division to early ascospore formation, and then back to a punctuate morphology during late ascospore differentiation. Those morphological changes suggest variations in the redox/oxidative state of peroxisomes during sexual development. Finally, we observed that peroxisome number decreases upon ascospore maturation, implying that specific stages of sexual development also require their removal. Our findings reveal a high regulation of peroxisome dynamics during the fungal life cycle that in coordination with the proteins that control peroxisome assembly likely defines the constitution and function of this organelle along development.

This research was supported by PAPIIT grant IA201815 from DGAPA-Universidad Nacional Autónoma de México.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M26

Monday 4th April
14:00 - 16:00

RAUDASKOSKI Marjatta *(1)*
(1) Molecular Plant Biology, Department of Biochemistry, University of Turku, Turku, Finland

Cell biology of living mono- and dikaryotic *Schizophyllum commune* hyphae expressing fluorescent proteins

While observations on nuclei and cytoskeletal elements labeled with fluorescent proteins in living hyphae have been very successful in filamentous ascomycetes, in filamentous basidiomycetes there are so far very few observations on this line. The present work describes the visualization of nuclei in the basidiomycete *Schizophyllum commune* in living monokaryotic (haploid) and dikaryotic hyphae by using the histone encoding gene h2b (Schco_3: PID2605148). The gene is expressed under its native promoter and the carboxyl terminus is tagged with a 30 bp linker, the *egf* gene and the *Sccdc42* gene terminator. Microtubules and microfilaments play a central role in nuclear division associated with septum formation in monokaryotic hyphae and with clamp cell formation in dikaryotic hyphae. Recently the labeling of microfilaments with the LifeAct-EGFP construct under *S. commune* beta-tubulin promoter has been successful. The nucleotide sequence of Lifeact encodes the N-terminal 17 amino acid peptide from budding yeast Abp40 [1]. In monokaryotic and dikaryotic hyphae, the construct visualizes microfilaments at hyphal tips and at the formation of septa. In order to be able to relate the different phases of nuclear division with actin filament polymerization during clamp cell formation, the h2b gene and the LifeAct nucleotide sequence, each, was fused with synthetic mRFPruby (monomeric red fluorescent protein), however visualization has not been successful yet. The possibility to follow nuclei with green and red fluorescent proteins during compatible mating could provide new information about the reciprocal nuclear exchange and migration during hyphal fusions necessary for sexual reproduction in *S. commune*

[1] Raudaskoski M. (2015) Fungal Biology Reviews 29: 179.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M27

Monday 4th April
14:00 - 16:00

SOUIBGUI Eytham *(1), DIERYCKX Cindy (2), BRUEL Christophe (2), LATORSE Marie-Pascale (3), POUSSEREAU Nathalie (2)*
(1) University Lyon 1, BAYER Cropscience, Lyon, France
(2) UMR CNRS 5240, University Lyon 1, Lyon, France
(3) BAYER Cropscience, Lyon, France

Role of clathrin in the phytopathogenic fungus *Botrytis cinerea*

Clathrin is a protein involved in the formation of coated vesicles. This process was observed at the plasma membrane for endocytosis, and on endosomes / Golgi apparatus during intracellular vesicles formation. Clathrin-mediated endocytosis is the most characterized process and is involved in nutriments uptake, receptors mediated endocytosis, regulation of signaling molecules, plasma membrane turnover, and cell polarity. The exploration of a *Botrytis cinerea* random mutant library allowed identifying a non-pathogenic clathrin-deficient mutant suggesting, for the first time, a role for this protein in fungal infection. A large number of studies has highligthed the importance of clathrin in mammalian, plant and yeast, but only few in filamentous fungi. In this study, different genetic approaches (reduced expression of clathrin, use of dominant negative clathrin mutant) were used to investigate the role of this protein in *B. cinerea* biology and pathogenicity. We have analyzed endocytosis using FM4-64 dye in wild type and our clathrin deficient mutant. Surprisingly, no important defect in FM4-64 uptake and incorporation in fungal intracellular membranes, was observed in the *B. cinerea* clathrin deficient mutants suggesting that this protein has no impact on FM64-related endocytosis. Secretion, another vesicle trafficking pathway involving clathrin, was investigated. The quantification of protein secretion of *B. cinerea* clathrin deficient mutants revealed a strong defect in protein secretion. A focus on plant cell wall degrading enzymes and other fungal components, known to be involved in pathogenicity was performed in order to understand the pathogenicity defect of the mutants. To establish the link between secretion defect and clathrin, isolation of clathrin coated vesicles is currently performed and the main results are presented.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M28

Monday 4th April
14:00 - 16:00

TROPPENS Danielle *(1), DIRNBERGER Benedict (1), BRAUS Gerhard (1)*
(1) Institute of Microbiology and Genetics, Department of Molecular Microbiology and Genetics, Georg-August University Göttingen, Göttingen, Germany

Investigating the potential of Hülle cells in *Aspergillus nidulans*

Filamentous fungi have an enormous potential to secrete proteins and are therefore widely used in biotechnology, whereas the secretion potential of yeasts as single cell fungi is often limited. It is our aim to explore whether we can use specialized cells of constitutively filamentous fungi as single cell tools for biotechnology. We started to address this issue by using Hülle cells. Hülle cells are a specialized and unique cell type that is specific to the genus Aspergillus. They are globose single cells that contain several nuclei. In the model organism *Aspergillus nidulans* Hülle cells emerge from hyphal tips after entering sexual development and form an envelope around the closed developing fruiting body (cleistothecium). In the absence of Hülle cells, cleistothecia do not reach maturity suggesting that they act as auxiliary nursing cells. Hülle cells express numerous hydrolyses which might produce building material for the fruiting body. They might also contribute to the defence against fungivore attack by secreting secondary metabolites. We are investigating (i) whether we can construct Hülle cells with different nuclei to expand the genetic potential, (ii) whether we can enrich Hülle cells i.e. obtain cultures with Hülle cells as the major growth form, (iii) whether we can use Hülle cells as tools for biotechnology to specifically produce secondary metabolite. We address these questions by investigating the role of Hülle cells in the development of the sexual fruiting body and their ability to germinate, to nurse and/or protect the cleistothecium. We are combining the analysis of deletion strains with altered Hülle cell formation with global systems biology analyses to obtain a comprehensive understanding of the biological role of Hülle cells. These findings should help answering the question whether and how Hülle cells as single cells of filamentous fungi can be used for biotechnological applications.

POSTER SESSION ABSTRACTS
Session CS1 Cell biology and traffic
CS1M29

Monday 4th April
14:00 - 16:00

GUIMARAES Sofia (1), SCHUSTER Martin (1), HIGUCHI Y. (1), BIELSKA E. (1), **STEINGERG Gero** (1)
(1) Biosciences, University of Exeter, Exeter, UK

Early endosomes organize the fungal cell

Filamentous fungi contain rapidly moving early endosomes. Their bi-directional motility is mediated by microtubules and associated kinesin-3 and dynein. Motor-driven motility of early endosomes consumes large numbers of ATP, and the question arises why the organelles are constantly moving. Recent work in the model fungus *Ustilago maydis* has revealed unexpected roles of fungal endosome motility in spatial organization of the translation machinery, peroxisomes, lipid droplets and endoplasmic reticulum. In addition, early endosomes participate in long-range signalling during plant infection. Thus, it emerges that early endosomes have key roles in spatially organizing the fungal cell.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M01

Monday 4th April
14:00 - 16:00

JOHN Evan *(1), LOPEZ-RUIZ Francisco (1), RYBAK Kasia (1), MOUSLEY Carl (2), OLIVER Richard (1), TAN Kar-Chun (1)*
(1) Centre for Crop Disease Management, Department of Environment and Agriculture, Curtin University, Perth, Australia (2) School of Biomedical Sciences, CHIRI Biosciences Research Precinct and Faculty of Health Sciences, Curtin University, Perth, Australia

Dissecting the role of Hog1 mitogen-activated protein kinase signalling in stress tolerance and pathogenicity of *Parastagonospora nodorum*

The Hog1 mitogen-activated protein kinase (MAPK) pathway is activated through two-component histidine kinase (HK) signalling. This pathway was first characterised in the budding yeast *Saccharomyces cerevisiae* as a regulator of osmotolerance. The fungus *Parastagonospora nodorum* is the causal agent of septoria nodorum blotch of wheat. This pathogen uses host-specific effectors in tandem with general pathogenicity mechanisms to carry out its infection process. Components of a Hog1 signalling pathway have been identified in *P. nodorum*. In this study, we examined the role of the pathway in the virulence of *P. nodorum* on wheat by disrupting putative pathway component genes; PnHog1 (SNOG_13296) Hog1 MAPK and PnNik1 (SNOG_11631) hybrid histidine kinase (HK). Mutants deleted in *PnNik1* and *PnHog1* were insensitive to dicarboximide and phenylpyrrole fungicides, but not a fungicide that targets ergosterol biosynthesis. Furthermore, both *nik1* and *hog1* mutants showed increased sensitivity to hyperosmotic stress. However, *PnHog1*, but not *PnNik1*, is required for tolerance to elevated temperatures. *PnHog1* deletion conferred increased tolerance to 6-methoxy-benzoxazolinone, a cereal phytoalexin. This suggests that the Hog1 signalling pathway is not exclusively regulated by *PnNik1*. Both *nik1* and *hog1* mutants retained the ability to infect and cause necrotic lesions on wheat. However, we observed that the *hog1* mutation resulted in reduced production of pycnidia, asexual fruiting bodies that facilitate spore dispersal during late infection. Our study demonstrated for the first time, the overlapping and distinct roles of a putative Hog1 MAPK and two-component HK signalling in *P. nodorum* growth and pathogenicity.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M02

Monday 4th April
14:00 - 16:00

LICHIUS Alexander *(1), GRUBER Sabine (2), REISMANN Alexander (3), BAUMGART Florian (3), SCHÜTZ Gerhard (3), ZEILINGER Susanne (1)*
(1) Institute of Microbiology, University of Innsbruck, Innsbruck, Austria
(2) Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria
(3) Institute of Applied Physics, Vienna University of Technology, Vienna, Austria

Visualisation of virulence-associated signalling complexes during mycoparasitic attack

Trichoderma atroviride is a potent mycoparasite of important crop pathogens, including *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani*, thus making it the most successful fungal biofungicide. Molecular details of how *T. atroviride* detects its prey fungus, initiates directed growth towards it and elicits the mycoparasitic attack are not well understood. Previous studies identified the 7-transmembrane receptor GPR1 as essential for virulence, and a trans-membrane domain protein of the SUR7-family as its potential interaction partner for host recognition signalling. To elucidate their functional interrelation, we expressed fluorescently labelled alleles of both proteins, and used confocal and TIRF single molecule microscopy to determine their dynamic distribution in the apical compartment of growing hyphae. Beside their expected residence in the plasma membrane, both proteins also localised to septa and compartments of the secretory and endocytic pathway. The localisation pattern of both proteins overlapped to a large extent making physical interaction possible, but notably, only SUR7 showed pronounced accumulation in tubular vacuoles and the Spitzenkörper. Initial single molecule data suggests directed diffusion of SUR7 molecules towards the growing tip apex. In parasite-host interaction assays - using differential fluorescent staining - we recognised tip growth arrest, tip swelling and lysis of host hyphae as the most reliable indicators of the process.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M03

Monday 4th April
14:00 - 16:00

TILEY Anna *(1), FOSTER Gary D. (1), BAILEY Andy M. (1)*
(1) The School of Biological Sciences, The University of Bristol, Bristol, UK

Investigating asexual sporulation in *Zymoseptoria tritici*, a pathogen of wheat

Zymoseptoria tritici is an ascomycete fungus that causes *Septoria tritici* blotch, a major disease of wheat. The fungus spreads to new hosts via asexual spores dispersed by rain splash. A potential way to control *Z. tritici* is to prevent asexual sporulation by inhibiting development of the asexual fruiting body and spores. There is currently limited understanding of *Z. tritici* at the molecular level, particularly the genetic pathways involved in asexual sporulation. Key genes already known to be important for asexual sporulation in other model ascomycete fungi were BLAST searched against the *Z. tritici* genome database to find potential orthologues, and their expression was assessed. Genes were selected for constructing knock-out mutants, including those known in *Aspergillus nidulans* such as *abaA, brlA* and *flbB*. Other candidate genes knocked-out share similarity to those with roles in light signalling, due to the importance of this pathway for sporulation in other fungi. The parameters needed for reliable sporulation of *Z. tritici* have also been investigated using a range of media and illumination conditions. This will help in the investigation of the knock-out mutants by uncoupling sporulation from other in planta phenotypes. To date, 8 genes have been disrupted with experiments ongoing. Current results suggest that the *A. nidulans* model of asexual sporulation does not apply to *Z. tritici*. The most recent results from these studies will be presented.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M04

Monday 4th April
14:00 - 16:00

PÉREZ-ARQUES Carlos *(1), NAVARRO Eusebio (1), TORRES-MARTÍNEZ Santiago (1), GARRE Victoriano (1)*
(1) Departamento de Genética y Microbiología, Facultad de Biología, Universidad de Murcia, Murcia, Spain

Identification of putative virulence factors regulated by RNAi in *Mucor circinelloides*

Mucor circinelloides is a filamentous basal fungus that is receiving exceptional attention as a model for studying mucormycosis, an emerging fungal infection caused by several Mucoralean species. Since endogenous small RNAs (esRNAs) generated by the RNAi pathway have been demonstrated to control multiple physiological and developmental processes in this fungus, we have investigated if they could also play a role in fungal pathogenesis. To do that, esRNAs from virulent and avirulent strains have been sequenced and their accumulation patterns have been compared to identify differentially expressed esRNAs. As avirulent strains, we have used a soil isolate and a null mutant for *M. circinelloides* white collar-1a gene, which encodes a member of WC-1 family of photoreceptors, previously shown to be involved in fungal pathogenesis. A large number of differentially accumulated esRNA producing loci, most of them corresponding to protein coding genes, were identified. Twenty eight of them share similar esRNAs accumulation patterns in both avirulent strains as compared with the virulent one, suggesting an implication in pathogenesis. Differential accumulation of esRNAs of several selected loci correlated inversely with mRNA levels of the corresponding target genes, indicating that these esRNAs regulate gene expression. One of these loci encodes a protein with ribonuclease features, which could regulate multiple virulence factors as described in other pathogenic fungi. These findings reinforce esRNA role in regulating fungal gene expression and point to an implication in pathogenesis, which will be confirmed by future research.

This research was funded by Fundación Séneca (19339/PI/14), MECD (FPU14/01983) and MINECO (BFU2015-62580-ERC and BFU2012-32246) co-financed by FEDER.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M06

Monday 4th April
14:00 - 16:00

NAVARRO-MENDOZA María Isabel *(1), LÓPEZ-MUÑOZ Azucena (2), HERNÁNDEZ-OÑATE Miguel Ángel (4), HERRERA-ESTRELLA Alfredo (3), MULERO Victoriano (2), TORRES-MARTÍNEZ Santiago (1), RUIZ-VÁZQUEZ Rosa M. (1), GARRE Victoriano (1), NICOLÁS Francisco E. (1)*
(1) Departamento de Genética y Microbiología, Universidad de Murcia, Murcia, Spain
(2) Departamento de Biología Celular e Histología, Universidad de Murcia, Murcia, Spain
(3) Laboratorio Nacional de Genómica para la Biodiversidad, CINVESTAV-IPN, Irapuato, Mexico
(4) Centro de Investigación en Alimentación y Desarrollo, Hermosillo, Mexico

Role of *Mucor circinelloides* ferroxidases in mucormycosis

The identification of new genetic determinants of virulence in mucormycosis, an emergent and lethal fungal infection caused by Mucorales, is the main purpose of this work. The availability of molecular genetic tools in *Mucor circinelloides* is attracting special attention to this fungus as a model to study mucormycosis. We chose zebrafish as a host model for infection with virulent and avirulent strains to carry out transcriptomic analyses during pathogenesis. One of the genes induced during infection, named *fet3a*, encoded a multicopper ferroxidase involved in the reductive iron uptake mechanism, and could be a putative virulence factor in *M. circinelloides*. A *fet3a* null mutant showed limited growth under low-iron conditions, however, it showed no virulence differences in the invertebrate model *Galleria mellonella*. Bioinformatics and expression analyses revealed two *fet3a* homologs, *fet3b* and *fet3c*, that are expressed in low-iron conditions and might be complementing the lack of function in *Δfet3a* strain. To confirm this hypothesis, *fet3b* and *fet3c* genes were silenced via RNAi in Δfet3a strain. Mutant and silenced strains virulence was compared to control strains in *G. mellonella*, showing that only the strain lacking activity of the three ferroxidases presented reduced virulence. These results revealed a redundant function between *fet3* genes in *M. circinelloides* and demonstrated their role as virulence determinants in mucormycosis.

This research is funded by Fundación Séneca (19339/PI/14), MECD (FPU14/01832) and MINECO (BFU2012-32246) co-financed by FEDER.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M07

Monday 4th April
14:00 - 16:00

THYNNE Elisha *(1), MCDONALD Megan C. (1), SOLOMON Peter S. (1)*
(1) Plant Sciences Division, Research School of Biology, The Australian National University, Canberra, Australia

HGT and gene loss have shaped the evolutionary path of three emerging fungal pathogens in Australia

In recent years, whole genome sequences for different fungal species have become ubiquitous on publically accessible sequence-databases. Increased access to gene sequences has heightened our ability to quickly and effectively study emerging fungal pathogens. We have utilised these resources to better understand a recently emerged phytopathogen complex that cause the disease known as white grain disorder (WGD) in wheat. We recently reclassified the causal agents of this disease as three separate Botryosphaeriaceae spp., *Eutiarosporella darliae*, *E. pseudodarliae*, and *E. tritici-australis*. It is unknown where these species originated, and how they are able to infect wheat. However, analysis of their genomes has revealed several horizontal gene transfer (HGT) of unique fungal secondary metabolite clusters, and a key gene loss event in *E. tritici-australis* that suggests a committed lifestyle shift to infection of wheat. One shared cluster we identified through the above analyses encodes a series of modular polyketide synthases (mPKSs). mPKS genes have traditionally only been found in bacteria and protists. As such, these are novel secondary metabolite key genes in fungi. We have identified orthologues in only one other fungal species *Macrophomina phaesolina*; a close relative and agricultural pathogen. We provide evidence that this gene was horizontally acquired from a bacterial or protist origin. We have also identified a hybrid PKS/non ribosomal peptide synthase (NRPS) gene cluster of interest, present in two of the three wheat pathogens, *E. darliae* and *E. pseudodarliae*. Orthologues of this cluster were previously shown to enable pathogenicity of fungi on woody plants; hosts more commonly associated with Botryosphaeriaceae spp. These results suggest that *E. darliae* and *E. pseudodarliae* were pathogens of woody plants at an earlier point in their evolutionary history. Comparison of the containing-genome region among the three species showed that most of the cluster has been lost in *E. tritici-australis*, the most infectious of the three species on wheat. Pathogenicity assays on the attached leaves of various woody plants showed that the two species whose genomes includes the PKS/NRPS are able to cause necrosis, whereas, *E. tritici-australis* was not. These initial findings have provided insight into the evolution of these emerging pathogens and indicated that through gene loss *E. tritici-australis* has made a committed lifestyle shift to infection of wheat.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M08

Monday 4th April
14:00 - 16:00

RODRÍGUEZ YON Yakelin *(1), DALPÉ Yolande (2), GONZÁLEZ Pedro J (1), FERNÁNDEZ Kalyanne (1), PÉREZ Eduardo J (1), MEDINA Laura R (1), QUIÑONES Madelaine (3), PETEIRA Belkis (3), VAN TUINEN Diederik (4), RIVERA Ramón A (1)*
(1) Instituto Nacional de Ciencias Agrícolas (INCA), Havana, Cuba
(2) Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, Canada
(3) Centro Nacional de Sanidad Agropecuaria (CENSA), Havana, Cuba
(4) Agroécologie, Pôle Interactions Plantes-Microorganismes, INRA, CNRS, Université de Bourgogne, Dijon, France

Cuban experience in the characterization and application of efficient strains of arbuscular mycorrhizal fungi

In Cuba, the use of biological products for production of healthy food, safe to man and to environment is a priority. The strategic projection of Mycorrhiza Program of the Cuban National Institute of Agricultural Science is particularly concerned by the manufacturing of bio-products, their application in agricultural practices and world marketing. Among the actually registered bio-products, highlight EcoMic® and LicoMic® which are based on three arbuscular mycorrhizal fungi (AMF) formulas. These strains were originally characterized through spore morphological studies and molecular analyses of V-H+ATPase and rDNA ITS regions. The three strains were placed in the Glomeraceae family of the *Glomeromycota phylum*. INCAM-4 strain was described as a new species: *Glomus cubense* (DAOM 241198), INCAM-2 strain was identified as *Funneliformis mosseae* and INCAM-11 strain belonging to Rhizophagus/Rhizoglomus genus as not yet been identified to species. The AMF bio-products have been largely tested over diverse economically important crops (tomato, wheat, yam, potato, rice, soybean, bean, banana, corn, pastures and others) under different agro-ecosystems. Results showed interesting yield increments (30-45 %), increase use of soil nutrients and reduction of chemical fertilizers (25-75 %) (NPK), decrease of crop damages caused by pathogens, increase plant tolerance to drought and to salt stress. As a relevant aspect, a highly efficient functioning of each strain was obtained at different soil pH. Based on those successful results and the opportunity to widespread AMF bio-product applications on cultivated land, we pay special attention to the evaluation of their ecological impact on agro-ecosystems. AMF bio-products development and application strengthened agricultural priority programs in Cuba by contributing to plant production, plant protection, mitigation of climate changes and food security.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M09

Monday 4th April
14:00 - 16:00

ERCHINGER Philipp *(1), WU Jinsong (2), KAHMANN Regine (1)*
(1) Max Planck Institute for Terrestrial Microbiology, Marburg, GERMANY
(2) Key Laboratory of Economic Plants and Biotechnology, Kunming, China

The effector protein Ten1 of *Ustilago maydis*

The fungus *Ustilago maydis* is a pathogen that establishes a biotrophic interaction with *Zea mays*. The interaction with the plant host is largely governed by more than 300 novel, secreted effector proteins, many of which are encoded in gene clusters. The deletion of cluster 10A consisting of 10 effector-encoding genes results in strongly reduced virulence after seedling infection. By generating subdeletions and by complementing the full deletion of cluster 10A with individual genes we could show that um03744 (termed ten1) has a major contribution to virulence. However, the single deletion of ten1 could not phenocopy the 10A cluster mutant suggesting that other genes of the cluster also contribute to virulence in the absence of ten1. Using self-assembling GFP we could show that Ten1 is secreted by the fungus in planta. Through a yeast 2-hybrid screen, ZmPP26, a maize protein phosphatase 2C, was identified as a potential interaction partner of Ten1. Interaction was further supported by Co-IP experiments after co-expression of Ten1 and ZmPP26 in *Nicotiana benthamiana*. Quantitative RT-PCR data showed that *ZmPP26* is upregulated in infected maize leaves and heterologously expressed ZmPP26 showed strong type 2C-specific phosphatase activity in vitro. Current work focuses on the recombinant production of Ten1 to test whether and how the interaction of both proteins alters phosphatase activity of ZmPP26.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M10

Monday 4th April
14:00 - 16:00

BARBETTI Martin *(1), GUNASINGHE Niroshini (1), CAWTHRAY Gregory (1), YOU Ming Pei (1)*
(1) School of Plant Biology and the UWA Institute of Agricultural Science, Faculty of Science, The University of Western Australia, 35 Stirling Highway, Crawley, Western Australia, Australia

Phenotypic, phylogenetic and mycotoxigenic variation within the crucifer white leaf spot pathogen *Pseudocercosporella capsellae*

Pseudocercosporella capsellae (white leaf spot disease) is an important disease across many crucifers. Isolates from *Brassica juncea* (Indian mustard), *B. napus* (oilseed rape), *B. rapa* (turnip), and *Raphanus raphanistrum* (wild radish) across Western Australia showed greatest virulence on *B. juncea*, least on *R. raphanistrum* and intermediate virulence on *B. napus*. While isolates collected from *B. napus*, *B. juncea* and *B. rapa* in general had a high degree of genetic similarity, isolates from *R. raphanistrum* were clearly differentiated from isolate groups collected from Brassica hosts. *P. capsellae* reference isolates from other countries generally grouped into a single separate cluster, highlighting the genetic distinctiveness of Western Australian isolates, and as occurs with other Brassica pathogens such as *Leptosphaeria maculans*, *L. biglogosa* and *Hyaloperonospora parasitica*. Subsequently, quantitative (thin-layer chromatography and high-performance liquid chromatography) and qualitative methods confirmed, for the first time, the production of cercosporin in *P. capsellae* and that cercosporin was positively correlated with virulence of isolates on *B. juncea* and *B. napus*. The enhanced virulence of *P. capsellae* in the presence of cercosporin and the ability of cercosporin per se to produce white leaf spot symptoms on host plants together highlight, for the first time, the important role of cercosporin as a pathogenicity factor in white leaf spot disease on Brassicaceae.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M11

Monday 4th April
14:00 - 16:00

LIANG Liang *(1), SCHIPPER Kerstin (2), LO PRESTI Libera (1), ZECHMANN Bernd (3), KAHMANN Regine (1)*
(1) Max Planck Institute for Terrestrial Microbiology, Dept. Organismic Interactions, Marburg, Germany
(2) Heinrich Heine University Düsseldorf, Dept. Microbiology, Düsseldorf, Germany
(3) Baylor University, Center for Microscopy and Imaging, Waco, USA

The role of Stp1, a secreted effector of *Ustilago maydis* essential for host colonization

Secreted effectors of phytopathogens play crucial roles during colonization. In the corn smut fungus *Ustilago maydis*, one of these essential effectors is stp1. stp1 mutants are non-pathogenic. Deletion analysis revealed that the N- and C-terminal domains of Stp1 are essential for function while the large central region is dispensable. In addition, co-expression of separated N- and C-terminal domains (fused to a signal peptide) of Stp1 could complement a ∆stp1 strain. To elucidate the function of Stp1, we have identified putative maize interactors (Sip proteins) of Stp1 by yeast two-hybrid screening. The activity of Sip3, a secreted maize cysteine protease, could be inhibited by both Stp1∆136-432 lacking the central domain and Stp1433-515 comprising the C-terminal domain of Stp1. Additionally, the activity of Sip19, a cytoplasmic serine/threonine-protein kinase could be inhibited by Stp1∆136-432. However, a newly established uptake assay based on transgenic maize expressing cytoplasmic BirA biotin ligase and preliminary result of immuno-EM yielded diverging results with respect to Stp1 localization. We are currently attempting to determine the localization of Stp1 via functional complementation of the ∆stp1 strain by transgenic maize expressing Stp1 in the cytosol or apoplast, respectively. In addition, we are trying to isolate interactors of Stp1 by performing Co-IP of tagged Stp1 (delivered by *U. maydis*) from infected maize tissue followed by mass-spectroscopic analysis. The results of these studies will be presented.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M12

Monday 4th April
14:00 - 16:00

YAN Xia *(1), LITTLEJOHN George R. (1), SOANES Darren M (1), TALBOT Nicholas J. (1)*
(1) School of Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, UK

Genome-wide characterization of novel effector-encoding genes in the rice blast fungus *Magnaporthe oryzae*

Rice blast disease is caused by the ascomycete fungus *Magnaporthe oryzae* and is one of the most devastating plant diseases threatening global rice production and food security. It is therefore important to investigate the interaction between rice blast and its host. Fungi secrete effector proteins into the apoplast and into plant cells to facilitate invasive hyphal growth and to suppress plant immunity responses. We used transcriptional profiling by RNA-seq to identify differentially expressed genes of *M. oryzae* during infection of rice. We identified 1825 genes that were significantly upregulated in at least one-timepoint during early host invasion. We classified 401 of these genes as candidate effector-encoding genes, including well characterized *AVR-PITA, AVR-PIK, SLP1, BAS1, BAS3* and *BAS4* effector genes. A total of 91 of these genes were co-ordinately expressed, including all previously identified effectors. Translational GFP fusions were expressed to visualize expression and localization of each effector by live-cell imaging. We have functionally analysed 31 novel *Magnaporthe* effector proteins (Meps) using targeted gene deletion and by identification of putative interacting partners. Apoplastic effectors outlined invasive hyphae while cytoplasmic effectors accumulated at the biotrophic interfacial complex (BIC). Interacting plant proteins recognized by these effectors suggest a range of putative functions during plant infection and will be described.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M13

Monday 4th April
14:00 - 16:00

GROGNET Pierre *(1), POHL Emma (1), KAHMANN Regine (1)*
(1) Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

Characterization of three putative mechano-sensitive ion channel encoding genes in *Ustilago maydis*

Ustilago maydis causes smut disease in maize. The infectious form is generated after mating of compatible haploids. Associated with mating on the leaf is the switch from the yeast to the hyphal form in response to sensing plant signals (hydrophobicity and hydroxy-fatty acids (HFA)). These two stimuli are sufficient to trigger filamentation and appressoria formation in vitro. Micro-array analysis, confirmed later by qPCR, showed that in these conditions, three putative genes, designated *msc1, msc2* and *msc3*, encoding mechano-sensitive (MS) channels are upregulated. To study the role of MS channels in *U. maydis* we used the inhibitor gadolinium chloride. Treatment of the solopathogenic strain SG200 with GdCl3 neither affected growth nor filamentation on charcoal plates. However, SG200 was unable to filament in response to hydrophobicity and HFA in presence of GdCl3. This suggested an involvement of putative MS channels during *U. maydis* development on the leaf surface. To connect such MS channels to infection-related development, we generated all single, double and the triple msc deletion strains. While strains carrying single and double msc gene deletions behaved like the parental strain, the triple deletion strain had largely lost virulence. This strain also displayed defects in filamentation and appressoria formation on parafilm. Based on these results, we propose a specific role of Msc channels in plant surface responses and/or during the early stages of infection.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M14

Monday 4th April
14:00 - 16:00

KROMBACH Sina *(1), REISSMANN Stefanie (1), BOCHEN Florian (1), KAHMANN Regine (1)*
(1) Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

The peroxisomal *Ustilago maydis* protein Scp2 is a virulence factor

It is firmly established for many pathogens that peroxisomes are crucial for successful host infection. Here we study the Scp2 protein of the biotrophic maize pathogen *Ustilago maydis*. Scp2 harbors a peroxisomal targeting signal and shares 38 % identity with the human sterol carrier protein 2 (SCP2). The *U. maydis* scp2 gene is up-regulated during early stages of plant colonization but shows a significant drop in expression two days post infection. Deletion of scp2 causes a virulence defect that appears to result from a reduced efficiency of plant cuticle penetration and the deletion strain is unable to form appressoria on artificial surfaces. This defect cannot be associated to a defect in peroxisomal Y-oxidation. Interestingly, we found small amounts of Scp2 to be unconventionally secreted with the help of colony secretion assays. However, infection experiments using a classical secreted version of Scp2 demonstrated that the observed virulence defect is not connected to the extracellular population of the protein. Surprisingly, infection of maize plants with a strain overexpressing scp2 under the cmu1 promoter, which is induced during infection and maintains high expression levels throughout biotrophic development, triggers strong plant defense reactions. We propose that Scp2 is involved in appressoria development, constituting a novel player in plant surface penetration.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M15

Monday 4th April
14:00 - 16:00

WALKOWIAK Sean *(1), ROWLAND Owen (2), SUBRAMANIAM Rajagopal (1)*
(1) Agriculture and Agri-Food Canada, Ottawa, Canada
(2) Carleton University, Ottawa, Canada

Whole genome sequencing and comparative genomics of closely related Fusarium Head Blight fungi: *Fusarium graminearum*, *F. meridionale* and *F. asiaticum*

We report on the first available genome sequences for *Fusarium asiaticum* and *Fusarium meridionale*, as well as additional genomes for *Fusarium graminearum*. These fungal species belong to the *Fusarium sambucinum* species complex and cause several diseases in economically important crops, including the disease Fusarium Head Blight of wheat. Despite being closely related, these species and individuals within the species have distinct phenotypic differences in toxin production and pathogenicity. Detailed comparison of genome structure and gene content across ten genomes for these species reveal greater than 95% genome alignment and gene conservation. We divided the genomes of these isolates into their «core» and «accessory» components to better classify the genetic overlap both within and between species. Genes that were poorly conserved between genomes were often co-localized in telomeric regions and select regions within chromosomes that also had increased single nucleotide polymorphisms, insertions, and deletions. Furthermore, we identified 12-92 genes unique to each isolate, over 100 unique genes to each of the three species, and over 1,000 genes that are poorly conserve in at least one genome. We also identified genes that are conserved across all genomes, but contain increased sequence variation and are under diversifying selection.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M16

Monday 4th April
14:00 - 16:00

LUDWIG Nicole *(1), LANVER Daniel (1), REIßMANN Stefanie (1), KAHMANN Regine (1)*
(1) Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

Functional characterization of the *Ustilago maydis* effectors stp2 and stp3

The fungus *Ustilago maydis* causes smut disease in maize. *U. maydis* is a biotrophic pathogen requiring living plant tissue for plant colonization. This is possible only if *U. maydis* manages to suppress plant defense responses and manipulate host physiology for its own benefit. To accomplish this, *U. maydis* secretes a cocktail of about 320 effector proteins. The majority of these proteins lack a functional annotation and their function remains to be uncovered. Our current work focuses on effectors expressed early during infection. Among these are the effector genes *stp2* and *stp3* (stop after penetration). *stp2* and *stp3* are highly conserved among related smut fungi infecting different hosts indicating a basic function. *U. maydis stp2* and *stp3* deletion strains are able to form appressoria that penetrate maize epidermal cells. In epidermal tissue *stp2* and *stp3* mutants both become growth arrested. However, both mutants differ with respect to the time point of the arrest and both mutants elicit distinct types of plant defense responses. We now use RNAseq to further analyze fungal and plant gene expression during the early stages of infection. In this approach the *stp2* or *stp3* deletion strains are compared to the wild type strain and other mutants which arrest plant colonization early. We expect this to reveal differences and similarities in plant responses to the different mutants which may provide leads to the function of these effector genes.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M17

Monday 4th April
14:00 - 16:00

HAN Xiaowei *(1), SCHUHMACHER Jan (2), GHOSH Anupama (1), DJAMEI Armin (1), KAHMANN Regine (1), BANGE Gert (2)*
(1) Max Planck Institute for Terrestrial Microbiology, Marburg, Germany
(2) LOEWE Center for Synthetic Microbiology (Synmikro), Marburg, Germany

Structure-function analysis of Cmu1, a secreted chorismate mutase in *Ustilago maydis*

The smut fungus *Ustilago maydis* codes for more than 300 secreted effectors which are mostly novel and contribute to the establishment of its biotrophic interaction with its host maize. These effectors exert their function either in the apoplast or translocate into the plant cells. The secreted chorismate mutase Cmu1 of *U. maydis* is one such translocated effector. Cmu1 is involved in suppressing host immunity by lowering salicylic acid levels. How Cmu1 is delivered into the plant cell is currently unknown. We have determined the structure of Cmu1 by X-ray crystallography using *E. coli* expressed protein and have initiated a detailed structure-based functional analysis. The structure revealed an acidic, surface-exposed domain. In addition, two conserved cysteine residues, which are unique in secreted chorismate mutases of smut fungi, are shown to form a disulfide bridge. We have followed up on the relevance of these and other features for uptake by plant cells, protein stability and allosteric regulation/catalytic activity of Cmu1, respectively, and will present our latest findings.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M18

Monday 4th April
14:00 - 16:00

SHOSTAK Kristina *(1), SUBRAMANIAM Gopal (2)*
(1) Agriculture and Agri-Food Canada, Ottawa, Canada
(2) Agriculture and Agri-Food Canada, Ottawa, Canada

Involvement of nitrogen metabolism in deoxynivalenol production through TRI gene regulation in *Fusarium graminearum*

Deoxynivalenol (DON) is a secondary metabolite extensively studied both due to its toxic properties and virulence function in *Fusarium graminearum*. Two genes essential for DON biosynthesis have been identified, TRI6 and TRI10, that were recently linked to regulation of several primary metabolic pathways in the fungus. Among these pathways, nitrogen metabolism is of particular interest because it is one of the environmental cues affecting DON production. The goal of the study was to construct and understand the metabolic interaction network in *Fusarium*, linking primary metabolic process of nitrogen response and secondary metabolic pathway of DON production. To identify nitrogen-responsive genes influenced by TRI6 and TRI10, analysis was done on general mRNA expression levels in *F. graminearum*. We compared early (6hr) and late (24hr) stages of DON induction in strains lacking functional TRI6 and TRI10. As well, a Tri6 deletion mutant with a constitutively expressed TRI6 vector was studied. The expression patterns highlighted changes in metabolic processes affected by the two genes, including nitrogen metabolism. We were able to identify expression profiles which were specific to TRI10 and TRI6, illustrating their functional differences. As well, RNA profiling in the strain where TRI6 is constitutively expressed reveals genes that are independent of nitrogen-limiting condition but regulated by TRI6. This data together with TRI6-Chromatin-IP experiments links the potential targets of TRI6 with their expression.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M19

Monday 4th April
14:00 - 16:00

TANAKA Shigeyuki *(1), RÖMER Mathis (1), LO PRESTI Libera (1), KAHMANN Regine (1)*
(1) Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

The putative host uptake motif in the Tin2 effector of *Ustilago maydis*

The secreted Tin2 effector protein of the corn smut fungus *Ustilago maydis* is taken up by plant cells and induces anthocyanin biosynthesis to promote virulence. Presently, it is unclear how Tin2 is taken up. We have mapped the putative uptake motif in Tin2 and found that the biological activity of Tin2 is severely reduced when negatively charged residues downstream of the signal peptide are substituted by alanine. On the other hand, the substitution of positively charged residues to alanine did not affect the biological activity of Tin2. Protein stability of Tin2 carrying the substitution of negatively charged residues during plant colonization was similar to the wild type Tin2 protein. We are currently assessing whether the reduced biological activity is due to reduced uptake by using a biotinylation-based assay we have recently developed.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M20

Monday 4th April
14:00 - 16:00

***SAKULKOO Wasin* (1)**, *LITTLEJOHN George R. (1), OSES-RUIZ Miriam (1), TALBOT Nicholas J. (1)*
(1) Biosciences, College of Life and Environmental Sciences, Exete University, Exeter, UK

The Pmk1 MAP kinase regulates cell-to-cell movement in rice tissue by the rice blast fungus

Rice blast disease, caused by a filamentous fungus *Magnaporthe oryzae*, poses a serious threat to global rice production. *M. oryzae* develops a specialised cell, called an appressorium, that generates enormous turgor to rupture and gain entry into plant cells. The Pmk1 Mitogen-Activated Protein Kinase (MAPK) cascade is essential for the formation and function of appressoria. Null mutants of *PMK1* cannot form appressoria, and are non-pathogenic, even when inoculated directly into wounded leaves. We generated an analogue-sensitive *pmk1*as mutant, with a point mutation in the gatekeeper residue causing the kinase to be selectively susceptible to an inhibitor, 1NA-PP1. We have used this novel mutant to characterise the role of Pmk1 in host tissue colonisation. Interestingly, when Pmk1 was inactivated with 1NA-PP1 after plant infection, fungal growth was restricted to the first plant cell occupied by the fungus and the plant immune response was also elicited, suggesting that Pmk1 MAPK is important for cell-to-cell movement by invasive hyphae of *M. oryzae* and for suppression of host immunity. To understand this process, Pmk1-dependent transcriptome analysis and quantitative live-cell imaging of biotrophic growth have been undertaken, revealing potential targets of Pmk1 MAPK regulation. The Pmk1 MAPK pathway is therefore a central regulator of plant infection by *M. oryzae* with a key role in host tissue invasion.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M21

Monday 4th April
14:00 - 16:00

GERVAIS Julie *(1), ROUXEL Thierry (1), FUDAL Isabelle (1), BALESDENT Marie-Hélène (1)*
(1) BIOGER, INRA, AgroParisTech, Thiverval-Grignon, France

Different waves of effector genes with contrasted genomic location are expressed by *Leptosphaeria maculans* during cotyledon and stem colonization

Leptosphaeria maculans, causal agent of blackleg disease, colonizes oilseed rape (*Brassica napus*) in two stages: a short and early colonization stage corresponding to cotyledon and leaf colonization leading to leaf spots development, and a late colonization stage during which the fungus colonizes systemically and symptomlessly the plant during several months before stem canker appears. To date, determinants of late colonization stage are poorly understood. Here, we hypothesized that *L. maculans* deployed effectors different from the one deployed in the early colonization and enabling the symptomless colonization. To get insight into these determinants, we performed a RNA-seq pilot project comparing fungal gene expression during the early colonization and the late colonization stages. Despite the low fraction of fungal material in infected tissues, enough fungal transcripts were detected to conduct a RNA-seq analysis focused on the discovery of new effector genes. 175 late effector candidates under-expressed in the early colonization stage and over-expressed in the infected stem were identified with this approach. These effector genes putatively involved in the systemic colonization are located in gene-rich regions, whereas the early effector genes involved in cotyledon colonization are located in gene-poor regions of the genome. Our analysis reveals a link between the expression pattern of effectors and their genomic location.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M22

Monday 4th April
14:00 - 16:00

ONUT BRÄNNSTRÖM Ioana *(1)*
(1) Uppsala University, Evolutionary Biology Centrem Department of Systematic Biology, Uppsala, Sweden

The lichen *Thamnolia vermicularis*, a lonely fungus with many green friends

The process of lichenization enables two habitat-dependent organisms, the fungi and the algae, to survive in environments hostile for its partners when isolated. The cosmopolitan, alpine and arctic lichen *Thamnolia vermicularis* represents one example of a successful colonizer. Nevertheless the assumed lichen spreading biology by fragments and the fact that its fungal partner seems to completely lack sexual reproduction the species huge geographic range is puzzling. We were able to confirm the species asexuality by sequencing the genomes and transcriptome of the fungal partner, and investigating the distribution of mating types in natural populations. First, we used the architecture of the mating-type idiomorphs to gain insight into the mating systems. The fungal genome harbors mating-type idiomorphs consistent with a heterothallic (i.e. self-incompatible) mating system. When screening a sample of 218 individuals from natural populations of *T. vermicularis*, we find only one of the mating types suggesting that sexual reproduction is constrained by the existence of a single mating type in natural populations. Even if fragment dispersal as holobiont can be advantageous in the short term, asexual lineages are considered evolutionary dead-ends. We hypothesize that the lichenized fungus of *T. vermicularis* adapts to a changing environment by associating with different algal species in different localities. If these algal haplotypes are adapted to a certain environment the switching to different photobionts might play a role by enabling the lichenized fungus to extend its geographic range and conquer new environments. Our data suggests that this might indeed be the case for the fungus of *T. vermicularis*. Using NGS sequencing data we discovered that on nutrient poor soils of Iceland, it shares the photobiont with two other genetically distant lichenized fungal species *Cetraria islandica* and *Cetraria aculeata*.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M23

Monday 4th April
14:00 - 16:00

DORLEKU Winfred-Peck (1), ***RIDENOUR John*** *(1), ATUNGULU Elizabeth (1), DUNKLE Larry D. (2), BLUHM Burton H (1)*
(1) Department of Plant Pathology, University of Arkansas, Division of Agriculture, Fayetteville, AR, USA
(2) Crop Production and Pest Control Research Unit, USDA-ARS, Purdue University, West Lafayette, IN, USA

The polyketide synthase, Ctb1, is required for cercosporin biosynthesis but not virulence in the maize pathogen *Cercospora zeae-maydis*

The genus *Cercospora* is one of the largest and most diverse assemblages of fungal foliar pathogens. Many *Cercospora* species are known to produce the polyketide-derived, secondary metabolite cercosporin. Cercosporin is a photoactivated perylenequinone, which absorbs light energy and generates reactive oxygen species. Recent work has elucidated the gene cluster required for cercosporin biosynthesis and demonstrated a role for cercosporin in virulence in multiple Cercospora species. However, it is not clear if cercosporin represents a virulence factor in all species of *Cercospora*. Considered one of the most important foliar pathogens of maize worldwide, *C. zeae-maydis* causes gray leaf spot of maize and is known to produce cercosporin. In this study, we identified an isolate of *C. zeae-maydis* that failed to produce cercosporin under favorable culture conditions yet was fully pathogenic on maize leaves. Thus, to clarify the involvement of cercosporin in grey leaf spot of maize, an ortholog of *CTB1* (a gene encoding a polyketide synthase that catalyzes formation of a key cercosporin precursor in *C. nicotianae*) was identified and disrupted via homologous recombination in *C. zeae-maydis*. Disruption of *CTB1* eliminated cercosporin production but did not affect fungal growth and development *in vitro*. Interestingly, pre-infectious development, including germination on the leaf surface, appressorium formation, and number of appressoria per germling, in the *CTB1* disruption strains was indistinguishable from the wild-type strain. Additionally, the *CTB1* disruption strains were able to form lesions and sporulate on maize leaves. Taken together, these findings suggest that cercosporin may be a component of a suite of as-yet unidentified virulence factors produced by *C. zeae-maydis* during infection of maize but, alone, is dispensable for pathogenesis.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M24

Monday 4th April
14:00 - 16:00

HERNANDEZ Julie *(1), HERNÁNDEZ-LEÓN Rocío (1), VALENCIA-CANTERO Eduardo (1), MORENO-HAGELSIEB Gabriel (2), SANTOYO Gustavo (1)*
(1) Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Mexico
(2) Wilfrid Laurier University, Waterloo, Ontario, Canda

Evaluation of biocontrol mechanisms and gene expression of antifungal metabolites by *Pseudomonas fluorescens UM270* against *Botrytis cinerea*

Botrytis cinerea was listed as the second scientifically/economically important pathogen in the world on 2012. This gray mould can infect more than 200 plant species, mainly affecting dicotyledonous hosts. Economic losses are huge and only fungicides are the common method to control Botrytis infection. These agrochemicals also represent a devastating damage to the environment and human health. Thus, an eco-friendly alternative to control phytopathogens is worldwide desired. The bacterial strain *Pseudomonas fluorescens UM270*, was isolated and tested for biocontrol activities against different phytopathogens and was found to be effective antagonist to *B. cinerea*. Several direct and indirect mechanisms were experimentally evaluated and some genes were bioinformatically identified, such as, antibiotics and siderophores, as well as production of volatile organic compounds and lytic enzymes involved in fungal inhibition. *P. fluorescens UM270* presented all those mechanisms. At expression level, Semi-quantitative PCRs of genes involved in the synthesis of different antibiotics were measured and compared, such as, phlD (2,4-diacetylphloroglucinol), phzCD (phenazines) and hcnAB (hydrogen cyanide). The experiment consisted in a confrontation assay between *UM270* and *B. cinerea* in a petri dish; after 48h of interaction, genomic RNA from strain *UM270* was isolated and synthesis of cDNA was carried out. Our results showed an increased expression level of phlD and hcnAB genes, respect to the 16s rDNA basal gene expression, being hcnAB the highest. Thus, suggesting these gene products as the main responsible of the inhibition. Following experiments are being performed and we expect to present them during the Conference.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M25

Monday 4th April
14:00 - 16:00

ESHER Shannon *(1), KOHLBRENNER Maria (1), OST Kyla (1), ALSPAUGH Andrew (1)*
(1) Duke University, Departments of Molecular Genetics and Microbiology/Medicine, Durham, NC, USA

Characterizing novel regulators of the *Cryptococcus neoformans* cell wall and their implications on immune recognition

Cryptococcus neoformans is an opportunistic fungal pathogen causing over 600,000 deaths per year in immunocompromised individuals. This fungus dramatically alters its cell wall, both in size and composition, upon entering the host. Through a genetic screen for fungal cell wall regulators, we have identified *C. neoformans* Mar1, a novel protein that mediates important cell wall phenotypes in response to the host environment. We have generated an independent loss-of-function mutant which displays reduced growth at elevated temperature and sensitivity to cell wall stressors. We have analyzed cell surfaces changes of the *mar1*Δ mutant and shown that Mar1 is required for capsule attachment. Using a combination of cell wall staining and biochemical assays we have observed an increase in the immunogenic cell wall components chitin and chitosan, specifically in host-like tissue culture conditions. We have observed that the *mar1*Δ mutant cell wall induces increased macrophage activation *in vitro*, and that Mar1 is required for virulence *in vivo*. In order to elucidate the innate immune receptor responsible for recognition the immunogenic *mar1*Δ mutant cell wall, we have shown that both MyD88 and Card9 are required. To determine the function of Mar1, we have fluorescently tagged this protein and observed localization to small, punctate structures throughout the cell. Finally in the absence of Mar1, the PKC1/cell wall integrity pathway is aberrantly activated. Ongoing work aims to define the innate immune response to the *mar1*Δ mutant, as well as identify the mechanism of Mar1 cell wall regulation.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M26

Monday 4th April
14:00 - 16:00

SPERSCHNEIDER Jana (1), *GARDINER Donald (2), DODDS Peter (3), TINI Francesco (4), COVARELLI Lorenzo (4), SINGH Karam (1), MANNERS John (3), TAYLOR Jennifer (3)*
(1) CSIRO Agriculture, Centre for Environment and Life Sciences, Perth, Australia
(2) CSIRO Agriculture, Queensland Bioscience Precinct, Brisbane, Australia
(3) CSIRO Agriculture, Black Mountain Laboratories, Canberra, Australia
(4) Department of Agricultural, Food and Environmental Sciences, University of Perugia, Perugia, Italy

EffectorP: Predicting fungal effector proteins from secretomes using machine learning

Eukaryotic filamentous plant pathogens secrete effector proteins that modulate the host cell to facilitate infection. Computational effector candidate identification and subsequent functional characterization delivers valuable insights into plant-pathogen interactions. However, effector prediction in fungi has been challenging due to a lack of unifying sequence features such as conserved N-terminal sequence motifs. Fungal effectors are commonly predicted from secretomes based on criteria such as small size and cysteine-rich, which suffers from poor accuracy. We present EffectorP which pioneers the application of machine learning to fungal effector prediction. EffectorP improves fungal effector prediction from secretomes based on a robust signal of sequence-derived properties, achieving sensitivity and specificity of over 80%. Features that discriminate fungal effectors from secreted non-effectors are predominantly sequence length, molecular weight, protein net charge as well as cysteine, serine and tryptophan content. We demonstrate that EffectorP is powerful when combined with in planta expression data for predicting high-priority effector candidates. We highlight future directions of EffectorP and the potential of machine learning for improved subcellular localization prediction of cytoplasmic effectors in plant cells and for effector prediction in oomycetes. EffectorP is available at http://effectorp.csiro.au.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M27

Monday 4th April
14:00 - 16:00

NAGY Gábor (1), *HASSAN Mohamed (2), KUMAR Dileep (1), VAZ Amanda Grace Leo (1), BARTHA Emese (1), CSERNETICS Árpád (1), VÁGVÖLGYI Csaba (1), VOIGT Kerstin (2), PAPP Tamás (1)*
(1) Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary
(2) Jena Microbial Resource Collection, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute and Friedrich Schiller University, Jena, Germany

Characterization of virulence genes in opportunistic human pathogenic Mucoralean fungi

Several members of Mucoromycotina are considered as opportunistic human pathogenic fungi, which can cause frequently fatal systemic infections in immunocompromised patients. In the recent years, the number of patient with mucormycoses has significantly increased worldwide. The aim of the present study is the functional characterization of selected genes, which may have role in the infection process of *Mucor circinelloides* and *Lichtheimia corymbifera*. Up and down regulated genes of *L. corymbifera* during interaction with murine alveolar macrophages (MH-S) were selected. Several genes involved in iron transport, encoding hydrophobic surface and heat stress proteins, ergosterol biosynthesis etc. were cloned and disrupted in *L. corymbifera* and mutants in the HMG-CoA reductase genes of *M. circinelloides* were also involved in the study. The interactions of *Lichtheimia* and *Mucor* mutants with murine alveolar macrophages are still in progress. Our results will greatly contribute to knowledge of the virulence of *Mucor* and *Lichtheimia*. Exploration of the role of the potential virulence factors in the infections would help to find new therapeutic targets against human pathogenic fungi. The applied comparative approach could reveal whether there are any differences and/or similarities in the pathogenicity and defensive ability of different Mucoromycotina species.

This research was supported by the grants OTKA NN106394 and TÉT_12_DE-1-2013-0009. T.P. is a grantee of J. Bolyai Scientific Scholarship of the Hungarian Academy of Sciences. Á.C. is supported by the Hungarian Academy of Sciences Postdoctoral Research Programme (Postdoc-16, 45.0.080).

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M28

Monday 4th April
14:00 - 16:00

YANG Shang-Wei (1), ***WANG Chih-Li*** *(1)*
(1) Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan

Colletotrichum higginsianum **ChHox1 is involved in modulating fungal morphology and virulence**

Colletotrichum higginsianum is the causal agent of crucifer anthracnose which affects many economically important crops of Brassica species and Raphanus species as well as *Arabidopsis thaliana*. The interaction of *C. higginsianum* and *A. thaliana* was proposed as a model pathosystem in studying the interaction between Colletotrichum and dicot plants. A transcriptome analysis of infected *A. thaliana* and our previous study on Chinese cabbage showed a transcription factor of *C. higginsianum*, ChHox1, containing a homeodomain was highly induced during the necrotrophic phase. In this study, we deleted *ChHOX1* and analyzed its functions. Interestingly, although the knockout strains of *ChHOX1,* Δ*Chhox1*, could form normal appressoria *in vitro* and *in vivo*, it showed differentiated virulence to two plant hosts. Δ*Chhox1* caused similar lesions as wild-type strain on *A. thaliana*, but only caused tiny sunken lesions or symptomless on Chinese cabbages. The infection course of Δ*Chhox1* was impeded at the biotrophic stage on Chinese cabbages. Moreover, Δ*Chhox1* showed altered morphological phenotypes, including slower and dense colony growth, lack of spiral growth on Czapek- Dox agar, longer and wider conidia, and hyperbranching mycelia on glass slides. Compared with the wild-type strain, Δ*Chhox1* was more tolerant to osmotic, oxidative and cell wall integrity stresses. ChHox1 is likely a transcription factor involved in the control of morphological development and of cellular responses to multiple stresses. The alteration of the two different functions involved in pathogenicity in Δ*Chhox1* may contribute to its differential virulence on different hosts.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M29

Monday 4th April
14:00 - 16:00

LONG Nanbiao (1), ***LU Ling*** *(1)*
(1) College of Life Sciences, Nanjing Normal University, Nanjing, China

The leucine-biosynthesis related, Zn2Cys6 transcript factor LeuB systematically regulates iron homeostasis in *Aspergillus fumigatus*

Aspergillus fumigatus has become one of the most prevalent opportunistic pathogen, causing severe and usually fatal invasive infections in immunocompromised hosts. During infection, iron acquisition is a critical factor for virulence of pathogens since host proteins tightly sequester the available iron from an essentially iron-free environment. Thus, the competition ability of pathogen with host for the iron acquisition is an important virulence determinant in *A. fumigatus*. However, the knowledge for the mechanism of iron regulation in *A. fumigatus* is still limited. Here, we demonstrated that the Zn2Cys6 transcription factor LeuB plays important roles in both iron regulation and leucine biosynthesis. Deletion of *leuB* greatly reduced the hyphal growth rates and sharply decreased conidiation under the iron starvation condition and/or in the absence of leucine. These defects could be completely recovered with the addition of both iron and leucine in media but addition of either one only can partly rescue the colony defect, indicating LeuB is involved in function of the iron uptake system and leucine biosynthesis in a independent manner. Most interestingly, nucleus-localized LeuB was able to systematically regulate siderophore-mediated iron acquisition components on transcription level but could not affect the transcription of hapX, a major transcription factor proved so far that regulate the production of the extracellular siderophore, triacetyl fusarinine C (TAFC) under the iron deficiency condition. Instead, LeuB is required for the stability of HapX such that deletion or defected leuB on the site of the Zn2Cys6 motif caused the completely degradation of HapX. However, addition of proteasome inhibitor MG132 was able to dramatically inhibit the HapX degradation, suggesting defect of LeuB transcription motif is involved in a ubiquitin-mediated proteasome degradation process. Further genetics studies collectively indicate that LeuB not only is able to influence the iron-excess-response transcription factor sreA under the iron sufficiency condition but also affect the stability of HapX to regulate TAFC production under the iron deficiency condition. To our knowledge, this is the first report for the function of LeuB on regulation the iron homeostasis in fungal pathogen *in vitro* and *in vivo*.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M30

Monday 4th April
14:00 - 16:00

HU Guanggan *(1), CAZA Melissa (1), BAKKEREN Erik (1), BAIRWA Gaurav (1), KRETSCHMER Matthias (1), KRONSTAD James (1)*
(1) Michael Smith Laboratories, The University of British ColumbiaVancouver, Canada

Cdc50 plays a role in protein trafficking, iron acquisition and virulence in *Cryptococcus neoformans*

The export of virulence factors, such as capsule polysaccharide, by exocytosis and specialized small vesicles, is critical in the pathogenicity in *C. neoformans*. We have previously showed that brefeldin A (BFA), an inhibitor targeting the anterograde transport of proteins between the ER and Golgi apparatus, significantly reduces capsule formation (Hu *et al*., 2007). By screening a collection of 30,000 Agrobacterium-mediated insertion mutants for growth defects on BFA, we identified a set of genes likely involved in trafficking and secretion of virulence factors. In this study, we characterized one of the genes, *CDC50*, in more detail; this gene is thought to encode an endosomal protein. Deletion of *CDC50* in a serotype A strain H99 resulted in increased sensitivity to inhibitors of protein trafficking including BFA and monensin, a Na+/H+ ionophore that blocks intracellular transport in both trans-Golgi and post-Golgi compartments. Unexpectedly, there was no significant difference in growth, capsule formation or melanin production between the wild type and *cdc50* strains at either 30^{o}C or 37^{o}C. However, deletion of *CDC50* caused a decrease in acid phosphatase activity and the mis-localization of Aph1, the major acid phosphatase that contributes to fungal growth during infections caused by *C. neoformans* (Lev *et al*, 2014). The *cdc50* deletion mutants also displayed reduced growth on heme and inorganic iron sources, indicating that Cdc50 plays a role in iron uptake and utilization. The mutants are also hypersensitive to a set of membrane targeting drugs (cinnamycin, duramycin, eldefosine and miltefosine), indicating that Cdc50 is involved in maintaining membrane phospholipid asymmetry. Cdc50 is also required for survival during interactions with macrophages. Furthermore, in a mouse inhalation model, *cdc50* mutants exhibited significant attenuated virulence compared to the wild-type strain. Taken together, Cdc50 contributes to virulence in *C. neoformans* through roles in the maintenance of phospholipid asymmetry, protein trafficking, and iron acquisition, but does not influence the deposition of the major virulence factors capsule and melanin outside of the cell.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M31

Monday 4th April
14:00 - 16:00

YU Pei-ling (1, 2, 3), CHANG Chai-Chi (1, 2, 3), WANG Chih-Li (1), CHEN Pei-Yin (1), **LEE Miin-Huey** (1, 2, 3)
(1) Department of Plant Pathology, National Chung-Hsing University, Taichung 402, Taiwan
(2) NCHU-UCD Plant and Food Biotechnology Center, National Chung-Hsing University, Taichung, Taiwan
(3) Agricultural Biotechnology Center, National Chung-Hsing University, Taichung, Taiwan

The transcription factor MfAP1-mediated redox sensing is crucial for a successful infection by *Monilinia fructicola*

Monilinia fructicola (G. Winter) Honey is a devastating pathogen on Rosaceae that causes blossom blight and fruit rot. Only a few of studies related to plant-pathogen interaction were published and knowledge about oxidative stress in relation to successful infection is highly limited in *M. fructicola*. In this study, we cloned and characterized a redox-responsive transcription factor MFAP1, a YAP1 homolog. *MfAP1*-silenced strains were generated by polyethylene glycol-mediated protoplast transformation and *Agrobacterium* T-DNA mediated transformation. Pathogenicity assay demonstrated that *MfAP1*-silenced strains caused smaller lesions on rose and peach petals. Transformants carrying extra copies of *MfAP1*driven by the native promoter were generated for *MfAP1* overexpression. Interestingly, *MfAP1*-overexpressing strains also caused smaller lesions on rose petal. Strains carrying two copies of *MfAP1* accumulated ROS at higher levels and delayed the accumulation of *MfAP1* transcripts compared to wild-type during pathogenesis. By analyzing reactive oxygen species (ROS) production and the expression patterns of redox- and virulence-related genes in the wild-type strain and an *MfAP1*-overexpressing strain, we found that *M. fructicola* wild-type strain could sense oxidative stress at the infection site, activate the expression of *MfAP1*, and then up-regulate the genes required for ROS detoxification and fungal virulence. In contrast, *MfAP1* expression in the *MfAP1*-overexpressing strain was suppressed after the induction of a strong oxidative burst at the infection site and thus altered the expression of ROS detoxification and virulence-related genes. Our results highlight the importance of *MfAP1* and ROS accumulation in the successful infection of *M. fructicola*.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M32

Monday 4th April
14:00 - 16:00

BAIRWA Gaurav *(1), TANG Richard (1), ZHANG Alicia (1), KRONSTAD James (1)*
(1) Michael Smith Laboratories, The University of British Columbia, Vancouver, Canada

Role of heme and iron acquisition functions in *Cryptococcus neoformans*-macrophage interactions

Cryptococcus species are the leading cause of fungal meningitis in the HIV/AIDS population with an estimated 1 million cases of cryptococcal meningitis per year. The majority of cases are caused by *Cryptococcus neoformans*. One critical aspect of diseases caused by fungal pathogens of humans is the ability to acquire nutrients in the host environment. Iron is one of the essential nutrients for both pathogen and host. We have identified important roles for both high and low-affinity iron acquisition pathways in the virulence of *C. neoformans*. However, our understanding of the functions of iron-acquisition pathways in fungal survival inside the host environment and immune cells is still in its infancy. In this study, we used a candidate gene approach to characterize the roles of both high and low-affinity iron acquisition pathways in *Cryptococcus* interactions with macrophages. We show that the *C. neoformans* is unable to survive in the murine macrophage-like cell line, J774A.1, upon iron-starvation, and prior iron-starvation of *C. neoformans* cell before macrophage infection also diminishes intracellular survival. Additionally, *C. neoformans* requires a siderophore transporter (encoded by *SIT2*), a ferric reductase (encoded by *FRE2*), and a putative extracellular hemophore (encoded by *CIG1*) to survive inside macrophages. The latter result indicates that heme may be one of the major iron sources inside the macrophage. We did not observe any growth inhibition for mutants defective in the high-affinity iron uptake system (encoded by *CFO1* and *CFT1*) inside the macrophages. Importantly, a mutant defective in the GATA-type, zinc-finger transcription factor, Cir1, which is a major regulator of iron-uptake genes and other virulence factors in *C. neoformans*, was found to be attenuated for growth in macrophages. We also conducted a large scale screen of a *C. neoformans* gene deletion library, representing half of the genes in the genome, to identify new candidates involved in heme use. The screen also identified mutants with altered susceptibility to non-iron metalloporphoryins, the toxic analogs of heme and potential anti-fungal drugs. Overall, our work on Cryptococcus-macrophage interactions and identifications of novel iron-uptake genes as drug targets will be presented.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M33

Monday 4th April
14:00 - 16:00

MAYER Francois *(1), KRONSTAD James (1)*
(1) Michael Smith Laboratories, University of British Columbia, Vancouver, Canda

Natural bacterium-mediated repression of virulence factor production by human fungal pathogens

Inter-species and inter-kingdom microbial interactions are ubiquitious in nature. As a consequence of competition for space and food, a considerable proportion of these interactions are antagonistic. The meningitis-causing human fungal pathogen *Cryptococcus neoformans* is acquired from the environment by inhalation of spores and/or desiccated yeast cells. In immunocompromised individuals these fungal cells are able to infect lung tissue and, eventually, to further disseminate to and infect the brain. Here, we hypothesized that *C. neoformans* is likely exposed to other microbes in nature that may possess anticryptococcal properties. We have identified the soil bacterium *Bacillus pumilus* as a potent inhibitor of *C. neoformans* melanization, capsule formation, urease secretion, and biofilm formation, all of which are key cryptococcal virulence factors. Interestingly, *B. pumilus* was also found to inhibit the yeast-to-hyphal transition in *Candida albicans*, another major human fungal pathogen that, in contrast to *C. neoformans*, is obligately associated with warm-blooded animals. In *C. neoformans*, the antifungal activity of *B. pumilus* appeared to be mediated by direct contact and live bacteria were required. We show that at least part of the antifungal activity appears to be the result of a targeted influence on the fungal cell wall. These results may help in the identification of new antibiotic drug targets, as well as in the discovery of novel antifungal mechanisms.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M34

Monday 4th April
14:00 - 16:00

BOHORQUEZ Julia *(1), JOHANSEN Renee (2), ROBERTSON Alastair (1), MILLNER James (1), STEPHENS Jonathan (3), ARCHER Richard (4)*
(1) Institute of Agriculture & Environment, Massey University, Palmerston North, New Zealand
(2) School of Biological Sciences, University of Auckland, Auckland, New Zealand
(3) Comvita Innovations, Institute for Innovation in Biotechnology, University of Auckland, Auckland, New Zealand
(4) Massey Institute of Food Science and Technology, Massey University, Palmerston North, New Zealand

Are arbuscular mycorrhizal fungi drivers of manuka performance and do they influence the quality of mānuka honey?

Leptospermum scoparium (mānuka) is a widespread indigenous shrub in New Zealand. There is lack of information regarding the presence and diversity of AMF in mānuka. Importantly, mānuka is valued for its medicinal honey and there is now a sudden interest in plantation mānuka to farm the plant for its honey. This research aims to explore the importance of mycorrhizal associations for the growth and survival of mānuka and the quantity and quality of the nectar. A pilot study was carried out to confirm the colonisation of mānuka roots by mycorrhizal fungi. Roots collected from different provenances of mānuka in the Wanganui Region confirmed the presence of ectomycorrhizal and endomycorrhizal mycorrhizal fungi. Next generation sequencing of the ITS region revealed many species belonging to the phyla Ascomycota, Basidiomycota and Glomeromycota. The closest BLAST matches indicate that the presence of *Rhizophagus*, *Glomus, Tormentella,* and *Mortirella*. These fungi could therefore play a crucial role in the development of the plant and consequently influence characteristics such as flower density and the nectar quality. The next steps are to broaden the molecular survey to a wider range of sites and to trial inoculating seedlings to improve establishment rates and vigour of planted mānuka.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M35

Monday 4th April
14:00 - 16:00

ZHANG Jianhua (1), SCHOUSTRA Sijmen (1), VERWEIJ Paul (2), MELCHERS Willem (2), ZWAAN Bas (1), ***DEBETS Fons*** *(1)*
(1) Laboratory of Genetics, Wageningen University, Wageningen, The Netherlands
(2) Department of Medical Microbiology, Radboud University Medical Centre, Nijmegen, The Netherlands

Resistance to medical azoles in *Aspergillus fumigatus* readily evolves experimentally under the selection pressure of agricultural fungicides

Azole resistance seriously hampers the treatment of invasive infections of the saprophytic mold *Aspergillus fumigatus* in the human lung. Resistance has emerged as a global health concern. Previous evidence indicated both clinical and environment routes of resistance selection. In order to assess the likelihood of both routes and their mechanistic bases, we performed an evolution experiment with *A. fumigatus* under selection pressure of five agricultural azoles and assessed the (cross)-resistance to agricultural and medical azoles. We showed that cross-resistance between the drugs is ubiquitous and evolves readily in only a limited number of generations. Moreover, we monitored the frequency of candidate gene (*cyp51* and *hapE*) mutations and phenotypic changes (morphotype and fitness) over evolutionary time. These analyses revealed a surprising variety in evolutionary trajectories and also that candidate gene mutations only partly explained phenotypic resistance. Our study indicates that experimental evolution can be a powerful tool to test scenario's for the emergence and persistence of clinical azole resistance. Moreover, the ongoing unravelling of the variation in evolutionary dynamics, the extent of cross resistance, and the (molecular) genetic mechanisms underlying the resistance has the potential to influence treatment protocols.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M36

Monday 4th April
14:00 - 16:00

CHANG Chia-Chi (1)
(1) Department of Plant Pathology, National Chung-Hsing University, Taichung, Taiwan

Agrobacterium T-DNA insertions identified genes involved in growth and pathogenicity of *Colletotrichum gloeosporioides* on mango

Warm and humid conditions favor the occurrence of mango anthracnose, caused by *Colletotrichum gloeosporioides* in Taiwan, which results in severe reduction on fruit quality and yield. To identify the pathogenicity factors of *C. gloeosporioides* on mango, *Agrobacterium tumefaciens*-mediated transformation (ATMT) was used to generate transformants of *C. gloeosporioides* TYC-2. Total 332 transformants were obtained. Southern blot analysis showed that 63.6% transformants carried single T-DNA insertion by analyzing 11 randomly selected transformants. Morphological examination determined that transformant C225 was an albino mutant and transformant I4 showed poor growth. Moreover, transformant I4 lost pathogenicity on mango leaf and displayed lower ability of sporulation and higher sensitivity to osmotic stress with sorbitol compared to the wild type strain. Transformant 48 and 76 showed reduced virulence and caused 0.7% and 21.4% of the lesion area generated by the wild type strain, respectively. Southern blot analysis indicated that transformant C225, 76 and I4 had only one T-DNA insertion event, whereas transformant 48 and C17 carried multiple copies of T-DNA. By inverse PCR, T-DNA flanking regions were recovered and sequence data showed that T-DNA inserted in the gene encoding a pH-response transcription factor (pacC) in transformant 76 and in between two genes encoding haloacid dehalogenase (HAD) and HNH endonuclease (HNH Ease) in transformant I4. Semi-quantitative RT-PCR showed that the expression of HAD and HNH Ease was affected in transformant I4. The function of these two genes and their homologs in fungi are unidentified. HAD gene knockout mutants, *HADKO2-1* and *HADKO4-2*, caused larger lesion size on mature mango leaf than the wild type strain. However, the coding region of a hypothetical protein located in the upstream of HAD gene was disrupted and the expression level of HNH Ease gene were influenced due to gene replacement in *HADKO2-1* and *HADKO4-2*. Therefore, single- and double-gene silenced strains were generated and characterized. Overall, we successfully generated transformants which were affected on pathogenicity or vegetative growth and identified genes involved in growth and pathogenicity of *C. gloeosporioides* on mango.

POSTER SESSION ABSTRACTS
CS2M37

Monday 4th April
14:00 - 16:00

MARTINEZ ROCHA Ana Lilia *(1), GLASENAPP Anika (1), BOENISCH Marike J. (1), MÜNSTERKÖTTER Martin (2), GÜLDENER Ulrich (2), SCHÄFER Wilhelm (1)*
(1) Molecular Phytopathology and Genetics, Biocenter Klein Flottbek, University of Hamburg, Hamburg, Germany
(2) Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München (GmbH), München, Germany

Identification of putative effector proteins during early *Fusarium graminearum* infection in wheat

The phytopathogenic fungus *Fusarium graminearum* causes devastating losses on cereal crops worldwide. Fungal plant pathogens secrete a repertoire of effector proteins to enable infection and manipulate the host immune response to their advantage. Until now, the only effector protein described for *F. graminearum* with a known function in virulence is a secreted lipase leading to suppression of wheat defence. We looked for small secreted proteins with unknown functions as putative effectors by comparing the differential gene expression from epiphytically growing runner hyphae and complex infection cushions produced during early infection of *F. graminearum* on wheat florets. The transcriptome analysis revealed 110 genes encoding for small secreted proteins that are up-regulated in infection cushions, from which 19 are *F. graminearum* specific. We functionally characterized the most up-regulated putative effector gene *FgEF1*. Expression analysis by qPCR and promotor fusion with mCherry revealed low expression of *FgEF1* in culture, a high expression in runner hyphae during infection, and an even stronger up-regulation in infection cushions. Although *FgEF1* is not essential for *F. graminearum* pathogenesis, its high expression during early wheat floret infection indicates a function during the initial infection process. Translational fusion with mCherry localized *FgEF1* near the fungal cell wall of runner hyphae and infection cushions during wheat leaf infection. This localization suggests a protective function against cell wall degradation and a possible evasion of host recognition during early infection.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M38

Monday 4th April
14:00 - 16:00

BECKER Yvonne *(1), BECKER Matthias (1), GREEN Kimberly (2), SCOTT Barry (2)*
(1) Leibniz Institute of Vegetabel and Ornamental Crops, Großbeeren, Germany
(2) Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand

Epichloë festucae **establishes an epiphyllous network on the surface of** ***L. perenne*** **leaves by an expressorium, an appressorium-like leaf exit structure.**

The biotrophic fungus *E. festucae* systemically colonizes the intercellular spaces of the aerial tissues of Festua and Lolium grasses, including leaf primordia, sheath and blade tissue. Besides forming an endophytic hyphal network, *E. festucae* also grows as an epiphyte but the mechanism whereby it establishes a network on the surface of the leaf is not known. Using a combination of confocal laser scanning (CLS), scanning electron- and transmission electron- microscopy we have identified a novel structure, which we have named an expressorium to distinguish it from the appressorium used by plant pathogens to enter plants, that allows endophytic hyphae to exit to the leaf surface. The expressorium is a swollen hyphal compartment, often delimited by two septa, that develops just below the cuticle after the hyphae have passed through the epidermis. CLSM analysis of aniline blue/WGA-AF488 co-stained samples revealed a major remodelling of the fungal cell wall following exit from the leaf. Only the septa of endophytic hyphae fluoresce with WGA-AF488 whereas the entire cell wall of epiphytic hyphae fluoresce, suggesting cell wall chitin is either absent or masked in the former but not the latter; results consistent with the need to avoid a host defence response. Given both the Nox1 (=NoxA) and Nox2 (=NoxB) NADPH oxidase complexes are required for the differentiation of appressoria in *M. oryzae*, as are NoxB and NoxR in *B. cinerea* we tested the effects of noxA, noxB, noxAB and noxR mutants on the development of expressoria in *E. festucae*. All of these mutants showed defects in the formation of expressoria and instead formed abundant hyperbranched sub-cuticular hyphae. These hyphae eventually breach the cuticle to form epiphyllous pseudo-networks of hyphae that fail to undergo cell-cell fusion (noxA, noxAB, noxR) but hyperconidiate (noxA, noxAB); phenotypes previously reported for these mutants in axenic culture. These results highlight the importance of the expressorium for *E. festucae* to transition from endophytic to epiphytic growth and the major cell wall remodelling that accompanies these distinct physiological states.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M39

Monday 4th April
14:00 - 16:00

FORD Kathryn *(1), GAMBLE Louise (1), GRIFFE Lucie (1), BEREPIKI Adokiye (1), EPIHOV Dimitar (1), KNOGGE Wolfgang (3), NEWTON Adrian (1), HAMMOND-KOSACK Kim (2), KANYUKA Kostya (2), AVROVA Anna (1)*
(1) Cell and Molecular Sciences, James Hutton Institute, Dundee, UK
(2) Wheat Programme, Rothamsted Research, Harpenden, UK
(3) Leibniz-Institute of Plant Biochemistry, Halle, Germany

A genomics approach to identify effectors in *Rhynchosporium commune* essential for pathogenicity on barley

The ascomycete pathogen *Rhynchosporium commune* is present in all barley-growing regions worldwide and is one of the most economically important constraints to barley production, causing yield reductions of up to 45% and a decrease in grain quality, leading to up to £7.2 million of losses in the UK annually. Disease management currently relies on fungicides and resistant cultivars, but *R. commune* populations can change rapidly, defeating new barley resistance (R) genes and fungicides within a few years. To provide more durable resistance for barley scald, we aim to elucidate effectors, including those that encode avirulence (Avr) genes, which may be essential for pathogenicity on barley. We have used a comparative genomics approach to analyse nine *R. commune* isolates in order to identify candidate effectors and expression analysis has identified candidates that are upregulated early in infection, suggesting importance in pathogenicity. Several approaches are being used to characterise these candidate effectors including association genomics, screening barley cultivars using Barley stripe mosaic virus (BSMV) for a cell death response indicating R-gene-mediated recognition of Avr effector proteins, heterologous expression of candidates in Pichia pastoris and targeted gene disruption and RNAi-mediated gene silencing of these candidates. Interesting candidates include those with LysM domains, similar to LysM effectors identified in other fungal pathogens that have a role in pathogenicity.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M40

Monday 4th April
14:00 - 16:00

HOANG XUAN Chien *(1), MARTINEZ-ROCHA Ana Lilia (1), SCHÄFER Wilhelm (1)*
(1) Molecular Phytopathology and Genetics, Biocenter Klein Flottbek, University of Hamburg, Hamburg, Germany

Maize resistant to fungal leaf pathogens by overexpression of the deoxyhypusine synthase

We strongly increased the resistance of maize towards the leaf pathogens *Bipolaris sorokiniana*, *Cochliobolus heterostrophus*, and *Colletotrichum graminicola*. In all cases the infection became clearly restricted compared to wild type infections, in spite of the different infection behaviour of the tested fungi. Plant resistance was achieved by changing the posttranslational modification of the eukaryotic translation initiation factor 5A (eIF5A) of maize. eIF5A is the only known protein to contain the unusual amino acid hypusine. The enyzmes deoxyhypusine (DHS) and deoxyhypusine hydroxylase (DOHH) modify a lysine to hypusine thereby activating eIF5A. Activated eIF5A is involved in mRNA shuttling, translation elongation and stress-induced regulation. We silenced and overexpressed DHS in *Zea mays*. Down and up regulation of DHS levels in transgenic plants was confirmed by qPCR. DHS-overexpressing lines are resistant to *Cochliobolus heterostrophus*, *Bipolaris sorokiniana* and *Colletotrichum graminicola* infections whereas DHS-silencing lines showed an increased susceptibility. We transformed the fungal strains to express gfp and monitored the reduction of infection with fluorescent stereomicroscope and confocal laser scanning microscopy. Quantification of fungal infection demonstrates the reduced amount of fungi on leaves of DHS-overexpressing lines in comparison with DHS-silencing lines and wildtype.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M41

Monday 4th April
14:00 - 16:00

HOANG Chien Xuan (1), MARTINEZ-ROCHA Ana Lilia (1), MÜNSTERKÖTTER Martin (2), GÜLDENER Ulrich (2), ***SCHÄFER Wilhelm*** *(1)*
(1) Molecular Phytopathology and Genetics, Biocenter Klein Flottbek, University of Hamburg, Hamburg, Germany
(2) Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, München, Germany

Gene expression of hypervirulent and avirulent mutants during the early infection phase of *Fusarium graminearum*

Fusarium graminearum is the causal agent of Fusarium Head blight of wheat. For successful penetration and invasion of the host plant, *F. graminearum* undergoes cellular differentiation to form complex infection cushions. We characterized the eukaryotic translation initiation factor 5A (eIF5A), whose post translational activation leads to the unique amino acid hypusine. Biosynthesis of hypusine requires two enzymes deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH). Both genes proved to be essential in *F. graminearum*. Overexpression of DHS or DOHH revealed opposite phenotypes during infection, namely hypervirulence or avirulence, respectively. Using scanning electron microscope and confocal laser scanning microscopy we observed an increase of infection structures formation in DHS overexpressing mutants in comparison to the wild type strain when inoculated on wheat florets. On the contrary, DOHH overexpressing mutants do not form infection structures. To identify genes necessary for pathogenesis and infection structures formation in *F. graminearum*, we isolated the fungal infection structures directly from the leaf using laser dissecting microscopy. Transcriptomic analysis was performed for: 1. mycelia grown in culture, 2. epiphytically growing runner hyphae or infection cushions of the wild type, the hypervirulent, and the avirulent mutants. We will present the genes specifically expressed during hypervirulence and avirulence during the early phase of leaf infection.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M42

Monday 4th April
14:00 - 16:00

HAUEISEN Janine *(1), GRANDAUBERT Jonathan (1), STUKENBROCK Eva (1)*
(1) Environmental Genomics, Christian-Albrechts University of Kiel & Max Planck Institute for Evolutionary Biology, Kiel & Plön, Germany

Combination of confocal microscopy and RNAseq reveals dramatic intra-species variation of *Zymoseptoria tritici* during wheat infection

The ascomycet *Zymoseptoria tritici* (syn. *Mycosphaerella graminicola*) is a global foliar pathogen with a complex and still poorly understood hemibiotrophic lifestyle. Although the fungus is specialized to infect wheat in a homogeneous agroecosystem we observe a surprisingly high degree of intra-species diversity between isolates. We compared genome data of three isolates originating from field populations in the Netherlands, Denmark and Iran. Their genomes show between 500,000 to 600,000 SNPs. Furthermore, they encode approx. 10,500 *Z. tritici* 'core' genes as well as up to 400 isolate-specific genes. The different isolates possess individual sets of small, dispensable chromosomes and show diverse *in-vitro* growth morphology as well as virulence phenotypes on the susceptible cultivar Obelisk. Our goal was to study the infection development from initial infection to pycnidia development of this three *Z. tritici* isolates. We inoculated seedling with the three isolates and analysed their intrafoliar infection morphology and development by confocal laser scanning microscopy. This approach was combined with stage-specific RNAseq to capture dynamics in fungal gene expression plus isolate-specific transcription profiles during wheat infection. Our microscopic analysis identified four distinct *Z. tritici* 'core' infection stages (A: Infection development, B: Biotrophic growth, C: Lifestyle transition and D: Necrotrophy and asexual reproduction) representing the hemibiotrophic infection sequence. However, during the individual stages morphology and spatial plus temporal infection development clearly differed between isolates. Differential gene expression (DGE) analyses based on RNAseq data showed that *Z. tritici* gene expression is highly dynamic during infections. Within the isolates ~10 % of the genes followed a stage-specific transcription profile between stages A and B. Moreover, we found the stage-specific genes to be significantly enriched in genes encoding secreted proteins and putative effectors. DGE analyses also revealed that many *Z. tritici* genes are isolate-specifically expressed. We found that during the infection stages 15 to 20 % of all transcribed genes are differentially expressed between the three isolates and an overlay of the stage-specific genes showed that 50 % of them also are isolate-specific. Our analyses reveal a 'core' *Z. tritici* infection program but also identify isolate-specific infection development and molecular host-pathogen interaction.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M43

Monday 4th April
14:00 - 16:00

CLARKSON John *(1), TAYLOR Andrew (1), JACKSON Alison (1), ARMITAGE Andrew (2), HARRISON Richard (2)*
(1) Warwick Crop Centre, School of Life Sciences, University of Warwick, Warwick, Warwickshire, UK
(2) East Malling Research, East Malling, Kent, UK

Genetic basis for pathogenicity in *Fusarium oxysporum f.sp. cepae* causing basal rot in onion

Basal rot caused by *Fusarium oxysporum f.sp. cepae* (FOC) is one of the most important diseases of bulb onions which are grown throughout the world and have an annual production of 83 Mt. FOC is a soilborne fungus and part of the *F. oxysporum* species complex, which includes other important pathogenic formae speciales (f.spp.) adapted to particular crop hosts, as well as non-pathogenic isolates. In the past, the genetic basis for pathogenicity in *F. oxysporum* has been poorly understood but recently 'secreted in xylem' (SIX) genes have been associated with virulence for several *f.spp.* A set of 32 *F. oxysporum* isolates from onion were tested for pathogenicity and whole genome sequencing and PCR approaches used to detect putative effector genes. Seven *SIX* genes and two other putative effectors were identified in isolates highly pathogenic on onion seedlings and bulbs which were all absent in non-pathogenic isolates. An isolate with an intermediate level of virulence was found to have just two of the *SIX* genes. The *SIX* genes and other putative effectors were also shown to increase in expression level in planta in a time course experiment over 96 hours following inoculation of onion seedlings in a sterile system. Different *SIX* gene complements were also identified in other f. spp. but none were identified in *F. avenaceum*, *F. proliferatum* and *F. redolens* which have also been reported to be pathogenic on onion and other crops. Further results from a comparative analysis of genomes from pathogenic and non-pathogenic isolates will also be described as well as the potential of SIX genes for the identification and quantification of FOC as a means of assessing disease risk in field or store.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M44

Monday 4th April
14:00 - 16:00

TÓTH Eszter Judit *(1), BOROS Éva (2), HOFFMANN Alexandra (1), VÁGVÖLGYI Csaba (1), NAGY István (2), PAPP Tamás (1)*
(1) Department of Microbiology, University of Szeged, Szeged, Hungary
(2) Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

Activation of cytokine, chemokine and chemotactic genes in THP1 cells in response to conidia and hyphae of *Curvularia* strains

Members of the genus *Curvularia*, *Bipolaris* and *Cochliobolus* are melanin producing ascomycetes fungi. Although *Bipolaris* and *Cochliobolus* species are saprotrophic or plant pathogenic fungi, *Curvularia* species have been recovered from human infections known as pheaohyphomycoses. These mycotic infections can manifest as fungal keratitis, sinusitis, cutaneous lesions or invasive infections with frequent involvement of the central nervous system. Opportunistic fungal infections represent a continuously increasing problem, because of the growing population with underlying conditions, difficulties of diagnosis and the high antifungal resistance of certain fungal agents. During our experiments strains of *Curvularia*, *C. lunata* and *C. spicifera* isolated from human eye infections, *C. hawaiiensis* from a systemic infection and, for comparison, *Cochliobolus carbonum* isolated from plant leaf were used. We have investigated the effect of the strains to the induction of cytokine and chemokine production and activation of THP1 monocytes. Relative transcription level of IL10, IL8, IL6, TNF-A, CXCL10, adhesion molecules, chemotactic molecules and their receptors was measured by real-time quantitative reverse transcription PCR in response to conidial and mycelial forms of the fungi. We blocked the DHN melanin biosynthesis during cultivation to analyse the role of melanin in hampering monocyte response to conidia.

This research was supported by the grants OTKA NN106394 and TÉT_12_DE-1-2013-0009. T.P. is a grantee of J. Bolyai Scientific Scholarship of the Hungarian Academy of Sciences.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M45

Monday 4th April
14:00 - 16:00

NOVAK Marusa *(1), KRAŠEVEC Nada (2), ČEPIN Urška (3), KOBAYASHI Toshihide (4), MAČEK Peter (1), ANDERLUH Gregor (2), SEPČIĆ Kristina (1)*
(1) University of Ljubljana, Biotechnical Faculty, Department of Biology, Ljubljana, Slovenia
(2) L11, National Institute of Chemistry, Ljubljana, Slovenia
(3) BioSistemika Ltd and National Institute of Biology, Ljubljana, Slovenia (4) Lipid Biology Laboratory, RIKEN, Hirosawa, Japan

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

Aegerolysins and membrane attack complex/perforin (MACPF) domain-containing proteins (Pfam06355 and 01823, respectively) are found in various kingdoms of life including fungi. In Basidiomycota, proteins of these two families seem to be involved in development of primordia and fruiting bodies, while in other filamentous fungi they might act as virulence factors. Various fungal members of both protein families have been shown to interact with biological and artificial lipid membranes, either sole or in combination with one another. It appears that the roles of these proteins are pleiotropic and adapted to the fungal life-style. *Aspergillus niger* is a saprophytic, filamentous fungus found around the world. In its genome, we identified two nucleotide sequences encoding aegerolysins and two nucleotide sequences encoding proteins with MACPF domain. Using qPCR, we showed that the increase of expression of all four target genes coincides with the beginning of conidiation in *A. niger*, and that the prevention of conidiation (either physical or genetic) alters the expression profiles and significantly downregulates their expression. Deletion of either of the aegerolysin genes did not affect the rate of conidiation, growth on different media and development of the fungus. Using polyclonal antibodies against recombinant aegerolysin (AspHS), we were able to confirm that aegerolysins in *A. niger* are secreted out of the fungus despite their lack of the corresponding signal peptide. Experiments employing surface plasmon resonance and vesicle sedimentation assay showed that AspHS protein can bind to lipid vesicles composed of ceramide phosphoethanolamine (CPE) and cholesterol (1:1, mol:mol), but is not able to permeabilize the membrane. Using cells derived from the insect *Spodoptera frugiperda* that contain considerable amounts of CPE we confirmed that fluorescently labelled AspHS bound to the cell membranes, but MTT test showed no toxic effects of the protein on these cells. Our results suggest that aegerolysins and MACPF domain-containing proteins are produced during conidiation of *A. niger*, but are not actively involved in this process, indicating that their role(s) might be related to some other physiological processes in the fungus, e.g. defence mechanisms, rather than development.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M46

Monday 4th April
14:00 - 16:00

BHADAURIA Vijai *(1), COHEN-SKALI Aurelie (1), MACLACHLAN Ron (1), POZNIAK Curtis (1), BANNIZA Sabine (1)*
(1) Crop Development Centre, Dept. of Plant Sciences, University of Saskatchewan, Saskatoon, Canda

Draft genome assembly of the lentil anthracnose pathogen *Colletotrichum lentis*

Colletotrichum lentis Damm causes anthracnose on various legumes, such as lentil, faba bean, pea and narrow-leaf vetch and is one of the most devastating pathogens of lentil (*Lens culinaris Medikus*) grown in western Canada. To gain insight into the molecular mechanisms governing pathogenicity/virulence and genome plasticity to counter resistance introgressed into lentil cultivars from wild species, the fertile and virulent *C. lentis* isolate CT-30 was sequenced on the Illumina HiSeq 2000 and MiSeq, and the Roche's 454 GS-FLX Titanium platforms. The draft assembly contains 50 scaffolds, which anchored 2980 contigs, and spans 56.1 Mb, with a scaffold N/L50 5/4.89 Mb and a contig N/L50 248/51.16 Kb. Around 4.5% repeated and low complexity sequences were detected in the genome using RepeatMasker with a fungal repeat library. The masked genome was used for ab initio gene prediction employing AUGUSTUS with pretrained parameter for *Magnporthe oryzae* and 20,602 ESTs generated from *C. lentis*-infected lentil tissues as a guide for gene structure. In total, 11,842 gene models and 17,758 protein models were predicted. tRNAscan and RNAmmer search revealed 329 gene models encoding tRNA and 54 genes encoding rRNA, respectively. The assembly was further assessed for gene space coverage and completeness using BUSCO, with the 50 scaffolds and 17,758 predicted protein models as an input, and a set of 1438 benchmarking universal single-copy orthologs from fungi as a profile. The assembly was found to be 96.94% complete, 2.6% fragmented and 0.42% missing. In addition, a genetic map with 12 linkage groups (equivalent to the number of chromosomes of C. lentis) was developed from a cross between the virulent race 0 isolate CT-30 and the less virulent race 1 isolate CT-21 to aid genome assembly. A total of 23 scaffolds ranging from 5 Kb to 6.76 Mb were ordered and orientated into 12 pseudomolecules/chromosomes.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M47

Monday 4th April
14:00 - 16:00

MORENO-SANCHEZ Ismael *(1), IBEAS Jose I. (1)*
(1) Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide-CSIC-Junta de Andalucía, Seville, Spain

Secreted and cell wall proteins involved in virulence in *Ustilago maydis*

Ustilago maydis has raised as an excellent model for the study of plant-pathogen interactions, and its relation with maize plant is one of the systems in which studies can be tackled from both plant and pathogen perspective (Dean *et al*., 2012; Djamei & Kahmann, 2012). *U. maydis* genome contains more than 500 putative secreted proteins, of which more than 50% doesn`t have known functional domains and many of these proteins have been related to infection process (Kämper *et al*., 2006). Protein N- and O- glycosylation are critical processes in host-pathogen relations, in fact mutations in genes pmt4, gls1 and gas2 compromise *U. maydis* virulence on maize, affecting different steps in the infection process (Fernandez-Alvarez *et al*., 2009; Fernandez-Alvarez *et al*., 2013). We have now performed a secretome analysis in order to identify proteins glycosylated by Pmt4 and/or Gls1, produced only when the virulence program is activated by over-expressing the transcription factor Biz1. Up today we have identified by mass spectrometry and MASCOT analysis two proteins glycosylated by Pmt4 and seven proteins glycosylated by Gls1, all dependent of the virulence program activation. These proteins are actually being characterized. Moreover we have also isolated cell wall proteins from wild-type, Δpmt4 and Δgls1 mutants that will be analysed in a similar way.

This work was funded by BIO2013-48858-P from MEC Spain.

Dean R, Van Kan JA, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J, Foster GD. (2012). Mol Plant Pathol. 13(7):804. Djamei A, Kahmann R. (2012). PLoS Pathog. 8(11):e1002955. Fernandez-Alvarez A, Elias-Villalobos A, Ibeas JI. (2009). Plant Cell. 21(10):3397-412. Fernandez-Alvarez A, Elias-Villalobos A, Jimenez-Martin A, Marin-Menguiano M, Ibeas JI. (2013). Plant Cell. Kämper J, Kahmann R, Bolker M, Ma LJ, Brefort T, *et al*. (2006). Nature 444: 97-101.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M48

Monday 4th April
14:00 - 16:00

TAKAHARA Hiroyuki (2), HACQUARD Stéphane (2), KOMBRINK Anja (3), HIRUMA Kei (4), ROBIN Guillaume (1), HALDER Vivek (2), HUGHES Bleddyn (2), SHINYA Tomonori (5), NEUMANN Ulla (2), ***O'CONNELL Richard*** *(1)*
(1) BIOGER, INRA, AgroParisTech, Thiverval-Grignon, France
(2) Department of Plant-Microbe Interactions, Max-Planck-Institute for Plant Breeding Research, Cologne, Germany
(3) Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands
(4) Graduate School of Biological Sciences, Nara Institute of Science and Technology , Nara, Japan
(5) Department of Life Sciences, School of Agriculture, Meiji University, Kawasaki, Japan

Colletotrichum higginsianum **extracellular LysM proteins play dual roles in appressorial function and suppression of chitin-triggered plant immunity**

The genome of the hemibiotrophic fungus, *Colletotrichum higginsianum*, encodes a large repertoire of secreted effectors (10) containing LysM domains, but the role of such proteins in pathogenicity is unknown for any Colletotrichum species. We characterized two effectors, ChELP1 and ChELP2, that are transcriptionally activated during the early biotrophic phase of infection. Immunocytochemistry showed ChELP2 is concentrated on the surface of bulbous biotrophic hyphae at the interface with living host cells but is absent from filamentous necrotrophic hyphae. In co-localization experiments with wheat germ agglutinin, the presence of ChELP2 was correlated with the absence of surface-accessible chitin, and vice versa. Recombinant ChELP1 and ChELP2 bound chitin and chitin oligomers *in vitro* with high affinity and specificity and both proteins suppress the chitin-triggered activation of two immune-related plant MAP kinases. Using RNAi-mediated gene silencing, we found ChELP1 and ChELP2 are essential for fungal virulence and appressorium-mediated penetration of both Arabidopsis epidermal cells and cellophane membranes *in vitro*. The data suggest a dual role for these LysM proteins as effectors for suppressing chitin-triggered immunity and as proteins required for appressorium function.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M49

Monday 4th April
14:00 - 16:00

RAMPITSCH Christof *(1), SUBRAMANIAM Rajagopal (2), FERNANDO Ursla (1), CHATUR Selima (2), FAN Tao (2), JOSHI Manisha (2), WANG Li (2)*
(1) Agriculture and Agrifood Canada, Morden, Canada
(2) Agriculture and Agrifood Canada, Ottawa, Canada

Redox-sensitive proteins are targeted by NADPH oxidase in *Fusarium graminearum*

Fungal NADPH oxidases (NoxA and NoxB) are carefully regulated to generate superoxide and ultimately hydrogen peroxide as an intracellular signal to target specific proteins with susceptible cysteine residues. Sulfhydryl groups on cysteine can be oxidized reversibly and depending upon their redox state can act as molecular switches to modulate protein function. We have used two strategies to label these reactive cysteines in *Fusarium graminearum*, either with biotin to facilitate affinity enrichment, or with monobromo bimane, a fluorescent label, to facilitate their detection on 2D gels. We then used LC-MS/MS to identify proteins and their modified cysteines in comparative analyses with a wild-type strain of *F. graminearum* and a ΔnoxAB deletion mutant. *F. graminearum* is a multicellular fungus that causes serious economic losses in cereal crops worldwide principally by contaminating grain with mycotoxins. The *ΔnoxAB* mutant is deficient in both *NoxA* and *B* and is phenotypically non-pathogenic. We reasoned that the level of oxidized cysteines in target proteins would be lower in the mutant as it produces less H_2O_2 and that we could detect this difference. The labelling approaches have yielded candidate redox-sensing proteins which are putative targets of redox signalling originating from NoxAB. To confirm their roles, we have constructed both deletion and substitution mutants (C to S) of some of these candidates and examined their phenotypes *in vitro* and *in planta*. We have thus far made six mutants and in one of them (FGSG 10089, a protein of unknown function) we have demonstrated that the deletion mutant is phenotypically similar to the *ΔnoxAB mutant*. More putative redox-sensing proteins are being investigated with the same mutagenesis strategy and the most recent results will be presented.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M50

Monday 4th April
14:00 - 16:00

UEHLING Jessie *(1), DIETRICH Fred (1), OHM Robin (3), GRIGORIEV Igor (3), NOLAN Matt (3), LABUTTI Kurt (3), LABBE Jessy (4), MARTIN Francis (5), BONITO Gregory (2), VILGALYS Rytas (1)*
(1) Duke University, Durham, NC, USA
(2) Michigan State University, East Lansing, MI, USA
(3) Joint Genome Institute, Walnut Creek, CA, USA
(4) Department of Energy, Oak Ridge National Laboratory, Oak Ridge, TN, USA
(5) INRA, Lorraine University, Champenoux, France

Transcriptome sequencing sheds light on host-symbiont interactions between zygomycete *Mortierella elongata* and endosymbiont *C. Glomeribacter sp.*

Several research groups have recently investigated the ubiquity and dynamics of bacterial endosymbionts living inside of fungal cells. There are a few studies on fungal hosts belonging to Asco- and Basidiomycota, however, the best-studied examples of endosymbiosis in fungi are from plant-associated zygomycetes. Best studied in the Arbuscular Mycorrhizal (AM) fungi, these endosymbionts are thought to be obligate, long-term residents of the intracellular environment where they may contribute to both fungal and plant health, although the direct or indirect nature of these interactions remain has yet to be experimentally evaluated. We isolated a strain of *Mortierella elongata AG77* (Zygomycota, Mucormycotina, Mortierellales) from the rhizopshere of *Populus deltoides*. We found that *M. elongata AG77* harbors a strain of *Candidatus Glomeribacter sp.*, and we have assembled and annotated genomes for both the host fungus and its resident endosymbiont. The genome of *C. Glomeribacter sp.* is similar to other publicly available genomes, but also has unique features potentially suggesting distinct functionality. In an attempt to understand the nature of the interaction between fungal host *M. elongata* and endosymbiont *C. Glomeribacter sp*. we used an antibiotic treatment to create a strain cleared of endosymbiont populations for comparative studies. Here we present comparative host fungal transcriptomes in the presence and absence of obligate endosymbiont *C. Glomeribacter sp*. The following questions were addressed through this work: Does endosymbiont presence affect fungal host functioning? Do host-endosymbiont dynamics of *Mortierella-Glomeribacter* differ from those of the obligate plant associated AM fungus *Gigaspora-Glomeribacter*?

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M51

Monday 4th April
14:00 - 16:00

CAZA Mélissa *(1), SALOU Viviane (2), HU Guanggan (1), PRICE Michael S. (3), PERFECT John R. (3), KRONSTAD James W. (1)*
(1) Michael Smith Laboratories, University of British Columbia, Vancouver, British Columbia, Canada
(2) Polytech Nice-Sophia, Université Nice Sophia Antipolis, Nice, France
(3) Duke University Medical Center, Duke University, Durham, North Carolina, USA

The zinc finger protein Mig1 regulates mitochondrial function and antifungal drug susceptibility in the fungal pathogen *Cryptococcus neoformans*

In recent years, efforts have been made to overcome the emergence of antifungal drug resistance in fungal pathogens of humans such as *Cryptococcus neoformans* by using combinations of antifungal with non-antifungal drugs. Mitochondrial dysfunction is associated with virulence and drug susceptibility in fungal pathogens such as *C. neoformans*, making this organelle an attractive potential new target for anti-fungal therapy. The antibiotic tetracycline that is believed to inhibit mitochondrial translation in eukaryotic cells also increases susceptibility to fluconazole and amphotericin B for *C. neoformans* and *Aspergillus fumigatus*. These observations imply that the mitochondrial role in antifungal tolerance might be a conserved mechanism in pathogenic fungi. To investigate the connection between mitochondrial function and azole drug susceptibility, we constructed a T-DNA insertion mutant library using *Agrobacterium tumefaciens*-mediated transformation and screened for mutants with increased susceptibility to fluconazole and tetracycline. We then used a whole genome sequencing approach to rapidly identify insertions in 48 genes whose functions may reveal molecular mechanisms linking mitochondrial function to azole drug susceptibility. An ortholog of the gene encoding Mig1 was identified among the candidates and this result fit with our ongoing characterization of the role of this protein in mitochondrial function. In previous studies, we found that the HapX component of the Hap complex represses genes encoding mitochondrial respiratory functions and TCA cycle components under low-iron conditions. In this study, our analysis revealed repression of *MIG1* by HapX and activation of *HAPX* by Mig1 in low iron conditions, and Mig1 regulation of mitochondrial functions including respiration, tolerance for reactive oxygen species, and expression of genes for iron-consuming and iron-acquisiton functions. Consistent with these regulatory functions, a *mig1Δ* mutant had impaired growth on inhibitors of mitochondrial respiration and inducers of ROS. Importantly, deletion of *MIG1* increased susceptibilty to fluconazole thus further linking azole antifungal drugs and mitochondrial function. Mig1 and HapX were also required for survival in macrophages, but loss of Mig1 alone had a minimal impact on virulence in mice. Overall, these studies reinforce the association between mitochondrial dysfunction and drug susceptibility that may provide new targets for the development of antifungal drugs.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M52

Monday 4th April
14:00 - 16:00

ROBIN Guillaume *(1), O'CONNELL Richard (1)*
(1) INRA, Thiverval-Grignon, France

Candidate effector proteins of the anthracnose pathogen *Colletotrichum higginsianum* target plant peroxisomes

Colletotrichum higginsianum causes anthracnose disease on cruciferous plants, including Arabidopsis. It uses a hemibiotrophic infection strategy, involving formation of a series of specialized cell types. After appressoria puncture host surfaces, bulbous biotrophic hyphae develop inside living host cells, surrounded by a modified host plasma membrane; finally, the fungus switches to destructive necrotrophy, associated with thin filamentous hyphae. The *C. higginsianum* genome encodes 365 putative secreted effectors (ChECs), of which 67 are highly expressed during infection in appressoria and/or biotrophic hyphae. Important clues to effector targets and functions may come from knowing their destination inside plant cells. We therefore transiently expressed these ChECs as N-terminal fusions with GFP in *Nicotiana benthamiana* leaf cells for confocal microscopy. Most proteins (38) were distributed between the plant cytosol and nucleus, similar to GFP alone. However, 11 targeted the plant nucleus and 12 labelled other plant organelles, including 3 specifically targeted to peroxisomes. Two of these have a functional C-terminal peroxisome targeting signal (PTS1) that is required for import into the peroxisome matrix, and one induces plant cell death. Progress towards the functional characterization of these peroxisome-targeted effectors will be presented.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M53

Monday 4th April
14:00 - 16:00

BRADSHAW Rosie *(1), KABIR Shahjahan (1), CHETTRI Pranav (1)*
(1) Bio-Protection Research Centre, Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand

Regulation and evolution of a virulence factor, the aflatoxin-like toxin dothistromin.

Dothistromin is a polyketide toxin that is chemically similar to the aflatoxin precursor, versicolorin A. It was recently shown to be a virulence factor of *Dothistroma septosporum*, the Dothideomycete pathogen that causes Dothistroma needle blight of pine trees (Kabir *et al*. 2015 Plant Pathol. 64, 225-234). Similar types of genes are involved in production of dothistromin in *D. septosporum* and aflatoxin in *Aspergillus spp.*, and an orthologous pathway regulator, AflR, regulates these genes (Chettri *et al*. 2013 Fungal Genet. Biol. 51, 12-20). However dothistromin biosynthesis involves two features that distinguish it from aflatoxin biosynthesis. Firstly, it is switched on during early exponential growth phase, instead of a later growth stage, concordant with dothistromin production during periods of rapid biomass growth in planta (Kabir *et al*. 2015 Forest Pathol. 45, 190-202). Secondly, the genes are dispersed in six loci across one chromosome instead of being clustered, with the *D. septosporum* AflR regulatory gene in a central location, rather than sub-telomeric as in most aflatoxin-producing fungi. Histone acetylation and methylation were monitored at different stages of growth in culture, revealing that the expression of AflR and early onset of dothistromin production are regulated at the chromatin level. We propose that the unusual arrangement of dothistromin genes may have adaptive significance for the pathogen by affecting the regulation of dothistromin production.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M54

Monday 4th April
14:00 - 16:00

BALESDENT Marie-Hélène (1), DUTREUX Fabien (1), CRUAUD Corinne (2), FUDAL Isabelle (1), MEYER Michel (1), DELOURME Régine (3), ***ROUXEL Thierry*** *(1)*
(1) BIOGER, INRA, AgroParisTech, Thiverval-Grignon, France
(2) Genoscope, CEA, Evry, France (3) INRA, Le Rheu, France

LeptoLife: deciphering the life and pathogenicity cycle of a fungal phytopathogen using metatranscriptomics

Leptosphaeria maculans, the agent of stem canker of oilseed rape (OSR, *Brassica napus*) displays an unusually complex pathogenic cycle and interactions with its host plant. Its life cycle encompasses a saprophytic stage on stem debris, during which sexual reproduction takes place, and contrasted pathogenicity programs including two biotrophic stages and two necrotrophic stages. One of the biotrophic stages consists of an exceptionally long endophytic life within the apoplast of plant tissues during which no symptoms are expressed and no apparent damages are caused to the plant. In addition, a closely related species, *L. biglobosa*, has a very similar pathogenic strategy and may interact with *L. maculans* within the plant. To have a complete insight into the different programs set up by the fungus to complete its life cycle, we undertook an extensive RNAseq analysis of the fungal life in isolated conditions or in multiple combinations of interactions with the plant or other fungi/microbes. A total of 420 biological samples corresponding to 11 distinct stages of the life/pathogenic cycle (and numerous time points) were obtained and submitted to RNAseq sequencing. The data are in the process of being analysed but already shade light on unexpected interaction between *L. maculans* and *L. biglobosa* in planta.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M55

Monday 4th April
14:00 - 16:00

DING Hao *(1), LIU Kexin (1), KRONSTAD James W. (1)*
(1) Michael Smith Laboratories, The University of British Columbia, Vancouver, Canda

Identification of genes involved in polysaccharide capsule production and regulation in *Cryptococcus neoformans*

The opportunistic human fungal pathogen *Cryptococcus neoformans* produces a polysaccharide capsule as one of its major virulence factors. Despite the identification of several genes involved in capsular material synthesis and secretion, the process of capsule production and its regulation remains poorly characterized. In this study, we first used a reverse genetics approach to examine the possible role of secretory autophagy in capsular material secretion by deleting early autophagy pathway genes (*ATG1, ATG7, ATG8* and *ATG9*) in *C. neoformans* strain H99. The autophagy mutants were defective for survival in a nitrogen starvation assay and showed 20-60% of the WT level of intracellular replication in a murine macrophage-like cell line. However, the autophagy mutants showed no difference *in vitro* from the wild type in producing virulence factors including capsule and melanin as well as in growth at 37°C. These results suggest that autophagy affects Cryptococcus virulence by reducing Cryptococcus replication inside phagocyte rather than by affecting the production of virulence factors. In a forward genetic approach, a screen was performed for abnormal capsule production in a T-DNA random insertional mutant library (> 19,000 mutants). A total of 50 such mutants (out of 4,500 screened) were identified. Further characterization of these capsule mutants is in progress.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M56

Monday 4th April
14:00 - 16:00

BENFIELD Aurelie (1), KAZAN Kemal (2), ***GARDINER Donald*** *(1)*
(1) Commonwealth Scientific and Industrial Research Organisation, Agriculture, Brisbane, Australia

Winner takes all: Barcoding reveals new insights into the colonisation behaviour of *Fusarium graminearum* during wheat head blight disease

DNA barcoding allows quantitative tracking of individual mutants within a pool of other mutants using amplicon sequencing. In yeast and bacterial pathogens of mammals, barcoding allows the function of essentially every gene in the genome to be assessed under any given condition. We have been developing a barcoded mutant collection for the wheat and barley pathogen *Fusarium graminearum* to exploit this technology and analyse competitiveness and colonisation behaviours of individual mutant strains. We found that when pools of barcoded mutants were co-inoculated into nutrient rich media, most mutants were recovered in a relatively unchanged proportion after extended culturing. In contrast, our initial data from point inoculations of wheat heads suggest that the in planta competition between mutants can be vastly different. As the disease progresses from the initial inoculation point, individual mutant strains dominated the colonised tissue. Interestingly, however, which particular mutant strain dominates on any particular head appears random and in most cases does not relate to the strain's genotype. Together our data suggest that multiple *F. graminearum* strains can simultaneously infect the florets; however, once an individual strain reaches the rachis, it rapidly colonises the head, excluding all other competitors. This finding has implications for the management of this important fungal pathogen.

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Session CS2 Pathogenesis and symbiosis
CS2M57

Monday 4th April
14:00 - 16:00

HANE James *(1), TESTA Alison (2), BERTAZZONI Stefania (2), HU Haifei (3), SYME Robert (2)*
(1) Curtin Institute for Computation, & CCDM Bioinformatics, Centre for Crop & Disease Managment, Department of Environment & Agriculture, Curtin University, Perth, Australia (2) Centre for Crop & Disease Management, Department of Environment & Agriculture, Curtin University, Perth, Australia (3) Pawsey Supercomputing Centre, Perth, Australia

Pathogen genome evolution-based approaches to pathogenicity effector gene prediction

Adaptation via genomic plasticity is key to the success of many fungal pathogens. Plant pathogens in particular are increasingly recognised to undergo rapid evolution. Whole-genome resources for Fungi have accumulated to the point where hundreds of genomes - representing a diverse range of lifestyles and taxa- are publicly available. Certain genome mutation processes and characteristics have been linked to genomic plasticity in Fungi, such as repeat-induced point mutation (RIP), lateral gene transfer (LGT), conditional dispensability of DNA segments or whole chromosomes, chromosome length polymorphism, transposon activity, and high rates of intrachromosomal inversion. Effector proteins play an critical role in interactions between fungal pathogen and host plant. While many have been identified, few exhibit strong sequence similarity - likely due to aforementioned genome mutation mechanisms. This presents a challenge to clearly defining conserved domains, motifs or other reliable means for identifying effector proteins *de novo*. Therefore, we applied various novel bioinformatic methods based on the tell-tale signatures of selected fungal genome mutation mechanisms to supplement our conventional effector prediction techniques. With particular emphasis on RIP, LGT and dispensability, we present new publicly available resources compiling effector predictions across a wide range of fungal species.

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CS2M58

Monday 4th April
14:00 - 16:00

WILLIAMS Angela *(1), THATCHER Louise (2), HANE James (3), SPERSCHNEIDER Jana (2), BUCK Sally-Anne (2), MA Li-Jun (4), VARSHNEY Rajeev (5), SINGH Karam (2)*
(1) The Institute of Agriculture, The University of Western Australia, Crawley,WA, Australia
(2) CSIRO Agriculture, Wembley, WA, Australia
(3) Curtin Institute for Computation, and CCDM Bioinformatics, Centre for Crop and Disease Management, Perth,WA, Australia
(4) University of Massachusetts, Amherst, Massachusetts, USA
(5) International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Telangana, India

Dispensome prediction in draft genome assemblies aids effector prediction in legume-infecting *Fusarium oxysporum* formae speciales

Fungi of the *Fusarium oxysporum* species complex are found in soils worldwide and cause devastating wilt disease on many crops. Significant yield reductions on pulses are caused by legume-infecting formae speciales (*ff. spp.*) including pathogens of chickpea, *F. oxysporum f. sp. ciceris* (Foc), and pea, f. sp. *pisi* (Fop).
We used genomic sequencing to investigate the relationship between three legume-infecting *ff. spp.*, Foc, Fop and *f. sp. medicaginis* (Fom), a pathogen of the model legume *Medicago truncatula* with which we developed a model legume pathosystem- supported by additional transcriptomic resources. Focusing on the identification of potential pathogenicity genes and dispensable sequences, we leveraged the well-studied core and dispensable chromosomes of model Fusarium pathogens *F. oxysporum f. sp. lycopersici* (Fol, tomato-infecting) and *F. solani* (pea-infecting). Variations in conservation of Fol and *F. solani* dispensable chromosomes were observed across the *F. oxysporum ff. spp*., however legume-pathogenic *ff. spp*. did not exhibit greater overall sequence conservation relative to non-legume pathogens, which may indicate the lack of a common ancestral source for legume pathogenicity. However we identified small genomic regions conserved across legume-infecting *ff. spp*., encoding genes expressed during Fom infection, including 10 candidate effectors. Four of these were most similar to proteins encoded by other legume-infecting *ff. spp*. - suggesting conserved roles relevant to legume pathogenicity.

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CS2M59

Monday 4th April
14:00 - 16:00

KIM Seongbeom *(1), PARK Sook-Young (2), KWON Seomun (1), CHOI Jaeyoung (3), JEON Junhyun (4), JEON Jongbum (1), KIM Ki-Tae (1), LEE Yong-Hwan (1)*
(1) Department of Agricultural Biotechnology and Center for Fungal Genetic Resources, Seoul National University, Seoul, South Korea
(2) Korean Lichen Research Institute, Sunchon National University, Sunchon, South Korea
(3) Samuel Roberts Noble Foundation, Ardmore, Oklahoma, USA (4) School of Biotechnology, Yeungnam University, Gyeongsan, South Korea

Characterization of effector candidates in the rice blast fungus, *Magnaporthe oryzae*

Effectors of plant pathogens modulate pathogenic environment in host plants. Many effectors have been characterized in the rice blast fungus, *Magnaporthe oryzae*. They play pivotal roles in disease establishment, but molecular mechanisms how they interact with corresponding components in the host cells remain mostly unsolved. Recently, novel TAL (Transcription activator-like) effectors that are directly transferred to host nuclei are characterized in plant pathogenic bacteria, *Xanthomonas oryzae*. They activate host susceptibility genes that are used to promote pathogenic development. However, information about this type of effector has been limited in plant pathogenic fungi. As a first step to identify putative TAL effectors in the rice blast fungus, 18 genes encoding transcription factor with signal peptide were identified from secretory proteins. Their in planta expression profiles were measured from RNA-seq data whose transcripts are collected from infected rice sheath. They are localized and accumulated at appressoria or BIC, a putative structure for effector secretion. Two of STFs are also detected in host nuclei, indicating that these are secreted and translocated into host nuclei. Molecular functional characterization of these genes are in progress.

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CS2M60

Monday 4th April
14:00 - 16:00

GAN Pamela *(1), NARUSAKA Mari (2), KUMAKURA Naoyoshi (1), TSUSHIMA Ayako (1), HIROYAMA Ryoko (1), TAKANO Yoshitaka (3), NARUSAKA Yoshihiro (2), SHIRASU Ken (1)*
(1) RIKEN Center for Sustainable Resource Science, Yokohama, Japan
(2) Research Institute for Biological Sciences Okayama, Okayama, Japan
(3) Graduate School of Agriculture, Kyoto University, Kyoto, Japan

Comparative genomics of *Colletotrichum* fungi reveals lifestyle-adapted fungal gene gain/loss and potential virulence-associated genes

Colletotrichum is a genus of fungi whose members adopt a range of infection lifestyles on a variety of hosts, including several agriculturally important crop and model plants. Recently, the genomes of several species with different host ranges have been sequenced. We performed a genus-wide comparative analysis revealing that *Colletotrichum* species have adapted carbohydrate-degrading enzyme profiles according to their preferred hosts. In addition, we show that positive selection acts on secreted and nuclear-localized proteins indicating that these may be important in speciation or adaptation to specific hosts. Further, comparative analysis of different isolates with different levels of virulence on the same host allowed the identification of potential virulence-associated pathogen genes.

This work was supported by the Programme for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry and Council for Science, Technology and Innovation (CSTI), Cross-ministerial Strategic Innovation Promotion Program (SIP) to K.S., Y.T. and Y.N.

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CS2M61

Monday 4th April
14:00 - 16:00

GREEN Kimberly *(1), BECKER Yvonne (1), TANAKA Aiko (2), LALUCQUE Hervé (3), SILAR Philippe (3), SCOTT Barry (1)*
(1) Massey University, Palmerston North, New Zealand
(2) Nagoya University, Nagoya, JAPAN
(3) University of Paris, Paris, France

Two *E. festucae* genes encoding putative membrane associated proteins are required for cell-cell fusion and *E. festucae-L. perenne* symbiosis.

Epichloë festucae is a filamentous fungus that forms a mutually beneficial symbiotic association with *Lolium perenne*. From a series of forward and reverse genetic screens we have shown that single gene disruptions of *noxA*, *noxR* and *racA*, encoding components of the NADPH oxidase complex, *proA*, encoding the transcription factor ProA, and mkkB and mpkA, encoding the MAPK kinase and MAP kinase components of the cell wall integrity signalling pathway, leads to a severe host interaction phenotype and a loss of hyphal fusion in culture. Interestingly, homologues of these genes in *N. crassa*, *S. macrospora* and *P. anserina* are required for cell-cell fusion and sexual fruiting body maturation, thereby establishing a link between self signalling and hyphal network formation during *E. festucae* symbiosis. The aim of this project was to test if *E. festucae* homologues of two recently identified *P. anserina* self signalling genes, *IDC2* (*N. crassa Ham-7*) and *IDC3*, which we have named *symB* and *symC*, are also required for symbiosis and hyphal network formation. In contrast to wild-type *ΔsymB* and *ΔsymC* mutants are totally deficient in cell-cell fusion, hyperconidiate and form intra-hyphal hyphae in culture. Within *L. perenne*, *ΔsymB* and *ΔsymC* mutants cause stunting, hypertillering, premature leaf senescence, vascular bundle colonization and a loss of hyphal network formation. These phenotypes are identical to those observed with *noxA, proA, mkkB* and *mpkA* mutants suggesting that SymB and SymC may interact to form a membrane associated sensor complex that regulates *Epichloë festucae* cell-cell fusion and hyphal network development via a currently undetermined pathway.

(1) Tanaka *et al*. (2006). Plant Cell 18: 1052-1066
(2) Tanaka *et al*. (2013) Mol Microbiol 90: 551-568
(3) Becker *et al*. (2015). Mol Microbe-Plant Interact.
(4) Lalucque *et al*. (2013). 27th Fungal Genetics Conference, Asilomar. p. 159.

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Session CS2 Pathogenesis and symbiosis
CS2M62

Monday 4th April
14:00 - 16:00

KROLL Kristin (1), SHEKHOVA Elena (1), MATTERN Derek (1), THYWISSEN Andreas (1), STRASSBURGER Maria (2), HEINEKAMP Thorsten (1), SHELEST Ekaterina (3), BRAKHAGE Axel A. (1), ***KNIEMEYER Olaf*** *(1)*
(1) Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI) and Institute of Microbiology, Friedrich Schiller University Jena, Jena, Germany
(2) Transfer Group Anti-infectives, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany
(3) Research Group Systems Biology and Bioinformatics, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI), Jena, Germany

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

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

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CS2M63

Monday 4th April
14:00 - 16:00

DERBYSHIRE Mark *(1), HEGEDUS Dwayne (2), ROLLINS Jeffrey (3), VAN KAN Jan (4), FAINO Luigi (4), SEIDL Michael (4), MBENGUE Malick (5), RAFFAELE Sylvain (5), HEARD Stephanie (6), OLIVER Richard (1)*
(1) Centre for Crop and Disease Management, Curtin University, Perth, AUSTRALIA
(2) Agriculture and Agrifood Canada, Saskatoon, Canda
(3) University of Florida, Gainesville, Florida, USA
(4) Wageningen University, Wageningen, Netherlands
(5) LIPM, INRA, CNRS, Toulouse University, Toulouse, France
(6) Rothamsted Research, Harpenden, UK

Improvement of the *Sclerotinia sclerotiorum* reference genome and annotation using PacBio and Illumina sequencing

Sclerotinia sclerotiorum is a major phytopathogen which infects over 400 plant species. Several of these are major crops including the first and second most widely cultivated oilseeds, soybean and canola, respectively. Recently, it has been shown that the closely related phytopathogen *Botrytis cinerea* can utilise small RNAs (sRNAs) to infect its hosts. By secreting sRNAs into the host cytoplasm, *B. cinerea* is able to reduce the expression of host genes involved in defence and thus facilitate infection. Though sRNAs have been identified in *S. sclerotiorum*, the link between them and pathogenicity has not been investigated. The genome sequences of *B. cinerea* and *S. sclerotiorum* were focal points for these analyses - as genome sequences often are for valuable bioinformatics and wet lab research. Though numerous genomes have been sequenced using Sanger and next generation sequencing, many contain large gaps due to the limitations of these technologies. Furthermore, gene models predicted either ab initio or reliant upon only limited evidence from expressed sequence tags and/or protein homology may be inaccurate. The recent advent of Pacific Biosciences Single Molecule Real-Time (PacBio) sequencing, which can produce reads up to 50 kb long, has allowed for the assembly of several complete genomes. Furthermore, incorporation of RNA sequencing data into gene calling pipelines has been shown to improve the accuracy of gene models. In order to improve the genome sequence of *S. sclerotiorum*, we sequenced the reference strain (known as ‘1980’) using PacBio sequencing at 36 x coverage. The version 2 genome sequence was assembled using the previously published optical map and sequencing errors (a known problem with PacBio sequencing) were corrected using highly accurate Illumina short reads. Gene models were produced based on extensive RNA sequencing data from numerous in planta and *in vitro* time points alongside existing expressed sequence tags, protein homology and ab initio gene predictions. Using the new genome sequence, further Illumina sequencing analyses were conducted on sRNAs in *S. sclerotiorum*, specifically with regards to their potential to target host transcripts. The new genome sequence of *S. sclerotiorum* contains approximately 1 Mb of additional sequence. 11,127 gene models were predicted and manually curated where necessary to produce a highly accurate genome annotation. In addition numerous sRNAs were identified, several of which exhibited complementarity to host transcripts involved in pathways potentially linked to plant defence. Several of these sRNA sequences were differentially expressed in planta relative to during growth *in vitro*. This work will provide an important starting point for future research on *S. sclerotiorum* and will serve as the basis for further testing of the role of sRNAs in infection.

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CS2M64

Monday 4th April
14:00 - 16:00

PALMA-GUERRERO Javier *(1), MA Xin (1), ZALA Marcello (1), TORRIANI Stefano (2), CROLL Daniel (1), MCDONALD Bruce (1)*
(1) Plant Pathology Group, ETH Zurich, Zurich, Switzerland
(2) Syngenta Crop Protection AG, Stein, Switzerland

Transcriptional profiling of four *Zymoseptoria tritici* isolates differing in virulence.

Zymoseptoria tritici is an ascomycete fungus that causes Septoria tritici blotch (STB), an important foliar disease on wheat. *Z. tritici infects* plants through stomata, rather than by direct penetration, and it exhibits a long latent period of up to 2-3 weeks following infection. After this latent period the fungus induces necrosis on the plant tissue corresponding with an increase in fungal biomass. The mechanisms involved in activating necrosis remain unknown. *Z. tritici* is a highly polymorphic species showing significant intraspecific variation for virulence. Our aim was to use this natural variation between isolates to identify genes involved in virulence. We generated a deep transcriptome sequencing dataset spanning the entire time-course of infection of four *Z. tritici* strains isolated from the same region but showing different levels of virulence. By comparing the transcriptional profiles of the four isolates at different time points we found that major components of the fungal infection transcriptome were conserved among the four strains. However, several genes showed strongly differentiated transcriptional profiles among isolates. Our analyses showed that heterogeneity in transcriptomes among isolates may explain some of the considerable variation in virulence and host specialization found within the species. We generated a list of genes that may play important roles in infection according to their differential expression profiles. Functional characterization of selected genes is now in progress.

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CS2M65

Monday 4th April
14:00 - 16:00

BOEDI Stefan *(1), BERGER Harald (2), SIEBER Christian (4), NUSSBAUMER Thomas (4), MUENSTERKOETTER Martin (4), SULYOK Michael (3), LEMMENS Marc (3), KUGLER Karl (4), GUELDENER Ulrich (4), STRAUSS Joseph (1)*
(1) Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences Vienna (BOKU), University and Research Center Tulln (UFT), Tulln, Donau, Autria
(2) Health and Environment Department, Austrian Institute of Technology GmbH, University and Research Center Tulln (UFT), Tulln,Donau, Austria
(3) Department for Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences Vienna (BOKU), Tulln,Donau, Austria
(4) Institute of Bioinformatics and Systems Biology,/ Helmholtz Zentrum Muenchen (GmbH), Neuherberg, Germany

In planta environment reverses reduced DON production in a *Fusarium graminearum* Heterochromatin protein 1 (Hep1) mutant revealing a hypervirulent phenotype

Heterochromatic marks have been shown to be involved in the regulation of secondary metabolite gene clusters in diverse fungi. We deleted the heterochromatin protein-1 homologue, Hep1, in *F. graminearum* Ph-1 background with the aim to examine the influence of chromatin-level regulation on toxin production and virulence of this prominent cereal pathogen. Upon axenic cultivation in presence of the mycotoxin inducing nitrogen source L- ornithine, the *hep1* deletion mutant showed an altered secondary metabolite profile including reduced levels of deoxynivalenol (DON) and its acetylated derivative 15-ADON. This finding was contrasted with a 1.5 fold increased infection rate on the susceptible wheat cv. Remus which was accompanied by increased production of DON, butenolide and culmorin mycotoxins in planta. Transcriptome analysis of the *hep1* deletion versus the Ph-1 wildtype strain during pathogenic growth and saprophytic growth on dead (non-responding) wheat heads and axenic samples, allows to distinguish gene response of the pathogen reacting on signals from the active, defending plant from those regulated by plant matrix effects or in vitro mimicked mycotoxin inducing conditions, providing insights into gene regulation underlying the observed hypervirulence. Notably, the most differential gene expression upon *hep1* deletion was observed during infection on living wheat heads, indicating that the impact of Hep1 function on gene regulation is most prevalent under pathogenic conditions. RNA-seq analysis of the complex infection samples will also reveal plant genes differentially responding to the *hep1* mutant.

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CS2M66

Monday 4th April
14:00 - 16:00

ORASCH Thomas *(1), PLEIFER Sophie (1), BINDER Ulrike (2), GSALLER Fabio (3), BROMLEY Michael (3), HAAS Hubertus (1)*
(1) Department of Molecular Biology, Medical University of Innsbruck, Innsbruck, Austria
(2) Division of Hygiene and Medical Microbiology, Medical University of Innsbruck, Innsbruck, Austria
(3) Manchester Academic Health Science Centre, The Manchester Fungal Infection Group, The University of Manchester, Manchester, UK

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

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

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CS2M67

Monday 4th April
14:00 - 16:00

PETIT Yohann *(1), DEGRAVE Alexandre (1), MEYER Michel (1), BLAISE Françoise (1), OLLIVIER Bénédicte (1), ROUXEL Thierry (1), FUDAL Isabelle (1), BALESDENT Marie-Hélène (1)*
(1) BIOGER, INRA, AgroParisTech, Thiverval-Grignon, France

A two genes – for – one gene interaction between *Leptosphaeria maculans* and *Brassica napus*

Leptosphaeria maculans is a hemibiotophic ascomycete which causes stem canker of oilseed rape. That phytopathogenic fungus interacts with its host (*Brassica napus*) according to the gene-for-gene concept. The most economically and environment friendly method of control of stem canker is the genetic control by using host resistance. Single gene resistance is extremely efficient, but races of the pathogen virulent towards a resistance gene can appear in a few years and necessitates continuously new breeding programs. Moreover, specific resistances are rare in oilseed rape, and a lot of efforts are made to find other resistance genes in other *Brassica* species. To date, 11 interactions were genetically characterized between *L. maculans* avirulence genes and corresponding resistance genes in *Brassica*, and 5 of those avirulence genes were cloned. Recently, the avirulence gene *AvrLm10* which is recognized by the resistance gene *Rlm10* of the black mustard (*Brassica nigra*) has been cloned. *AvrLm10* corresponds in fact to two avirulence genes *AvrLm10_1* and *AvrLm10_2* which are located in the same AT-rich genomic region. They encore for small secreted proteins (SSP), are co-regulated and over-expressed 7 days post-infection. Each of them is necessary but not sufficient to induce resistance towards Rlm10. Silencing of one of those genes is sufficient to abolish recognition by *Rlm10*. Silencing by RNA interference of *AvrLm10-1* induces an increase of lesion size on oilseed rape leaves while silencing of *AvrLm10-2* has no major effect on aggressiveness of the fungus. That interaction of two avirulence genes against one resistance gene is therefore different from the classical gene-for-gene concept. It suggests that AvrLm10_1 and AvrLm10_2 could directly interact and / or that they could target the same plant protein. A Y2H screen suggested a direct interaction between AvrLm10-1 and AvrLm10-2. This interaction was confirmed with Bimolecular Fluorescence Complementation (BiFC) experiments. Coimmunoprecipitation experiments are also in progress to confirm this interaction.

POSTER SESSION ABSTRACTS
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CS2M68

Monday 4th April
14:00 - 16:00

NORDZIEKE Daniela *(1), TURRÀ David (1), DI PIETRO Antonio (1)*
(1) Universidad de Córdoba, Córdoba, Spain

Link between ROS and MAPK signaling in peroxidase-induced chemotropism of *Fusarium oxysporum*

Chemotropism, the ability to re-orient hyphal growth in response to chemical cues, is critical for many aspects of the fungal lifestyle such as colony establishment or location of host organisms. We use the root-infecting pathogen *Fusarium oxysporum* as a model to study various aspects of chemotropic signaling. Recently, we showed that class III peroxidases secreted by plant roots function as chemoattractants to direct hyphal growth towards the host plant, and that signal perception is mediated by the conserved fungal cell wall integrity mitogen-activated protein kinase (MAPK) cascade. Since peroxidases catalyze the reductive cleavage of reactive oxygen species (ROS), we examined here the role of the fungal ROS-generating enzyme NADPH oxidase (Nox) in chemotropic sensing of *F. oxysporum*. Using deletion mutants in *noxA*, *noxB* and their regulator *noxR* we found that the encoded proteins NoxB and NoxR, but not NoxA, are specifically required for peroxidase-induced directed growth whereas the Mpk1 MAPK cascade is needed both for the chemotropic response to peroxidase and to A-pheromone. To further elucidate the interplay between ROS and MAPK signaling, we established an experimental system for monitoring Mpk1 phosphorylation status in response to peroxidase treatment. Additionally, we are studying the subcellular localization of NoxA, NoxB and NoxR, as well as their role in diverse aspects of the fungal life cycle such as hyphal fusion, invasive growth and plant infection.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M69

Monday 4th April
14:00 - 16:00

NIZAM Shadab *(1), QIANG Xiaoyu (1), ZUCCARO Alga (1)*
(1) Institute for Genetics, University of Cologne, Cologne, Germany

Suppression of extracellular ATP (eATP) triggered defense response in host by the root endophytic fungus *Piriformospora indica*

The extracellular ATP (eATP) works as an extracellular signaling molecule in plants and animals. In plants, eATP triggers the production of reactive oxygen species (ROS), nitric oxide, callose deposition, transient phosphorylation of MPK3 and MPK6, and expression of genes involved in plant stress response and immunity. Furthermore, eATP is actively released from plant cells in response to abiotic stresses, fungal elicitors, and mechanical stimuli. Recently, transcript profiling has indicated that there is a considerable overlap of genes induced by ATP and by pathogens. Therefore, it appears that eATP plays a central role in mediating the plant immune response and thus can serve as a prime target for pathogen assault. Indeed, it was shown that a *Phytophthora brassicae* secreted effector protein (IPI-O), containing the RXLR-dEER, targets LecRK-I.9 (also known as DORN1, a recently identified ATP receptor in *Arabidopsis thaliana*). *LecRK-I.9* mutant plants showed enhanced susceptibility to the oomycete pathogen, whereas its over-expression resulted in increased resistance. However, nothing is clear about the role of eATP and its suppression during plant-fungus interactions. The filamentous root endophyte *Piriformospora indica* colonizes the roots of a wide variety of unrelated plants, including the dicot model plant *A. thaliana* and the monocot cereal crop *Hordeum vulgare* (barley). The fungus displays a biphasic colonization strategy with initial biotrophic phase followed by a cell death associated phase. Colonization by *P. indica* exhibits various beneficial effects on the host plant such as enhanced growth, tolerance to abiotic stresses, resistance against pathogens, and enhanced assimilation of nitrate and phosphate. Our results suggest that colonization of *P. indica* in *A. thaliana* and barley triggers the release of eATP. Furthermore, colonization of *P. indica* in the *A. thaliana* eATP receptor DORN1 (*dorn1*) mutant line clearly indicated that *dorn1* mutant supports significantly higher fungal colonization with respect to Col-0 control. Hence we expect that *P. indica* uses some intrinsic mechanism to hydrolyze the plant generated eATP, which eventually allows it to counteract ATP-mediated host immune responses. Here we report a *P. indica* effector candidate possibly involved in controlling host eATP homeostasis.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M70

Monday 4th April
14:00 - 16:00

RAJ Sumit *(1), RANI Mamta (1), KUMAR Manoj (2), JOHRI Atul Kumar (1)*
(1) Jawaharlal Nehru University, New Delhi, India (2) Indian Institute of Toxicology Research, Lucknow, India

Cross regulation of hexose and phosphate transporters in endophytic fungus *Piriformospora indica*

To develop sustainable agricultural system, a major research focus is on symbiotic organism especially Arbuscular mycorrhizal fungus (AMF). The symbiosis between plants and arbuscular mycorrhizal (AM) fungi is arguably the world's most prevalent mutualism. In exchange, AM fungi provide their host plants with mineral nutrients [e.g., phosphorus (P)] and other benefits such as protection against biotic (pathogens and herbivores) and abiotic (e.g., drought) stresses (Smith and Smith, 2011). This partnership is credited with driving the colonization of land by plants, enabling massive global nutrient transfer and critical carbon sequestration (Smith and Smith, 2011; Bonfante and Genre, 2010). It is generally established that there is reciprocal benefit to the partners, due to the exchange of plant-derived carbohydrates for amino acids and nutrients supplied by the fungus (Plett and Martin, 2011; Bonafante and Genre, 2010). It has been proposed that exchange across the root-fungus interface generally involve the passive diffusion of Pi and carbohydrates through the fungal and plant plasma membranes into the interfacial apoplast and then the active absorption of nutrients by both partners driven by H+-ATPase(s) (Smith and Read, 2008). However, how carbohydrates are transported through the symbiotic interface is still unknown. Presently we know very little about the regulation of exchange processes occurring in a mycorrhiza and the mechanisms involved in polarizing the transfers. Recently *P. indica* has been characterized as a plant growth promoting, salt stress tolerance, disease resistance and phosphate enriching fungus. Phosphorus is a major macronutrient for plant health and development. Because of its role in plant nutrition and key role in the symbiosis, symbiotic phosphate transport has been well characterized (Kumar et al., 2011; Yadav et al., 2010). By contrast, carbon (C) transport to the fungus is less well understood, and fungal transporters involved have not been identified except in *Glomus* sp DAOM 197198. In the present study we show that phosphate is supplied to the host plant by the phosphate transporter PiPT of the fungus *P. indica*. Knockdown of PiPT decreases the supply of phosphate to the host plant. Further, the expression of carbohydrate transporters involved in the C uptake from host plant is increased. Subsequently, a highly expressed carbohydrate transporter has been characterized. In silico analysis shows that it belongs to MFS sugar family. Substrate specificity assay shows that it has high affinity for glucose. The optimum transport is observed between pH 4.5 and 5.5. It has been established that it is a H+/Glucose co-transporter and that transport is inhibited by proton gradient uncoupler. Symbiotic interaction is dependent on the plant for carbon supply in the form of photosynthates, mediated by carbohydrate transporter. At the symbiotic interface, transport proteins specific to the exchange of sugar and phosphate regulate the transfer. Cooperation is only stable because both partners are able to preferentially reward the other.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M71

Monday 4th April
14:00 - 16:00

GARZOLI Laura *(1), POLI Anna (1), GNAVI Giorgio (1), PRIGIONE Valeria (1), VARESE Giovanna Cristina (1)*
(1) Mycotheca Universitatis Taurinensis (MUT), Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy

Taxonomical and ecological unresolved issues on marine fungi from the Mediterranean Sea

Marine fungi are a great challenge for fungal genetists, since their evolution, biodiversity and physiology are still mainly unknown. In the last decade, the Mycotheca Universitatis Taurinensis (MUT) carried out research programs with the intent of filling this lack of information, establishing a collection of around 1,500 marine fungal strains from different substrates. A case study is presented here. Three different substrates growing in close proximity have been deeply studied: the macrophyte *Posidonia oceanica*, a brown algae (*Padina pavonica*) and a green algae (*Flabellia petiolata*). Although samples were collected from the same area (Elba Island), their mycobiota were very different. This may suggest a different «recruiting system» of fungi from the surrounding environment, which may involve still unknown «metabolic dialogues». Macro and microorganisms can establish interrelationships that may lead to symbiosis and/or parasitism by means of biosignaling and others metabolic mechanisms (i.e. production of antifouling and antimicrobial substances). The genetic markers (ITS, LSU and SSU rDNA) of many isolates, showed low homology with sequences retrieved from public databases. Phylogenetic analyses support the hypothesis of new lineages within Dothideomycetes, Sordariomycetes and Eurotiomycetes. Revealing the diversity of marine fungi is of fundamental importance to understand the complexity of marine communities, thus representing additional models to study life in extreme environments.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M72

Monday 4th April
14:00 - 16:00

VAN DAM Peter *(1), FOKKENS Like (1), VAN DER GRAGT Michelle (1), TER HORST Anneliek (1), REP Martijn (1)*
(1) Molecular Plant Pathology, University of Amsterdam, Amsterdam, Netherlands

SMRT-sequencing of an extraordinarily virulent strain of *Fusarium oxysporum* reveals a novel large pathogenicity chromosome

Fusarium oxysporum f.sp. *radicis-cucumerinum* (Forc) is an unusual member of the *F. oxysporum* (Fo) species complex in that its host range is broader than that of other formae speciales. It is able to cause severe root and shoot rot in various cucurbitaceous plant species, whereas most wilt-inducing Fo strains specifically cause disease in a single species. We decided to sequence the genome of a strain of Forc with SMRT sequencing, resulting in a genome assembly of 52.4 Mb with a total of only 41 contigs. 11 of these contigs correspond to the previously described Fo f.sp. *lycopersici* (Fol) core chromosomes and one has been identified as a circular mitochondrial DNA sequence. In total, we find 25 telomere repeats and find centromeres for most contigs in the assembly. One of the non-core contigs has a size of 2.1 Mb (comparable to the pathogenicity chromosome 14 of Fol) and contains all identified candidate effector genes, including homologs of Fol *SIX6*, *SIX9*, *SIX11* and *SIX13*, as well as a secreted metalloprotease and a polygalacturonase. Because this contig is also very rich in transposable elements, it is likely that it encompasses (a large part of a) pathogenicity chromosome in Forc. Individual knockouts of several candidate effectors do not result in a large reduction in pathogenicity. We now aim to investigate the contribution of the whole chromosome by selecting for its loss using fluorescence assisted flow cytometry (1). Together with RNA-sequencing of Forc-infected plants, these data shed light on the molecular basis for infection in this remarkable pathogen.

(1) Vlaardingerbroek I, et al. Fluorescence Assisted Selection of Transformants (FAST): Using flow cytometry to select fungal transformants. Fungal Genet Biol. 2015;76: 104-109.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M73

Monday 4th April
14:00 - 16:00

RECH Gabriel *(1), SANZ-MARTIN José M. (1), SUKNO Serenella A. (1), THON Michael (1)*
(1) Instituto Hispano-Luso de Investigaciones Agrarias (CIALE), Department of Microbiology and Genetics, University of Salamanca, Salamanca, Spain

The genome-wide mutational landscape of wild isolates of the maize pathogen *Colletotrichum graminicola*

Comparative genomic studies of filamentous fungi have revealed an association between repetitive sequences (RS) and genes involved in pathogenicity. Since RS are usually related with genomic regions with high mutational rates, such association will encourage faster evolutionary adaptations of those genes interacting with the host defenses. *Colletotrichum graminicola* genome sequencing has revealed that RS are found throughout the genome and no correlation was found between TEs and candidate effectors or secondary metabolism genes. In this work we phenotypically characterized and performed whole-genome sequencing of seven field isolates of *C. graminicola* from different regions of the world. We analyzed patterns of large mutations (such as gene/exon loss), medium size mutations (deletions and translocations) and small mutations (small INDELs and SNPs) and determined those genes putatively affected by mutations in each isolate. We found that the amount of genomic variation in each chromosome is positively correlated with the amount of repetitive DNA, however we did not find evidence of a bipartite architecture in the genome. When considering the degree of virulence of the isolates we have found two different scenarios: phenotypically-similar isolates showing large genetic differences and phenotypically-different isolates showing few genetic differences. In the first case, this discordance may indicate that the *C. graminicola* genome has the capability to tolerate a large number of mutations without altering the phenotype (buffering). In the second case, the results may indicate that specific mutations at key genes involved in pathogenicity may result in drastic reductions of virulence. We found that the impact of mutations differs across different gene categories related with pathogenicity. Virulence factors, transporters and transcription factors are usually less affected by high impact mutations, while secondary metabolism and genes upregulated during the infection process are more affected by moderate impact mutations. Overall, this study helps to improve our understanding of *C. graminicola* genome dynamics and provides a valuable resource for selecting targets for further functional analyses aimed at identifying genes involved in the development of maize anthracnose.

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CS2M74

Monday 4th April
14:00 - 16:00

SANZ-MARTIN José M. *(1), PACHECO Ramón (1), THON Michael R. (1), SUKNO Serenella (1)*
(1) Instituto Hispano-Luso de Investigaciones Agrarias (CIALE), Department of Microbiology and Genetics, University of Salamanca, Salamanca, Spain

A highly conserved metalloprotease effector enhances virulence in the maize anthracnose fungus *Colletotrichum graminicola*

Fungal pathogens secrete a wide range of enzymes and effector proteins to interact with their hosts and manipulate the plant immune system. In this study, we describe a Zn dependent fungalysin metalloprotease (Cgfl), a 640 aa secreted protein from the maize pathogen *Colletotrichum graminicola*, with a role in virulence. Members of this family have been previously shown to bind to plant produced class IV chitinases and induce post-translational modifications. Phylogenetic analyses show that fungalysins are evolutionary conserved and are present in most fungi. Transcriptional profiling experiments using different time points during leaf anthracnose as well as live-cell imaging show that gene expression is activated at the late biotrophic stage, specifically when the fungus switches to necrotrophic growth. To confirm its role in pathogenesis, we constructed null mutants tagged with GFP by gene replacement using DelsGate methodology and performed pathogenicity assays in maize. The null mutants produce reduced lesion sizes on leaves and reduced colonization of roots, showing that Cgfl has a role in virulence. Biochemical analyses of proteolytic and chitinase activities with null mutants confirm the lack of ability to degrade these substrates. Several fungal effectors that contain LysM domains have been identified that interfere with chitin-triggered immunity (CTI), such as Ecp6 from *Cladosporium fulvum*. Similarity searches, phylogenetic analyses and transcriptional profiling show that *C. graminicola* encodes two LysM domain containing homologs of Ecp6, suggesting that this fungus employs multiple strategies to control chitin mediated signaling. Our results show that Cgfl is a broadly conserved fungal effector that plays a role in plant infection and host colonization and could be another mechanism by which fungi control chitin-mediated activation of host defenses.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M75

Monday 4th April
14:00 - 16:00

FUKADA Fumi *(1), KUBO Yasuyuki (1)*
(1) Kyoto Prefectural University, Kyoto, Japan

Proper G1/S progression regulated by a two-component GAP and a GTPase is essential to establish plant infection in *Colletotrichum orbiculare*

The cucumber anthracnose fungus *Colletotrichum orbiculare* establish hemibiotrophic infection by coordinating proper infection-related morphogenesis and cell cycle progression. Here, we show that a two-component GAP CoBub2/CoBfa1 regulates G1/S progression via GTPase CoTem1 during appressorium formation. In a random insertional mutagenesis screen of infection-related morphogenesis, we identified a homolog of *Saccharomyces cerevisiae* Bub2p, which negatively regulates mitotic exit forming a GAP complex with Bfa1p. Unlike *S. cerevisiae*, disruption of either *CoBUB2* or *CoBFA1* decreased the time of phase progression from G1 to S during conidial germination, determined by a LacO/LacI-GFP chromosome tagging system. Accordingly, live-cell imaging of M phase in Δ*cobub2* or Δ*cobfa1* showed its earlier onset. In *S. cerevisiae*, a Bub2p/Bfa1p GAP complex negatively regulates GTPase Tem1p that controls mitotic exit. Introducing the dominant-negative form of CoTem1 into Δ*cobub2* or Δ*cobfa1* complemented the defect in G1/S progression, indicating that CoBub2/CoBfa1 regulates G1/S progression via CoTem1. Furthermore, Δ*cobub2* and Δ*cobfa1* showed reduced virulence by attenuating infection-related morphogenesis and enhancing the plant defense response. Thus, CoBub2/CoBfa1 regulates cell cycle progression to arrest in G1 phase during conidial germination, and we propose that this regulation has been adapted to fulfill specific plant-pathogenic roles in virulence-associated processes.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M76

Monday 4th April
14:00 - 16:00

NOVOHRADSKÁ Silvia *(1), VIEDIERNIKOVA Iuliia (1), HILLMANN Falk (1)*
(1) Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute and Friedrich Schiller University, Jena, Germany

Fungivorous amoeba impose predatory selection pressure on environmentally acquired pathogenic fungi

Systemic mycoses are severe infections of immunocompromised patients and frequent nosocomial threats. In many cases the causative yeasts or filamentous fungi are coined "opportunistic" and represent classical examples of environmentally acquired pathogens. How such free-living organisms could have evolved multifactorial virulence strategies effective against higher eukaryotes is not well understood. In their natural environment, fungi are exposed to the selection pressure from the side of their predators, such as soil amoebae which graze on bacteria, yeasts and filamentous fungi. The well-studied soil amoeba, *Dictyostelium discoideum* served as an initial model for investigating general virulence determinants of the filamentous fungus *Aspergillus fumigatus*. During *in vitro* confrontations with fungal conidia, we could demonstrate that phagocytic interactions between both organisms showed similarities to encounters with macrophages. While white, unmasked conidia of a *pksP* mutant were rapidly ingested by *Dictyostelium*, uptake of those covered with the green pigment DHN-melanin was drastically reduced. Besides such phagocytic interactions, both amoeba and fungus secreted cross-inhibitory compounds of which gliotoxin had the highest amoebacidal activity. To further expand our model and to demonstrate that such predatory interactions with fungi actually occur in natural habitats, we have recently isolated and identified the widely spread soil amoeba *Protostelium mycophaga*. The amoeba is characterized by a phagocytic and strictly fungivorous life-style. Feeding experiments of *P. mycophaga* with different pathogenic fungi revealed a broad food fungal prey spectrum including most *Candida* species, but also some filamentous fungi. Such phagocytic interactions primed some yeast species such as *C. parapsilosis* or *Rhodotorula mucilaginosa* to escape predation by the formation of invasive colonies. Our results suggest that formation of such invasive filaments is at least one adaptive strategy to escape phagocytic predation in natural environments.

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Session CS2 Pathogenesis and symbiosis
CS2M77

Monday 4th April
14:00 - 16:00

SHARPEE William *(1), OH Yeonyee (1), YI Mihwa (2), JEON Jongbum (3), FRANCK William (1), EYRE Alex (1), OKAGAKI Laura (1), VALENT Barbara (2), LEE Yonghwan (3), DEAN Ralph (1)*
(1) North Carolina State University, Raleigh, USA
(2) Kansas State University, Manhattan, USA
(3) Seoul National University, Seoul, Korea

Identification and characterization of Suppressors of Plant Cell Death (SPD) genes from *Magnaporthe oryzae*

Phytopathogenic microorganisms secrete a myriad of effector proteins to facilitate infection. *Magnaporthe oryzae*, a filamentous fungus, is the causative agent of the most destructive disease of rice: rice blast disease. Through a forward genetics screen, we identified 11 suppressors of plant cell death (SPD) effectors from *M. oryzae* that are able to block the host cell death reaction induced by the *BAX* and/or the *NPP1* genes within *N. benthamiana*. Four of the 11 *SPD* genes were previously shown as either essential for pathogenicity of *M. oryzae*, secreted into the plant during disease development, or homologues of other characterized suppressors. Additionally, of the 7 previously uncharacterized, we demonstrated one is also secreted into the plant during infection. Phylogenetic analysis of 43 sequenced *M. oryzae* genomes revealed that another suppressor has high level of nucleotide diversity in addition to presence/absence polymorphisms, suggesting that this gene is undergoing selection to avoid recognition by the host. RNAseq of *M. oryzae* infected rice tissue indicated that these genes are expressed at various times during infection likely due to them acting at different stages of the infection process. Taken together, this study demonstrates function to candidate effectors that have been previously identified as well as novel effectors that contribute to virulence of *M. oryzae*.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M78

Monday 4th April
14:00 - 16:00

JESSY Labbé *(1), YANG Yongil (1), MUCHERO Wellington (1), TUSKAN Gerald (1), JAY Chen (1)*
(1) Oak Ridge National Laboratory, Oak Ridge, USA

Genome-wide Analysis of Lectin Receptor-like Kinases in the *Populus-Laccaria* symbiosis

The Lectin Receptor-like Kinases (LecRLKs) play important roles in plant development, innate immunity and stress responses. LecRLKs are typically consisted of an N-terminal lectin domain, an intermediate transmembrane domain and a C-terminal kinase domain. There are 75 and 173 LecRLKs in *Arabidopsis* and rice, respectively. However, little is known about LecRLKs in the woody model plant *Populus*. Here we report the genome-wide analysis of classification, domain structure and expression of LecRLKs in *Populus*. We found that the LecRLK family has been significantly expanded in *Populus*. The total number of LecRLKs in *Populus* is three time that in *Arabidopsis*. Moreover, some of *Populus* LecRLKs possess novel domain structures that have not been previously reported in any other plant species. A number of *Populus* LecRLKs are present as tandem repeats derived from whole-genome duplication. Expression analysis indicated that some LecRLKs are tissue-specific. These studies offer a comprehensive view of LecRLKs in the woody model plant *Populus* and provide a foundation for functional characterization of this important family of receptor-like kinases.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M79

Monday 4th April
14:00 - 16:00

ZHANG Feng *(1), CHAMPION Charlotte (2), HAON Mireille (2), KEMPPAINEN Minna J (3), PARDO Alejandro G (3), FOURREY-VENEAULT Claire (1), KOHLER Annegret (1), BERRIN Jean-Guy (2), MARTIN Francis (1)*
(1) INRA, Université de Lorraine, Laboratoire d'Excellence ARBRE, , Champenoux, France
(2) Biodiversité et Biotechnologie Fongiques, INRA, Polytech Marseille, Faculté des Sciences de Luminy, Marseille, France (3) Laboratorio de Micología Molecular, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Provincia de Buenos Aires, Argentine

Role of symbiosis-regulated plant cell wall degrading enzymes from *Laccaria bicolor* in ectomycorrhiza development

Plant-associated fungi have evolved a repertoire of enzymes acting on plant polysaccharides to colonize their hosts. The genomes of the ectomycorrhizal fungi sequenced so far have a reduced complements of plant cell wall degrading enzymes (PCWDEs). Using comparative analyses of available genomics and transcriptomics data, we have identified a set of carbohydrate-active enzymes (CAZymes) that are released by the ectomycorrhizal basidiomycete *Laccaria bicolor* upon symbiosis development. The few retained genes coding for PCWDEs are acting on pectins (GH28, GH88 and CE8), hemicelluloses (GH30) and cellulose (GH5_5 with a CBM1 domain and LPMOs) and are upregulated in ECM root tips. They likely modify the plant cell walls during colonization of the host root apoplastic space. To characterize the enzyme activity and substrate(s) of the symbiosis-induced PCWDEs, we are producing the recombinant proteins for the unique glycosyl hydrolase 5 (GH5) with a carbohydrate-binding motif CBM1, an expansin-like protein and the polygalacturonase GH28. As of today, the GH5-CBM1, its catalytic motif, and the expansin-like protein have been produced in the yeast *Pichia pastoris*. The recombinant proteins are used for assaying the enzyme activity, determining the protein 3D structure and to elicit antibodies for further protein immunolocalization in ectomycorrhizal roots. This project will elucidate how symbiotic fungi modify plant cell walls to successfully establish within host tissues. In addition, it may generate new enzymatic tools for green chemistry.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M80

Monday 4th April
14:00 - 16:00

KEBDANI-MINET Naïma *(1), GOURGUES Mathieu (1)*
(1) UMR Institut Sophia Agrobiotech, Université de Nice Sophia Antipolis-CNRS-INRA, 400 route des chappes, BP 167, 06903 Sophia Antipolis, France, Sophia Antipolis, France

The PPSCPL1 protein from the oomycete *Phytophthora parasitica* is expressed during early infection of plant roots and favors plant infection

Oomycetes are major crop pests which cause million dollars losses every year. To date, only a few efficient chemicals are available against these filamentous microorganisms. A better understanding of the molecular events that occur during plant-oomycete interactions will help to propose new strategies for crop protection. Using the root-infecting oomycete *Phytophthora parasitica* and *Arabidopsis thaliana* as a model for studying plant-oomycete interactions, we performed a transcriptional analysis of the first hours of infection. Transcripts with a transient accumulation during penetration of the first host cells were identified among which three sequences encoding proteins from the SCP-like extracellular family of proteins. These small secreted cystein rich proteins are found in a large variety of organisms among which eucaryotes and prokaryotes. In mammals, they are involved in various biological processes such as adhesion but also cellular differentiation. Molecular function of these proteins remains unclear but members were reported to act as proteases, protease inhibitors or inhibitors of ions channels. The expression pattern of the three genes, named *PPSCPL1*, *PPSCPL2* and *PPSCPL3* was confirmed by quantitative RT-PCR and for *PPSCPL1* by using transgenic *P. parasitica* lines expressing a fusion with GFP and GUS reporters. The functional characterization of the PPSCPL1 protein was initiated. When expressed in planta, this protein was shown to favor plant infection by *P. parasitica* in *A. thaliana* and *Nicotiana benthamiana* but also by the Tobacco Rattle Virus in *N. benthamiana*. Agroinfiltration experiment showed that PPSCPL1 protein alters plant defense responses without affecting canonical hormonal signaling pathways associated to plant defense. Interestingly, a high expression of the PPSCPL1 protein triggers plant cell death in *N. benthamiana* suggesting a strong effect on plant functions. Taken together, these results suggest that PPSCPL1 protein is secreted during penetration of the first host cells to favor plant infection. Analysis of additional members of this family is required but one can suppose that SCP like extracellular proteins constitute a new class of effectors mobilized by oomycete during the onset of infection.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M81

Monday 4th April
14:00 - 16:00

BARENSTRAUCH Margot *(1), NAY Bastien (1), PRADO Soizic (1), MANN Stéphane (1), KUNZ Caroline (2)*
(1) Sorbonne Universités, Muséum National d'Histoire Naturelle, CNRS, Laboratoire Molécules de Communication et Adaptation des Microorganismes (MCAM), UMR 7245 CNRS-MNHN, CP54, 57 rue Cuvier, 75005 Paris, Paris, France
(2) Sorbonne Universités, UPMC Universités Paris 06, UFR 927, Paris, France, Paris, France

Oxylipin-mediated regulation of mycotoxins in *Fusarium* phytopathogen species during the interaction with the endophyte *Paraconiothyrium variabile*

Endophytic fungi, involved in mutualistic associations with plants, have been shown to provide fitness benefits to their host, such as protection against phytopathogens. Indeed, they produce many metabolites displaying fungicidal or antibacterial activity. Studying the cultivable fungal diversity of the Asian conifer *Cephalotaxus harringtonia*, our team found an Ascomycete known as *Paraconiothyrium variabile*, exhibiting a strong antagonistic activity towards the ubiquitous soil-borne phytopathogen *Fusarium oxysporum*. During competition between both microorganisms, two oxylipins identified as 13-oxo-9,11-octadecadienoic acid (13-KODE) and 13-hydroperoxy-9,11-octadecadienoic acid (13-HpODE) are synthesized. Besides, the amount of beauvericin, one of the most potent mycotoxins of *F. oxysporum*, is significantly reduced. Previous results showed that exogenous application of 13-KODE lowers beauvericin production, pointing out a probable role of endophytic oxylipins in this beauvericin downregulation (1). We want to explore whether an endophytic strain reduced in oxylipins production can still affect mycotoxins biosynthesis by *F. oxysporum* and other *Fusarium* species. Thus, we started engineering oxylipin mutants in the endophyte *P. variabile*. Furthermore, analysis of RNA transcripts in both fungi will allow us to provide genetic evidences that endophytic oxylipins are involved in *F. oxysporum* gene expression changes, leading to possible alterations of its pathogenicity towards the plant and/or the endophyte.

(1) Combès, A. et al. Chemical Communication between the Endophytic Fungus *Paraconiothyrium Variabile* and the Phytopathogen *Fusarium oxysporum*. PLoS ONE 7, e47313 (2012)

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M82

Monday 4th April
14:00 - 16:00

SJÖKVIST Elisabet *(1), MCGRANN Graham (1), BLAXTER Mark (2), HAVIS Neil (1)*
(1) Scotlands Rural College, Edinburgh, UK
(2) University of Edinburgh, Edinburgh, UK

The genome of the barley leaf spot *Ramularia collo-cygni* and phylogenomic analyses of kingdom fungi

Ramularia collo-cygni is a fungal pathogen of barley, and the causal agent of *Ramularia* leaf spot (RLS). The fungus has a long asymptomatic growth stage, typically causing disease after the switches from vegetative to reproductive growth. The genome of one isolate of *R. collo-cygni* from Denmark has been sequenced. The genome is 30.3 Mb, with 11617 predicted genes. Phylogenetic analyses based on 1026 single copy proteins from 26 Dothidiomycetes + outgroups places *R. collo-cygni* in the Capnodiales, sister to *Zymoseptoria tritici*. A putative secretome of 1053 genes were identified, of which 150 of were identified as potential effectors. Seventy three proteins matched known pathogenicity determinants associated with toxin production in other Dothidiomycetes. *R. collo-cygni* has approximately twice as many polyketide synthase genes compared to other species of Capnodiales. Some of these may be involved in the biosynthesis of the toxin rubellin which has been identified in the fungus. We are now investigating the secretome across the kingdom fungi using phylogenomics.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M83

Monday 4th April
14:00 - 16:00

ALTAMIRANO Sophie *(1), FANG Diana (1), SIMMONS Charles (1), SRIDHAR Shreyas (2), SANYAL Kaustuv (2), KOZUBOWSKI Lukasz (1)*
(1) Department of Genetics & Biochemistry, Clemson University, Clemson/South Carolina, UNITED STATES
(2) Molecular Mycology Laboratory, Molecular Biology & Genetics Unit, JNCASR, Bangalore, India

Elucidating the mechanism(s) of fluconazole based aneuploidy in *Cryptococcus neoformans*

Cryptococcus neoformans is a major human fungal pathogen that causes meningitis in mostly immunocompromised individuals. There are approximately 1 million cases of cryptococcal meningitis each year, with over a half occurring in AIDS patients in Sub-Saharan Africa. The current treatment for cryptococcosis consists of flucytosine, amphotericin B, and fluconazole (FLC). Amphotericin B and flucytosine are more toxic and relatively expensive. FLC, an inhibitor of the ergosterol pathway, is considerably less toxic and cheaper, but its use leads to increased resistance. Resistance to FLC has been associated with aneuploidy. *C. neoformans* is typically a haploid yeast, but under FLC treatment a population of resistant cells with disomy of certain «beneficial» chromosomes gets selected. Although specific genes amplified in the FLC resistant aneuploids have been characterized, the underlying mechanisms responsible for aneuploid formation remain elusive. The aim of this study was to explain how FLC leads to aneuploidy. We examined the effect of FLC on the ergosterol content in the plasma membrane at a single cell level based on staining with the fluorescent dye filipin and correlated these results with the effects of FLC on cell growth and nuclear division using a series of fluorescent markers. We have also utilized cell sorting coupled with quantitative microscopy to better assess physiological processes that fail under FLC treatment and may be critical to aneuploidy formation. Interestingly, treatment with FLC led to the emergence of a population of multibudded cells as a result of cytokinesis failure and possibly unequal partition of the chromatin between the mother and daughter cells. Resulting multimeras consisted of cells with unequal ergosterol content that correlated with mis-segregation of chromatin. Based on our results, we hypothesize that FLC leads to a slow depletion of ergosterol, which affects budding, nuclear division, and cytokinesis. We postulate that all three defects contribute to aneuploidy. Furthermore, the multibudded state is likely a morphology associated with aneuploidy that allows for subsequent selection of FLC resistant cells.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M84

Monday 4th April
14:00 - 16:00

LAROCHE André *(1), FRICK Michele (1), BUCKOLL Paul (1), AMUNDSEN Eric (1), PUCHALSKI Brent (1), RANDHAWA Harpinder (1), GRAF Robert (1), DENIS GAUDET Denis (1)*
(1) Agriculture and Agri-Food Canada, Lethbridge, AB, Canada

Regulation of gene expression in incompatible/compatible reactions between stripe rust isolates from western Canada and wheat

We evaluated the interaction at the transcriptome level between two *Puccinia striiformis f.sp. tritici* (Pst) western Canadian isolates, LSW3_2012_SP2 and SWS484_SPF, with resistant and susceptible hosts. The number of clean Illumina paired-ends reads obtained from inoculated tissues in compatible and incompatible reactions with isolates SWS484 and LSW3 ranged from 78 M to 184 M. To validate Pst transcripts involved in the infection process, over 2.9 M reads were obtained from intact RNA isolated from LSW3 infected haustoria. Mapping of reads against the PST-78 transcripts reference reveals that 54% of the reads are mapped in the compatible reaction while only 0.5% did map in the incompatible reaction. The proportion of the reads mapping to cereal transcriptomes was 28% and 86% for compatible and incompatible reactions. These results show an important differential regulation of genes in both the fungal pathogen and the wheat host. Genes differentially regulated between the incompatible and compatible reactions were characterized by annotation and GO term classification. A list of potential effectors present in both LSW3 haustoria-enriched tissues and infected leaf tissues will be presented. As we used Illumina Mate Pair (MP) and PacBio technologies to obtain larger contigs and scaffolds for these two Pst isolates, the distribution of putative effectors has been mapped to the scaffolds and results will be presented in elucidating sequences involved in these specific reactions.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M85

Monday 4th April
14:00 - 16:00

FOKKENS Like *(1), VAN DAM Peter (1), REP Martijn (1)*
(1) Molecular Plant Pathology, University of Amsterdam, Amsterdam, Netherlands

Host-specific regions in the pangenome of *Fusarium oxysporum* reveal evolutionary trajectories of host-switches

The *Fusarium oxysporum* species complex (FOSC) consists of both pathogenic and non-pathogenic isolates that occur in large populations. Pathogenic isolates enter the vascular system of host plants through the root and cause wilting or root rot disease symptoms. Individual isolates can be grouped into host-specific formae speciales, but collectively the species complex has a wide host range. Host-specificity is polyphyletic and virulence towards a specific host can be transferred through the exchange of (parts of) chromosomes. To assess the evolutionary mechanisms underlying host-switches, we selected genomes of 87 FOSC isolates infecting different hosts and belonging to distinct phylogenetic clades. For 18 of these we used SMRT sequencing to obtain near complete assemblies, which significantly augmented identification of host-specific regions. In each assembly, approximately one third (~18Mb) of the genome is sparsely present in other isolates and can thus be considered. We found both clade-specific and host-specific contigs. For all formae speciales, we observed footprints of chromosome exchange between isolates that are in different phylogenetic clades but infect the same host. For tomato-infecting strains, gain of virulence can be completely explained by gain of (parts) of two chromosomes. In contrast, for the cucumber, melon and watermelon infecting isolates we observed several scenarios, indicating that switches between different cucurbit hosts occurred on several occasions via distinct evolutionary mechanisms. Interestingly, all cucurbit-infecting isolates possess a genomic region that is absent in other formae speciales and is likely to contain cucurbit-specific effector genes.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M86

Monday 4th April
14:00 - 16:00

DUPLESSIS Sebastien *(1), DE MITA Stephane (1), HALKETT Fabien (1), FREY Pascal (1)*
(1) INRA, Unité Mixte de Recherche INRA/Université de Lorraine 1136 Interactions Arbres/Micro-organismes, Centre INRA Nancy Lorraine, CHAMPENOUX, France

Update on *Melampsora larici-populina* genomics

The genome of the poplar rust fungus *Melampsora larici-populina* has been sequenced almost 10 years ago by an international consortium in the frame of the Community Sequencing Program of the US DoE Joint Genome Institute. The detailed analysis of the genome content and gene annotation, along with comparison to the cereal rust fungus *Puccinia graminis* f.sp. *tritici*, has revealed the singular profile of rust fungal genomes (1). Similar to other obligate biotrophic fungi (e.g. powdery mildews), rust fungi exhibit a large genome size (>80Mb) and an important content in repeat elements (~45%). In contrast, they possess a large gene complement (>15,000 genes) marked by the presence of many expanded gene families specific to the Pucciniales order or the corresponding families (*i.e.* Melampsoraceae or Pucciniaceae). Since, more rust genomes have been sequenced, analyzed and published, confirming these general trends and opening great perspectives for comparative rust genomics (2). The genome of *M. larici-populina* has been instrumental to explore the genetic diversity of the fungus through re-sequencing of isolates sampled through the years in natural populations. Beside, gene expression during poplar leaf infection or throughout the lifecycle has helped to build a comprehensive transcriptomic analysis of the rust fungus. More recently, a genetic map of the poplar rust fungus has been obtained from a self-cross of the reference genome isolate. All these data have led to the version 2 of the genome now anchored to 18 linkage groups, including a refined gene annotation available online on the MycoCosm webite (3). We will provide here a complete update on the poplar rust genome and an overview of ongoing works to decipher the biology of this fascinating and devastating rust fungus.

(1) Duplessis, Cuomo, et al. (2011). PNAS 108:9166-9171
(2) Duplessis, Bakkeren & Hamelin (2014) Adv Bot Res 70: 173-209
(3) Mycocosm http://genome.jgi.doe.gov/programs/fungi/index.jsf

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M87

Monday 4th April
14:00 - 16:00

GSALLER Fabio *(1), FURUKAWA Takanori (1), JAVIER Javier (2), MACDONALD Darel (1), BECKMANN Nicola (3), EL-ZEIHERY Dalia (4), GILSENAN Jane (1), OLIVER Jason (3), BIRCH Michael (3), BROMLEY Michael (1)*
(2) Universitat Rovira i Virgili, Reus, Spain
(3) F2G Ltd, Manchester, UK
(4) Pharmacelsus GmbH, Saarbrücken, Germany

Identification of a novel antifungal compound as a potent inhibitor of ∆9-desaturase in *Aspergillus fumigatus*

The health risk of fungal infections is still heavily underestimated. Annually around 1.5 million deaths are caused by fungal infections, which exceeds the number of people being killed by malaria and tuberculosis. The azoles represent the gold standard for treatment of infections caused by the fungal pathogen *Aspergillus fumigatus*. Resistance to this class is growing due to prolonged exposure of patients to these drugs and the widespread use of azoles in agriculture. The mortality rate for individuals who are infected with a resistant isolate exceeds 85%. Novel antifungal drugs are urgently needed. In this study we identify and validate the molecular target of a novel and potent antifungal agent. A variety of genetic, genomic as well as biochemical approaches including chemical genomics, transcriptional profiling and, phenotypic profiling were used to identify ∆9-desaturase (sdeA) as the likely drug target. We confirmed the association between the antifungal compound and sdeA with both targeted gene expression analysis and quantification of the substrate:product ratio (stearic acid:oleic acid) of ∆9-desaturation in cells exposed to the compound. To further analyse the suitability of ∆9-desaturase as potential drug target in *A. fumigatus*, a tetracycline/doxycycline inducible sdeA strain was created. The TET-sdeA isolate was unable to grow in the absence of the inducer doxycycline indicating that sdeA is essential for viability. Supplementation with oleic acid only partially reversed the growth defect of the strain. The TET-sdeA isolate was avirulent in both an insect (*Galleria melonella*) and a murine model of infection. This lack of virulence was reversed by doxycycline treatment. Taken together, this study shows that sdeA is the target of the novel antifungal compound and demonstrates the target is critical for virulence.

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CS2M88

Monday 4th April
14:00 - 16:00

HAWKINS Wayne *(1), BORGES Leandro (1), DHILLON Braham (1), RAMEGOWDA Yamunarani (1), RIDENOUR John (1), ZACCARON Marcio (1), RUPE John (1), BLUHM Burton (1)*
(1) University of Arkansas, Fayetteville, USA

HAP3 of *Phomopsis longicolla* influences growth, development, and pathogenesis

Phomopsis longicola (Hobbs) causes *Phomopsis* seed decay, one of the most prevalent and potentially damaging diseases of soybean seed. Currently, little is known about the molecular basis of pathogenesis in *P. longicolla*, in part because crucial tools for molecular genetics, such as targeted gene deletion, have not been demonstrated in this organism. In other filamentous fungi, the heterotrimeric CCAAT-binding complex is involved in diverse aspects of growth and development, including secondary metabolism, morphogenesis, and pathogenesis. In this study, a putative component of the CCAAT-binding complex (HAP3) was identified in *P. longicolla* and characterized through functional genomics. The *HAP3* gene was successfully deleted via homologous recombination, and the mutant was genetically complemented via reintroduction of the wild-type gene. Deletion of *HAP3* substantially impaired radial growth and induced the formation of rhizomorphic (rope-like) hyphae on defined culture media. Experiments are being conducted to evaluate the importance of the *P. longicolla* CCAAT-binding complex during pathogenesis. This study demonstrates the feasibility of targeted gene deletion in *P. longicolla* and will elucidate the involvement of the CCAAT-binding complex during the initiation and development of *Phomopsis* seed decay. Phenotypic similarities between *HAP3* deletion mutants of *P. longicolla* and other plant pathogenic fungi potentially indicate broad involvement of the CCAAT-binding complex in plant pathogenesis.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M89

Monday 4th April
14:00 - 16:00

HEIMEL Kai *(1), DOEHLEMANN Gunther (2), JAKOBI Mareike (1), SCHMITZ Lara (1), HAMPEL Martin (1)*
(1) Georg-August-University, Göttingen, Germany
(2) University of Cologne, Cologne, Germany

The unfolded protein response coordinates developmental progression and virulence factor delivery in *Ustilago maydis*

Pathogenic development of the corn smut fungus *Ustilago maydis* requires the coordination of various signaling pathways. To cause disease virulence factor delivery has to be aligned with fungal development and sensing of environmental stimuli. We identified the unfolded protein response (UPR), a pathway to counteract secretion stress, as a regulatory hub connecting these processes. During pathogenic development the UPR is specifically activated after plant penetration. Premature UPR-activation interferes with morphogenetic switching and pathogenic development. By contrast, plant colonization is strictly dependent on an active UPR pathway. The physical interaction between the Hac1-like UPR regulator in *U. maydis* (Cib1), and the central regulator of fungal development in planta, Clp1, triggers fungal proliferation *in planta* and allows continuous UPR activation during biotrophic development. Importantly, expression of secreted virulence factors is not only regulated on posttranscriptional but also on transcriptional level by the UPR. Hence, the UPR is used to synchronize developmental progression and secretion of virulence factors for a rapid establishment of the compatible biotrophic interaction.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M90

Monday 4th April
14:00 - 16:00

GAO Yuanyuan *(1), LIU Zhaohui (1), FARIS Justin D. (2), RICHARDS Jonathon (1), BRUEGGEMAN Robert S. (1), OLIVER Richard P. (3), MCDONALD Bruce A. (4), FRIESEN Tim (1)*
(1) Department of Plant Pathology, North Dakota State University, Fargo, USA
(2) USDA-ARS, Northern Crop Science Lab, Cereal Crops Research Unit, Fargo, ND, USA
(3) Centre for Crop and Disease Management, Department of Environment and Agriculture, School of Science, Curtin University, Perth, WA, Australia
(4) Institute of Integrative Biology, Plant Pathology Group, Swiss Federal Institute of Technology, Zürich, Switzerland

Identification of necrotrophic effectors in *Parastagonospora nodorum* using genome wide association studies

Parastagonospora nodorum is a necrotrophic fungal pathogen and the causal agent of Septoria nodorum blotch (SNB) on wheat. *P. nodorum* produces necrotrophic effectors (NEs) that are critical to the virulence of the pathogen. The NEs produced by *P. nodorum* are recognized directly or indirectly by dominant sensitivity genes contributing to susceptibility in wheat. Nine NE-host dominant sensitivity gene interactions have been identified, and three NE genes have been cloned, including *SnToxA*, *SnTox1* and *SnTox3*. Since sexual populations of *P. nodorum* are difficult to develop under lab conditions, bi-parental populations have not been used to genetically locate genes involved in virulence, namely genes involved in NE production. Genome wide association studies (GWAS) are an alternative method to identifying genomic regions associated with traits using natural populations. In fungi, only a few studies using GWAS analysis have been reported and therefore the level of marker saturation necessary for GWAS analysis remains unknown. To identify additional NE gene regions, we developed a GWAS strategy using 191 global isolates (13 US states, Australia, Denmark, Finland, Latvia, Lithuania, Norway, Sweden, Brazil, Switzerland, China, South Africa, and Iran). Genotypic data of the 191 isolates were generated using a restriction site associated DNA-genotype by sequencing (RAD-GBS) approach to identify single nucleotide polymorphism (SNP) markers across the genome. Phenotypic data was collected by spore inoculation of the 191 isolates on wheat susceptible line Sumai3 (Tsn1, Snn3) and the North Dakota hard red spring wheat cultivar Alsen (Tsn1, Snn). Absence/ presence polymorphism in *SnToxA* and *SnTox3* genes were used as positive controls. Strong marker trait associations (MTA) were identified for *SnToxA* on Sumai3 and Alsen, and for *SnTox3* on Sumai3. A locus showing multiple strong MTAs with SNB on Alsen and Sumai3 was identified indicating a novel NE gene is present in this genomic region and four candidate genes are being characterized. These results show that GWAS is a powerful tool that can be used to identify genomic regions associated with *P. nodorum* virulence. Additionally, this study has begun to show the level of marker saturation necessary to associate quantitative genes with phenotypic traits.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M91

Monday 4th April
14:00 - 16:00

LIAO Hui-Ling (1), CHEN Yuan (1), BRUNS Tom D. (2), PEAY Kabir G. (3), TAYLOR John W. (2), BRANCO Sara (2), TALBOT Jennifer M. (4), GREGORIEV Igor (5), BARRY Kerrie (5), HOYT David W. (6), NICORA Carrie D. (6), PURVINE Samuel O. (6), CHEN Ko-Hsuan (1) and VILGALYS Rytas (1)
(1) Duke University, NC, USA
(2) UC Berkeley, CA, USA
(3) Standford University, CA, USA
(4) Boston University, MA, USA
(5) JGI, CA, USA
(6) EMSL, WA, USA

Common and unique (host-specific) genes involved in ectomycorrhzal symbiosis between *Pinus* and *Suillus*

Suillus species are members of the boletes (Suillaceae) that form host-specific ectomycorrhizal (EM) associations with conifer tree species in the family Pinaceae. *Suillus* species play critical roles in the function of forest ecosystems by providing their plant hosts with resources as well as protection from pathogens in exchange for plant-derived sugars. Our research aims at understanding of coevolution and genomics between plants and mutualistic fungi of *Suillus*. We investigated the genetics of EM symbiosis and host-specificity by cross-inoculation of different *Pinus* hosts with basidiospores of different *Suillus* species with varying natural host ranges. We assessed the outcome of these interactions using comparative genomics, metatranscriptomics, metabolomics and proteomics. Several *Suillus* spp. including *S. granulatus*, *S. pictus*, and *S. americanus* readily form ectomycorrhizae (compatible reaction) with plant hosts belonging to *Pinus* subgenus *Strobus* (*P. strobus* and *P. monticola*), but were incompatible with other '2-needle pine' hosts belonging to subgenus *Pinus* (*P. taeda*, *P. banksiana*). Meta0mics of inoculated roots revealed that both plants and fungi express distinctive responses during incompatible vs. compatible mycorrhizal pairings. Using RNA-Seq, we identified common and unique patterns of plant-fungal gene expression for compatible/incompatible *Pinus-Suillus* pairings. Compatible mycorrhizal interactions are further characterized by distinct metabolomic signatures for trehalose, mannitol and arabitol. Functional annotation of highly expressed genes reveals that the *Suillus-Pinus* respond via similar highly conserved gene regulatory networks involving coordinated responses by both fungal and plant genomes, including G-protein signaling and secretory pathways. *Pinus* spp. activate a core set of gene products that mediate *Suillus*-specific recognition, including leucine-rich repeat and pathogen resistance proteins. We are developing the *Pinus-Suillus* symbiosis as a model for understanding plant-fungal communication and other aspects of EM function. Current studies employ full genome analysis, transcriptomics and metabolomics to investigate molecular mechanisms of EMF-plant co-evolution.

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CS2M92

Monday 4th April
14:00 - 16:00

MARTINO Elena *(1), GRELET Gwen (3), MORIN Emmanuelle (2), KOHLER Annegret (2), DAGHINO Stefania (1), HENRISSAT Bernard (5), KUO Alan (4), GRIGORIEV Igor (4), MARTIN Francis (2), PEROTTO Silvia (1)*
(1) Department of Life Sciences and Systems Biology, University of Torino, 10125, Turin, Italy
(2) INRA, Université de Lorraine, Lab of Excellence ARBRE, Champenoux, France
(3) Landcare Research, Manaaki Whenua, Lincoln, New Zealand
(4) U.S. Department of Energy, Joint Genome Institut, Walnut Creek, CA, USA
(5) CNRS, Aix-Marseille Université, Marseille, France

New insights from genome and transcriptome analyses of four ericoid mycorrhizal fungi

Ericoid endomycorrhizal symbiosis (ERM) involves few soil fungi and members of a single plant family, the Ericaceae, distributed worldwide. Based on the age of the Ericaceae family, ERM is considered to be the youngest of all mycorrhizal types. ERM plants typically colonize harsh habitats with acidic soils, very low nutrient availability and slow decomposition of soil organic matter. In such environments, ERM fungi are instrumental for plant survival and contribute to both mobilisation and accumulation of soil C. Better knowledge of the ERM saprotrophic abilities and their regulation during the symbiosis may hold interesting keys to understand the mechanisms shaping the evolution of mycorrhizal symbioses. Fungi currently known to form ERM include mainly Ascomycetes in the Leotiomycetes. We sequenced the genomes of 4 ERM fungi (*Meliniomyces bicolor*, *M. variabilis*, *Oidiodendron maius* and *Rhizoscyphus ericae*) and compared them with the genome of other Leotiomycetes as well as with other 50 fungi, chosen because of their different lifestyles (i.e. ecto- and orchid mycorrhiza, endophytes, saprotrophs, pathogens). All ERM genomes feature a high number of CAZymes coding genes, acting on a wide range of substrates. Based on the total CAZymes repertoire MDS analysis placed ERM fungi nearer to saprotrophs and pathogens, and well separate from ECM, white and brown rot fungi. MDS analysis on genes coding for lipases and proteases placed ERM fungi quite distant from all other ecological strategies for both total and secreted lipases and for total proteases, while for secreted proteases ERM fungi grouped with saprotrophs and pathogens. For secondary metabolism genes, MDS analysis indicated a closely related gene signature for ERM fungi, pathogens and saprotrophs. Gene expression during ERM symbiosis was assessed by RNASeq for the model fungus *O. maius*. Around 6% of the total *O. maius* genes were up-regulated in symbiosis. Among them are CAZymes (19%), transporters (12%), SSPs (9%), lipases (2%) and proteases (3%). 66% of the induced CAZymes are secreted and they could be used to penetrate the thick host cell wall during root colonization, but they may also facilitate nutrient mobilisation from organic substrates thus supplementing host plant photosynthesis with fungal-derived carbon. ERM traits are thus suggesting a behaviour that is still bordering between saprotrophy and symbiosis.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M93

Monday 4th April
14:00 - 16:00

EBERT Malaika *(2), THOMMA Bart (2), BOLTON Melvin (1)*
(1) USDA - ARS, Fargo, USA
(2) Wageningen University, Wageningen, The Netherlands

Characterization of a novel *Cercospora beticola* effector proteins

Cercospora Leaf Spot (CLS), caused by the hemibiotrophic fungus *Cercospora beticola*, is the most destructive foliar disease of sugar beet worldwide. Plant pathogens secrete effectors that help establish disease during infection. Although many effectors have been characterized in several pathosystems, no *C. beticola* effector proteins have been reported to date. To identify *C. beticola* proteins involved with virulence, we grew *C. beticola in vitro* under specific conditions and tested culture filtrate for necrosis-inducing activity by infiltration into sugar beet leaves. Culture filtrate from one growth condition reliably caused necrosis in sugar beet leaves within 24 h. Treatment of culture filtrate with a mixture of proteases abolished necrosis-inducing activity, confirming that the *C. beticola* effector(s) responsible for necrosis was proteinaceous in nature. Active culture filtrates were partially purified using liquid chromatography (LC). A single (LC) fraction was repeatedly identified that caused necrosis upon infiltration into host tissue. MS/MS analysis of this fraction identified three *C. beticola* proteins. Each protein exhibited classic effector characteristics, including secretion signal, high cysteine content and low molecular weight (6 to 11 kDa). Candidate effector proteins were produced in *Pichia pastoris*. Infiltration of the candidate effector CbNIP10 caused necrosis in sugar beet leaves, while the other two effector candidates did not. A detailed characterization of CbNIP10 will be presented. Gaining an understanding of *C. beticola* effector biology will give new insight in *C. beticola* pathology and may lead to novel CLS control measures.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M94

Monday 4th April
14:00 - 16:00

HACQUARD Stéphane *(1), HIRUMA Kei (1), KRACHER Barbara (1), GERLACH Nina (2), SACRISTÁN Soledad (3), NAKANO Ryohei Thomas (1), THON Michael R. (4), BUCHER Marcel (2), O'CONNELL Richard J. (5), SCHULZE-LEFERT Paul (1)*
(1) Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, Germany
(2) Botanical Institute, Cologne Biocenter, CEPLAS, University of Cologne, Cologne, Germany
(3) Centro de Biotecnología y Genómica de Plantas (UPM-INIA) and E.T.S.I. Agrónomos, Universidad Politécnica de Madrid Campus de Montegancedo, Madrid, Spain
(4) Instituto Hispano-Luso de Investigaciones Agrarias (CIALE), Departamento de Microbiología y Genética, Universidad de Salamanca, Villamayor, SPAIN
(5) BIOGER, INRA, AgroParisTech, Thiverval-Grignon, France

Survival trade-offs in plant roots during colonization by closely related beneficial and pathogenic fungi

Although most characterized species of the fungal genus *Colletotrichum* are destructive pathogens, we found recently that *C. tofieldiae* (*Ct*) is an endemic endophyte in natural *A. thaliana* populations in Central Spain. Colonization by *Ct* initiates in roots, but can also spread systemically into shoots. *Ct* transfers the macronutrient phosphorus to shoots, promotes plant growth and increases fertility only under phosphorus-deficient conditions, a nutrient status that might have facilitated the transition from pathogenic to beneficial lifestyles. Comparative genome and transcriptome analyses between *Ct* and its closely related pathogenic species *C. incanum* (*Ci*) identified genomic signatures reflecting this recent transition from pathogenic to beneficial lifestyles, including a narrowed repertoire of secreted effector proteins, expanded families of secondary metabolism-related proteins, and limited activation of pathogenicity-related genes *in planta*. Analysis of the *Arabidopsis* transcriptome during root colonization by *Ct* revealed that beneficial responses are prioritized under phosphorus-deficient conditions whereas defense responses, involving ethylene and glucosinolate pathways, were activated under phosphorus-sufficient conditions. These data, together with the analysis of *Arabidopsis* mutants that are impaired in indole glucosinolate metabolism and the phosphate starvation response (PSR), provide evidence for a specific coordination between the PSR, the plant immune system and invasive fungal growth during beneficial interaction with *Ct*. Importantly, *Arabidopsis* immune responses were retained in phosphate-starved roots colonized by pathogenic *Ci*, illustrating the extraordinary ability of plants to maximize survival in response to conflicting stresses.

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CS2M95

Monday 4th April
14:00 - 16:00

ZACCARON Marcio *(1), SHARMA Sandeep (1), FAGUNDES Wangner (1), HAWKINS Wayne (1), LAWSON Nicholas (1), RIDENOUR John (1), SMITH Jonathon (1), DHILLON Braham (1), RUPE John (1), BLUHM Burton (1)*
(1) University of Arkansas, Fayetteville, USA

A forward genetic screen in *Phomopsis longicolla* provides unique insights into pathogenesis

Phomopsis longicola (Hobbs) causes *Phomopsis* seed decay of soybean (*Glycine max*), and also causes lesions on soybean stems, pods, and petioles. *P. longicola* can also exist endophytically within soybean stems, which has given rise to the hypothesis that pathogenesis occurs when the pathogen transitions from endophytic or hemibiotrophic growth to necrotrophy. In this study, a forward genetic screen was performed to elucidate genetic mechanisms underlying necrotrophy, and a RAD-seq approach was adapted to characterize genomic lesions in selected mutants. A collection of 1114 tagged, insertional mutants was created via *Agrobacterium*-mediated transformation, and mutants were evaluated individually in soybean stems for their ability to induce necrosis and form pycnidia. Six mutants induced significantly less necrosis in soybean stems than the wild-type strain, and four formed significantly fewer pycnidia. No gain-of-function mutations (increased necrosis) were observed. A modified RAD-seq protocol developed to identify the site of insertion was applied to a mutant significantly reduced in its ability to cause stem necrosis; a single copy of the disruption cassette integrated into the presumed promoter region of a cellobiohydrolase. This study describes new tools to dissect the interaction between *P. longicola* and soybean, and provides new insight into conserved mechanisms underlying stem, pod, and seed necrosis caused by *P. longicola*.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M96

Monday 4th April
14:00 - 16:00

WEIBERG Arne (1)
(1) Ludwig-Maximilians University, Munich, Germany

Small RNAs: a common weapon of plant pathogens?

Botrytis cinerea is a destructive plant pathogenic fungus that causes the grey mold disease in many field and glasshouse crops. We discovered that *B. cinerea* uses mobile small RNAs that translocate into host plants during infection and hijack the plant RNA interference (RNAi) pathway to silence host immunity genes (1). So far, *B. cinerea* is the only reported example that employs natural cross-kingdom RNAi as an effective virulence mechanism (2,3). Currently, we are investigating whether other classes of plant pathogens evolved similar small RNA-based infection strategies, which would establish cross-kingdom RNAi a common model in host-microbe interaction. The oomycete downy mildew pathogen *Hyaloperonospora arabidopsidis* (*Hpa*) is highly adapted to its host *Arabidopsis thaliana* representing an obligate biotroph pathogen. Our genetic screenings in combination with small RNA high-throughput sequencing point out that *Hpa* produces host gene suppressive small RNA effectors. By comparing small RNA effectors delivered either by *B. cinerea* or *Hpa*, with their respective *Arabidopsis* host targets we are uncovering a novel layer of rapid host-pathogen co-evolution on RNA level. Furthermore, commonalities and differences of small RNAs effectors and their respective host plant targets that are produced by these distinct plant pathogens as well as by an arbuscular mycorrhiza fungus (symbiosis) will be discussed.

(1) Weiberg, A. et al. Science 342, 118-123, (2013).
(2) Weiberg, A., Wang, M., Bellinger, M. & Jin, H. 52, 495-516, (2014).
(3) Weiberg, A. & Jin, H. Curr Opin Plant Biol 26, 87-94, (2015).

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M97

Monday 4th April
14:00 - 16:00

JUNG Boknam *(1), PARK Jungwook (2), YOUN Kihun (1), LI Taiyang (1), KIM Sunyoung (2), SEO Young-Su (2), LEE Jungkwan (1)*
(1) Department of Applied Biology, Dong-A University, Busan, South Korea
(2) Department of Microbiology, Pusan National University, Busan, South Korea

Interaction between the plant pathogenic fungus *Fusarium graminearum* and the bacterial pathogen *Burkholderia glumae*

The fungal pathogen *Fusarium graminearum* causes rice head blight which resembles the rice grain rot caused by the bacterial pathogen *Burkholderia glumae*. Previously, we showed that *F. graminearum* is resistant to toxoflavin produced by *B. glumae* while other fungal genera are sensitive to the toxin. We have tried to elucidate the resistant mechanism of *F. graminearum* against toxoflavin and interaction between the two pathogens in nature. We identified one transcription factor deletion mutant that is sensitive to toxoflavin, and analyzed transcriptomes of the wild-type strain compared to the mutant strain under either absence or presence of toxoflavin. Under the criteria with more than two-fold changes, 1,440 genes were up-regulated and 1,267 genes were down-regulated in wild-type strain than mutant strain in response to toxoflavin treatment. A comparison of gene expression profiling between the wild type and mutant through gene ontology analysis showed that genes related to metabolic process and oxidation-reduction process were highly enriched in the mutant strain. Supplement of toxoflavin in *F. graminearum* resulted in increased conidia and trichothecene production. Co-cultivation of two pathogens allowed bacterial cells to tightly attach to fungal conidia. Chemotaxis analysis showed that the bacterial cells move toward the fungal culture. These results suggested that coexistence of the two pathogens in rice provides mutual benefits to both pathogens for survival and dispersal.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M98

Monday 4th April
14:00 - 16:00

CARLA MICHELLE Aponte Lopez *(1), CAFARO Matías J. (1), RIOS-VELAZQUEZ Carlos (1), WESSEL-BEAVER Linda (2)*
(1) Department of Biology University of Puerto Rico, Mayagüez Campus, Mayagüez, Puerto Rico, USA
(2) Department of Crops and Agro-Environmental Sciences, University of Puerto Rico at Mayagüez, Mayagüez, Puerto Rico, USA

Local mycorrhizal diversity associated with *Capsicum chinense* and its response to Different inoculation treatments

Capsicum chinense is widely cultivated in the Caribbean region. Most types are pungent, such as 'Scotch Bonnet' and 'Habanero'. In Puerto Rico, non-pungent types are preferred and referred to as "ají dulce" or sweet chili pepper. Sweet chili pepper is one of the main crops cultivated on the island and is consumed as part of the local cuisine. It is also known for being a good source of vitamins A, C, E and for its antioxidant properties. Currently, in commercial agriculture, the use of chemical fertilizers dominates the local market, while biological ones are overlooked. Symbiotic relationships between mycorrhizal fungi and the roots of vascular plants can serve as biological crop enhancers. The purpose of this research was to determine, characterize and identify the mycorrhizae associated with locally grown *C. chinense* in the western area of Puerto Rico. We collected «ají dulce» roots and surrounding soil from plants growing in experimental plots at UPRM. Roots were cleared in KOH 10% at 60°C for one hour and 2% HCl for 20 minutes, stained using Trypan blue 0.4% for 72 hours and preserved in glycerol. After processing samples and staining, fungi were morphologically identified using taxonomic keys. Among the genera identified so far we found *Glomus* and *Acaulospora*. We also grew «ají dulce» under six treatments using commercial Promix® BX for general purpose, Promix® Mycorrhizae (*Glomus intraradices*), Promix® with 25% soil from the UPRM, greenhouse Promix® with fertilizer, Promix® Mycorrhizae (*Glomus intraradices*) with fertilizer and Promix® with 25% soil from the UPRM greenhouse with fertilizer. Significant differences were obtained when compared using ANOVA-test, obtaining a p-value <0.0001 between Promix® Mycorrhizae and Promix® BX treatments where the first one resulted in greater stem length, fruit and leaves number. Molecular aspects for the diversity of the mycorrhizal community had been done. Currently, cloning samples are been processing for lighten this path. The genera expected will be *Glomus*, *Acaulospora*, *Gigaspora* and *Archaespora*.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M99

Monday 4th April
14:00 - 16:00

SANCHEZ VALLET Andrea *(1), MEILE Lukas (1), STEWART Ethan (1), CROLL Daniel (1), MCDONALD Bruce (1)*
(1) Dep. of Environmental Systems Science, ETH, Zurich, Switzerland

Zymoseptoria tritici pathogenicity is determined by isolate specific virulence factors

Zymoseptoria tritici is a major pathogen of wheat around the world and the causal agent of septoria tritici blotch. Understanding the molecular basis of *Z. tritici* virulence and its genetic regulation will aid to design better control methods. However, to date only a few virulence factors have been characterized by means of gene disruption. Remarkable differences in virulence levels and in host specificity occur among *Z. tritici* isolates. Therefore, exploring the genetic bases of phenotypic variability is a promising strategy to identify new virulence factors. In fact, a single quantitative trait locus (QTL) for virulence was identified to explain the genetic differences between a highly virulent (3D7) and a non-virulent (3D1) isolate. Gene re-annotation of the QTL-region in the 3D7 genome identified 37 candidate genes, including 5 encloding putative effectors and a major facilitator superfamily transporter. Interestingly, the genomic region where the QTL was mapped is rich in tranposable elements and the synteny between the parental isolates is not conserved. Two big insertions of around 30 and 40 kb were identified in 3D7, but not in the 3D1 genome. Two genes encoding two highly polymorphic putative effectors were identified in between the two regions. The localization of these two genes in a genomic region undergoing rearrangements points them as candidate effector genes of *Z. tritici*.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M100

Monday 4th April
14:00 - 16:00

SPADARO Davide *(1), MATIC Slavica (1), SICILIANO Ilenia (1), AMARAL CARNEIRO Greice (1), BAGNARESI Paolo (2), BISELLI Chiara (2), ORRÙ Luigi (2), VALÉ Giampiero (3), GARIBALDI Angelo (1), GULLINO Maria Lodovica (1)*
(1) Università di Torino, Grugliasco , Italy, Grugliasco , Italy
(2) CREA,Genomics Research Centre, Fiorenzuola d'Arda , Italy
(3) CREA, Rice Research Unit, Vercelli, Italy

Transcriptional changes, phytohormones and phytoalexins modulate the response of rice to *Fusarium fujikuroi*

Fusarium fujikuroi, causal agent of bakanae disease, is the main seedborne pathogen on rice. Molecular and chemical mechanisms regulating rice defence responses towards this fungus are not yet fully known. Different rice genotypes were screened against bakanae disease leading to the identification of Selenio and Dorella as the most resistant and the most susceptible cultivars, respectively. In order to identify transcriptional mechanisms and underpinning Selenio resistance, a RNA-seq based comparative transcriptome profiling was conducted on infected seedlings of both genotypes. Dynamic profiles and possible interactions of defence-related phytohormones and phytoalexins were also determined. Gene ontology (GO) enrichment analyses detected 46 common enriched GO terms in both genotypes, some of them associated with general plant response on fungus attack. Some GO terms related to fungal infection were Selenio-specific. Further induced genes in the resistant genotype included *PR1* and germin-like protein genes, glycoside hydrolases, MAP kinases, and WRKY transcriptional factors. In the resistant cultivar Selenio the presence of pathogen induced high production of phytoalexins, mainly sakuranetin, and symptoms of bakanae were not observed. On the contrary, in the susceptible genotype Dorella, the pathogen induced the production of gibberellin and abscisic acid, inhibited jasmonic acid production, phytoalexins were very low and bakanae symptoms were observed. Phytoalexin synthesis is an important factor for rice resistance against bakanae disease. Our results indicate also that hypersensitive response, response to chitin and jasmonic acid dependent signalling might be the main pathways controlling rice resistance to bakanae disease.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M101

Monday 4th April
14:00 - 16:00

DE GUILLEN Karine *(2), ORTIZ Diana (1), GRACY Jerôme (2), PADILLA André (2), KROJ Thomas (1)*
(1) BGPI, INRA, CIRAD, SUPAGRO, Montpellier, France
(2) Centre de Biochimie Structurale, INSERM, CNRS, Université Montpellier, Montpellier, France

Structure analysis uncovers a highly diverse but structurally conserved effector family in phytopathogenic fungi

Phytopathogenic ascomycete fungi possess huge effector repertoires that are dominated by hundreds of sequence-unrelated small secreted proteins. The molecular function of these effectors and the evolutionary mechanisms that generate this tremendous number of singleton genes are largely unknown. To get a deeper understanding of fungal effectors, we determined by NMR spectroscopy the 3-dimensional structures of the *Magnaporthe oryzae* effectors AVR1-CO39 and AVR-Pia. Despite a lack of sequence similarity, both proteins have very similar 6 Y-sandwich structures that are stabilized in both cases by a disulfide bridge between 2 conserved cysteins located in similar positions of the proteins. Structural similarity searches revealed that AvrPiz-t, another effector from *M. oryzae*, and ToxB, an effector of the wheat tan spot pathogen *Pyrenophora tritici-repentis* have the same structures suggesting the existence of a family of sequence-unrelated but structurally conserved fungal effectors that we named MAX-effectors (*Magnaporthe* Avrs and ToxB like). Structure-informed pattern searches strengthened this hypothesis by identifying MAX-effector candidates in a broad range of ascomycete phytopathogens. Strong expansion of the MAX-effector family was detected in *M. oryzae* and *M. grisea* where they seem to be particularly important since they account for 5-10% of the effector repertoire and 50% of the cloned avirulence effectors. Expression analysis indicated that the majority of *M. oryzae* MAX-effectors are expressed specifically during early infection suggesting important functions during biotrophic host colonization. We hypothesize that the scenario observed for MAX-effectors can serve as a paradigm for ascomycete effector diversity and that the enormous number of sequence-unrelated ascomycete effectors may in fact belong to a restricted set of structurally conserved effector families.

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Session CS2 Pathogenesis and symbiosis
CS2M102

Monday 4th April
14:00 - 16:00

LORRAIN Cécile *(1), PETRE Benjamin (2), SAUNDERS Diane (3), SKLENAR Jan (2), WIN Joe (2), HECKER Arnaud (4), KAMOUN Sophien (2), DUPLESSIS Sébastien (1)*
(1) INRA, Lorraine University, Lab of excellence Arbre, Champenous, France
(2) The Sainsbury Laboratory, Norwich Research Park, Norwich, UK
(3) The Genome Analysis Centre, Norwich Research Park, Norwich, UK
(4) Université de Lorraine, INRA, Lab of excellence Arbre, Vandoeuvre-lès-Nancy, France

Functional characterization of *Melampsora larici-populina* candidate effectors

The poplar leaf rust fungus, *Melampsora larici-populina* has been established as a tree-microbe interaction model. In biotrophic plant-parasites, effectors are known to condition host cell colonization. Effectors are molecules that interfere with host cell mechanisms and immune system. One of the key questions in effector biology is to understand the role of these effectors during host infection. We developed an effectoromic pipeline to select, clone and expressed 20 candidate effectors in *Nicotiana benthamiana* leaf cells to (i) determine their subcellular localisation and (ii) to identify plant proteins interactors. We performed coimmunoprecipitation and mass spectrometry to identify plant proteins associating with 5 candidate effectors. Using confocal microscopy, we report 6 candidate effectors localizing in particular cell compartments such as nucleus, nucleoli, chloroplasts and mitochondria (1). In particular, one effector candidate targets plant chloroplasts and mitochondria utilizing a transit peptide cleaved after entrance in the stroma of chloroplasts. This candidate effector named CTP1 (Chloroplast Targeted Protein 1) is part of a Melampsoracae-specific family of polymorphic and modular small-secreted proteins that also localise in chloroplasts (2). Ongoing research aimed at elucidating the CTP family function will be presented.

(1) Petre, B., Saunders, D.G.O., Sklenar, J., Lorrain, C., Win, J., Duplessis, S., and Kamoun S., (2015). MPMI
(2) Petre, B., Lorrain, C., Saunders, D.G.O., Duplessis, S., and Kamoun S., (2015). Cellular Microbiology.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M103

Monday 4th April
14:00 - 16:00

KRAUSE Katrin *(1), HENKE Catarina (1), ASIIMWE Theodore (1), BOLAND Wilhelm (2), KOTHE Erika (1)*
(1) Friedrich Schiller University, Institute of Microbiology, Microbial Communication, Jena, GERMANY
(2) Max Planck Institute for Chemical Ecology, Jena, Germany

Modulation of *Tricholoma vaccinum* ectomycorrhiza by indole-3-acetic acid

The growth of plants and its roots is affected by the auxin indole-3-acetic acid (IAA), which is a long time known phytohormone. Here, we report on fungus-derived IAA, causing morphological changes in the development of ectomycorrhiza. Both partners, tree and fungus are affected. The biosynthesis in fungal hyphae, excretion, induced ramification in fungal cultures, and enhanced Hartig" net formation in mycorrhiza were observed. In addition, gene expression, labeled IAA precursors, aldehyde dehydrogenase ald1 overexpressing *Tricholoma vaccinum* and heterologous expression of a transporter were used to study the effects in molecular detail. In *T. vaccinum*, IAA is produced from tryptophan via indole-3-pyruvate and indole-3-acetaldehyde finally oxidized by an aldehyde dehydrogenase. Upregulation of *ald1* was found in ectomycorrhiza and by external supplementation with indole-3-acetaldehyde. In mycorrhization studies, *ald1* overexpressing *T. vaccinum* showed an increased width of the apoplast between the cortical cells of the Hartig" net, as well as upregulation of the multidrug and toxic extrusion (MATE) transporter *Mte1*, involved in the export of IAA from fungal cells. External supply of IAA and its precursors induced elongation and increased branching in mycorrhizal fungi, whereas no morphogenetic changes were observed in saprobic fungi like *Schizophyllum commune*. These findings indicate a crucial role for IAA in the regulation of ectomycorrhiza formation and morphology.

Henke C, Jung E-M, Voit A, Kothe E, Krause K (2016). JBM. 56 (2):in print.
Krause K, Henke C, Asiimwe T, Ulbricht A, Klemmer S, Schachtschabel D, Boland W, Kothe E (2015). Appl Environ Microbiol. 81(20):7003-7011.
Schlunk I, Krause K, Wirth S, Kothe E (2014). Environ Sci Pollut R.

ESPR-D-14-03482R2.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M104

Monday 4th April
14:00 - 16:00

DAGUERRE Yohann *(1), SCHELLENBERGER Romain (1), WITTULSKY Sebastian (1), PLETT Jonathan (2), KOHLER Annegret (1), VENEAULT-FOURREY Claire (1), MARTIN Francis (1)*
(1) INRA, Lorraine University, Lab of Excellence ARBRE, Champenoux, France
(2) Hawkesbury Institute for the Environment, University of Western Sydney, Richmond, NSW, Australia

The use of the fungal effector MiSSP7 to decipher the jasmonic acid pathway in *Populus trichocarpa*.

Roots of most trees form symbiosis with mutualistic soil-borne fungi. The crosstalk between the two partners is fundamental for the timing, establishment and maintenance of beneficial relationships. However, very little is known about how symbiosis is initiated by both partners. We previously showed that the ectomycorrhizal basidiomycete *Laccaria bicolor* (Maire) P.D. Orton relies on Mycorrhizal-induced Small Secreted Proteins (MiSSP) to establish the interaction (1). In particular MiSSP7 interacts with the jasmonic acid (JA) co-receptor PtJAZ6 of *P. trichocarpa*, blocking JA signaling and promoting mutualism (2). JAZ proteins are known to interact with NINJA and TOPLESS proteins as well as bHLH transcriptional factor in leaves of *Arabidopsis*. We aim identifying the proteins interacting with PtJAZ6 in roots of *P. trichocarpa*. Using Yeast Two Hybrid (Y2H) screen, we show that PtJAZ6 interacts with PtNINJA proteins and bHLH transcription factors PtMYC2 and PtJAM1. Next step will be the validation of these interactions by Bimolecular Fluorescence Complementation (BiFC) and the identification of genes targeted by the bHLH transcription factors, using in silico prediction and ChipSeq assay in order to fully understand mechanism underlying ectomycorrhizal ontogenesis.

(1) Plett et al . Current Biology (2011), 21(14):1197-203.
(2) Plett et al.. PNAS (2014), 111(22):8299-304.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M105

Monday 4th April
14:00 - 16:00

DESIRÒ Alessandro *(1), ZHEN Hao (1), VANDE POL Natalie (1), BENNING Nils (1), TORRES CRUZ Terry (2), PORRAS-ALFARO Andrea (2), BONITO Gregory (1)*
(1) Department of Plant Soil and Microbial Sciences, Michigan State University, East Lansing, MI, USA
(2) Department of Biological Sciences, Western Illinois University, Macomb, IL, USA

Endobacterial diversity in the genus *Mortierella*

Plant roots are intimately associated with a myriad of microorganisms which play key-roles in plant health and nutrition. One of the most important elements of this tangled belowground interaction is represented by mycorrhizal fungi that, in turn, can be associated with other microorganisms such as endobacteria. One of the most investigated example of fungal-bacterial interaction is represented by arbuscular mycorrhizal fungi (Glomeromycota). This group of fungi are a niche for two types of endobacteria: a Y-proteobacterium called *Candidatus Glomeribacter gigasporarum* (*CaGg*) and a still undescribed group of Mollicutes/Mycoplasma-related endobacteria (MRE). MRE have been also reported within the fruiting bodies of Endogone (Mucoromycotina), while an endobacterium closely related to *CaGg* has been detected within the mycelium of *Mortierella elongata* (Mortierellomycotina). *Mortierella* is a diverse genus of early diverging fungi that is widespread in plant rhizospheres and soils. Because *Mortierella* is phylogenetically related to mycorrhizal fungi such as Glomeromycota and Endogone, both of which contain Glomeribacter-related endobacteria (GRE) and/or MRE, we chose to assess the presence and identity of these two endobacteria types dwelling within the mycelium of different *Mortierella* lineages. In total, more than 150 strains belonging to *Mortierella* and collected worldwide were screened. We confirmed the presence of different GRE phylotypes (4) and two main MRE phylogroups encompassing novel phylotypes closely related to the Glomeromycota-associated MRE. Our results show for the first time that different GRE exist and they can be found in association with a variety of *Mortierella* taxa, which can also host MRE. With these findings, the view of endobacteria as a common element, perhaps a synapo- or plesiomorphic trait shared by early divergent fungi can be also extended to the Mortierellomycotina, and, therefore, to the MMG clade. While we can observe phenotypic changes of the fungal host when inhabited or not by the endobacteria, the evolutionary and ecological role of GRE and MRE still remains a central question. Finally, the saprotrophic and plant-associated nature of *Mortierella* and the possibility to obtain «cured» cultures devoid of endobacteria make this fungus an excellent candidate for further study of fungal-bacterial and plant-fungal-bacterial interactions.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M106

Monday 4th April
14:00 - 16:00

CHUJO Tetsuya *(2), LUKITO Yonathan (1), EATON Carla (1), DUPONT Pierre-Yves (1), JOHNSON Linda (3), COX Murray (1), SCOTT Barry (1)*
(1) Massey University, Palmerston North, New Zealand
(2) National Institute of Agrobiological Sciences, Tsukuba, JAPAN
(3) AgResearch Grasslands, Palmerston North, New Zealand

Heterochromatin protein I regulates alkaloid biosynthesis and the mutualistic symbiotic interaction between *Epichloë festucae* and its host *Lolium pere*

Genes for the synthesis of ergot alkaloid (*eas*) and indole-diterpene (*ltm*) bioprotective metabolites are organised as clusters in the sub-telomeric regions of the genome of *Epichloë festucae*, a fungal symbiont of *Lolium perenne*. These genes are highly expressed *in planta* but not expressed in axenic culture. We recently showed that the levels of histone H3 lysine 9 and lysine 27 trimethylation are reduced at these loci *in planta* compared to axenic culture (Chujo & Scott 2014). Here we investigate the role of *E. festucae* HepA, a homolog of Heterochromatin Protein I, in regulating expression of these alkaloid cluster genes and establishment of a mutualistic symbiotic interaction. Deletion of *hepA* led to derepression of *ltm* and *eas* gene expression under non-symbiotic culture conditions in a manner similar to deletion of *clrD*, which encodes the cognate H3K9 methyl transferase. Plants infected with the *hepA* mutant were stunted and underwent early senescence. Hyphae of the *hepA* mutant had a proliferative pattern of growth within the leaves of *L. perenne* with increased colonization of the intercellular spaces and vascular bundles. To gain further insight into possible mechanism(s) underlying some of these changes we compared the transcriptomes of wild-type and *hepA* mutant symbiota using RNAseq. An analysis of the fungal transcriptome data set showed that 894 genes (~11%), including *ltm* and *eas* cluster genes, were differentially expressed (DE) between the two samples. Changes in expression of the alkaloid genes were verified by qRT-PCR. Levels of the alkaloids found in these tissues, as determined by LC-MS/MS analysis, correlated with the changes in gene expression observed. We also observed that 111 of the 182 differentially expressed genes (DEG) common to three other (*noxA*, *proA* and *sakA*) symbiotic mutant associations (Eaton et al. 2015) were DE in the *hepA* association. We also found that the *hepA* DEG were significantly enriched at AT-isochores whereas DEG in the *sakA*, *noxA* and *proA* mutant data sets were not. These results are consistent with the hypothesis that AT transposon-rich regions of the genomes preferentially form heterochromatin, and that regulation of gene expression in these regions by HepA protein is important for alkaloid biosynthesis and the mutualistic symbiotic interaction.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M107

Monday 4th April
14:00 - 16:00

GAGIC Milan *(1)*
(1) AgResearch, Palmerston North, New Zealand

Effect of host genotype on vertical transmission of *Epichloë* endophytes

Epichloë endophytes colonise and live asymptomatically in the intercellular spaces of the grass species. They have an important role in agriculture and forage based industries. Despite the importance of seed transmission for asexual *Epichloë*, research in this area is lacking. Synthetic *Epichloë*-grass associations have lower endophyte transmission frequency with seed transmission failing at various stages of the symbiotic lifecycle. The objective of our study is to conduct multifaceted research into endophyte transmission to identify transmission failure points and the genetic markers driving this process. Five independent ryegrass populations infected with *E. festucae* were screened to develop a panel of host genotypes that vary with respect to endophyte transmission. Significant differences were found between the five populations. Environmental differences were shown not to be a factor in endophyte transmission, but morphological study of the contrasting associations showed significant differences in endophyte quantity and distribution. Marker discovery through the use of Genotyping by Sequencing (GBS) technology has been used to identify marker indices associated with low and high endophyte transmission. GBS markers identified will be correlated with gene expression experiments utilising RNA-Seq and expression of candidate genes monitored throughout the life cycle of the host. This integrated approach will enable us to verify potential host markers that underlie endophyte transmission.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M108

Monday 4th April
14:00 - 16:00

KHANG Chang-Hyun *(1), JONES Kiersun (1), SHIPMAN Emma (1), JENKINSON Cory (1), ZHU Jie (1), KIM Dong Won (1)*
(1) University of Georgia, Athens, GA, USA

Dynamics of blast effectors and rice cell membranes during hemibiotrophic invasion by *Magnaporthe oryzae*

Hemibiotrophic *Magnaporthe oryzae* causes rice blast disease using an appressorium to produce invasive hyphae (IH) that infect rice cells. During biotrophic colonization, IH are surrounded by host-derived extra invasive hyphal membrane (EIHM) and are associated with biotrophic interfacial complexes (BICs). IH secrete effectors that enter the host cytoplasm likely via BICs across the EIHM; other effectors remain in the EIHM matrix. Using quantitative fluorescence live-cell imaging along with our newly developed fluorescent reporters, we investigated the development of the BIC-associated cell, the regulation of effector gene expression, and host cellular responses during the IH proliferation. We found that a filamentous primary IH extends from the appressorium to a consistent distance and then swells asymmetrically at the apex. The nucleus in the appressorium divides, and one nucleus moves down the primary IH into the swollen portion. Septation occurs in the filamentous region of the primary IH to produce the first bulbous BIC-associated cell. The expression of the effector gene *PWL2* is strongly induced in the appressorium immediately after penetration into living cells and in the BIC-associated cell but reduces in subsequently grown IH cells. IH proliferation leads to the sequential disruption of EIHM, host vacuole, host plasma membrane and nuclear envelope. Our studies provide a detailed framework for future genetic understanding of hemibiotrophic invasion of *M. oryzae*.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M109

Monday 4th April
14:00 - 16:00

GUTHRIDGE Kathryn *(1), SPANGENBERG German (1)*
(1) AgriBio, Centre for AgriBioscience, Department of Economic Development, Jobs, Transport and Resources, La Trobe University, Bundoora, Australia

Novel endophyte discovery and characterisation for pasture grasses

A limited number of novel fungal endophyte strains with desirable metabolite profiles have been advanced into commercial production of pasture grass germplasm such as perennial ryegrass, tall fescue and brachiaria. An integrated systems biology approach for discovery of novel endophytes based on exploitation of genotypic information has been implemented across a broad range of pasture grass-endophyte germplasm. Genetically novel endophyte strains with favourable alkaloid profiles were identified in studies of global genetic diversity using DNA-based markers combined with metabolic profiling. Fungal endophytes were isolated from their endogenous host and subjected to *in vitro* characterization for bioactivity and genome survey sequencing. The genetic basis for alkaloid production was determined by correlating observed metabolic profiles of candidate endophytes with presence and copy number of alkaloid biosynthesis genes. Pan-genome analysis has enhanced understanding of genomic variation amongst pasture grass endophytes and enabled molecular marker discovery and development. Phylogenomics confirmed the distinct taxonomic status of newly identified endophyte taxa. Transcriptomics identified endophyte-derived genes associated with novel metabolites, as well as genes associated with the host-endophyte association. A novel method for inoculation of endophytes into meristem culture-derived callus tissue of single genotypes from multiple perennial ryegrass cultivars was developed to allow isogenic comparisons in respect to both host and endophyte genotype. Beneficial toxin profiles were confirmed for associations formed with the grass genotypic panel, and semi quantitative metabolite analysis provided evidence for genotype-specific effects of both host and genotype on levels of alkaloid production. Vegetative stability was also assessed over both shorter- and longer-term time periods. A substantial number of candidate novel endophytes have been discovered that are suitable for deployment in pasture grass breeding programs.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M110

Monday 4th April
14:00 - 16:00

GAULIN Elodie *(1), CAMBORDE Laurent (1), PEL Michiel J.c. (1), RAMIREZ-GARCÈS Diana (1), DUMAS Bernard (1)*
(1) LRSV, CNRS, UPS, Castanet-Tolosan, France

Crn13 effector from the legume pathogen *Aphanomyces euteiches* triggers plant DNA damage response

Microbial pathogens translocate effectors inside host cells to subvert cellular functions and suppress immune responses. Oomycetes secrete two large groups of effectors: RXLR and CRN (Crinkler) proteins. RXLRs and CRNs are modular proteins with conserved N-termini and highly diverse C-terminal effector domains. We recently obtained the genome sequence of the legume root pathogen *Aphanomyces euteiches* (ATCC201684, AphanoDBv2.0; https://www.polebio.lrsv.ups-tlse.fr/aphanoDB/). This data revealed the absence of RXLR effectors and the presence of over 150 putative CRN effectors in the genome of this pathogen. We have been able to show that one of the CRN effectors, CRN13, localizes in the plant nucleus where it triggers cell death. Further, we found that the CRN13 homolog of the fungal amphibian pathogen *Batrachochytrium dendrobaditis* is able to cause a similar response in both plant and amphibian cells. Additionally, we demonstrated that both CRN13s are able to bind DNA and cause plant DNA damage *in vivo*. Altogether, this work reveals that CRN effectors produced by unrelated plant and animal pathogens bind DNA and trigger host DNA damage probably to interfere with host cell development.

Ramirez et al.,. New Phytol, in press.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M111

Monday 4th April
14:00 - 16:00

PRUSKY Dov *(1), BI Fangcheng (2), BARAD Shiri (1), MENT Dana (1), LURIA Neta (1), DUBEY Amit (1), CASADO Virginia (3), MÍNGUEZ Jose Diaz (3), ESPESO Eduardo A. (4), FLUHR Robert (5)*
(1) Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, the Volcani Center, Bet Dagan, Israel
(2) Institute of Fruit Tree Research, Guangdong Academy of Agricultural Sciences, Guangzhou, China
(3) Department of Microbiology and Genetics, CIALE, Universidad de Salamanca, Salamanca, Spain
(4) Department of Molecular and Cellular Biology, Centro de Investigaciones Biológicas, Madrid, Spain
(5) Department of Plant and Environmental Sciences, Weizmann Institute of Science, Rehovot, Israel

Carbon regulation of environmental pH by secreted small molecules that modulate pathogenicity in phytopathogenic fungi

Fruit pathogens can contribute to acidification or alkalization of the host environment. This capability has been used to divide fungal pathogens into acidifying and/or alkalizing classes. Here we show that diverse classes of fungal pathogens - *Colletotrichum gloeosporioides*, *Penicillium expansum*, *Aspergillus nidulans*, and *Fusarium oxysporum* - secrete small pH-affecting molecules. These molecules modify the environmental pH that dictates acidic or alkaline colonizing strategies and induce the expression of PACC-dependent genes. We show that in many organisms, acidification is induced under carbon excess, i.e. 175mM sucrose (the most abundant sugar in fruits). In contrast, alkalization occurs under conditions of carbon deprivation, i.e. less than 15mM sucrose. The carbon source is metabolized by glucose oxidase (gox2) to gluconic acid, contributing to medium acidification, whereas catalyzed deamination of non-preferred carbon sources, such as the amino acid glutamate, by glutamate dehydrogenase 2 (gdh2) results in the secretion of ammonia. Functional analyses of Δ*gdh2* mutants showed reduced alkalization and pathogenicity during growth under carbon deprivation, but not in high-carbon media or on fruit rich in sugar, whereas analysis of Δ*gox2* mutants showed reduced acidification and pathogenicity under conditions of excess carbon. The induction pattern of gdh2 was negatively correlated with the expression of the zinc finger global carbon catabolite repressor creA. The present results indicate that differential pH modulation by fungal pathogens in fruit is affected by host sugar content, which modulates environmental pH to enhance fruit colonization.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M112

Monday 4th April
14:00 - 16:00

DJAMEI Armin *(1), BINDICS Janos (1), UHSE Simon (1), STIRNBERG Alexandra (1), NAVARRETE Fernando (1)*
(1) Gregor Mendel Institute of Molecular Plant Research, Vienna, Austria

Dissecting the effectome of the maize pathogen *Ustilago maydis*

Biotrophic plant pathogenic fungi employ a battery of small secreted molecules, so called effectors, to suppress host defense responses and to redirect the host metabolism in favor of the invader. Although effector proteins are shaping the interaction between the pathogen and the host, their specific function stay often elusive as they largely show no sequence homology to proteins with known functional domains. The smut fungus *Ustilago maydis* causes galls on all aerial parts of the host plant maize. This basidiomycete became in the past decade a model to study biotrophic plant fungal interactions. In a systematic approach we functionally elucidate the effectome comprising several hundred putative small secreted proteins of *Ustilago maydis*, to learn more about specific effector functions and their plant target proteins. A new group of effectors that target plant growth hormone signaling in the host plant, their influence on virulence, host sided target and likely place of action will be presented.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M113

Monday 4th April
14:00 - 16:00

PETRE Benjamin *(1), WIN Joe (1), SCHATTAT Martin (1), ABD-EL-HALIEM Ahmed (2), SKLENAR Jan (1), BOZKURT Tolga (1), DAGDAS Yasin (1), SCHORNACK Sebastian (1), JONES Alex (1), VOSSEN Jack (2)*
(1) The Sainsbury Laboratory, Norwich, UK
(2) Wageningen UR Plant Breeding, Wageningen, The Netherlands

Members of the *Phytophthora infestans* PexRD12 effector family target host endomembrane compartments

Filamentous plant parasites engage in intimate contact with host cells through specialized infection structures called haustoria. In plant cells haustoriated by the Irish potato famine pathogen *Phytophthora infestans*, endomembrane trafficking pathways are perturbed. We hypothesize that *P. infestans* delivers effector proteins into host cells to manipulate endomembrane trafficking. Recent in planta screens of RXLR effectors revealed the PexRD12 effector family, whose members accumulate in endomembrane compartments and modify the number of FYVE-labeled vesicles in plant cells. We report that members of the PexRD12 family accumulate in distinct endomembrane compartments. During *P. infestans* infection, PexRD12 family members label the extra-haustorial membrane as well as vesicles that accumulate around haustoria. The identification of host proteins associated with PexRD12 family members is ongoing, and may help revealing the mechanism by which *P. infestans* manipulates host vesicular trafficking.

Additional authors:
11. ROBATZEK, Silke, The Sainsbury Laboratory
12. KAMOUN, Sophien, The Sainsbury Laboratory (correspondence)

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M114

Monday 4th April
14:00 - 16:00

SALVIOLI Alessandra *(1), BONFANTE Paola (1), GHIGNONE Stefano (2), LIPUMA Justine (3), DUPONT Laurence (3)*
(1) Department of Life Sciences and Systems Biology, Torino, Italy
(2) IPSP, Torino, Italy
(3) Institut Sophia Agrobiotech , INRA CNRS, Université de Nice, Sophia Antipolis, France

The genome of the obligate endobacterium of an AM fungus reveals an interphylum network of signal and nutrient exchanges

The Gram-negative bacterium *Candidatus Glomeribacter gigasporarum* is an obligate endobacterium living inside the cytoplasm of the arbuscular mycorrhizal fungus *Gigaspora margarita*. We sequenced its genome, leading to a 1.8-Mb final assembly. Phylogenetic analyses placed *Ca. G. gigasporarum* among Burkholderiaceae, whereas metabolic network analyses clustered it with insect endobacteria, revealing that it has undergone convergent evolution to adapt to an intracellular lifestyle. *Ca. G. gigasporarum* has an extreme dependence on its host for nutrients and energy, whereas the fungal host is itself an obligate biotroph that relies on a photosynthetic plant. It is thus surprising that, notwithstanding its reduced genome, the endobacterium possesses genes related to the production of secondary metabolites. It retains indeed the whole biosynthetic pathway for vitamin B12, as well as two large genes coding for nonribosomal peptide synthase and polyketide syntase. These features may contribute to the fungal host''s ecological fitness. *Ca. G. gigasporarum* proliferates inside a fungal phagosome/vacuole, suggesting that its communication with the fungal host should be mediated by some, still unknown, molecular determinants. A few sequences that are likely to code for putative secreted effectors were identified, and their potential role in regulating the bacterial-fungal interaction is being to be further investigated. Lastly, many toxin-antitoxin (TA) modules were found in the *Ca. G. gigasporarum* genome, and their expression was monitored over the fungal life cycles' stages. For one of them the toxic activity was demonstrated heterologously. These data open the question whether TA systems might play a role in the functioning of such fungal-bacterial symbiosis.

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CS2M115

Monday 4th April
14:00 - 16:00

BORAH Nilam *(1)*
(1) RWTH Aachen University, Microbial Genetics, Aachen, Germany

Determining host specificity of *Sporisorium reilianum*

Most smut fungi have a very narrow host range and can infect only one or two different plant species. The molecular reasons for this highly specific host adaptation are not well understood. The biotrophic smut fungus *Sporisorium reilianum* exists in two host-adapted varieties (SRS and SRZ) that cause disease either on sorghum (SRS) or on maize (SRZ). On each other's hosts, both fungi can colonize but do not cause smut disease. To understand the genetic mechanism of host selection, we generated a population of about 500 meiotic progeny (SRSZ) of a mating event between SRS and SRZ that contained different genome complements of their parental strains. In order to correlate phenotype with genotype, we first tested each individual strain for its virulence potential on sorghum. Of 500 tested strains, 36 (7.2%) showed full virulence on sorghum and were selected for genotype analysis. 170 (42.5%) were non-virulent on sorghum. To ensure that they are non-virulent because of the presence or absence of specific genes involved in host selection (and not because of the accidental destruction of genes involved in general processes like spore formation), we tested each sorghum non-virulent SRSZ strain for virulence on maize. Of the 170 strains, 122 were virulent on maize and were selected for genotype analysis. Selected strains will be sequenced and the parental origin of their genomes compared to correlate phenotype with genotype and identify genes associated with host adaptation.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M116

Monday 4th April
14:00 - 16:00

WIDINUGRAHENI Sri *(1), VAN DER DOES H.c. (1), SANCHEZ Jonathan N. (2), ORDONEZ Nadia R. (3), BASTIDAS Fernando G. (3), KEMA Gert (3), SUBANDIYAH Siti (4), KISTLER Corby (2), REP Martijn (1)*
(1) SILS-University of Amsterdam, Molecular Plant Pathology, Amsterdam, The Netherlands
(2) USDA-ARS Cereal Disease Laboratory Saint Paul, Minnesota, USA
(3) Plant Research Institute, WUR, Wageningen, The Netherlands
(4) KNAW-SPIN Project, Faculty of Agriculture, Gadjah Mada University, Jl. Flora Bulaksumur, Yogyakarta, Indonesia

In search of Panama disease effectors

Fusarium oxysporum f.sp cubense (Foc) is the causal agent of Panama disease in banana. Foc has evolved into several different races, namely Race 1, Race 2 and race 4, the latter being divided into Sub-tropical Race 4 (ST4) and Tropical Race 4 (TR4) based on the geographical region. In the *Fusarium oxysporum*, f.sp lycopersici (Fol)-tomato system, small-secreted proteins (Six, Secreted in xylem) can act as virulence factors, but may also trigger resistance in the host. In tomato, three resistance genes are known, I-1, I-2 and I-3, and the corresponding AVR genes SIX4(AVR1), SIX3 (AVR2), and SIX1 (AVR3) have been identified. In Foc, however little is known about the effectors necessary to infect banana. We identified 11 candidate effector genes in the genome sequence of a Foc TR4 strain (II5, sequenced and annotated by the Broad Institute) based on homology to SIX genes and other characteristics. Here we present result of our initial characterization of these candidate effector genes. We saw that three homologs of SIX1 are present only in the single TR4 clonal line, and only one putative effector, FocEf3 is present in all Foc races and clonal lines. Further investigation is being done through gene-knock out and complementation

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CS2M117

Monday 4th April
14:00 - 16:00

YADAV Usha *(1)*
(1) JAWAHARLAL NEHRU UNIVERSITY, New Delhi, India

Functional characterization of CaGpi12, N-acetylglucosaminylphosphatidylinositol de-N-acetylase from *C. albicans*

GPI anchored proteins are known to be very important for adhesion, virulence and pathogenesis of *C. albicans.* Gpi12/PIG-L catalyses the second step of GPI anchor biosynthesis. It de-N-acetylates N-acetylglucosaminylphosphatidylinositol (GlcNAc-PI) to generate glucosaminyl-phosphatidylinositol (GlcN-PI). To study the *C. albicans* ortholog, CaGPI12, we made a heterozygous *Cagpi12/CaGPI12* mutant by disrupting one allele of *CaGPI12* with *HIS1* and a conditional null mutant *Cagpi12/ PMET3-CaGPI12* by placing the other allele under a regulatable *MET3* promoter. *CaGPI12* mutants show reduced GlcNAc-PI de-N-acetylase activity besides growth defects, altered cell wall phenotypes and hypofilamentation. CaGpi12 shows optimal activity at 30 °C and pH 7.5. Detergents at concentrations close to their CMC values do not significantly affect the activity of the enzyme. *CaGPI12* partially complements *ScGPI12* function in a *S. cerevisiae GPI12* knock-down mutant. Deletion of either N-terminus or C-terminus hydrophobic regions of *CaGpi12* could not restore growth defects in *ScGPI12* mutant, suggesting that both regions are critical for the function of the enzyme in the cell. Our results put together suggests that *CaGPI12* is the only gene coding for GlcNAc-PI de-N-acetylase in *C. albicans* and is important for cell growth, normal morphology and GPI anchor biosynthesis.

POSTER SESSION ABSTRACTS
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CS2M118

Monday 4th April
14:00 - 16:00

VENICE Francesco *(1), GHIGNONE Stefano (2), SALVIOLI Alessandra (1), NOVERO Mara (1), BONFANTE Paola (1)*
(1) Department of Life sciences and System Biology, University of Turin, Turin, Italy
(2) IPSP, CNR, Turin, Italy

Symbiosis between an endobacterium and a mycorrhizal fungus has an impact on the trascriptional profile of the plant partner

Arbuscular mycorrhizal fungi (AMF) are crucial drivers of plant evolution: as obligate biotrophs, they have a key role in plant health. Some AMF possess endobacteria, whose genome sequencing revealed a reduced genome and a dependence on fungal host. We previously combined transcriptomics and cell biology to demonstrate that *Candidatus Glomeribacter gigasporarum* (CaGg) positively influences the physiology of its fungal host, *Gigaspora margarita*. We demonstrated how CaGg rises host's bioenergetic potential in terms of ATP production, enhancing its capability in detoxifying endogenous reactive oxygen species (Salvioli et al., 2016). Starting from these results we wondered whether such a positive impact of the bacterial presence on the fungal physiology may be transmitted to the host plant. With this aim, we performed an RNA-seq analysis of *Lotus japonicus* roots colonized by both the B+ (containing CaGg) and B- (without CaGg) *G. margarita* lines after 28 days. As expected, only a few genes (112) resulted to be differentially expressed: among them plant genes involved in hormones signaling, flavonoid biosynthesis and mineral exchange resulted to be sensitive to the presence or absence of the endobacterium inside the AMF. Interestingly, some genes involved in defense response to pathogens, such as PR10, were up-regulated in the B- colonized roots. The results suggest that the intracellular fungal microbiota may influence plant responses.

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CS2M119

Monday 4th April
14:00 - 16:00

MOUYNA Isabelle *(1), AIMANIANDA Vishukumar (1), HARTL Lukas (1), PREVOST Marie-Christine (1), LOCKER Jacomina Krijnse (2), LATGÉ Jean-Paul (1)*
(1) Unité des Aspergillus, Institut Pasteur, 25 rue du Docteur Roux, Paris, France
(2) Plateforme UltraPole, Institut Pasteur, 25 rue du Docteur Roux, Paris, France

GH16 and GH81 family beta-(1,3)-glucanases in *Aspergillus fumigatus* are essential for conidial cell wall morphogenesis

Softening of the cell wall is an essential phenomenon during fungal morphogenesis, wherein rigid cell wall structures are cleaved by glycosylhydrolases. The rigidity of the cell wall is due to fibrillar and branched beta-(1,3)-glucan linked to chitin. In fungi, beta-(1,3)-glucan constitutes a major cell wall component, accounting for ~40% of the cell wall dry mass. During the search for glycosylhydrolases acting on beta-(1,3)-glucan, we identified seven genes in the *A. fumigatus* genome, coding for potential endo-beta-(1,3)-glucanase; ENGL1 belongs to GH81 family while ENG2 to ENG7, to GH16 family. ENGL1 and four GH16 (ENG2-5) were expressed in the resting conidia as well as during germination, suggesting an essential role during *A. fumigatus* morphogenesis. Here we report the effect of sequential deletion of *ENG2-5* (GH16 family members) followed by the deletion of *ENGL1* (GH81 family member) in the *Af-eng2-5* mutant. The quadruple Afeng2-5 mutant showed conidial cell wall defects and there was increase in the defective phenotype in the quintuple *Af-eng2-5/engl1* mutant. Both quadruple *Af-eng2-5* and quintuple *Af-eng2-5/engl1* mutants showed fragile cell wall in the swollen conidia, a morphotype showing the initiation of germination process. Additionally, quintuple *Af-eng2-5/engl1* mutant showed conidiation defects, with linear chains of conidia unable to separate. These results show, for the first time in a filamentous fungus, that beta-(1,3)-glucanases are essential for proper cell wall assembly both during conidiation and morphogenesis

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CS2M120

Monday 4th April
14:00 - 16:00

TARANTO Adam *(1), MCDONALD Megan C. (1), SOLOMON Peter S. (1)*
(1) The Australian National University, Canberra, Australia

Cross-Cultivar Transcriptomics: a novel method for comparing pathogen-induced gene expression between non-model and polyploid host varieties

Following infection, gene expression of a plant host can reveal pathways and processes targeted by a successful fungal pathogen. Equally, plant genes deployed in a successful defence response may betray fungal weaknesses, and provide clues for breeding durable resistance. Accurate prediction of differentially expressed genes between cultivars relies on identification of equivalent transcripts. For many crop species reference genomes are not available and generation of near-isogenic lines is impractical; necessitating the use of de novo transcriptome assemblies. Traditional identity based clustering methods face a trade-off in polyploid species; strict clustering allows true divergent homologues between cultivars to group together, but risks over-clustering of biologically relevant homeologs, paralogues and splice variants. We employ a recently developed hierarchical, shared-read, clustering method to assign equivalent transcripts between independently assembled de novo transcriptomes. This method is superior to pooled-read assemblies, and allows preservation of splice-variants with distinct expression profiles, while minimising homeologue collapse. Two allohexaploid wheat (*Triticum aestivum*) cultivars, with differential susceptibility to the necrotrophic fungal pathogen *Parastagonospora nodorum* were infected, and their transcriptomes sequenced. This work highlights several known components of anti-fungal plant defence at play in susceptible and non-susceptible varieties, as well as suggesting a role for novel candidates.

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CS2M121

Monday 4th April
14:00 - 16:00

TANGUAY Philippe *(1), FOSTER Adam (1), SÉGUIN Armand (1)*
(1) Laurentian Forestry Centre, Canadian Forest Service, Natural Resources Canada, Québec, Canada

Tree-pathogen interactions: RNA-seq disentangled differential interactions in three related *Sphaerulina* spp. poplar pathosystems

While *Sphaerulina* spp. are common and benign foliar pathogens on multiple poplar trees, one species, *Sphaerulina musiva*, is of primary economic importance for the intensive cultivation of fast growing hybrid poplar clones where it can cause canker responsible of stem breakage and plantation failure. RNA-seq was used to gain a better understanding of the different responses of poplar species to infection caused by their naturally co-evolved *Sphaerulina* species. Transcript profiles of in vitro leaves of *Populus deltoides*, *P. balsamifera* and *P. tremuloides* respectively infected with *S. musiva*, *S. populicola* and a new undescribed species (Ston1) were studied in a time course experiment. Compared to *P. balsamifera* and *P. tremuloides*, the appearance of disease symptoms, the pathogen growth and the expression of poplar defensive genes were delayed in *P. deltoides*. Leaf abscission appears to be a mechanism of *P. balsamifera* to contain *S. populicola*, while defense in *P. deltoides* correlate with lignification of cell walls surrounding leaf infection spots. On the pathogen side, transcript abundance of the RNA samples only allowed the study of the *S. populicola* gene expression. Several fungal genes that appear to be exclusively expressed in planta were identified. Results from this study represent one of the most comprehensive analysis of gene expression ever conducted on a forest pathogen during leaf infection.

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CS2M122

Monday 4th April
14:00 - 16:00

POLONI Alana *(1), SCHIRAWSKI Jan (1)*
(1) RWTH Aachen University, Aachen, Germany

The smut fungus *Sporisorium reilianum* shows distinct host specificity mechanisms in maize and sorghum.

Sporisorium reilianum is a biotrophic plant pathogen that occurs in two formae speciales adapted to cause head smut on sorghum (*S. reilianum* f. sp. reilianum; SRS) or on maize (*S. reilianum* f. sp. zeae; SRZ). To understand the factors responsible for host specificity in this organism, we investigated the disease progression in both hosts when inoculated with SRS and SRZ. For that, we compared fungal growth by microscopy and fungal proliferation by gDNA quantification, as well as induced plant defense reactions by histology and plant transcriptome responses by RNA-seq. On sorghum, SRS penetrated seedling leaves and hyphae spread through vascular bundles to the apical meristems, inducing spore formation in the inflorescence. SRZ was able to penetrate and multiply in sorghum leaves, but could not reach meristems and did not sporulate. SRZ-infection was accompanied by strong defense reactions, including oxidative burst, callose formation and phytoalexin deposition. SRZ-infected plants showed induction of innate immune responses, such as flavonoid biosynthesis and generation of pathogenesis related proteins, whereas SRS specifically induced genes involved in plant cell multiplication, such as DNA replication and chromatin remodeling. On maize, both formae speciales were able to penetrate and spread to the apical meristems but only SRZ formed spores in inflorescences. Infection with SRS led to a low incidence of leaf-like structures at the place of floral organs. Defense responses were weakly activated and similar for both strains. Transcriptome analysis showed the induction of specific genes belonging to related processes, including oxidoreduction and iron binding. However, SRS remarkably induced a suite of pentatricopeptide proteins of unknown function. Taken together, our results indicate that the mechanisms of host selection by *S. reilianum* strains differ between maize and sorghum.

POSTER SESSION ABSTRACTS
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CS2M123

Monday 4th April
14:00 - 16:00

BEAUVAIS Anne *(1), VALSECCHI Isabel (1), BEAUSSART Audrey (2), AIMANIANDA Vishukumar (1), DUPRES Vincent (3), LAFFONT Frank (3), GUIJARRO Inaki (1), BARRY Jagadesh (4), DUFRÊNE Yves F. (2), LATGÉ Jean-Paul (1)*
(1) Institut Pasteur, Paris, France
(2) Université catholique de Louvain, Louvain-la-neuve, Belgium
(3) Institut Pasteur, Lille, FRANCE
(4) Inserm, Paris, France

Role of hydrophobins in *Aspergillus fumigatus*

Aspergillus fumigatus is the most ubiquitous airborne fungal pathogen. The airborne spores, called conidia, are inhaled by the human population and can cause diseases, from common allergies to fatal infections in immunocompromised individuals. Conidia resistance to desiccation and their capacity to reach the alveoli, are due to the presence of a highly hydrophobic layer that covers the conidial surface. This layer, which shows rodlet morphology, is exclusively composed of a protein from the hydrophobin family called RodA. Hydrophobins are low molecular weight proteins, characterized by their hydrophobicity profiles and an idiosyncratic pattern of eight conserved cysteine residues that form four disulfide linkages. Hydrophobins are secreted in a soluble form that self-associates into amphipathic layers at hydrophobic/hydrophilic or air/water interfaces. Based on their hydrophobicity pattern, the morphology of the monolayers they form and their solubility in detergents, hydrophobins are divided in two classes. Class I hydrophobins form functional amyloid fibers organized in layers with rodlet morphology while Class II hydrophobin layers show no defined morphology in general. Recently, an intermediate class of hydrophobins (Class III) has been described. In *A. fumigatus*, there are seven hydrophobins (RodA-RodG). The in silico analyses include RodA, B, C and possibly E in Class I. RodD, F and G belong to the intermediate Class (III). Among them, RodA is the best characterized. We demonstrated that the conidial surface rodlet layer formed by RodA, masks conidial recognition by the host immune system. Our interest was to understand what makes the *A. fumigatus* conidial surface rodlet layer immunologically inert: the RodA sequence, its structure or its aggregation into rodlets. Accordingly, we determined the 3D solution structure of RodA and the importance of conserved cysteine residues in the RodA sequence, and we identified the amino acids responsible for self-association into rodlets. We demonstrated that the hydrophobins RodB to RodG do not play a role in the conidia-mycelia strengthening of the cell wall, nor to the hydrophobicity of the conidia and aerial myceli, in spite that RodC is localized in conidia and RODB gene is the most expressed under biofilm conditions. The characteristics of a *A. fumigatus* mutant deleted in all hydrophobins is currently studied.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M124

Monday 4th April
14:00 - 16:00

PEDRO Helder *(1), KERSEY Paul (1), MAHESWARI Uma (1), STAINES Daniel (1), MCDOWALL Mark (1)*
(1) European Bioinformatics Institute, Cambridge, UK

PhytoPath, an integrative resource for phytopathogen genomics

Achieving sustainable increases in the yield of crop plants will depend on pesticide research and plant breeding. For this, an understanding of gene function is critical, as it determines the total genetic reservoir available to plant breeders. Integrating resources from Ensembl Genomes and PHI-base, PhytoPath (www.phytopathdb.org) organizes (for fungal, oomycete and bacterial pathogens) genome sequence data, genetic variation, and (DNA and peptide-centric) comparative analyses, and phenotypic data to facilitate research on plant pathogenesis. Molecular data is visualized using the Ensembl software suite to provide a highly functional genome browser and a number of alternative routes for programmatic data access; while linkage from genes to disease progression is provided using literature-curated data from the PHI-base resource. Currently PhytoPath houses more than 100 plant pathogen genomes, from which more than 1700 genes have been associated with disease phenotypes in PHI-base. A simple but powerful «query builder» style interface allows users to progressively select and combine gene-centric data associated with particular hosts, phenotypes, or molecular characterizations.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M125

Monday 4th April
14:00 - 16:00

VRABKA Josef *(1), MLYNARČÍKOVÁ Eva (1), HRADILOVÁ Michaela (1), PĚNČÍK Aleš (2), HINSCH Janine (3), TUDZYNSKI Paul (3), GALUSZKA Petr (1)*
(1) CRH Department of Molecular Biology, Palacký University Olomouc, Olomouc, Czech Republic
(2) CRH Department of Chemical Biology and Genetics, Palacký University Olomouc, Olomouc, Czech Republic
(3) Institute of Plant Biology and Biotechnology, Westfälische Wilhelms-University Münster, Münster, Germany

Auxin role and production in *Claviceps purpurea*

Biotrophic fungus *Claviceps purpurea* is known as a parasite of monocots, invading mainly rye florets (Secale cereale). The late infection is characteristic by the formation of dark purple sclerotia, which are the source of ergot alkaloids, substances used in pharmaceutical industry. During the infection, host plant does not show any symptoms of immune response and fungal hyphae grow unrecognized through the plant tissue. Plant defence responses are driven by phytohormone crosstalk and the fungus might utilize its own phytohormone biosynthesis for the host manipulation. Preliminary results showed that mycelium of Claviceps purpurea contains high amount of major auxin- indole-3-acetic acid (IAA). The aim of this work is to characterize an auxin biosynthesis in *Claviceps purpurea* and to determine a role of fungi-borne auxin in *Claviceps* infection strategy. Genome data mining and measurements of auxin biosynthesis intermediates identified two possible pathways of IAA biosynthesis in *Claviceps purpurea* based on plant and bacterial models. Putative genes participating on different auxin biosynthesis pathways were deleted by the homologous recombination and transformants have been characterized upon the infection and in axenic cultures. Detailed characteristic of several deletion mutants and WT showed interested features indicating that auxin is secreted from fungal tissue upon the infection.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M126

Monday 4th April
14:00 - 16:00

SBRANA Cristiana *(1), GIOVANNETTI Manuela (2)*
(1) Institute of Agricultural Biology and Biotechnology, UOS Pisa, CNR, Pisa, Italy
(2) Dept of Agriculture, Food and Environment, University of Pisa, Pisa, Italy

A database of arbuscular mycorrhizal fungal diversity obtained from field data for genetically modified plants impact assessment

The cultivation of genetically modified plants (GMPs) may affect natural and agricultural ecosystems and particularly the complex network of interactions among plants and soil microrganisms. Variations in structure, function and diversity of soil and rhizosphere microbial communities, which provide essential ecosystem services, may change soil fertility parameters and ultimately impact plant nutrition and productivity. Several experimental studies investigated the effects of GMPs on soil and rhizosphere microrganisms, including arbuscular mycorrhizal fungi (AMF), beneficial symbionts of most agricultural plants, and produced a large body of data on AMF species occurrence in different agroecosystems. Public access to biodiversity databases generated by the scientific community may synergize individual efforts and represent a reference for GMPs cultivation impact assessment studies. Here we collected available data on AMF diversity, based on molecular and morphological studies, as affected by GMPs cultivation in the field. The assembled database reports: different data sources, GMP events considered, scale of the study (in terms of space and time), sampling data, and a description of the methods used to build a relational database. Statistical treatments of data organised in the database may also help planning experimental design and data collection in studies targeting AMF diversity as one of the key soil indicators for a comprehensive assessment of GMPs impact.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M127

Monday 4th April
14:00 - 16:00

SCHMIDPETER Johannes *(1), DAHL Marlis (1), KOCH Christian (1)*
(1) Friedrich-Alexander Universitaet Erlangen-Nuernberg, Erlangen, Germany

Mob2 binds to Cbk1 and is required for pathogenicity in *Colletotrichum higginsianum*

The hemibiotrophic ascomycete fungus *Colletotrichum higginsianum* causes anthracnose disease on brassicaceae like *Arabidopsis thaliana*. Using random insertional mutagenesis by ATMT we generated a collection of 75 virulence mutants and characterized their genotypes and phenotypes. In particular, mutant vir-88 produces less and smaller conidia, less primary and secondary hyphae upon infection and often forms misshaped appressoria. Vir-88 was found to be a hypomorphic allele of an essential gene encoding for the potential ortholog of *S. cerevisiae MOB2*. All observed phenotypes of vir-88 could be complemented by reintroduction of a functional *ChMOB2* allele. ChMob2 was identified by co-immunoprecipitation and mass spectrometry as a binding partner of the potential kinase ChCbk1 and may activate it by direct binding. We analyzed potential targets of the Mob2-Cbk1 complex in *C. higginsianum* and will present their role in pathogenicity. Furthermore, the genome of *C. higginsianum* encodes for two more Mob proteins. While deletion of *ChMOB3* did not produce any phenotype, *ΔChmob1* mutants showed defects in growth, conidiation and pathogenicity.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M128

Monday 4th April
14:00 - 16:00

Spadaro Davide *(1), PRENCIPE Simona (1), BANANI Houda (1), GULLINO Maria Lodovica (2), GARIBALDI Angelo (2)*
(1) Dept. Agricultural, Forestry and Food Sciences (DISAFA), University of Torino, Grugliasco, Italy
(2) AGROINNOVA, University of Torino, Grugliasco, Italy

Evaluation of the suppressiveness of Italian soils and selection of potential biocontrol agents against *Fusarium oxysporum* f.sp. lycopersici on tomato

The soil-borne pathogen *Fusarium oxysporum* f.sp. lycopersici (FOL) is responsible for vascular wilts on tomato plants, causing severe economic losses. Biotic and abiotic components of soil could influence the disease development. The use of suppressive soils in the control of a large number of diseases is known, as well as their use for screening potential biocontrol agents (BCAs). The suppressiveness against FOL of two soils from northern Italy was compared with an artificial substrate based on peat and perlite, on susceptible seed of tomato (*Lycopersicon esculentum*) cv Cuore di Bue. Twenty-day-old seedlings were transplanted into soils inoculated with 3x104 chlamydospores per gram of soil. The disease index, ranging 0 to 100, was assigned 35 days after transplant and inoculation. Sterilized inoculated and not inoculated soils were used as control. One soil (Leca), previously cultivated with basil, significantly decreased the severity of the disease compared to the second one. When both soils were sterilized, the Leca one completely lost its suppressiveness against fusarium wilts. Forty fungal isolates were collected and tested, in in vitro and in vivo assays, as potential BCAs against FOL. Some strains of *Fusarium* spp. and *Trichoderma* spp. associated to the soil Leca partially explained the suppressiveness of the soil. Some BCAs were able to inhibit the growth and the conidia germination of the pathogen and to reduce the wilt symptoms on the tomato plants. The effect of some BCAs on the modulation of pathogenicity genes of FOL was also considered.

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Monday 4th April
14:00 - 16:00

KUNZ Caroline *(1), BARENSTRAUCH Margot (2), MANN Stephan (2), PRADO Soizic (2), NAY Bastien (2)*
(1) Sorbonne Universités, UPMC Paris 06, UFR de Biologie, Paris, France
(2) Sorbonne Universités, MNHN CNRS, Laboratoire Molécules de Communication et Adaptation des Microorganismes (MCAM), CNRS, MNHN, Paris, France

Paraconiothyrium variabile, a non-Clavicipitaceous endophyte, a novel model to study molecular interactions with the host plant and the microbiota

Fungal, non-symptomatic, endophytes are promising micro-organisms in terms of discovering new biologically active compounds that can be used in medicine as well as in crop-plant protection. The endophytes themselves, also, have potential uses in the control of plant pests. However, little is known about the interactions between the plant, the endophytes and the phytopathogens, especially in the interactions including foliar non-Clavicipitaceaous fungal endophytes. We have at our disposition a range of isolated fungal endophytes from the conifer tree *Cephalotaxus harringtonia* (Langenfeld et al. 2013, Fungal Biol. 117, 124-136) and in particular, a fairly dominant foliar species, *Paraconiothyrium variabile* belonging to the Pleopsporales (Dothidiomycetes). Initial research on this endophyte indicates antagonistic activity towards phytopathogens, inhibition of mycotoxin production in *F. oxysporum* (Combès et al. 2012, PLoS ONE 7, e47313) and plant metabolite biotransformation to the advantage of the endophyte (Tian et al. 2014, Phytochemistry 108, 95-101). First results also indicate that oxylipins are involved in the inter-species cross-talk between *P. variabile* and *F. oxysporum* (see Poster M . Bärenstrauch). It is more and more evident that in order to study plant fungal infection processes, one has to take into account the presence of third protagonists, the endophytic and epiphytic microbiota. How fungal endophytes communicate with invading plant pathogens, the microbiota and the host plant is the topic of our research project. We chose *P. variabile*, as a novel model to study these molecular interactions in particular the role of oxylipins in the chemical language. The genome of *P. variabile* was sequenced and we are currently establishing molecular tools (genetic transformation, gene expression, gene cloning) as well as metabolomic approaches (LC/MS). Another challenging approach is the study of the infection cycle of the endophyte *P. variabile* and we are currently infecting the host plant *C. harringtonia* with *P. variabile* alone but also with reconstructed *C. harringtonia* endophyte communities. In parallel infection studies have started on the model plant *Arabidopsis thaliana*. Colonisation of the plant is followed using classic isolation methods and microscopy approaches. In the long term, we envisage plant phenotyping of colonised and endophyte free plants using metatranscriptomics, metagenomics and metabolics.

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Monday 4th April
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ATANASOVA Lea *(1), DUBEY Mukesh (2), GRUJIĆ Marica (1), GUDMUNDSSON Mikael (3), DRUZHININA Irina S. (1), SANDGREN Mats (3), KUBICEK Christian P. (1), FUNCK JENSEN Dan (2), KARLSSON Magnus (2)*
(1) Research Area Biotechnology and Microbiology, Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria
(2) Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden
(3) Chemistry and Biotechnology, Swedish University of Agricultural Sciences, Uppsala, Sweden

Functional characterization of *Clonostachys rosea* pectate lyase PEL1 in *Trichoderma reesei*

Pectin is one of the major and most complex plant cell wall components present in the middle lamella in the non-woody parts of terrestrial plants. Comparative genomic studies revealed an expansion of the PL1 pectin/pectate lyase gene family in the genome of the mycoparasitic and plant-beneficial fungus *Clonostachys rosea* (Bionectriaceae, Hypocreales) compared to ecologically similar fungi. In order to understand the function of pectin degrading enzymes in *C. rosea*, we studied their expression and secretion during growth on apple pectin. To our surprise, out of 17 PL1 enzymes, only two were secreted under these conditions and confirmed by MS/MS analysis. One of them, encoded by the pel1 gene, was also found to be induced during hyphal contact with *Botrytis cinerea* in a RNA-seq transcriptome study. In order to study the function of PEL1 we expressed it in *Trichoderma reesei* (Hypocreaceae, Hypocreales) capable of mycoparasitism but with no PL1 genes present in its genome and thus not able to grow on pectin. *T. reesei* pel1OE mutants revealed the direct involvement of PEL1 in utilization of pectin, D-galacturonic acid and D-arabinose as a carbon source. Such *T. reesei* mutants also influenced tomato root development and showed higher in vitro antagonism against the plant pathogenic fungi *Fusarium oxysporum* and *Rhizoctonia solani* in comparison to the wild type strain. This study thus broadens our understanding of the biological role of pectinolytic enzymes in filamentous fungi.

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Monday 4th April
14:00 - 16:00

YOUNG Carolyn *(1), CHARLTON Nikki D. (1), MATTUPALLI Chakradhar (1), WOOD Bruce (2), BOCK Clive (2)*
(1) The Samuel Roberts Noble Foundation, Ardmore, Oklahoma, USA
(2) USDA-ARS-SEFTNRL, Byron, Georgia, USA

The mating type idiomorphs *of Fusicladium effusum*: identification, frequency and spatial distribution in the southeastern USA

Fusicladium effusum is the causal agent of pecan scab, the most prevalent disease of pecan (Carya illinoinensis) in the southeastern USA. Infection by the pathogen can result in serious and even catastrophic yield loss when conditions are favorable for an epidemic. Despite earlier efforts to determine a sexual stage, *F. effusum* is currently known only by its asexual (conidial) stage. The degree and distribution of genetic diversity observed within and among populations of *F. effusum* are typical of a sexually reproducing fungal pathogen, and comparable to other dothideomycetes that are known to have a sexual stage, including the closely related apple scab pathogen, *Venturia inaequalis*. The mating type mtAA (mat1-1) idiomorph was identified in a draft genome of *F. effusum* flanked by two conserved genes encoding a DNA lyase (apnB) and an uncharacterised PH domain-containing protein. The mating type locus, spanning across the flanking genes, was amplified and sequenced in 14 isolates, which represented isolates from different geographic locations and cultivars, revealing that only 50% of the samples contained the mtAA idiomorph and are considered mating type A (MTA). The remaining samples contained the mtBA (mat1-2) idiomorph and are considered mating type B (MTB). A multiplex PCR screen was developed to amplify a conserved housekeeping gene (tubB), mtAA and mtBA, and was used to screen 1203 *F. effusum* isolates collected from 13 pecan populations across the southeastern USA. A hierarchical sampling protocol representing regional, orchard, tree and leaflet was followed at all sites so the mating type structure at different spatial scales could be assessed. Analysis of this collection revealed the frequency of the mating type idiomorphs is in a 1:1 equilibrium. The apparent equilibrium of the mating type idiomorphs provides impetus to a renewed effort to search for a sexual stage of *F. effusum*.

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Monday 4th April
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GUTHRIDGE Kathryn *(1), EKANAYAKE Piyumi (1), HETTIARACHCHIGE Inoka (1,2), KAUR Jatinder (1), MANN Ross (1), GOMES-CARDOSO Patricia (1), SPANGENBERG German (1,2)*
(1) AgriBio, Centre for AgriBioscience, La Trobe University, Bundoora, Victoria, Australia
(2) School of Applied Systems Biology, La Trobe University, Bundoora, Victoria, Australia

Fungal endophytes for novel trait delivery: phytopathogen resistance and cross-species compatibility of endophytes from *Brachiaria*

Brachiaria is a pan-tropical grass genus comprising c. 100 species, of which several are economically important forage pasture crops. Although widely used for pasture-based agriculture in tropics, *Brachiaria* exhibits a number of shortcomings that constrain both its use and genetic improvement. Endophytes provide an excellent mechanism for novel trait delivery in *Brachiaria* improvement. In excess of 90 fungal endophyte isolates derived from 11 Brachiaria species have been identified in a global study of 281 accessions from 23 countries. Ribosomal DNA sequence based analysis identified seven distinct clades, with members showing genetic similarity to a variety of Ascomycota. Endophytes with anti-fungal properties may benefit host plants by preventing pathogenic organisms from colonisation and disease development. Isolates that exhibited broad spectrum bioactivity against phytopathogenic fungi were identified in an in vitro bioassay. Nine brachiaria endophytes representing 6 distinct taxa were selected for inoculation into a brachiaria host panel. Endophyte inoculation frequency was determined for each endophyte using strain specific DNA-based SSR markers. Cross-species compatibility was observed. Novel associations are the subject of evaluation for vegetative and intergenerational stability as well as fungal disease resistance.

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PESICOVA Kamila *(1), PAŽOUTOVÁ Sylvie (1), KOSTOVČÍK Martin (1), STODŮLKOVÁ Eva (1), ČMOKOVÁ Adéla (1), FLIEGER Miroslav (1), VESELSKÁ Tereza (1), KOLAŘÍK Miroslav (1)*
(1) Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic

Evolutionary history of the genus *Claviceps*

The Bayesian analysis based on sequences of the five loci (rDNA, Mcm7, EF-1A, tubulin and RPB2) together with the knowledge of the geographic distribution, of the host range and of the secondary metabolites spectrum was used for the reconstruction of evolution of the genus *Claviceps*. The consensual tree shows the relationships of 42 *Claviceps* species (including the newly described species from South Africa) and the most related genera *Aciculosporium*, *Balansia*, *Cepsiclava*, *Corallocytostroma*, *Epichloë*, *Myriogenospora*, *Neoclaviceps* and *Periglandula*. Besides the basal lineages such as *C. citrina* and *C. paspali*, two main clades are in the *Claviceps* genus. The first one is *C. purpurea* group and the second clade contains *C. maximensis* group, *C. pusilla* group, *C. africana* group, *C. fusiformis* group and *C. digitariae*. The species in *C. purpurea* group have many unique features which facilitate the cosmopolitan distribution, e.g. ergotoxine production and certainly widest host spectrum encompassing almost all *Poaceae* subfamilies (Panicoideae*, Chloridoideae, Arundinoideae, Pooideae* and *Ehrhartoideae*) and *Cyperaceae* family. Whereas, *Panicoideae* grasses alone with rare exceptions are the hosts of all other groups.

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Monday 4th April
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NÉMETH B. Julianna *(1), NÉMETH Z. Márk (2), HEGEDÜS A. Panna (1), TAMÁS László (3), KNAPP G. Dániel (1), KOVÁCS M. Gábor (1)*
(1) Eötvös Loránd University, Institute of Biology, Department of Plant Anatomy, Budapest, Hungary
(2) Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Science, Budapest, Hungary
(3) Eötvös Loránd University, Institute of Biology, Department of Plant Physiology and Molecular Plant Biology, Budapest, Hungary

Intraspecific differences in expression of genes related to symbiotic and saprobic life style of a dark septate endophytic fungus

Dark septate endophytes (DSE) are worldwide distributed root colonizing fungi. Although DSE are common root-associated fungi in many ecosystems, their role in ecosystem functioning and their functional diversity are poorly understood. Our aim was to study the intraspecific functional diversity of a widely distributed DSE fungus *Periconia macrospinosa* (Pleosporales), a general root colonizer in (semi)arid grasslands. We aimed to screen the expression levels of genes related to symbiotic and saprobic life styles. We studied nine isolates of *P. macrospinosa* originating from a semiarid sandy grassland. Free hyphae were grown on the same medium used in symbiosis experiments with barley. After two weeks the inoculated roots were harvested, total RNA was extracted, just like from free hyphae, and the transcript levels were screened by qPCR. Symbiosis related target genes (e.g. sugar transporters) were selected from the complete genome of *P. macrospinosa* DSE-2036 based on similarity search of the symbiosis upregulated genes of *Tuber melanosporum*. Putative proteins related to saprobic life style of the DSE fungus were selected from literature data. Significant differences in gene expressions of the different *P. macrospinosa* isolates were detected. Some isolates showed strikingly upregulated symbiosis related genes with barley, and similar differences were detected in expression of genes related to saprobic life style. The results presented show obvious intraspecific functional diversity of this DSE and this phenomenon may have a great importance in their role in ecosystem functioning.

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Monday 4th April
14:00 - 16:00

RAHIMI Mohammad Javad *(1), DRUZHININA Irina (1)*
(1) Research Area Biotechnology and Microbiology, Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria

Impact of natural associated bacteria on mycoparasitism of *Trichoderma* and it's relevance for plant protection

Species of *Trichoderma* (*Hypocreales*, *Ascomycota*) are the best known biofungicides used for biological control of plant pathogenic fungi. The most notable role of *Trichoderma* in community is its ability to parasitize on fungi (mycoparasitism). This property is universal for the genus that, in turn, evolved from an entomopathogenic ancestor. The resulting heritage provides *Trichoderma* with a genetic basis for an outstanding environmental opportunism such as ability to saprotrophy, mutualistic interactions with plants and parasitism on animals. We discovered that species of *Trichoderma* frequently co-occurs with a nitrogen-fixing bacteria from such genera as *Burkholderia* and *Achromobacter* (Burkholderiales). The later funding is in line with emerging evidences of intensive fungal bacteria interactions (FBI) in nature. We investigated the cross-talk between plant pathogenic fungi and strains of *T. harzianum* and *T. reesei* in presence of *A. xylosoxidans* and *B. tropica*, respectively. Results show a multifactorial and highly dynamic interaction network regulated by nutrients, biotic and abiotic factors. The generalization allows assigning A. xylosoxidans as antagonist of *Trichoderma* while *B. tropica* inhibited growth of plant pathogenic fungi and favored *Trichoderma* development, in particular in N-limiting conditions. However exceptions were noted. The role of fungal proteins modulating properties of hyphal surfaces (hydrophobins and cerato-platanins) in FBI will be demonstrated.

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Monday 4th April
14:00 - 16:00

SEO Jeong-Ah *(1), CARROLL Emily (1), HEO Neung-Kang (1), KIM Seong-Mi (2), KIM Young-Suk (2)*
(1) School of Systems Biomedical Science, Soongsil University, Seoul, South-Korea
(2) Department of Food Science & Engineering, Ewha Womans University, Seoul, South-Korea

Analysis of fungal spoilage and volatile compound production on apples

Postharvest spoilage of apples is a major concern of apple producers, and swift screening and removal of spoiled apples is an important part of the packaging process. Filamentous fungi have been known as causative agents in the spoilage of fruits and vegetables. These fungi produce unique volatile organic compounds (VOCs) that could be used as indicators when sorting out spoiled fruits. In this study, two major varieties of apples produced in Korea, Fuji and Hongro, were collected at varying stages decay postharvest to set up the decay index. The apple"s rate of decay was recorded and the spoiling filamentous fungi were identified. Simultaneously, the volatile compounds produced at each stage of decay were analyzed and profiled. A 5-point decay index (index 1~5) was used to determine the spoilage scale of apple (0%~50). The Fuji apples reached an index value of 5 much faster than Hongro apple variety. Based on the spoilage symptoms of each apple sample, the spoiling filamentous fungi were isolated and identified by differences in their rDNA sequences as well as macroscopic and microscopic observations. *Alternaria alternata* and *Botryosphaeria dothidea* were prevalent on the Fuji apples while *Cladosporium* and *Alternaria* species were prevalent on the Hongro samples. The VOC of the spoiled Fuji apples were profiled at each decay point using GC-MS combined by solid-phase microextraction (SPME). During decay, four VOCs including ethyl acetate and ethanol increased gradually while five VOCs (butyl acetate, sweet and fruity flavor) decreased according the decay period. Over the course of decay, 40% of five compounds were drastically decreased and at the final point of decay butyl butylate was not detected. Based on the relationship between apple spoilage and VOC changes we will be able to determine marker VOCs of spoilage and develop a sensory device for detecting apple spoilage on-site.

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Monday 4th April
14:00 - 16:00

MARQUES FORTUNA Taiadjana *(1), HOOD Michael (2), SNIRC Alodie (1), RAQUIN Christian (1), BADOUIN Hélène (1), BÜKER Britta (3), BERGEROW Dominik (3), SHYKOFF Jacqui (1), GIRAUD Tatiana (1)*
(1) Université Paris-Sud, CNRS, Laboratoire d'Ecologie, Systematique et Evolution, Orsay, France
(2) College Amherst, Dept. Biology, Amherst, USA
(3) Ruhr-University Bochum, Dept. Geobotany, Bochum, Germany

Pathogen competition and virulence in multiple infections by the anther-smut fungus *Microbotryum*

Theory predicts that parasite virulence should increase when multiple strains regularly compete within hosts. Under conditions of multiple infections, parasites that rapidly exhaust limiting host resources, and thus decrease host fitness, produce more offspring than more prudent ones. However, virulence can be reduced in hosts with multiple infections if competitors inhibit each other. We study multiple infections in the phytopathogenic fungi *Microbotryum*, responsible for the anther-smut disease in *Caryophyllaceae* plants. Previous studies have shown that multiple infections of *M. lychnidis-dioicae* occur frequently in natural populations of *Silene latifolia*, and that competitive inhibition and exclusion occur between unrelated strains within the infected plant. Here, we assessed the frequency of multiple infections of *M. saponariae* infecting *Saponaria* officinalis plants in natural populations, and we studied the competitive mechanisms of genotype interaction within host. New microsatellite markers were developed to identify fungal genotypes. Controlled inoculations were carried out in the greenhouse to investigate how fungal genotypes interact within plants and the effects of multiple infections and pathogen relatedness on infection success and virulence. *M. saponariae* hyphae can grow in vitro, hence we measured hyphae behavior, length and density using the inverted microscope and scanning electron microscope (SEM) to elucidate the proximal mechanisms of fungal interactions.

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Monday 4th April
14:00 - 16:00

SOUZA Elaine *(1), MOTA Suellen (1), GONÇALVES Paulo (1), BARCELOS Quélen (2), ANDRADE Mariana (1)*
(1) Universidade Federal de Lavras, Lavras, Brazil
(2) Universidade Federal de Mato Grosso, Sinop, Brazil

Understanding the behavior and relationship of *Colletotrichum* spp. strains from common bean

Colletotrichum and *Glomerella* (teleomorph) strains have been isolated from anthracnose and scab lesions on common bean in Brazil. The behavior of *Colletotrichum* and *Glomerella* strains has been evaluated by cytological, molecular, karyotype, pathogenicity, sexual and asexual recombination analyses. Comparison among *Colletotrichum* and *Glomerella* different strains have been carried out and revealed wide variability and the occurence of conidial anastomosis tubes (CATs). Molecular phylogeny analysis is currently being carried out. These information provide insight about evolution of these species, virulence and can assist common bean breeding program.

Acknowledgments: Fapemig, PACCSS/Capes-Fapemig for financial support.

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Monday 4th April
14:00 - 16:00

BROWN Daren *(1), KELLY Amy (1), PROCTOR Robert H. (1)*
(1) USDA-ARS, Peoria, USA

Aromatic polyketide synthases from 127 *Fusarium*: pas de deux for chemical diversity

Fusarium species collectively cause disease on almost all crop plants and produce numerous natural products (NPs), including mycotoxins, of great concern. Many *Fusarium* NPs are derived from polyketide synthases (PKSs), large enzymes that catalyze the condensation of simple carboxylic acids. To gain insight into the biosynthesis of aromatic polyketide-derived NPs in *Fusarium*, we retrieved 340 non-reducing PKS (NR-PKS) genes from 127 *Fusarium* genome sequences. Phylogenetic analysis resolved the 340 PKSs into 19 clades of which nine are new. Six clades, resolved in a larger, monophyletic clade, include PKSs required for multi-aromatic fused rings (pigments). The remaining 13 clades were polyphyletic but most are likely required for NPs with a single aromatic ring. The latter NR-PKSs were present in 54% of the *Fusarium* examined and in at least one member of each of the 20 *Fusarium* species complexes surveyed. The NR-PKSs in 7 clades are flanked by a reducing PKS (R-PKS). The R-PKSs in NR-/R-PKS sets characterized synthesize a C-8 to C-12 carbon chain that is used by the NR-PKS as a starter unit to synthesize a larger polyketide (e.g. zearalenone). Comparison of *Fusarium* PKSs to PKSs from other *Ascomycetes* provides genetic evidence that *Fusarium* has the potential to synthesize an NP previously reported in another fungus. The multiple, evolutionary origins of NR-/R-PKS pairs and their broad distribution attest to their important contribution to *Fusarium* chemical diversity.

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Monday 4th April
14:00 - 16:00

MEAD Oliver *(1)*
(1) Australian National University, Canberra, Australia

Gamma-aminobutyric acid: a regulator of asexual reproduction and pathogenicity in the wheat pathogen, *Parastagonospora nodorum*

Gamma-aminobutyric acid (GABA) is a non-proteinaceous amino acid produced in a chemical shunt from the Krebs cycle. This molecule and its associated pathway have long been known in animal and plant systems as a neurotransmitter and abiotic stress regulator respectively. Through the GABA shunt A-ketoglutarate is drawn from the Krebs cycle and returned as succinate, bypassing a key ATP producing reaction. Despite the expense at which GABA is produced, the enzymes required are ubiquitous across the fungal kingdom but its function is yet to be determined. The dothideomycete *Parastagonospora nodorum* is a wheat pathogen and the causal agent of the disease Septoria Nodorum Blotch. This disease is prevalent worldwide, causing significant economic losses to growers in Europe, the U.S.A, the Middle East and Australia. This project utilized the amenability of *P. nodorum* to genetic transformation and ‘Omics techniques to investigate the role of GABA in a fungal plant pathogen. Essential enzymes in the GABA pathway were disrupted, which dramatically reduced virulence and increased growth rate of *P. nodorum*. Conversely, exogenous addition of GABA to fungal growth media induced a state of hyper sporulation. To explore the influence of GABA on sporulation further, a combined transcriptomic and metabolomic approach was employed. Here we describe the changes of gene expression and metabolites in important sporulation pathways that are induced when GABA is added to fungal growth media.

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Monday 4th April
14:00 - 16:00

GOUT Lilian (3), CONFAIS Johann (1), MASSOT Marie (1), DUCASSE Aurélie (1), VALADE Romain (2).

(1) BIOGER, INRA, AgroParisTech, Thiverval-Grignon, France,

(2) ARVALIS, Pathologie Végétale, Thiverval-Grignon, France,

(3) BIOGER, AgroParisTech, INRA, Thiverval-Grignon, France

Population genetic structure and host specialization in the fungal plant pathogen *Zymoseptoria tritici*

Zymoseptoria tritici causes Septoria tritici blotch of wheat, one of the most important diseases of this crop worldwide. Yield losses can reach 50% in disease-conducive climates and epidemics occur regularly both on hexaploid bread wheat (*Triticum aestivum*) and tetraploid durum wheat (*Triticum turgidum ssp. durum*). Isolates of *Z. tritici* exhibit both cultivar specificity (ability to infect only some cultivars of either bread or durum wheat) and host species specificity (ability to only infect one or the other wheat species). In France, the major bread wheat growing areas are located in the northern regions of the country whereas durum wheat is mainly cropped in the southern regions. Besides these traditional growing regions, both species are also cultivated in the 'Centre' region and in the southwestern region of France. In this study, we exploited these contrasted agrosystems to investigate, at a population level, the extent of host specialization in *Z. tritici* and to test whether this specificity towards bread or durum wheat had a significant effect on the genetic structure of the fungal populations. A set of 700 isolates were sampled from naturally infected fields of bread and durum wheat in four main French growing regions and genotyped using 12 microsatellite markers. The level of host specialization of 72 isolates from these populations was also determined by cross inoculation experiments on a panel of bread and durum wheat varieties.

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Monday 4th April
14:00 - 16:00

HABIG Michael *(1), QUADE Jakob (1), STUKENBROCK Eva H. (1)*
(1) Christian-Albrechts University of Kiel, Environmental Genomics, & Max Planck Institute for Evolutionary Biology, Kiel, Plön, Germany

Functional studies on the accessory chromosomes of *Zymoseptoria tritici*

The accessory chromosomes of several fungal plant pathogens like *Nectria haemotococca* and *Fusarium oxysporum* (f. sp. lycopersici) play a role in host specificity and pathogenicity. The genome of the wheat pathogen *Zymoseptoria tritici* (synonym *Mycosephaerella graminicola*) includes several small accessory chromosomes, however the role of these is so far unknown. The accessory chromosomes of *Z. tritici* are characterized by a high degree of absence/presence polymorphism and structural variation. It has been hypothesized that high levels of plasticity may enhance rapid adaption to different hosts and environmental conditions. Nevertheless, the accessory chromosomes contain only very few genes that in general are silenced or only expressed at low levels during host infection. Functional studies are needed to obtain insight into the role of genes located on the accessory chromosomes. We deleted full chromosomes in the reference strain IPO323 to assess their importance for normal growth and pathogenicity. We generated viable strains in which each of the single accessory chromosomes were deleted. The deletion frequencies differed significantly among the individual accessory chromosomes with chromosome 15 being overrepresented and chromosome 19 underrepresented suggesting that some chromosomes are more stable than others. The phenotypic consequences of single accessory chromosome deletions were characterized in vitro and in planta. Overall, our new strain collection provides a valuable resource for the in-depth assessment of the functional role of accessory chromosomes in this important wheat pathogen.

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CS2M143

Monday 4th April
14:00 - 16:00

RUUD Anja Karine *(1), FICKE Andrea (2), FRIESEN Tim (3), LILLEMO Morten (1)*
(1) Norwegian University of Life Sciences, ÅS, Norway
(2) Norwegian Institute for Bioeconomic Research, ÅS, Norway
(3) Department of Plant Pathology, North Dakota State University, FARGO, ND, USA

Necrotrophic effectors and sensitivity genes in the wheat-*Parastagonospora nodorum* pathosystem under field conditions

Leaf blotch diseases in wheat can cause significant yield losses and reduce grain quality. In Norway, *Parastagonospora nodorum* is the dominant causal agent. Research spanning the last decade suggests that very specific, inverse gene-for-gene actions are involved in the pathosystem. Many host-selective necrotrophic effectors (NEs) and corresponding sensitivity (Snn) genes in the host have already been identified. The effectors induce cell death in the host, which enables the pathogen to invade the dead tissues. The focus of our project is to identify and map NE/Snn-interactions in Norwegian pathogen population and wheat material, and to determine the importance of these interactions under field conditions. An genome wide association (GWAS) mapping panel of Norwegian commercial lines, breeding material and international cultivars (MASbasis) and several bi-parental spring wheat populations including SHA3/CBRD x Naxos and Soru#1 x Naxos have been screened in a mist irrigated field nursery over several years, and resistance data corrected for confounding traits (height and earliness). QTL have been detected on chromosomes 1B, 2AS, 3B, 5BS, 5BL, 7A and 7B in SHA3/CBRD x Naxos, and on 1A, 2B, 4B, 7A and 7B in Soru#1 x Naxos. Greenhouse trials have showed that some of these QTL also are significant at seedling stage and caused by NEs including Tox3, and possibly also host-nonspecific mechanisms. Results also indicate that QTL on 7A and 7B may be validated in Soru#1 x Naxos and that a QTL on 7B in the seedling stage may be caused by a novel NE. Screening of Norwegian *P. nodorum* isolates revealed a higher proportion of ToxA-producing isolates than for previously published data from Europe. The results from Tox-screening and association mapping of MASbasis will explain whether this deviation is caused by Tsn1-sensitivity in Norwegian commercial cultivars. Further work will include characterization of putative new NEs, fine mapping of the chromosomal regions of interest and development of diagnostic markers for practical breeding.

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CS2M144

Monday 4th April
14:00 - 16:00

FRIESEN Tim *(2), WYATT Nathan A. (1), BRUEGGEMAN Robert S. (2), FARIS Justin D. (3)*
(1) Biosciences Research Laboratory, USDA-ARS, FARGO, ND, USA
(2) Department of Plant Pathology, North Dakota State University, Fargo, ND, USA
(3) USDA-ARS, Northern Crop Science Lab, Cereal Crops Research Unit, Fargo, ND, USA

Unraveling the molecular mechanism of virulence in *Pyrenophora teres f. teres*

Net form net blotch is an economically important foliar disease of barley (Hordeum vulgare) caused by the necrotrophic fungal pathogen *Pyrenophora teres f. teres.* Yield losses have been reported in the range of 10-40% and complete yield loss has been observed under environmental conditions highly favorable to the pathogen. Little is known as to the molecular mechanisms involved in the host-pathogen interaction. Therefore, a mapping population was developed from a cross between two California *P. teres f. teres* isolates that showed a differential response on barley lines Rika and Kombar, with 15A being virulent on Kombar and 6A being virulent on Rika. Two virulence QTL conferred by 6A (VR1 and VR2) that contribute to virulence on Rika and an additional two virulence QTL conferred by 15A (VK1 and VK2) that contribute virulence on Kombar have been identified. To further investigate the genes underlying these loci, we have generated a reference-quality assembly based on the Canadian *P. teres f. teres* isolate 0-1, and resequencing efforts are underway to obtain near reference quality assemblies of parental isolates 6A and 15A along with RNA sequencing efforts to annotate the regions of interest. Previous studies have shown necrotrophic effectors to be small secreted proteins. Current annotation has predicted a total of 20 genes encoding small (<50kDa) secreted proteins (SSP) with proximity to the most closely associated markers; two, two, six, and one of the predicted SSP genes are at the VR1, VR2, VK1, and VK2 loci, respectively. Further validation and characterization of these genes will give valuable insight into the barley *P. teres f. teres* host-pathogen interaction.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M145

Monday 4th April
14:00 - 16:00

FRIESEN Tim *(1), CARLSEN Steve (1), NEUPANE Anjan (1), RICHARDS Jonathon (1), XU Steven (2), FARIS Justin D. (2), BRUEGGEMAN Robert S. (1)*
(1) Department of Plant Pathology, North Dakota State University, Fargo, ND, USA
(2) USDA-ARS, Northern Crop Science Laboratory, Cereal Crops Research Unit, Fargo, ND, USA

A *Pyrenophora teres f. maculata* mapping population reveals the complexity of virulence in spot form net blotch

Pyrenophora teres f. maculata is a major pathogen of barley worldwide, however, little is known about the virulence underlying this disease. Therefore, a mapping population was developed using a cross between *P. teres f. maculata* isolates FGOB10Ptm-1 (North Dakota) and SG1 (Australia) to derive 105 progeny. The population was phenotyped on four barley lines (Skiff, 81-82/033, TR326, PI 392501) shown to have strong differential reactions to the parental isolates. The FGOB10Ptm-1 × SG1 population was subsequently genotyped using a restriction associated DNA-genotype by sequencing (RAD- GBS) approach developed for the Ion Torrent PGM. A SNP calling pipeline identified a total of 983 informative markers that were used to develop the first genetic map of *P. teres f. maculata*. The 983 markers were distributed across 16 linkage groups generating a map size of 1650 cM. Using phenotypic and genotypic data, quantitative trait loci (QTL) analysis identified eight genomic regions significantly associated with *P. teres f. maculata* virulence. QTL associated with individual barley lines ranged from two to four, with each line showing a different QTL pattern. Generation of a reference quality sequence of parental isolate FGOB10Ptm-1 allowed for candidate gene identification under the eight genomic regions identified in QTL analysis. Based on the current annotation, the number of predicted small secreted proteins in each QTL region ranged from five to fourteen. Among the genes encoding predicted small secreted proteins, 14 are within an 80 kb range of the QTL peaks, making them strong effector candidates. The number of genomic regions associated with virulence and the QTL pattern across the different barley lines indicates a high level of complexity in pathogen virulence.

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CS2M146

Monday 4th April
14:00 - 16:00

SEE Pao Theen *(1), MOFFAT Caroline (1), OLIVER Richard (1)*
(1) Centre for Crop and Disease Management, Department of Environment and Agriculture, School of Science, Curtin University, Perth WA6102, Perth, Australia

Evaluation of Australian spring wheat seedling resistance to ToxA knockout *Pyrenophora tritici-repentis* race 1

The foliar wheat disease tan spot, also known as yellow leaf spot is caused by the necrotrophic dothideomycete pathogen *Pyrenophora tritici-repentis*. Since the emergence of tan spot disease globally in 1970s and 1980, this disease has become one of the most concerned crop diseases in Australia causing up to 30% yield lost in susceptible varieties. *P. tritici-repentis* produces multiple host-selective toxins (HST) and one of the HST that is well-studied in this pathogen is ToxA, a proteinaceous effector. The sensitivity of ToxA in the host is conferred by a single gene Tsn1. The significance of ToxA-Tsn1 interaction in the development of tan spot disease is well-documented in the *P. tritici-repentis*-wheat pathosystem. Studies have shown that host sensitivity to ToxA has been found to be associated with disease susceptibility. Conversely, insensitivity to ToxA has been associated with tan spot resistance. In this study, commercial hexaploids spring wheat varieties were evaluated for their disease response to the race 1 *P. tritici-repentis* mutant strain lacking the ToxA gene. Observation of tan spot symptoms between wild-type and toxa mutant strains revealed complex pathogen-host interactions with the outcome of the disease responses that are dependent on the wheat genotypes.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M147

Monday 4th April
14:00 - 16:00

ELLWOOD Simon *(1), SCHWEIZER Patrick (2), GE Xintian (1)*
(1) Curtin UNiversity, Perth, AUSTRALIA
(2) IPK Gatersleben, Saxony-Anhalt, Germany

A *mlo-11* variant provides effective broad-spectrum resistance to barley powdery mildew without deleterious side effects

Recessive mutations in the Mlo gene confer broad spectrum resistance in barley (Hordeum vulgare) to powdery mildew (Blumeria graminis f. sp. hordei). All alleles discovered to date provide comprehensive resistance but also show deleterious pleiotropic effects. Recessive resistance was discovered in Eth295, an Ethiopian landrace, which was developmentally controlled and quantitative without spontaneous cell wall appositions or extensive necrosis and loss of photosynthetic tissue. This resistance is determined by only two copies of the naturally occurring *mlo-11* repeat units, compared to 11-12 in commonly grown cultivars and was designated *mlo-11* (cnv2). Copy number-dependent methylation governing relative Mlo transcript expression corresponded to the observed cytological and macroscopic phenotypic differences. The methylation is stable rather than de novo. *mlo-11* (cnv2) is the only example of a moderated mlo variant discovered to date and may be a result of natural selection against the deleterious effects of standard *mlo-11*.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M148

Monday 4th April
14:00 - 16:00

GKARMIRI Konstantia (1), *FINLAY Roger (1), ALSTROM Sadhna (1), THOMAS elisabteh (1), CUBETA Marc (1), HOGBERG Nils (1).*
(1) Swedish University of Agricultural Sciences, Uppsala, Sweden

Insight into the defence of the fungal pathogen *Rhizoctonia solani* against bacterial antagonists pave the way towards new biocontrol methods

Soil living pathogenic fungi are problematic for agriculture as they cannot be efficiently dealt with by chemical control methods. Great hope is put to the possibility to develop biological agents from bacteria naturally antagonistic to the fungi. The interaction between the pathogen *Rhizoctonia solani* and two of its bacterial enemies, *Serratia plymuthica* and *S. proteamaculans*, is hereby investigated. To develop as efficient biocontrol agents as possible, it is crucial to understand the interaction between fungi and bacteria. In this antagonism, the behavior of the bacteria has received most of the scientific attention. However in this study focus is put on the fungal response to two bacterial species, *Serratia proteamaculans* and *S. plymuthica* that been respectively shown to be a stronger and a weaker antagonist towards *R. solani*. The fungus full transcriptome, i.e. all its expressed genes, in response to exposure to either bacteria and when growing undisturbed has been studied. A large change in gene expression after exposure to the bacteria (almost 10% of the whole fungal transcriptome) compared to prior to exposure was observed. In addition, a small difference in the fungal responses when comparing the bacterial treatments, with a slightly larger battery of genes activated against the stronger antagonist, *S. proteamaculans* was found. Differential expression of genes associated with general stress responses was evident in both cases. More precisely, a) arrested growth of the fungus and changes in hyphal morphology, b) defence against bacterial stress through the production of antioxidants, xenobiotic degradation and environmental alterations and c) attack involving toxin production and oxidative stress were observed. The findings in this study will be beneficial for further research on biological control and in depth exploration of bacterial-fungal interactions in the rhizosphere.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M149

Monday 4th April
14:00 - 16:00

HAHN Matthias *(1), KNÜPPEL Nathalie (1), LEROCH Michaela (1)*
(1) University of Kaiserslautern, Dept. of Biology, Kaiserslautern, Germany

The Sho1 membrane sensor protein is important for organic acid secretion, host tissue acidification and pathogenesis of *Botrytis cinerea*

B. cinerea is a necrotrophic pathogen with a wide range of host plants. Germination, appressorium formation and host penetration is triggered by plant surface signals. For successful completion of these differentiation processes, the BMP1 MAP kinase cascade is required. We have recently found that the mucin like protein Msb2 is involved in hard surface recognition of *B. cinerea* germlings and MAP kinase activation prior to penetration. Another plasma membrane sensor protein, Sho1, is known to be involved in hyperosmotic stress adaptation in yeast and in host surface sensing and penetration in *Ustilago maydis* and *Magnaporthe oryzae*. *B. cinerea sho1* mutants revealed only minor defects in appressorium penetration and formed primary lesions with slight delay compared to the wild type. However, *sho1*-induced lesions rarely expanded, irrespective of the host tissue. Measurements of pH changes in the inoculation droplets and in liquid medium revealed that the wild type acidified the host tissue and the growth medium, whereas the *sho1* mutant did not. Whereas the wild type secreted citrate as major organic acid, the *sho1* mutant secreted mainly malate. Oxalate, a putative virulence factor in the closely related fungus *Sclerotinia sclerotiorum*, was not found in significant amounts in *B. cinerea*-infected tomato leaf tissues. RNAseq analyses with *Botrytis*-infected leaf tissue revealed dramatic downregulation in the *sho1* mutant, of genes encoding proteases, pectinases, toxin biosynthesis enzymes and other proteins with putative virulence functions. In contrast, the pH regulatory gene pacC, and genes of the velvet complex (vel1, vel2 and lae1) were upregulated in the *sho1* mutant. Taken together, Sho1 appears to be a major upstream component in the regulation of necrotrophic development of *Botrytis*.

POSTER SESSION ABSTRACTS
Session CS2 Pathogenesis and symbiosis
CS2M150

Monday 4th April
14:00 - 16:00

SIMBAQUEBA Jaime *(1), GONZALEZ Carolina (2), CATANZARITI Ann Maree (1), JONES David (1)*
(1) The Australian National University, Canberra, ACT, Australia
(2) Tibaitatá Research Center, Colombian Corporation for Agricultural Research (CORPOICA), Mosquera, Cundinamarca, Colombia

Analysis of *Fusarium oxysporum* SIX gene function in cape gooseberry and tomato

The *Fusarium oxysporum* species complex is a group of polyphyletic fungal lineages, many of which are the causal agent of vascular wilt disease in a broad range of plants, including economically important crops such as banana, cotton, melon, tomato and recently, cape gooseberry. *F. oxysporum f. sp. lycopersici* (Fol) causes wilt disease on tomato. Fourteen small Fol proteins secreted in the xylem (SIX) during infection have been identified. Five are associated with virulence and three are recognised by resistance proteins in the host. However the function of most of these SIX proteins remains unclear. In this study, homologues of five SIX genes (named SIX1a, SIX1b, SIX7, SIX10 and SIX12) have been identified in *F. oxysporum f. sp. physali* (Foph), which causes wilt disease on cape gooseberry. These genes were identified by mapping Foph RNAseq data against the Fol transcriptome and were found to encode proteins with 70 to 100% identity to their Fol counterparts, suggesting their function may be conserved in Foph. SIX1a and SIX1b have been tested in a ΔSIX1 strain of Fol to see if they can complement the virulence function of Fol SIX1 in tomato. Preliminary results show a partial restoration of virulence by three SIX1a transformants, and several SIX1b transformants are currently being analysed. To investigate the function of SIX7, SIX10 and SIX12, this cluster of genes will be knocked out in Fol and complemented with their Foph counterparts to assess their role in virulence.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T1

Tuesday 5th April
14:00 - 16:00

CHAILLOT Julien (1), MALLICK Jaideep (2), TEBBJI Faiza (1), COOK Michael A (3), TYERS Mike (2), ***SELLAM Adnane*** *(1)*
(1) Infectious Diseases Research Centre (CRI), CHU de Québec Research Center (CHUQ), Université Laval, Québec, Canada
(2) Institute for Research in Immunology and Cancer (IRIC), Université de Montréal, Montréal, Canada
(3) Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Canada

p38/HOG pathway, more than a stress-activated protein kinase (SAPK) signalling circuit

The basis for commitment to cell division in late G1 phase, called Start in yeast and the Restriction Point in metazoans, is a critical but still poorly understood aspect of eukaryotic cell proliferation. Most dividing cells accumulate mass and grow to a critical cell size before traversing through cell cycle. This size threshold couples cell growth to division and thereby establishes long-term size homeostasis. So far, mechanisms involved cell size homeostasis in fungal pathogens are not known. In this study, we performed a quantitative genome-wide survey of the size phenome in the pathogenic yeast *Candida albicans* to delineate the architecture and dynamics of regulatory circuits coordinating growth and division in this fungal pathogen. We provided evidence of a novel stress-independent function of the p38/HOG MAPK pathway in cell size and thus in coupling cell growth to cell division. HOG/p38 pathway is one of the best-studied MAPK signaling modules in eukaryotic organisms and it is a central signaling mediator to respond to different kind of stress in fungi and metazoans. This novel role of the HOG pathway relies on its basal phosphorylation activity (and not stress inducible activity) and emphasizes a new paradigm in MAPK signalling. Using genetic epistasis we found that the type 2C Ser/Thr phosphatases, Ptc1 and Ptc2 control Start onset by sustaining the basal phosphorylation activity of Hog1. We found that both the MAP2K Pbs2 and the MAPK Hog1 interact genetically and physically with the SBF (Swi4-Swi6) transcriptional complex controlling the G1 phase of the cell cycle. This finding demonstrate that HOG pathway is a critical regulator of Start and acts as inhibitor of the SBF complex and G1/S transition. Furthermore, we found that the HOG pathway controls the ribosome biogenesis machinery through the master transcriptional regulator, Sfp1 in a TOR (Target Of Rapamycin)-dependant manner. Together, this comprehensive mechanistic investigation demonstrates that the HOG signalling pathway is the linchpin in *C. albicans* size control, coordinating both growth potential and cell cycle commitment. Interestingly, while the role of the HOG pathway in coordinating growth and division was specific to *C. albicans* as compared to the model yeast *Saccharomyces cerevisiae*, recent work has shown that the deletion of Hog1 MAPK ortholog in metazoans, p38b resulted in a reduced cell and organism size in *D. melanogaster*. It is tempting to speculate that the role of basal activity of HOG/p38 pathway in controlling Start or the restriction point is evolutionary conserved. Ongoing investigations in our group are exploring the contribution of the four-p38 paralogs in size homeostasis in different human cell lines.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T2

Tuesday 5th April
14:00 - 16:00

JACOB Stefan *(1), BOHNERT Stefan (1), THINES Eckhard (2)*
(1) Institut für Biotechnologie und Wirkstoff-Forschung gGmbH, Kaiserslautern, Germany
(2) Johannes Gutenberg University Mainz, Institut für Mikrobiologie und Weinforschung, Mainz, Germany

Regulation dynamics in the HOG-signalling pathway in filamentous fungi

Perception and transduction of environmental signals is vital for microorganisms in order to adapt to changing external conditions during their life cycles. For this purpose, signaling pathways such as the high osmolarity glycerol (HOG) signaling pathway responsible for osmoregulation of fungi appear to be essential for rapid adaptation to changing environmental conditions. This signal cascade extensively studied in the model yeast *Saccharomyces cerevisiae* comprises a phosphorelay system linked to a MAPK cascade [4] . In its phosphorelay system the histidine kinase Sln1p was found to be the sole osmosensor and inactivation of the corresponding gene SLN1 is lethal in yeast [3]. In filamentous pathogenic fungi osmoregulation differs significantly from yeast, e.g. the phosphorelay system comprises more sensor components and elements specific for filamentous fungi. One example is the sensor hybrid histidine kinase MoHik1p in the rice blast fungus *Magnaporthe oryzae*. We intensively studied the HOG pathway in *M. oryzae* and found among others the signaling cascade sensing environmental signals such as salt and sugar stress by different sensor kinases MoSln1p and MoHik1p [1,2]. These signals were both identified to be transferred via the MAPK MoHog1p by means of phosphorylation resulting in an osmotic stress response. However, the pre- and posttranslational regulation of MoHog1p as part of the cellular response to osmotic stress has not been studied to large detail in the past. Therefore, we compared the *M. oryzae* wildtype strain to a mutant strain with inactivated HOG signaling cascade in a time course experiment in order to identify genes encoding proteins contributing to the transcriptional regulation by RNAseq (NGS) analysis. Analysis of the resulting data revealed a set of significantly regulated genes/factors with putative functions in osmoregulation which have not been linked to the HOG pathway before. Furthermore, we will display initial findings concerning the regulatory network and crosstalk of the HOG pathway in filamentous fungi.

[1] Jacob, S., Foster, A., Yemelin, A., und Thines E. (2015) Fungal Biology. 2015, 119 (7), 580 – 594.
[2] Jacob S., Foster A., Yemelin A., und Thines E. (2014). Microbiology Open 2014; 3(5), 668 – 687.
[3] Maeda, T., Wurgler-Murphy, S.M., und Saito, H. (1994). Nature 369, 242-245.
[4] Suescún-Bolívar L.P. and Thomé P.E. (2015). World journal of microbiology & biotechnology, 31(3), 435-43.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T3

Tuesday 5th April
14:00 - 16:00

PEREZ-LLANO Yordanis *(1), BATISTA-GARCÍA Ramón Alberto (1), SÁNCHEZ CARBENTE María Del Rayo (2), FOLCH-MALLOL Jorge Luis (2)*
(1) Instituto de Investigación en Ciencias Básicas y Aplicadas, Centro de Investigación en Dinámica Celular. Universidad Autónoma del Estado de Morelos (UAEM), Cuernavaca, Morelos, Mexico
(2) Centro de Investigación en Biotecnología (CEIB), UAEM, Cuernavaca, Morelos, Mexico

Identification and characterization of a hydrophobin gene in the moderate halophile *Aspergillus caesiellus*

Hydrophobins (HFB) are small secreted proteins produced by filamentous fungi. These proteins self-assemble at hydrophilic-hydrophobic interfaces forming amphipathic films that are involved in a broad spectrum of functions in fungal growth and development. For instance, HFB enable hyphae to breach the interface and to grow into the air and are also thought to alter cell wall permeability to water and solutes. Our lab has isolated and characterized the halophile ascomycete *Aspergillus caesiellus* with potentials for the discovery of salt resistant proteins of biotechnological interest. There are previous reports of an increase in the number of HFB genes from a halophile basidiomycete (*Wallemia ichthyophaga*) compared to other halophile fungi, with some of these genes being differentially expressed in different salt concentrations (Zajc et al., Environ. Microbiol. 2013, 80:247–256). As we aim to elucidate the physiological traits that allow *A caesiellus* halophilic behavior, we were interested in characterizing the differential expression of HFBs in media with different salt concentration. Because HFB are characterized by a specific pattern of cysteines and have little sequence similarity between homologs, we implemented a regular expression search strategy using *Aspergillus* genomic sequences from the MycoCosm database to identify all possible HFB genes. Using the identified conserved sequence regions within these, we designed CODEHOPs primers. HFB genes were amplified from the mRNA of *A. caesiellus* cultured in media with different NaCl concentration (no salt, 0.5M and 2.0M NaCl). We sequenced, cloned and expressed one gene of class I HFB that was observed to be differentially expressed in high salinity. Using the identified HFB linked to FITC, we showed (preliminarily by fluorescence microscopy) that this protein localize to the hyphae cell wall when added to the culture of *A. caesiellus*. The subsequent characterization of this HFB and its role in cell wall permeability could delineate a mechanism of halophilia in this fungal strain.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T4

Tuesday 5th April
14:00 - 16:00

VASKOVICOVA Katarina (1), AWADOVA Thuraya (1), VESELA Petra (1), BALAZOVA Maria (2), MALINSKY Jan (1), ***OPEKAROVA Miroslava*** *(1)*
(1) Institute of Experimental Medicine, Prague, Czech Republic
(2) Institute of Animal Biochemistry and Genetics, Ivanka pri Dunaji, Slavakia

A new type of regulation of mRNA decay: requirement of eisosome.

The yeast plasma membrane is compartmented into functional microdomains. Intense search for physiological significance of such compartmentation has been revealing its involvement in stress response, cell signaling and regulation. We showed previously that the main *S.cerevisiae* 5"-3" mRNA exo-ribonuclease Xrn1 associates with a specialized plasma membrane compartment, eisosomes, under conditions of glucose deprivation. In this study, we document that the Xrn1 activity in glucose-grown cultures is controlled by its actual localization within a cell which is changing during the cell development. Notably, we show that Xrn1 activity is down-regulated in postdiauxic cells, where the protein is sequestrated to eisosomes, and Pil1 is a prerequisite for this sequestration. In *Pil1*Δ cells, Xrn1 does not localize to the cell cortex and its activity is not down-regulated after diauxic shift. At eisosomes, Xrn1 retains its functionality since an addition of glucose to such cells leads to both release to cytoplasm and recovery of the enzyme activity. Most importantly, our data document that the regulation of Xrn1 activity can be achieved by a different mechanism than via the mRNA deadenylation and decapping, namely by a change in the enzyme localization. Thus our findings reveal a novel mechanism of post-translational regulation of Xrn1 activity.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T5

Tuesday 5th April
14:00 - 16:00

KUROKI Misa *(1), SHIGA Yuriko (1), OKAUCHI Kana (1), NARUKAWA Megumi (1), SAITOH Ken-Ichiro (2), TERAOKA Tohru (2), KAMAKURA Takashi (1)*
(1) Tokyo University of Science, Noda, Chiba, Japan
(2) Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan

How the chitin-deacetylase homologous protein, Cbp1, behaves during appressorium formation in *Pyricularia oryzae*?

Magnaporthe oryzae (*Pyricularia oryzae*) causes rice blast and inflicts enormous damage on rice crop. *P. oryzae* differentiates melanized appressorium to infect host cells. We focused on Chitin Binding Protein 1, Cbp1, that seems to express specifically in germling forming appressorium. cbp1 deletion mutants delay appressorium formation on hydrophobic cover glass, but the delay is restored by adding cutin monomer or inhibitor of cAMP degradation. These data suggest that Cbp1 has a role in induction of appressorium formation. On the other hand, Cbp1 looks like a chitin-deacetylase (CDA), because the central domain of Cbp1 is highly homologous to CDAs of other species. From this property, Cbp1 seems to be a cell-wall modifier which can make infecting fungi stealthy. We observed fluorescence intensity of chitosan in cell surface stained by eosin Y or OGA488 during appressorium formation. cbp1 deletion mutants have low level of chitin-deacetylation compared with wild type strains. We estimated CDA active center from known CDAs of other species and constructed substitution mutants of the active center. These mutants form appressorium at the same level of cbp1 deletion mutants. *P. oryzae* has 7 CDA homologous proteins, but Cbp1 seems to keep a distance from other 6 CDAs because Cbp1 have signal peptides and S/T cluster. We presumed that Cbp1 localizes cell surface and locally converts chitin into chitosan. Furthermore, expression level of other CDAs and amounts of chitosan in appressorium reduced by deletion of CBP1.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T6

Tuesday 5th April
14:00 - 16:00

SCALA Valeria *(1), BECCACCIOLI Marzia (1), LUDOVICI Matteo (1), FANELLI Corrado (1), REVERBERI Massimo (1)*
(1) Department of Environmental Biology, University of Rome "Sapienza", Roma, Italy

Linoleate diol synthase-oxylipins as intracellular and extracellular signal in *Fusarium verticillioides*

Fusarium verticillioides is a fungal pathogen of maize, producer of fumonisins, secondary metabolites harmful to humans and animals (classified as IARC2B). As shown in previous studies, the production of some mycotoxins (e.g. fumonisins) is related to fatty acids metabolism and oxylipins signaling. Different authors assessed that fungal lipids play a crucial role in regulating the fungal growth and the interaction with the host. In *F. verticillioides*, the linoleate diol synthase, LDS1, is involved in the oxylipin pathway and by a gene deletion approach the role of this enzyme was clearly shown to be involved in controlling fungal growth and reproduction, in switching on secondary metabolism and in controlling the interaction with the host. The molecular and metabolic characterization of the *lds1*-deletion mutant pinpointed that the deletion of this single gene has pleiotropic effects. It strongly influenced the polyunsaturated fatty acid (PUFA) amount in the fungal cell and affected the variation rate of the fungal genome.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T7

Tuesday 5th April
14:00 - 16:00

SHALABY Samer (1), ***HORWITZ Benjamin*** *(1)*
(1) Faculty of Biology, Technion, Israel Institute of Technology, Haifa, Israel

MAP kinase signaling pathways of the maize pathogen *Cochliobolus heterostrophus*

The genome of *Cochliobolus heterostrophus*, a necrotrophic Dothideomycete causing Southern corn leaf blight, encodes three mitogen-activated protein kinases (MAPKs). ChPmk1 (Chk1) is required for virulence and development: mutants lack conidia, appressoria and a cross between two mutants is infertile. ChSlt1 (Mps1), ortholog of cell integrity MAPKs, is required for full virulence and for conidiation. Mutants lacking ChHog1 show decreased virulence and are hypersensitive to osmotic and oxidative stresses (Lev et al. 1999, PNAS; Igbaria et al. 2008, MPMI). Although all three MAPK pathways are clearly involved in development and virulence, a direct relationship between the signals that the fungus actually responds to during interaction with the plant, and the phosphorylation state of the MAP kinases, has remained elusive. 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 structure-activity relationships of a series of phenolics showed an inverse correlation between two classes of compounds: (1) those that induce expression of enzymes for their metabolism by the beta-ketoadipate pathway, and (2) more toxic compounds that promote nuclear retention of the oxidant-responsive transcription factor ChAP1 (Shalaby et al. 2012, MPMI). Focusing on the group of compounds that cause a stress response, we found that ferulic acid (FA) caused damage to the membrane as shown by permeability to trypan blue, and hyphal shrinkage to nearly half the normal diameter. Mutants lacking ChHog1 and ChPmk1 are hypersensitive to FA, and exposure of the wild type to FA caused rapid dephosphorylation of both ChHog1 and ChPmk1. Mutants lacking ChSlt2 were not hypersensitive to phenolics, and exposure of wild type to FA increased ChSlt2 phosphorylation. These results show involvement of the three known MAPK pathways in the stress response to phenolics. FA, which provides a host-derived small-molecule signal, modulates the phosphorylation level of all three MAPKs.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T8

Tuesday 5th April
14:00 - 16:00

VIRGILIO Stela *(1), CUPERTINO Fernanda Barbosa (1), BERNARDES Natália Elisa (2), FREITAS Fernanda Zanolli (1), TAKEDA Agnes Alessandra Sekijima (2), FONTES Marcos Roberto De Mattos (2), BERTOLINI Maria Célia (1)*
(1) Departamento de Bioquímica e Tecnologia Química, Instituto de Química, UNESP, Araraquara, São Paulo, Brazil
(2) Departamento de Física e Biofísica, Instituto de Biociências, UNESP, Botucatu, São Paulo, Brazil

Molecular components of the *Neurospora crassa* pH-signaling pathway and their regulation by pH and by the PAC-3 transcription factor

Extracellular pH has an important role in cell biology as it regulates gene expression and influencing growth, differentiation, development and pathogenicity. In *Aspergillus nidulans*, the central regulator PacC is processed by the pH-dependent pal genes cascade followed by a second proteolytic processing pH-independent. In *Saccharomyces cerevisiae*, Rim101p, the PacC orthologue, undergoes only one proteolytic cleavage and is activated by the pH-dependent RIM genes cascade. The *Neurospora crassa* genome has all *A. nidulans* pal gene homologues. Recently, the DNA binding preference of the *N. crassa* PAC-3 transcription factor (5"-BGCCVAGV-3"), the PacC homologue, was identified and the sequence was found in the promoters of the pal and pac-3 genes. Here, we performed the characterization of the *N. crassa* pal and pac-3 genes and their participation in the pH cascade regulation by the activation of PAC-3. The pal mutant strains showed high melanin production and normal growth at pH 5.8. However, they were unable to grow at alkaline pH (7.8). The tyrosinase gene, which encodes the rate-limiting enzyme controlling melanin production, was over-expressed in the pal mutant strains and regulated by alkaline pH. The expression of the pac-3, pal-1, pal-2, pal-6, and pal-9 genes was regulated by pH 7.8 and by PAC-3. ChIP-PCR analysis demonstrated that PAC-3 bound to all pal and pac-3 promoters under alkaline pH, confirming their expression regulation by PAC-3. We also showed that PAC-3 undergoes only one pH-dependent proteolytic processing although PAC-3 could be processed in a pH-independent manner. Using ITC (isothermal titration calorimetry) assay, we observed that the putative PAC-3 NLS (Nuclear Localization Signal) has a strong affinity with the *N. crassa* Importin-A protein (NcImpA), which suggests that PAC-3 may require NcImpA to translocate to nucleus. Finally, using fluorescence microscopy, we demonstrated that PAC-3 shuttles between nucleus and cytoplasm being localized mainly in the nucleus under alkaline pH. The results indicated that the *N. crassa* pal genes play a role in the pH-signaling pathway leading to PAC-3 activation, which in turn regulates the pH cascade genes and melanin biosynthesis. Some aspects of this pathway are *N. crassa*-specific and the advances in the understanding of PAC-3/PacC/Rim-mediated functions may lead to potential drug targets for antifungal strategies. Financial support: FAPESP and CNPq.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T9

Tuesday 5th April
14:00 - 16:00

MIRALLES-DURÁN Alejandro (1), SÁNCHEZ-ROMERO Mª Antonia (1), ***CORROCHANO Luis*** *(1)*
(1) University of Seville, Seville, Spain

The photoreceptor WcoB of *Phycomyces blakesleeanus* accumulates in the cytoplasm and interacts with enzymes for the biosynthesis of beta-carotene

Phycomyces blakesleeanus is sensitive to 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 transcription factor complex composed of MadA and MadB. MadA and MadB are homologs of WC-1 and WC-2 from *Neurospora crassa*. The *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 for chromophore binding, but lacks the Zn finger domain. 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 grown 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 and is located in the cell membrane. In order to identify proteins that interact with WcoB we performed immunoprecipitation assays. Candidate proteins were excised from the electrophoresis gel and sequenced. Several proteins were immunoprecipitated with WcoB: HMG-CoA, CarRA, CarB and CarS. These proteins participate in the biosynthesis of beta-carotenes and the trisporic acid pheromones. We have expressed madA, madB and wcoB in A. nidulans to confirme these interactions using the Bimolecular Fluorescence complementation system (BiFC). Our results suggest that WcoB is a photoreceptor for the regulation of the enzymes that that participate in the biosynthesis of beta-carotene and pheromones.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T10

Tuesday 5th April
14:00 - 16:00

GIL-SÁNCHEZ María Del Mar (1), LUQUE Eva M. (1), ***CORROCHANO Luis*** *(1)*
(1) University of Seville, Seville, Spain

Localization and stability of the regulator VE-1 during conidiation in *Neurospora crassa*

The *N. crassa* ve-1 gene is a homolog of veA in *Aspergillus nidulans*. In *A. nidulans* mutations in veA results in constitutive conidiation that is independent of light, and the VeA protein 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 a deletion in ve-1 and in the wild type for a comparison. A ten-fold reduction in sensitivity was observed in the ve-1 mutant, an indication for a role of VE-1 in light sensing in *N. crassa*. VE-1 is a protein with a nuclear localization signal and a velvet factor domain that is highly conserved in fungi. We observed a minor increase in the accumulation of ve-1 mRNA after light exposure in vegetative mycelia (30 min), which did not lead to a major change 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 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 the cytoplasm. We detected VE-1 in vegetative mycelia in the dark but light promoted the accumulation of VE-1 in vegetative mycelia, aerial hyphae and conidia through the activity of the WC complex. We didn't observe any major changes in the accumulation of ve-1 mRNA during conidial development but exposure to light modify the stability of VE-1. The light-dependent degradation of VE-1 requires CSN-5 and FWD-1 and the activity of the WCC. We propose that the absence of VE-1 in aerial hyphae is a key step in the regulation of conidial development.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T11

Tuesday 5th April
14:00 - 16:00

SANTOYO Francisco (1), RAMIREZ Lucía (1)
(1) Genetics and Microbiology Research Group, Department of Agrarian Production, Public University of Navarre, Pamplona, Spain

Allelic imbalance in the Basidiomycete *Pleurotus ostreatus* genes is determined by the particular position of each gene in the genome

Allelic imbalance is a situation where the two alleles of a given gene are expressed at different levels in a given cell. There are a variety of reasons why the expression may vary between the alleles. Using the relative expression levels of two SNP alleles of a gene in the same sample is an effective approach for identifying the gene allelic imbalance. Basidiomycete fungi are an attractive model system for the study of basic questions in genetics and genomics. During most of their life cycle, sexually competent basidiomycetes grow as dikaryons instead of the diploid nuclear structure found in most other organisms. The dikaryotic configuration maintains the two parental nuclei independent in the cytoplasm providing, at the same time, haploid and diploid properties to the organism. This offers a unique possibility for the functional study of the two cell genomes as separate nuclei and forming a dikaryon. *Pleurotus ostreatus*, is an attractive model organism for genetics studies due to be, as a typical *Basidiomycota,* a multicellular organism with a clear division between the haploid and diploid life cycle condition. The genomes of the two nuclei present in the dikaryotic strain N001 have been sequenced and assembled independently making *P. ostreatus* the first organism for which the two haplotypes have been effectively sequenced in a given individual. We have used these genome sequences as template for the annotation of the whole transcriptome analysis data produced by monokaryons derived from each of the two N001 nuclei and for the N001 dikaryon itself. In order to better understanding how both nuclei behave within the dikaryotic cell we conducted a second RNA -Seq experiment with the main objective of study how the allelic imbalance behaved on three growth temperatures in three dikaryons with a big difference in the growth rate character. We observed gene clusters with a predominant allelic imbalance sense to each of the parental monokaryon nuclei. We also observed that in on one of the dikaryon the allelic imbalance variation with respect to N001, the sense of the average change always coincided with one of the nucleus allelic state on the region. We concluded therefore that on that dikaryon there is a gene nuclei dominance mechanism, whereas this does not happen on the other studied dykaryon.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T12

Tuesday 5th April
14:00 - 16:00

HELLER Jens *(1), ZHAO Jiuhai (1), ROSENFIELD Gabriel R. (1), KOWBEL David J. (1), GLASS N. Louise (1)*
(1) Plant and Microbial Biology Depertment, University of California at Berkeley, Berkeley, CA, USA

Characterization of greenbeard genes involved in long distance kind recognition in *Neurospora crassa*

Microorganisms are capable of communication and cooperation to perform social behaviors that are of benefit to the species. In filamentous fungi, cell fusion occurs through all stages of life, making soma a public good. In *Neurospora crassa*, genetically identical germinated asexual spores (germlings) communicate and fuse in a highly regulated process, which is associated with fitness benefits during colony establishment. Recognition and chemotropic interactions between isogenic germlings requires oscillation of signal transduction components consisting of a MAPKKK (NRC-1), a MAPKK (MEK-2), a MAPK (MAK-2) and a scaffold protein (HAM-5) to specialized fusion structures termed conidial anastomosis tubes (CATs). Using a population of 110 wild *N. crassa* isolates, we investigated germling fusion between genetically unrelated individuals and discovered that chemotropic interactions are regulated by kind discrimination. Distinct communication groups (CGs) were identified, where germlings within one CG communicated at high frequency, while germlings of different CGs avoided each other. Bulk segregant analysis (BSA) followed by whole genome re-sequencing identified three linked genes (doc-1, doc-2, and doc-3) associated with CG phenotype. Alleles at doc-1, doc-2, and doc-3 fell into five haplotypes that showed trans-species polymorphism and were necessary and sufficient to confer CG. During chemotropic interactions, DOC-1 oscillated with MAK-2 to CATs, while DOC-2 was statically localized to the plasma membrane; DOC-1 and DOC-2 were shown to interact with each other and with components of the MAK-2 pathway. Our data indicate that doc-1, doc-2, and doc-3 function as «greenbeard» genes, involved in mediating long distance kind recognition that involves actively searching for one"s own type, resulting in cooperation between non-genealogical relatives. Our findings serve as a basis for investigations into the mechanisms associated with attraction, fusion and kind recognition in other eukaryotic species.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T13

Tuesday 5th April
14:00 - 16:00

N'GUYEN Guillaume (1), MARCHI Muriel (1), MOCHES Chloe (1), BATAILLÉ-SIMONEAU Nelly (1), GUILLEMETTE Thomas (1), ***SIMONEAU Philippe*** *(1)*
(1) IRHS, Université d'Angers, INRA, Agrocampus-Ouest, Angers, France

Responses of a seed-borne fungus to hydric stress

Seed-borne necrotrophic fungi are exposed to a stressful environment when colonizing the host plant reproductive organs. Indeed, they have to overcome both chemical (plant defence metabolites) and physical (low water availability) barriers to actively reach the seeds. Using *Alternaria brassicicola* as fungal model, we previously showed that components of the cell wall integrity (Joubert et al., 2011, Cell. Microbiol. 13:62) and unfolded protein response (Joubert et al., 2011, Mol. Microbiol. 79:1305) pathways play a key role in the fungal protection against host plant antimicrobial metabolites. We also showed that fungal mutants defective for the class III Histidine kinase (HK) AbNik1 were strongly impaired in their ability to colonize seeds suggesting that a functional high osmolarity pathway is required for efficient infection of maturing fruits and seeds (Pochon et al., 2012; Plant Methods 8:16). To gain further insights into the response of the fungus to hydric stress, we analysed changes in the transcriptomic profiles of germinated spores from the wild-type and the AbNik1-deficient strains in response to exposure to either high sorbitol concentrations (osmotic stress) or low relative humidity (desiccation stress). Both common and specific responses were observed when comparing the two stresses. For instance, genes involved in protein biosynthesis and maturation were, as expected, down-regulated during osmotic stress while they were up-regulated during desiccation stress. Common expression patterns concerned genes involved in the synthesis of several small glycine-rich intrinsically disordered proteins belonging to the hydrophilin-like superfamily and genes encoding eisosomal proteins. Interestingly, we observed that the expression of the latter was AbNik1-dependent suggesting a close link between eisosome organization and the osmolarity pathway. We are currently phenotyping mutants deficient for either hydrophilins or eisosomal proteins to better understand their role in the response to hydric stress and possible involvement in fungal transmission to seeds.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T14

Tuesday 5th April
14:00 - 16:00

ZHANG Jianhua *(1), SCHOUSTRA Sijmen (1), VERWEIJ Paul (2), MELCHERS Willem (2), ZWAAN Bas (1)*
(1) Laboratory of Genetics, Wageningen University; Wageningen, The Netherlands,
(2) Department of Medical Microbiology, Radboud University Medical Centre, Nijmegen, The Netherlands

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

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

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T15

Tuesday 5th April
14:00 - 16:00

CERVANTES-CHÁVEZ José Antonio *(1)*
(1) Universidad Autónoma de Querétaro. Facultad de Ciencias Naturales, Querétaro, Mexico
(2) Tecnológico Superior de Ciudad Hidalgo, Michoacán, Mexico

The metacaspase Mca1 is involved in the response to stress in vitro and cell viability in the fungal plant pathogen *Ustilago maydis*

The metacaspases are arginine-lisine proteases, their function is involved to carry out the process of proggramed cell death or «apoptosis», mainly in response to several stress conditions or senescence. Related to fungal plant pathogens, after and at the time of plant penetration, the fungus is attacked by the plant defense system, being the most important the oxidative burst, also changes in the plant cell wall composition are observed such as callose deposition imposing an osmotic stress to the fungus. The role of this kind of metacaspases, have been studied in fungi like *Candida albicans*, *Saccharomyces cerevisiae*, and *Podospora anserina*. Mutants in this gene showed increased resistance to several conditions related to oxidative, acid or osmotic stresses. Particularly, it was reported that *C. albicans* deleted in the unique Mca1 gene were highly resistance to oxidative stress-induced dead. In most of the studied fungi only one Mca1 gene is identified by in silico analysis, but two functional genes were studied in *P. anserina* PaMca1 and PaMca2, which are involved in cell longevity. *Ustilago maydis* is a biotrophic basidiomycota fungus whose life cycle in nature involves the alternation of two stages: saprophytic haploid yeasts and dikaryotic parasitic hypha that infects maize plants producing the corn-smut disease. Keeping in mind the importance of the metacaspase protein regulating response to stress and cell death reported in some fungi, we pursued to study its role in *U. maydis*. Accordingly, we deleted the unique Mca1 gene presented in *U. maydis* genome using the DelsGate technology. The mca1 mutants are able to grow under different carbon sources tested to a similar rate as the wild-type. But under growth stress conditions, mca1 strains showed to be more resistance to oxidative, acid stresses, and also to cell wall stressor agents, as compared with the wild-type strain. The expression of MCA1 gene was induced under similar stress conditions. Even the increased resistance observed during the in vitro experiments, the virulence in mca1 mutants was not affected. Currently, we are conducting experiments to reveal the importance of Mca1 during the death of *U. maydis* strains under osmotic and oxidative stresses.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T16

Tuesday 5th April
14:00 - 16:00

PIETRICOLA Chiara *(1), IORI Angela (2), FANELLI Corrado (1), SCARPARI Marzia (1), REVERBERI Massimo (1), SCALA Valeria (1)*
(1) Department of Environmental Biology, University of Rome "Sapienza", Roma, Italy
(2) CREA, CRA, QCE Roma, Italy

Use of *Trametes versicolor* for eliciting defence reactions in wheat plants against foliar diseases

Durum and common wheat are amongst the most cultivated cereals worldwide. Their grains represent the base for human food and used in livestock industry. Up to 20-30% of worldwide cereals production is wasted because of foliar diseases due to pathogenic fungi such as *Zymoseptoria tritici* and *Parastagonospora nodorum*. Phytochemicals treatment in field can partially control these diseases but concomitantly provide environmental pollution and health hazards. Moreover, starting from 2014, EC has banned several pesticides (EC/129/2009) posing severe constraint to cereal farmers for using such products. If confirmed, the application of this directory will worst the current situation concerning the wheat production leading to concrete and severe losses in yield. The aim of our study is to exploit the eliciting aspect of *T. versicolor* and notably, Trametano®, an exo-polysaccharide produced by this mushroom, for priming the defences of wheat against causal agents of the septoriosis complex disease.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T17

Tuesday 5th April
14:00 - 16:00

JUNG Sascha *(1), SCHÄPE Paul (1), PAEGE Norman (1), NITSCHE Benjamin M. (1), MEYER Vera (1)*
(1) Institute of Biotechnology, Department Applied and Molecular Microbiology, Berlin University of Technology, Berlin, Germany

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

Although *Aspergillus niger* is used since decades in industrial biotechnology for the production of organic acids and proteins, it largely depicts a black box and we are far from understanding how most of the internal cellular processes work on the molecular level. However, the availability of its genome sequence and hundreds of microarray data for this fungus make it now feasible to shed light into this black box. Our interest in AnAFP is due to the fact that the growth-inhibitory effect of the protein and its homologs from other filamentous Ascomycetes seems to be restricted to fungi. No detrimental effects have been observed against bacterial, plant and mammalian systems, making this group of proteins interesting for application in red, green and yellow biotechnology. We have recently established a database that stores 377 high-throughput microarray data for *A. niger*. The database includes 158 different cultivation conditions related to carbon source and carbon availability, nitrogen metabolism, conditions related to stress, temporal and spatial stages during its asexual life cycle and many more. We have performed a transcriptome-meta analysis of this database, which enabled us to zoom into the gene expression networks and physiological processes under which AnAFP is expressed. The corresponding transcriptome meta-analysis of *A. niger* suggests a novel prominent biological role of AnAFP. Remarkably, anafp gene expression is apparently regulated in a non-defense manner. Instead, upon carbon starvation, anafp is strongly upregulated and its expression profile resembles that of genes involved in nutrient mobilization and with a predicted role for autophagy. In addition, anafp expression strongly increases when the mycelium becomes committed to asexual development. Compared to the wild type, its expression is more than two- to tenfold upregulated in both a ΔbrlA or ΔflbA background, respectively. As the flbA mutant depicts an autolytic phenotype, we propose AnAFP has a function during the asexual life cycle of A. niger and is somehow linked to autophagic processes during normal development. Our in-house transcriptomic database depicts a valuable tool which enabled us to zoom into the gene expression networks and physiological processes of *A. niger*. Further analysis of this database will definitely help to increase our knowledge of the complex regulation of *A. niger* gene network.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T18

Tuesday 5th April
14:00 - 16:00

ADAM Alexander (1), BRYCH Annika-Helen (2), TRINKS Nora (1), BATSCHAUER Alfred (2), ***TERPITZ Ulrich*** *(1)*
(1) Department of Biotechnology and Biophysics, Julius-Maximilian University, Biocenter Am Hubland, Würzburg, Germany
(2) Department of Plant Physiology and Photobiology, Philipps-University, Marburg, Germany

Rhodopsins in phytopathogenic basidiomycetes – lessons from the corn smut provoking fungus Ustilago maydis

Fungi are often exposed to intense light, especially when they are associated to plants, and thus, they are equipped with photoreceptors which regulate important physiological processes and allow the fungus to adapt to different light intensities. Rhodopsins are membrane proteins using the carotenoid retinal as chromophore. Though it is known, that rhodopsins perceive green light, their biological role in fungi is not well understood yet. Recently CarO, a proton-pumping rhodopsin from the filamentous ascomycete *Fusarium fujikuroi*, was shown to slow down conidia germination in the light. BLAST analysis of the genome of the basidiomycete *Ustilago maydis*, a phytopathogenic fungus provoking corn smut, revealed the occurrence of three different rhodopsins, UmOps1 (UM02629), UmOps2 (UM00371), and UmOps3 (UM04125). In the present work we combined phenotypic analysis of rhodopsin knock-out mutants with electrophysiological and fluorescence microscopic techniques to reveal the function (ion-pump or sensory protein) and biological role of these rhodopsins in *U. maydis*. In patch-clamp experiments we found UmOps1 and UmOps2 to behave as ion pumps, while no light-dependent ion pumping was observed with UmOps3. Rhodopsins are highly abundant in plant pathogens, therefore we also investigate a potential role of rhodopsins in the virulence of this fungus in plant-infection experiments.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T19

Tuesday 5th April
14:00 - 16:00

RUGER-HERREROS Macarena (1), AVALOS Javier (1), ***LIMON MIRON M Carmen (1)***
(1) Department of Genetics. University of Seville, Seville, Spain

The RING finger protein CarS of *Fusarium fujikuroi* is related to stress response

Carotenoids are terpenoids pigments with different functions in microorganisms, including protection against oxidative stress. In *Fusarium fujikuroi*, carotenoids are accumulated in large amounts in deep-pigmented carS mutants. CarS mediates the repression of a gene cluster that includes the first two structural genes of the carotenoid pathway. It was formerly known that transcription of the cluster is induced by light and nitrogen starvation, and it is derepressed in the carS mutants. Here we show that expression of the cluster, as well as that of gene carS, is also regulated by heat shock and exposure to hydrogen peroxide. The effect of carS mutation on *F. fujikuroi* transcriptome was studied by RNAseq and the results were validated by RT-PCR. Interestingly, the carS mutation affects also the expression of several genes related with oxidative stress response, but unexpectedly, the carS mutation results in higher sensitivity to hydrogen peroxide. The protein CarS contains two RING finger domains, typical of E3-type ubiquitin ligases, and a LON domain. A search of putative CarS targets achieved through the screening of a cDNA expression library with a yeast two-hybrid method found three positive candidates: a beta-glucosidase, a putative Zinc-finger transcription factor and a CWH (calcofluor white hypersensitive) related protein, the latter showing the strongest interacting signal. Possible connections of these proteins with oxidative stress response remain to be elucidated.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T20

Tuesday 5th April
14:00 - 16:00

FISCHER Juliane *(1), GACEK-MATTHEWS Agnieszka (2), MÜLLER Sebastian (3), PEZZINI Francesco (1), SCHERLACH Kirstin (1), SHELEST Ekaterina (1), HERTWECK Christian (1), STRAUSS Joseph (2), BRAKHAGE Axel A. (1)*
(1) Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany
(2) Universität für Bodenkultur, Universitäts- und Forschungszentrum Tulln, Tulln,Donau, Austria
(3) Department of Plant Sciences, University of Cambridge, Cambridge, UK

Genome-wide mapping of *Aspergillus nidulans* and *Streptomyces* interaction

Microbial communication has a pivotal role in nature as, among other things, it can lead to the activation of otherwise silent fungal secondary metabolite (SM) gene clusters (1). Hence, the exploration of such communities provides a very promising approach to discover novel natural products (2). We have been studying the interaction of the fungus *Aspergillus nidulans* and the bacterium *Streptomyces rapamycinicus* (3). Interestingly, co-cultivation of these two organisms led to the activation of the silent orsellinic acid (ors) gene cluster. Crucial for this interaction is the activity of the acetyl transferase GcnE (Gcn5 in *Saccharomyces cerevisiae*) of *A. nidulans*, which primarily acetylated lysine 9 and 14 of histone H3 during the co-cultivation (4). In order to demonstrate the relevance of these modifications for gene expression and production of SMs we exchanged several amino acids of histone H3 of *A. nidulans*. Major changes for the penicillin, sterigmatocystin and orsellinic acid biosynthesis in mutants mimicking non-acetylated H3 lysine 9 and 14 were detected (5). Therefore, we initiated genome-wide ChIPseq to analyse the distribution of these acetylations upon co-cultivation. Interestingly, numerous genes associated with the nitrogen metabolism of the fungus were found to be reduced in acetylation during interaction which correlated with a decreased expression of the respective genes.

(1) Brakhage (2013) Nat Rev Microbiol
(2) Netzker et al. (2015) Front Microbiol
(3) Schroeckh et al. (2009) PNAS
(4) Nützmann et al. (2011) PNAS
(5) Nützmann, Fischer et al. (2013) AEM

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T21

Tuesday 5th April
14:00 - 16:00

MISSLINGER Matthias *(1), MAIR Katharina (1), BECKMANN Nicola (1), WERNER Ernst R. (2), HAAS Hubertus (1)*
(1) Department of Molecular Biology, Medical University of Innsbruck, Innsbruck, Austria
(2) Division of Biological Chemistry, Medical University of Innsbruck, Innsbruck, Austria

The monothiol glutaredoxin GrxD is essential for growth and iron homeostasis in *Aspergillus fumigatus*

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

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T22

Tuesday 5th April
14:00 - 16:00

KNABE Nicole *(1), BREITENBACH Romy (1), DEMENTYEVA Polina (1), GORBUSHINA Anna A. (1)*
(1) Federal Institute for Materials Research and Testing, Department 4 (Materials & Environment), Berlin, Germany

Genetic manipulation of protective pigments in a rock-inhabiting model fungus *Knufia petricola* A95

Black ascomycetous microcolonial fungi (MCF) are persistent inhabitants of rock surfaces, but are especially conspicuous in hostile environments like cold and hot deserts. These ascomycetes are also ubiquitous in such wide-spread terrestrial ecosystems like sub-aerial material surfaces. It is in these niches where MCF have evolved mechanisms to cope with multiple stresses like high solar irradiation, temperature extremes, low water activity and spare nutrient availability. The unique robustness of MFCs is supported by protective pigments, like melanin and carotenoids. We study the role of these pigments in the stress resistance of the model rock fungus *Knufia petricola* (Chaetothyriales) strain A95. For this non-pathogenic fungus (that possesses all characteristic features of MCF, including meristematic growth, melanised cell-walls and extensive secondary metabolite production) several mutants have been recently produced. The melanin knockout mutants dSDH and dPKS were compared concerning physiology, stress resistance to H2O2 and UV radiation. TEM of the cell wall and analysis of the extracellular polysaccharides (EPS) were further used to elucidate mechanisms of cell wall maturation. Deletion of the polyketide synthase type I (dPKS) in K. petricola leads to a complete loss of melanin, showing the carotenoids which are normally hidden beneath the melanin. Colonies of scytalone dehydratase mutants (dSDH) appear darker than dPKS strains because of the incomplete disruption of the melanin synthesis pathway. In comparison to the wild type strain, treatment with the oxidative agent H2O2 (up to 30 mM) did not show any dose-dependent growth inhibition in any melanin mutant strain. This phenotype may be supported by the fact that melanin mutants demonstrate a significantly thickened cell wall (proven by TEM) and an increased development of EPS (demonstrated by biochemical analysis).

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T23

Tuesday 5th April
14:00 - 16:00

MEIJUEIRO OROZCO Martha Lucía *(1), PISABARRO DE LUCAS Antonio Gerardo (1)*
(1) Universidad Pública de Navarra, Pamplona, Spain

Identification of genes who expression is regulated by light and darkness in the basidiomycetes *Pleurotus ostreatus*

Pleurotus ostreatus is a basidomycete widely used in food industry and of great importance as well in the field of biotechnology (pharmaceutical industry, bioremediation and medicine)[1,2]. Previous studies have determined the relationship between light in fungal development and the activation of metabolic pathways [3]. Using a functional genomics approach (transcriptomics), we intend to evaluate the effect of visible light and darkness during fungal culture in gene expression in the basidiomycete *Pleurotus ostreatus*, and identify differentially expressed genes. First, we show the differential expression of wild type vs. blind (i.e. light insensitive) mutants of the sequenced monokaryotic strain PC9, and then we expand the analysis to other sibs to identify genes differentially expressed in different genetic backgrounds. The results obtained so far indicate that light inhibits the linear growth of the mycelium as reported also by other authors [4], and there are more than 100 genes with differential expression in light vs. darkness. When the blind mutant is analyzed, no changes in the growth rate are seen and the number of genes differentially expressed is reduced to near 10. Among the genes with differential expression, there is an abundance of genes coding for members of the cytP450 family. In this analysis, we have studied the response of sib monokaryons to distinguish the expression differences due to the different genetic backgrounds from that corresponding to a more general light response. We discuss the relevance of using sibs in the interpretation of global transcriptomic studies.

[1] Cohen R, Persky L, Hadar Y. (2002). Appl Microbiol Biotechnol. 2002 Apr;58(5):582- 94
[2] Eichlerová I1, Homolka L, Nerud F, Zadrazil F, Baldrian P, Gabriel J. Biodegradation. 2000;11(5):279-87.
[3] Schmoll M. Adv Appl Microbiol. 2011;76:27-78
[4] Nakano Y, Fuji H, Kojima M. Biosci Biotechnol Biochem. 2010 74 (10) 2160-5

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T24

Tuesday 5th April
14:00 - 16:00

COHRS Kim *(1), SCHUMACHER Julia (1)*
(1) Institute of Plant Biology and Biotechnology, WW University of Münster, Germany

The role of photoreceptors in the opposing effect of near-UV and blue light on the conidiation of *Botrytis cinerea*

Light is an important cue for fungi to sense their environment and adapt to their surroundings by triggering developmental processes and setting the circadian rhythm. *Botrytis cinerea* is a necrotrophic plant pathogen that affects a broad spectrum of more than 200 host species. When exposed to light, *B. cinerea* forms (macro-) conidia, while sclerotia are formed in the dark. By genome-wide expression analyses and targeted mutation approaches we were able to identify the light-responsive transcription factor (TF) BcLTF2 as major regulator of conidiation in *B. cinerea*. Deletion of bcltf2 causes the loss of conidiation, whereas overexpression is sufficient to induce the formation of conidia in darkness and suppress sclerotial development. Not only its presence, but also the quality of light plays a role in photomorphogenesis. *B. cinerea* reacts to near-UV (NUV), blue, red and far-red light. Exposure to blue light inhibits conidiation and leads to the development of sterile aerial hyphae, an effect that can be reverted by exposure to NUV light (Tan, 1975; Trans Br Mycol Soc 64:215-222). To sense light, *B. cinerea* possesses a total of eleven putative photoreceptor-encoding genes – the expression of nine of them is induced by light (Schumacher et al. 2014; PLoS Genet 10:e1004040). BcWCL1 is a blue light receptor and a GATA TF, which together with another GATA TF, BcWCL2, forms the White Collar complex (WCC). Deletion of bcwcl1 causes the uncoupling of conidiation from light, leading to an always conidia phenotype. In accordance with this observation, the WCC acts as a negative regulator of bcltf2 expression in both light and dark (Canessa et al. 2013; PLoS One 8:e84223). Moreover, the WCC positively regulates the expression of bcvvd1 in the light. BcVVD1, another blue light receptor, in turn negatively affects WCC activity. To gain insight into the role of the two putative NUV light-sensing cryptochromes/photolyases in the regulation of developmental programs in *B. cinerea*, we generated deletion and overexpression mutants for bccry1 and bccry2 and studied the differentiation phenotypes and the transcriptional light responses in the corresponding mutants.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T25

Tuesday 5th April
14:00 - 16:00

MEYER Michel *(1), BOURRAS Salim (2), PLISSONNEAU Clémence (1), GERVAIS Julie (1), BALESDENT Marie-Hélène (1), ROUXEL Thierry (1)*
(1) BIOGER, INRA, AgroParisTech, Thiverval-Grignon, France
(2) Institute of Plant Biology, University of Zürich, Switzerland

Biotic and abiotic factors influence the expression of effectors in *Leptosphaeria maculans* during axenic growth

Plant pathogens secrete effector proteins into host tissues to promote infection through the manipulation of host processes. Sequencing and analyses of the genomes of fungal phytopathogens have shown that they contains tens to hundreds of genes predicted to encode putative effectors. Moreover, global analyses of gene expression revealed that several waves of concerted expression of effector genes take place during host invasion. In sharp contrast with the situation described in-planta, the expression of the effectors is difficult to detect and quantify in axenic cultures because their genes are expressed at a very low level. In the present study, we investigate biotic and abiotic factors that may relieve suppression of expression of effectors during axenic growth. Biotic factors (such as carbon source, nitrate source, antibiotics) as well as abiotic factors (pH, temperature) can influence their expression. Of major interest, incubation of the fungal mycelium with 1ug/ml of an antibiotic of the aminoglycoside family allowed an increase of effector gene expression 20-fold to 60-fold compared to regular axenic growth. An RNAseq analysis aiming at identifying the set of effectors up- and down-regulated in a culture medium supplemented or not with the antibiotic has been performed and results obtained will be presented. This simple system could be a good starting point to characterize the plant signals that trigger fungal effector gene expression.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T26

Tuesday 5th April
14:00 - 16:00

KNABE Nicole (1), BREITENBACH Romy (1)(2), DEMENTYEVA Polina (1), ***GORBUSHINA Anna*** *(1)*
(1) Federal Institute for Materials Research and Testing, Department 4 (Materials & Environment), Berlin, Germany
(2) Freie Universität Berlin, Department of Biology, Chemistry and Pharmacy, Berlin, Germany
(3) Freie Universität Berlin, Department of Earth Science, Berlin, Germany

Genetic manipulation of the synthesis of protective pigments in the rock-inhabiting model fungus *Knufia petricola* A95

Black ascomycetous microcolonial fungi (MCF) are persistent inhabitants of rock surfaces, but are especially conspicuous in hostile environments like cold and hot deserts. MCF are ubiquitous and inhabit wide-spread terrestrial ecosystems including sub-aerial material surfaces. In these niches MCF have evolved mechanisms to cope with multiple stresses including high solar irradiation, temperature extremes, low water activity and spare nutrient availability. This robustness of MFCs is partly due to protective pigments, such as melanin and carotenoids. In Berlin we study the role of these pigments in the stress resistance of the model rock fungus *Knufia petricola* (Chaetothyriales) strain A95. A95 is non-pathogenic and possesses all characteristic features of MCF, including meristematic growth, melanised cell-walls and extensive secondary metabolite production. Disruption of genes («knockout») in melanin sythesis A95DSDH and A95DPKS were compared to the native strain in terms of physiology as well as stress resistance to H2O2 and UV radiation. Transmission electron microscopy (TEM) of the cells and analysis of the extracellular polysaccharides (EPS) were further used to elucidate mechanisms of cell wall maturation. Deletion of polyketide synthase type I (A95DPKS) in *K. petricola* leads to complete loss of melanin, revealing the carotenoids which are normally hidden by the melanin. Colonies of scytalone dehydratase mutants (A95DSDH) appear darker than A95DPKS strains because the melanin synthesis pathway is not completely disrupted. In comparison to the wild type strain, treatment with H2O2 (up to 30 mM) did not inhibit growth in a dose-dependent manner in either mutant strain. We suggest that this is because melanin-deficient mutants have significantly thickened cell wall as seen in TEM as well as increased extracellular polysaccharide synthesis.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T27

Tuesday 5th April
14:00 - 16:00

DE LA TORRE Antonio Luis *(1), PERÉZ-MARTÍN José (1)*
(1) Institute of Functional and Genomic Biology, CSIC−USAL, , Salamanca, Spain

Role of TOR pathway during the formation of the infective filament in the phytopathogen *Ustilago maydis*.

Ustilago maydis, the causal agent of corn smut disease, infects corn plants by using a specialized structure called the infective hypha. This structure consists of a single dikaryotic cell, which has its cell cycle arrested in G2. In spite of to be unable of proliferation on the plant surface, this hypha enlarges to produce a filament circa 100µm in length (while vegetative cells are about 17 µm in length). It is unknown how this dramatic cell growth is regulated. In eukaryotic organisms the TOR complexes (TORC1 and TORC2) regulate transcription and translation processes in order to promote cell growth. We are trying to address whether the TOR complexes are involved in some way in the formation of the infective filament as well as which role they might have. For that we have characterized the TORC1 and TORC2 complexes in *U. maydis*. Our results indicated that both complexes are essential for proliferation in *U. maydis*. By using chemical inhibitors as well as conditional mutants, we have found that TOR kinase activity is required for the proper formation of the infective hypha. Interestingly, unscheduled activation of TORC1 disables the ability of infective hyphae to properly enlarge. Our results suggest a way to connect the formation of the infective hypha with the metabolic status of the cell.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T28

Tuesday 5th April
14:00 - 16:00

TAYYROV Annageldi *(1), SCHMIEDER Stefanie S. (1), GORYACHKIN Aleksandr (1), STANLEY Claire E. (2), DEMELLO Andrew J. (2), AEBI Markus (1), KÜNZLER Markus (1)*
(1) Institute of Microbiology, Department of Biology, ETH Zürich, Zurich, Switzerland
(2) Institute for Chemical and Bioengineering, Department of Chemistry and Applied Biosciences, ETH Zürich, Zurich, Switzerland

Identification of novel effector proteins of multicellular fungi against nematodes by challenge of vegetative mycelia with fungivorous nematodes

Multicellular fungi evolved different strategies to defend themselves against antagonists. In addition to physical defense lines such as the buildup of melanized outer layers or chitinous cell walls, fungi possess a chemical defense mediated by effector molecules (toxins). Characterized classes of fungal defense effectors include secondary metabolites, peptides and proteins. The best known examples of these classes are penicillin, alpha-amanitin and fungal pore-forming toxins, respectively. Most likely, this list of fungal defense effectors is not complete and, thus, novel approaches for the identification of such molecules are needed in order to explore the fungal defensome. It has been shown before that genes coding for fungal defense effectors can be identified on the bases of differential gene expression upon challenge with antagonists. Hence, in this study we conduct a genome-wide analysis of transcription (RNA sequencing) of the model mushroom *Coprinopsis cinerea* challenged by the fungivorous nematode *Aphelenchus avenae* in a tailor-made microfluidics device. Based on the expression profile of genes coding for already known anti-nematode effector proteins of *C. cinerea*, we set up a list of putative novel classes of anti-nematode effector proteins of this fungus. These proteins were expressed in *Escherichia coli* and tested for toxicity towards the bacterivorous model nematode *Caenorhabditis elegans*. Preliminary results suggest that cytoplasmic lipases may constitute a novel type of fungal effector proteins against nematodes.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T29

Tuesday 5th April
14:00 - 16:00

OH Yeon Yee *(1), Jennifer Parker (2), David Muddiman (2), Ralph A. Dean (1)*
(1) Center for Intergrated Fungal Research, Department of Plant Pathology, North Carolina State University, Raleigh, USA
(2) W.M. Keck Fourier Transform Mass Spectrometry Laboratory, Department of Chemistry, North Carolina State University, Raleigh, USA

Comparative transcriptome and proteome analysis of Magnaporthe oryzae in response to nitrogen starvation.

Rice blast is the most important disease of rice worldwide, and is caused by the filamentous ascomycete fungus, Magnaporthe oryzae. During the infection process, the fungus experiences starvation conditions and efficient regulation of nutrient sources including nitrogen plays an important role in fungal pathogenicity. To learn about regulation of nitrogen metabolism in the rice blast pathogen, we performed a whole proteome analysis as well as genome-wide analysis of gene expression under nitrogen-limiting conditions. Using liquid chromatography-tandem mass spectrometry (LC-MS/MS), we analyzed expression of more than five thousand proteins and compared the data with gene expression. About 16 % of the identified proteins were upregulated and 18 % of the proteins were down regulated. A detailed analysis of differentially expressed proteins suggested major biological pathway changes during nitrogen starvation including protein translation, secondary metabolism and signal transduction. The results from this study provide a better understanding of fungal pathogenesis, provide the opportunities to suppress fungal disease and help improve crop production.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T30

Tuesday 5th April
14:00 - 16:00

WANG Zheng (1), LI Ning (1), LI Jigang (2), DUNLAP Jay (3), TRAIL Frances (4), ***TOWNSEND Jeffrey*** *(1)*
(1) Yale University, New Haven, CT, USA
(2) China Agricultural University, Beijing, China
(3) Geisel School of Medicine at Dartmouth, Hanover, NH, USA
(4) Michigan State University, East Lansing, MI, USA

The fast-evolving gene phy-2 modulates sexual development in response to light in *Neurospora crassa*

Rapid responses to changes in incident light are critical to the guidance of behavior and development in most species. Phytochrome light receptors in particular play key roles in bacterial physiology and plant development, but their functions and regulation are less well understood in fungi. Nevertheless, genome wide expression measurements provide key information that can guide experiments that reveal how genes respond to environmental signals and clarify their role in development. We performed functional genomic and phenotypic analyses of the two phytochromes in *Neurospora crassa*, a fungal model adapted to a post-fire environment that experiences dramatically variable light conditions. Expression of phy-1 and phy-2 was low in early sexual development, and in the case of phy-2, increased in late sexual development. Under light stimulation, strains with the phytochromes deleted exhibited increased expression of sexual development related genes. Moreover, under red light, the knockout strain of phy-2 commenced sexual development early. In the evolution of phytochromes within ascomycetes, at least two duplications have occurred, and the faster-evolving phy-2 has frequently been lost. Additionally, the three key cysteine sites that are critical for bacterial and plant phytochrome function are not conserved within fungal phy-2 homologs. Through the action of phytochromes, transitions between asexual and sexual reproduction are modulated by light level and light quality, presumably as an adaptation for fast asexual growth and initiation of sexual reproduction of *N. crassa* in exposed post-fire ecosystems.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T32

Tuesday 5th April
14:00 - 16:00

RODE Nicolas *(1)*
(1) AGAP, INRA, CIRAD, Montpellier, France

Genotype x genotype indirect genetic effects affect mycelium growth rate in the filamentous fungus *Aspergillus nidulans*

Indirect genetic effects (IGEs) occur when genes carried by an individual affect the phenotype of another individual. IGEs can be decomposed into additive IGEs when a neighbour genotype has the same effect on different genotypes and non-additive IGEs when a neighbour genotype has different effects on different genotypes. Relatively little in known regarding the genetic architecture of and the effect of relatedness on additive and non-additive IGEs. Using the filamentous fungus *Aspergillus nidulans*, we investigated the occurence of air-borne IGEs between genotypes growing on different plates within a stack. We quantified the relative importance of IGEs in natural populations using wild British isolate. We also investigated the effect of random mutations on IGEs using closely related lab strains and mutation accumulation (MA) lines. We focused on mycelium growth rate a fitness-related trait both in lab and field conditions. Using quantitative genetic models, we found strong non-additive IGEs between a focal genotype and its neighbour genotypes growing one petri dish above or below it, within the same stack. Overall, non-additive IGEs accounted for more than 10% of total phenotypic variation. Variation in IGEs was similar across MA lines and wild isolates, suggesting that IGEs are determined by many loci. Non-additive IGEs were not affected by the genetic distance between interactants. These results suggest that air-borne interactions have been underappreciated in fungi and that, when not accounted for, between-plate IGEs are likely to lead to biased results in fungal growth experiments.Indirect genetic effects (IGEs) occur when genes carried by an individual affect the phenotype of another individual. IGEs can be decomposed into additive IGEs when a neighbour genotype has the same effect on different genotypes and non-additive IGEs when a neighbour genotype has different effects on different genotypes. Relatively little in known regarding the genetic architecture of and the effect of relatedness on additive and non-additive IGEs. Using the filamentous fungus *Aspergillus nidulans*, we investigated the occurence of air-borne IGEs between genotypes growing on different plates within a stack. We quantified the relative importance of IGEs in natural populations using wild British isolate. We also investigated the effect of random mutations on IGEs using closely related lab strains and mutation accumulation (MA) lines. We focused on mycelium growth rate a fitness-related trait both in lab and field conditions. Using quantitative genetic models, we found strong non-additive IGEs between a focal genotype and its neighbour genotypes growing one petri dish above or below it, within the same stack. Overall, non-additive IGEs accounted for more than 10% of total phenotypic variation. Variation in IGEs was similar across MA lines and wild isolates, suggesting that IGEs are determined by many loci. Non-additive IGEs were not affected by the genetic distance between interactants. These results suggest that air-borne interactions have been underappreciated in fungi and that, when not accounted for, between-plate IGEs are likely to lead to biased results in fungal growth experiments.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T33

Tuesday 5th April
14:00 - 16:00

MARTORANA Domenica *(1), HAMPEL Martin (1), HEIMEL Kai (1)*
(1) Georg-August-University Goettingen, Goettingen, Germany

Addressing the consequences of UPR hyperactivation in the corn smut fungus *Ustilago maydis*

The unfolded protein response (UPR) is a conserved eukaryotic signaling pathway, which regulates endoplasmic reticulum (ER) homeostasis during ER stress. Erroneous UPR signaling is associated with developmental, metabolic and neurodegenerative disorders, such as cancer, diabetes and Alzheimer"s disease. UPR activation is triggered upon sensing of un- or misfolded proteins in the ER by the transmembrane kinase/RNase Ire1. Activated Ire1 mediates unconventional cytoplasmic splicing of the HAC1/cib1/XBP1 mRNA, leading to the production of the corresponding UPR key regulator (Hac1 – yeast, Cib1 – *Ustilago maydis*, XBP1 – higher eukaryotes) to activate UPR target gene expression. Although UPR activation ensures cell survival under ER stress conditions, extended, high-level UPR activation is known to trigger cell death in higher eukaryotes, whereas much less is known in fungi. We are studying the cellular and molecular consequences of UPR hyperactivation in the corn smut fungus *U. maydis*. Induced expression of constitutively spliced cib1s mRNA effectively suppresses *U. maydis* cell growth. Interestingly, toxicity of cib1s depends on the genetic background, indicating that mechanisms to counter the deleterious effects of a hyperactive UPR exist in *U. maydis*.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T34

Tuesday 5th April
14:00 - 16:00

CAMPOS ROCHA Marina *(1), FABRI João (1), GODOY Krissia (1), CASTRO Patricia (2), HORI Juliana (3), BOM Vinícius (2), CUNHA Anderson (1), FILL Taícia (4), GOLDMAN Gustavo (2), MALAVAZI Iran (1)*
(1) Federal University of São Carlos, Department of Genetics and Evolution. Sao Carlos, Brazil
(2) Department of Pharmaceutical Sciences, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil
(3) Department of Pharmacology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil
(4) Institute of Chemistry, State University of Campinas, Campinas, São Paulo , Brazil

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

Aspergillus fumigatus is an allergen of mammals and an important opportunistic pathogen that causes invasive pulmonary aspergillosis in immunosuppressed individuals. The ability to handle different stress conditions is essential to the survival and virulence of the pathogen inside the host. Therefore, environmental changes are sensed by invading microorganisms and transduced through signaling transduction pathways that lead to cell adaptation strategies. The Cell Wall Integrity Pathway (CWIP) is a signaling cascade primarily activated in fungal cells under conditions of synthesis and/or remodeling of the cell wall. In *S. cerevisiae*, CWIP is launched by the activation of the Protein Kinase C (PKC1), which interplays with a MAP kinase cascade (MAPK) leading to the phosphorylation of the associated RLM1 transcription factor. We have demonstrated that *A. fumigatus* rlmA homologue plays an important role in the maintenance of the cell wall and the *ΔrlmA* strain is avirulent in a mouse model infection of invasive pulmonary aspergillosis. Here, we observed that *rlmA* deletion led to increased TNF-A production by bone marrow derived macrophages and higher detection of beta-1,3 glucan in the fungal cell wall in comparison to the wild-type and complementing strain, suggesting that the *ΔrlmA* mutant had altered cell wall organization. In addition, we analyzed the genetic interactions of the *A. fumigatus* rlmA with the other components of the CWIP in *A. fumigatus*, i. e., PKC1 and MAPK1 during the cell wall stress. Our results indicate that there is an epistatic relationship between these genes indicating that the CWIP organization in *A. fumigatus* resembles that of *S. cerevisiae*. Additionally, we verified that rlmA are involved in the production of gliotoxin and fumiquinazoline C since the *ΔrlmA* mutant presented lower concentration of these secondary metabolites. Consistently, the expression of the genes in the fumiquinazoline cluster (fmqA-E) was down regulated in *ΔrlmA* mutant during the asexual development. Our results show that canonical CWIP is conserved in this pathogen and plays an important role in virulence, host recognition and production of secondary metabolites.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T35

Tuesday 5th April
14:00 - 16:00

TENORIA Maria (1), ***PEREZ-MARTIN Jose*** *(1)*
(1) Institute of Functional and Genomic Biology, CSIC, USAL, , Salamanca, Spain

An error-prone DNA polymerase protects mitochondrial DNA from oxidative stress during the induction of the infective hypha in *Ustilago maydis*

The phytopathogenic fungus *Ustilago maydis* produces a specific structure called the infective filament consisting of a dikaryotic hypha, which is required to penetrate the plant tissue. Although able to grow, this filament is cell cycle arrested on the plant surface. The formation of the infectious hypha in *U. maydis* depends on an intricate transcriptional program that primarily involves a transcriptional regulator called b-factor. Strikingly, one of the direct targets of this transcriptional factor is a gene, polX, encoding a putative DNA polymerase that belongs to the X family (Brachmann et al., Mol Microb 42, 1047). These DNA polymerases are devoted to DNA repair processes. We have analyzed the ability of polX mutant dikaryotic hyphae to cope with DNA damaging agents and we have found only slight defects in response to oxidative stress. Interestingly we have found that PolX seems to be required for the ability of the dikaryotic hyphae to resume proliferation once the b-program is repressed. We have also found that PolX is a mitochondrial protein and that most likely it is involved in the repair of damaged mitochondrial DNA. In our communication we will discuss why the induction of the infective hypha needs the presence of an error-prone DNA polymerase to protect the mitochondria.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T36

Tuesday 5th April
14:00 - 16:00

KIRTZEL Julia *(1), GUBE Matthias (2), KRAUSE Katrin (1), KOTHE Erika (1)*
(1) Microbial Communication, Friedrich Schiller University, Jena, Germany
(2) Soil Science of Temperate Ecosystems, Georg August University, Goettingen, Germany

Heavy metal release by fungal induced black slate degradation

Microorganisms are important factors in the degradation of rocks. Especially fungi promote the biological decomposition of lithic substrates. These processes are of high ecological relevance because they increase carbon dioxide concentration in the atmosphere and cause damages at historical buildings. Other major difficulties are waste dumps. Once the rock material has been lifted to the earth`s surface, microbial activity may release embedded heavy metals from rocks. The filamentous fungus *Schizophyllum commune* is a widespread basidiomycete and has the ability to degrade wood. In doing so, it secretes many enzymes like unspecific oxidases such as laccases, which are responsible for lignin degradation. Many enzymes involved in wood decomposition are assumed to be implicated in organic carbon release from rocks like black slates.
In this study, the connection between fungal caused rock degradation and heavy metal release is investigated. Furthermore, a laccase overexpressing strain and a control are compared to verify the contribution of laccases on black slate decomposition. For these experiments, the fungal strains were grown in liquid medium with and without grounded black slate for up to 14 days. Afterwards, metals in the medium were analyzed using inductively coupled plasma mass spectrometry (ICP-MS). The results show a significant involvement of *S. commune* on the release of e.g. Fe, Pb, and U. However, in some cases the metal concentration in the medium is higher in the absence of the fungus. This indicates that these metals are bioavailable, possibly essential for the fungus, and so taken up by it. Considering the role of laccases, the overexpressing strain sometimes shows higher release of metals compared to the control and vice versa. The study suggests a high ecological importance of *S. commune* as a rock-degrading fungus. It decomposes the slate to use containing organic carbon and nutrients for its energy generation and nutrition. During the process of biological weathering, it could be shown, that metals are released to the surrounding. In nature, this could cause serious problems in matters of soil and water contamination. Further experiments will be performed to test the bioavailability of released metals.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T37

Tuesday 5th April
14:00 - 16:00

ZHANG Jian *(1), MIAO Youzhi (1), ATANASOVA Lea (2), YANG Dongqing (1), JAVAD RAHIMI Mohammad (2), S. DRUZHININA Irina (2), SHEN Qirong (1)*
(1) Jiangsu Key Lab for Organic Waste Utilization and National Engineering Research Center for Organic-based Fertilizers, Nanjing Agricultural University, Nanjing, China
(2) Microbiology Group, Research Area Biotechnology and Microbiology, Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria

The function of reactive oxygen species in the mycoparasitic attack of *Trichoderma guizhouense* on *Fusarium oxysporum*

Trichoderma guizhouense NJAU 4742 (Harzianum clade) can combat the causative agent of banana wild disease *Fusarium oxysporum* f. sp. *cubense* 4 (Foc4) and kill a broad range of other fungi including many plant pathogens. At early stages of the interactions, when NJAU 4742 hyphae riches the colony of Foc4, it produces the droplets of the yellowish exudate. The GC-MS analysis identified that the exudate contained almitic and stearic acids, several hydrolytic enzymes (mainly chitinases and proteases) and an essential amount of H2O2. It allowed us to assume that these compounds also contribute to killing the Foc4 as reactive oxygen species (ROS) produced by the NADPH oxidase (NOX) complex is known to be involved in defense in fungi. To test the function of ROS in mycoparasitism we deleted the NADPH oxidase 1 (*nox1*) gene in NJAU 4742 and also overexpressed it under a strong constitutive promoter cDNA1P. The Δ*nox1* mutant had reduced ability to attack Foc4 and many other fungi while nox1OE mutants showed stronger mycoparasitic potential compared to the wild type phenotype as they produced the measurably higher amounts of $O2^{\cdot-}$and H2O2. Moreover, the action of ROS and peroxide correlated with increased transcription of catalase and superoxide dismutase encoding genes in Foc4 in response to *nox1OE* mutants of NJAU 4742. In order to investigate the genome-wide effect of nox1 deletion we applied deep RNA sequencing technology to respective mutant and wild type strains of NJAU 4742 at different stages of its interactions with Foc4. In this presentation we will demonstrate the specific tools used by T. guizhouense to kill its prey fungus Foc4 and compare them with defense mechanisms against other fungi.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T38

Tuesday 5th April
14:00 - 16:00

BAZAFKAN Hoda (1), STAPPLER Eva (1), BÖHMDORFER Stefan (2), OBERLECHNER Josua (2), ***SCHMOLL Monika*** *(1)*
(1) AIT Austrian Institute of Technology GmbH, Department Health and Environment, Bioresources, Konrad-Lorenz Strasse 24, Tulln, Austria
(2) University of Natural Resources and Life Sciences Vienna, Department of Chemistry, Division of Chemistry of Renewable Resources, Tulln, Austria

Functions of SUB1 and its position in the light signaling cascade in *Trichoderma reesei*

Light regulated processes are involved in regulation of growth, development and enzyme production in *Trichoderma reesei*. We therefore investigated strains bearing diverse combinations of photoreceptor mutantions for their relevance in asexual and sexual development. Additionally, we analyzed the function of SUB1 (homologue of *Neurospora crassa* SUB-1), a crucial transcription factor in the light response pathway. SUB1 influences growth and conidiation in dependence of the carbon source and was found to be a regulator of secondary metabolism in *T. reesei*. In contrast to its homologue in *N. crassa*, SUB1 is not essential for fruiting body formation in *T. reesei*, but required for female fertility. Additionally, SUB1 is involved in regulation of the pheromone system of *T. reesei*. Confrontation of strains lacking sub1 results in growth arrest prior to contact of the potential mating partners, which is at least in part due to altered secondary metabolite production. Female sterility of mutants lacking env1 is rescued in triple mutants of blr1, blr2 and env1, but not in double mutants of these genes. However, female sterility caused by deletion of sub1 is not rescued in combinations with blr1 or blr2 or both. Moreover, morphological effects of different mating partners were observed that suggest an involvement of the photoreceptors and sub1 in the complex communication upon sexual development.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T39

Tuesday 5th April
14:00 - 16:00

FLAK Michal *(1), KÖNIG Claudia (1), NETZKER Tina (1), FISCHER Juliane (1), VALIANTE Vito (1), SCHROECKH Volker (1), BRAKHAGE Axel A. (1)*
(1) Leibniz Institute for Natural Product Research and Infection Biology Hans Knöll Institute (HKI), Jena, Germany

Molecular signaling mechanisms of bacteria-induced fungal silent gene clusters

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

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T40

Tuesday 5th April
14:00 - 16:00

CASAS-FLORES Sergio *(1), OSORIO-CONCEPCIÓN Macario (1), CRISTOBAL-MONDRAGÓN Gema Rosa (1)*
(1) División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica, San Luis Potosí, Mexico

The histone deacetylase HOS-2 and the blue light regulator-1 and -2 differentially regulate development and oxidative stress in *Trichoderma atroviride*

In the filamentous fungi *Trichoderma spp*., light influences on physiological and developmental processes. The BLR-1 and -2 proteins regulate gene transcription, mycelial growth and asexual development in these fungi. The balance between histone acetylation and deacetylation is a key regulatory mechanism for gene expression. In this work we show that the histone deacetylase HOS-2 encoding gene is induced by light, H2O2 and menadione, and its lack led to altered development, and high sensitivity to oxidative stress, whereas the Dblr mutants exhibited the opposite phenotype. Expression of ROS-related genes under oxidative stress was highly induced in the wild-type and ∆blr mutants, whereas ∆hos-2 showed low levels. Transcription of blr-1 was high in ∆hos-2, whereas hos-2 showed low levels in the ∆blr. Furthermore, the ∆blr mutants displayed high transcription of ROS-related genes, which is in agreement with their high resistance to oxidative stress. These results led us to propose a negative and positive roles for BLR and HOS-2 to contend against oxidative stress.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T41

Tuesday 5th April
14:00 - 16:00

SO Yee-Seul *(1), IANIRI Giuseppe (2), IDNURM Alex (2), BAHN Yong-Sun (1)*
(1) Department of Biotechnology, Center for Fungal Pathogenesis, Yonsei University, seoul, South Korea
(2) Division of Cell Biology and Biophysics, School of Biological Sciences, University of Missouri-Kansas City, Missouri 64110, USA

Unravelling of the TOR signaling pathway in *Cryptococcus neoformans*

Tor1 is a serine/threonine protein kinase that is widely conserved across eukaryotic species. Tor1 was first identified in *Saccharomyces cerevisiae* as a target of rapamycin (TOR). The TOR pathway has been implicated in regulating cellular responses to nutrients, proliferation, translation, transcription, autophagy, and ribosome biogenesis. Here we identified two homologues of *S. cerevisiae* Tor protein, CNAG_06642 (Tor1) and CNAG_05220 (Tlk1, TOR-like kinase 1), in *Cryptococcus neoformans* causing a life-threatening fungal meningoencephalitis. Both Tor1 and Tlk1 have rapamycin-bindidng (RB) domains but Tlk1 has truncated RB form. To study the TOR-signaling pathway in the fungal pathogen, we attempt to construct the tor1 and tlk1 deletion mutant and phenotypically analyze them. Although we fail to consturct the tor1 deletion mutant, we successfully construct the tlk1 deletion mutant. The tlk1 deletion mutant does not exhibit any discernable phenotypes, suggesting that Tlk1 is dispensable in *C. neoformans*. The essentiality of TOR1 is independently confirmed by constructing the TOR1 promoter replacement strain by using a copper transporter 4 (CTR4) promoter and the TOR1/tor1 heterozygous mutant in diploid C. neoformans strain background followed by sporulation analysis. To further analyze the fuction of Tor1, we construct TOR1 overexpression mutant using a constitutively active histone H3 in *C. neoformans*. We find that the Tor1 overexpression mutant is resistant to rapamycin but the tlk1 deletion mutant does not exhibit any altered resistance to rapamycin, futher confirming that Tor1, but not Tlk1, is critical for TOR signaling. Futhermore, we found that Tor1 is involved in response to diverse stresses, including genotoxic stress, oxidative stress, thermo-stress, antifungal drug treatment, and production of melanin. To identify any TOR-related transcription factors, we screened *C. neoformans* transcription factor library that we constructed in our previous study and identified several potential downstream factors of Tor1, including Atf1, Crz1 and Bzp1. In conclusion, the current study provides insight into the role of the TOR signaling pathway in human fungal pathogens as well as *C. neoformans*.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T42

Tuesday 5th April
14:00 - 16:00

BARAD Shiri (1), ESPESO Eduardo A (2), SHERMAN Amir (3), ***PRUSKY Dov*** *(1)*
(1) Department of Postharvest Science of Fresh Produce, ARO, The Volcani Center, Israel, Bet Dagan, Israel
(2) Department of Molecular and Cellular Biology, Centro de Investigaciones Biológicas , Madrid, Spain
(3) Genomics Unit, ARO, The Volcani Center, Bet Dagan, Israel

Ammonia activates pacC and patulin accumulation in acidic environment during apple colonization by *Penicillium expansum*

Penicillium expansum, causal agent of blue mold rot, causes severe postharvest fruit maceration simultaneously with secretion of D-gluconic acid (GLA) and the mycotoxin patulin in colonized tissue. The factor(s) inducing patulin biosynthesis during colonization of the host acidic environment is unclear. During colonization of apple fruit in vivo and growth in culture, *P. expansum* secretes pH-modulating GLA and ammonia. While patulin and its possible opportunistic precursor GLA accumulate together during fungal development, ammonia is detected on the colonized tissue's leading edge and after extended culture, close to patulin accumulation. Here we demonstrate ammonia-induced transcript activation of the global pH modulator PacC and patulin accumulation in the presence of GLA by: i. direct exogenous treatment of *P. expansum* growing on solid media, ii. colonizing apple tissue, iii. growth under self-ammonia-production conditions with limited carbon, and iv. analysis of the patulin-biosynthesis cluster's transcriptional response to ammonia. Ammonia induced patulin accumulation concurrently with transcript activation of pacC, and patulin-biosynthesis cluster genes, indicating ammonia's regulatory effect on pacC transcript expression under acid conditions. Electrophoretic mobility shift assays using *P. expansum* PacC and antibodies to the different cleaved proteins showed that PacC is not protected against proteolytic signaling at pH 4.5 compared to pH 7.0, but NH4 addition did not further enhance its proteolytic cleavage. Ammonia did enhance activation of palF transcript in the PaL pathway under acidic conditions. Ammonia accumulation in the host environment by the pathogen under acid pH may be a regulatory cue for pacC activation, toward accumulation of secondary metabolites such as patulin.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T43

Tuesday 5th April
14:00 - 16:00

MARTINEZ-ROSSI Nilce Maria *(1), JACOB Tiago (1), PERES Nalu (2), MARTINS Maíra (1), LANG Elza (1), SANCHES Pablo (1), ROSSI Antonio (1)*
(1) São Paulo University / Ribeirão Preto Medical School / Department of Genetics, Ribeirao Preto / SP, Brazil
(2) Federal University of Sergipe / Department of Morphology, Aracaju / SE, Brazil

HSPs as molecular markers in the development of novel drugs for the treatment of dermatophytosis

Heat shock proteins (HSPs) are molecular chaperones highly conserved among different organisms exerting many cellular functions. In fungi, the functioning of these proteins has been implicated in morphogenesis, pathogenicity, stress responses and drug resistance, among others. HSP90 mainly plays central roles in the cell, modulating the activities of regulators and signaling networks, thus emerging as a molecular target for antifungal therapy. Here, we analyzed the hsp genes in the dermatophyte *Trichophyton rubrum*, which is a keratinolytic fungus and the primary cause of skin and nail mycoses in humans. Transcriptional analyses showed that some hsp and related genes were modulated according to particular environmental challenges, such as nutrient sources, interaction with cells and molecules of the host tissue, and drug exposure. Blocking Hsp90 function by chemical inhibition affected the transcription profile of some of these genes, decreased the growth of *T. rubrum* in an ex vivo model of nail infection, and increased the susceptibility of the fungus to some antifungal. Together, our results suggest the involvement of Hsp90 in the regulation of other HSPs, in the pathogenicity and drug susceptibility of *T. rubrum*. Moreover, the synergism observed between the inhibition of Hsp90 and the effect of itraconazole and micafungin in reducing the fungal growth on *T. rubrum* growth, raise Hsp90 as the potential target to treat dermatophytosis.
Financial support: FAPESP, CNPq, CAPES, FAEPA.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T44

Tuesday 5th April
14:00 - 16:00

SOULIE Marie-Christine *(1), JASSON Agathe (2), COURTIAL Julia (2), FAGARD Mathilde (2)*
(1) Sorbonne Université, UPMC Univ Paris06, UFR 927, Paris, France
(2) Institut Jean-Pierre Bourgin, UMR 1318 INRA-AgroParisTech, Versailles, France

Impact of nitrogen during the interaction *Arabidopsis thaliana-Botrytis cinerea*

Nutritional state of plant plays a key role during plant disease and it is not surprising that nitrogen, the fourth most abundant element in plant, can influence many plant-pathogen interactions. Indeed, the application of fertilizer nitrogen can increase or decrease plant susceptibility towards pathogens, depending on the pathosystem (Huber and Thomson, 2007). Generally, N fertilizers increase the diseases due to biotrophic fungi whereas they reduce those due to necrotrophs. But the situation is more complex in particularly with the necrotophic fungus *Botrytis cinerea*. Depending on the plant host, the strain's virulence and the choice of inoculum (mycelium or spores), nitrogen supply enhanced the spreading lesions or did not affect infection. We have studied the impact of nitrogen on the interaction *Arabidopsis thaliana- B. cinerea* and compared the effect of 4 strains with different aggressiveness. We have established that N limitation (0,5 and 2mM NO3) increased the resistance of the plant model *A. thaliana* only when we applied mycelium inoculum (Fagard et al, J. Exp. Botany, 2014). In order to better understand how host susceptibility or resistance is established, we have analyzed gene expression, some from pathogenicity factors of *B. cinerea* (pectinolytic enzymes, toxins) the others from plant defenses (PAD3, CHIB, PDF1). Transcriptomic analysis is under investigation

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CS3T45

Tuesday 5th April
14:00 - 16:00

ARENTSHORST Mark *(1), PARK Joohae (1), LAGENDIJK Ellen (1), DE LANGE Davina (1), RAM Arthur (1)*
(1) Molecular Microbiology and Biotechnology, IBL, Leiden University, Leiden, Netherlands

Galactofuranosyltransferase GfsA and GfsB have a redundant function in biosynthesis of galactofuranose-containing glycostructures in *Aspergillus niger*

Galactofuranose (Galf)-containing glycostructures are important to secure the integrity of the fungal cell wall. Golgi-localized Galf-transferases have recently been identified in *A. nidulans* and *A. fumigatus*. BLASTp searches using the GfsA proteins of *A. nidulans* and *A. fumigatus* identified three putative Galf-transferases in *A. niger*. Phylogenetic analysis showed that the three putative Galf-transferases group in three distinct phylogenetic groups. Characterization of the three Galf-transferases in *A. niger* by constructing single, double and triple mutants revealed that gfsA is most important for Galf biosynthesis. The phenotype of the ΔgfsA mutant is less severe than the phenotype of the ΔgfsAΔgfsB mutant, indicating that GfsA and GfsB have redundant functions. Deletion of gfsC did not result in any growth defect. Combining ΔgfsC with other deletion mutants (ΔgfsAC, ΔgfsBC or ΔgfsABC) did not exacerbate the phenotype of the single mutantsΔgfsA, ΔgfsB or double mutant ΔgfsAB, respectively. The phenotype of the ΔgfsAB double mutant resembled the phenotype of the ugmA mutant in respect to Calcofluor White-sensitivity, indicating that GfsA and GfsB together are responsible for galactofuranosylation of glucostructures in *A. niger*.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T46

Tuesday 5th April
14:00 - 16:00

BINDER Ulrike *(1), MAURER Elisabeth (1), MÜLLER Christoph (2), BRACHER Franz (2), PÜMPEL Thomas (3), LASS-FLÖRL Cornelia (1)*
(1) Division of Hygiene and Medical Microbiology, Medical University Innsbruck, Innsbruck, Austria
(2) Department of Pharmacy, Ludwig Maximilians University Munich, München, Germany
(3) Institut für Mikrobiologie, Leopold-Franzens University Innsbruck, Innsbruck, Austria

Combinatorial effects of azoles and hypoxia on sterol biosynthesis and sterol composition of *Aspergillus spp*.

Invasive Aspergillosis represents a serious life threatening disease in patient cohorts with impaired immune response. During infection, fungal pathogens must adapt to microenvironmental stresses, and such conditions are usually not taken into account in the assessment of antifungal sensitivities. Hypoxia is one stress which occurs during fungal infection in vivo. Ergosterol, the most important fungal cell membrane component, or it biosynthesis are the main targets of azoles, and additionally, the ergosterol pathway is dependent on oxygen availability. We therefore investigated the influence of azole treatment together with hypoxia on (1) the effectivity of azoles, (2) transcriptional changes of ergosterol biosynthetic genes (qPCR), (3) total ergosterol amount and (4) sterol accumulation in clinically relevant Aspergilli. A significant decrease of total ergosterol was observed during azole treatment in normoxia, while no changes were visible under hypoxic conditions. Additionally, azole treatment led to changes in the relative amount of sterols at both oxygen concentrations compared to untreated controls. Interestingly,changes were detected with lower abundance in hypoxia, indicating lower efficacy of azoles under low oxygen conditions. Azole exposure elicited increased levels of erg11A, erg11B, erg6 and erg7C mRNA abundance in normoxia, while transcript levels in hypoxia were not altered in comparison to untreated controls. These observations are well correlating with results from GC-MS and HPLC.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T47

Tuesday 5th April
14:00 - 16:00

SANCHEZ Olivia *(1), SOID-RAGGI Gabriela (1), RAMOS-BALDERAS Jose Luis (1), AGUIRRE Jesús (1)*
(1) Instituto de Fisiologia Celular, UNAM, Ciudad de Mexico, Mexico

The adenylate-forming enzymes AfeA and TmpB are involved in *Aspergillus nidulans* self-communication during asexual development

Aspergillus nidulans asexual sporulation (conidiation) is triggered by different environmental signals and involves the differentiation of specialized structures called conidiophores. The elimination of genes flbA-E, fluG and tmpA results in a fluffy phenotype characterized by delayed conidiophore development and decreased expression of the conidiation essential gene brlA. While flbA-E encode regulatory proteins, fluG and tmpA encode enzymes involved in the biosynthesis of independent signals needed for normal conidiation. Here we identify afeA and tmpB as new genes encoding members the adenylate-forming enzyme superfamily, whose inactivation cause different fluffy phenotypes and decreased conidiation and brlA expression. AfeA is most similar to unknown function coumarate ligase-like (4CL-Lk) enzymes and consistent with this, a K544N active site modification eliminates AfeA function. TmpB, identified previously as a larger homolog of the oxidoreductase TmpA, contains a NRPS-type adenylation (A) domain. A high degree of synteny in the afeA-tmpA and tmpB regions in the Aspergilli suggests that these genes are part of conserved gene clusters. afeA, tmpA and tmpB double and triple mutant analysis as well as afeA overexpression experiments indicate that TmpA and AfeA act in the same conidiation pathway, with TmpB acting in a different pathway. Fluorescent protein tagging shows that functional versions of AfeA are localized in organelle-type bodies and the plasma membrane, while TmpA and TmpB are localized at the plasma membrane. We propose that AfeA participates in the biosynthesis of an acylated compound, either a p-cuomaryl type or a fatty acid compound, which might be oxidized by TmpA and/or TmpB, while TmpB A domain would be involved in the adenylation of a hydrophobic amino acid, which in turn would be oxidized by the TmpB oxidoreductase domain. Both, AfeA-TmpA and TmpB signals are involved in self-communication and reproduction in *A. nidulans*.

This work was funded by grants CB-2010-01-153256 and CB-2014-01-238492 from CONACYT México, IN207913 and IN208916 from PAPIIT-UNAM.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T48

Tuesday 5th April
14:00 - 16:00

MURRY Reyna *(1), KOTHE Erika (1)*
(1) Department of Microbial Communication, Institute of Microbiology, Friedrich Schiller University of Jena, Jena, Germany

Inositol phosphate signaling in the basidiomycete *Schizophyllum commune*

The basidiomycete *Schizophyllum commune* has been studied for tetra-polar mating since the early 1900s. Intracellular signal transduction after recognition of mating pheromones involves MAPK, cAMP and Ras signaling. In addition, inositol monophosphatase (IMPase) in inositol signaling is a second messenger which is specifically inhibited by lithium. In *S. commune*, aberrant morphology, growth inhibition, down-regulation of imp gene expression, and lower enzyme activity has been observed under lithium presence. Furthermore, imp gene expression is down-regulated in a Ras dependent manner, indicating there is a crosstalk between Ras and inositol phosphate signaling cascades. A two dimensional gel-based proteomic approach under LiCl effect was performed in both wild type and constitutively active Ras mutant strains. In the presence of LiCl, inositol phosphatase/fructose-1,6-bisphosphatase (FBPase), Ran BP1, and actin are down regulated in Ras dependent manner strain. FBPase is known to shares similar sequence motifs (Asp-Pro-Ile/Leu-Asp-Gly/Ser-Thr/Ser) with IMPase and known to be inhibited by lithium, Ran BP1 is an important precursor in G-protein Ran signaling cascade which is essential for the translocation of RNA and proteins through the nuclear pore complex. Actin plays a control role in cell polarity, tip growth and long-distance intracellular transport, its down-regulation seems to be linked to growth reduction and hyphal morphology alteration. Besides, we have performed gain-function analysis of inositol monophosphatase, a second messenger of inositol signaling pathway via imp overexpression to know the function of IMP in the fungus. Two imp overexpresion transformants showed higher mRNA level expression of imp in comparison to the wildtype. The activity of imp will be further measured and the signals of pheromone recognition will be analyzed on a functional level and correlated with phenotypic changes in cell biology and fruiting body development.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T49

Tuesday 5th April
14:00 - 16:00

YOSHIMI Akira *(1), FUKUMA Yasuyuki (2), HAGIWARA Daisuke (3), FURUKAWA Kentaro (4), MIDORIKAWA Yura (2), NAKAYAMA Mayumi (2), HASEGAWA Fumihiko (1), ABE Keietsu (1)*
(1) New Industry Creation Hatchery Center, Tohoku Univ., Sendai, Japan
(2) Grad. Sch. Agric. Sci., Tohoku Univ., Sendai, Japan
(3) MMRC, Chiba Univ., Chiba, Japan
(4) Grad. Sch. Med. & Dent. Sci., Niigata Univ., Niigata, Japan

The relationships between the lethality and the constitutive activation of HogA pathway through the downregulation of YpdA protein in *A. nidulans*

Fungal histidine-to-aspartate (His-Asp) phosphorelay systems consist of three types of common signal transducers: Histidine kinase (HK), a histidine-containing phosphotransfer intermediate (HPt), and a response regulator (RR). Generally, HPt acts as an intermediate between HK and RR, and is indispensable for inducing appropriate responses to environmental stresses through His-Asp phosphotransfer signaling. In the model filamentous fungus *Aspergillus nidulans*, the osmoadaptative response system is relatively well characterized by genetic approaches: HK (NikA), HPt (YpdA), and RR (SskA and SrrA) are critical for the response of high-osmotic and oxidative stresses. In addition, it is considered that the deletion of the ypdA gene is lethal. Although the function of YpdA should be important for the signaling system, the molecular mechanisms underlying the essentiality of YpdA remain unclear. In this study, we constructed the conditional-ypdA (CypdA) strain in which ypdA expression was conditionally regulated under the control of the alcA promoter. We also constructed several CypdA strains with the deletion of RR genes (CypdA/ΔsskA, CypdA/ΔsrrA, CypdA/ΔsskAΔsrrA), and analyzed their phenotypes to elucidate the relationships between the YpdA and RRs. Western blot analysis revealed that YpdA protein was undetectable in all CypdA strains in line with downregulation of ypdA expression. In addition, the ypdA downregulation induced phosphorylation of HogA MAP kinase in the CypdA and CypdA/ΔsrrA strains. Interestingly, the phosphorylation of HogA continued at least from 12 to 36 hours. These observations suggested that the abnormal activation of HogA was dependent on SskA pathway and was partly responsible for the lethality of YpdA defection. Although the growth retardation of the CypdA strain under ypdA downregulation was partly restored by shutting off the SskA or SrrA pathway, the retardation of CypdA growth was completely suppressed by shutting off both RR pathways. We further carried out the microscopic observation in the CypdA strains to assess the morphological effects by the ypdA downregulation. Here, we discuss the relationships between the lethality and the downregulation of ypdA expression.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T50

Tuesday 5th April
14:00 - 16:00

ZACCARIA Marco *(1), COMITO Giusy (2), CARY Jeffrey William (3), BHATNAGAR Deepak (3), SANSEVERINO Walter (4), AIESE CIGLIANO Riccardo (4), FABBRI Anna Adele (1), FANELLI Corrado (1), CHIARUGI Paola (2), REVERBERI Massimo (1)*
(1) Department of Environmental and Evolutionary Biology, "La Sapienza" University of Rome, Roma, Italy
(2) Department of Biomedical, Experimental and Clinical Sciences, University of Florence, Florence, Italy
(3) USDA, ARS, Southern Regional Research Center, New Orleans, Louisiana, USA
(4) Sequentia Biotech, Campus UAB, CRAG Bellaterra, Cerdanyola del Vallès 08193, Barcelona, Spain

Cancer and filamentous fungi: convergent evolution of survival strategies

With this work, we delve into the evolutionary relations between two apparently distant biological systems: Fungi and cancer. Cancer is a living tissue that differentiates within an animal organ, de facto defying its original histological characteristics. Cancer has an undifferentiated and continuous growth, a pro-glycolytic metabolic phenotype, an intense replication rate and a high dispersion potential. In Fungi, Ascomycota in particular, we find organisms that lead a kindred «lifestyle», displaying several phenotypic affinities to cancer in response to the same environmental stresses, such as hypoxia or oxidative insult. In order to investigate how deep such affinities go, we chose *Aspergillus flavus* as the model organism for fungi; for cancer, we employed A375 malignant human melanoma cell line. We performed transcriptome analysis, enzymatic assays and microbiological tests to evaluate several parameters on samples grown in normoxic and hypoxic (O2 1%) environments. We intend to evaluate markers for cellular growth, dispersion (conidia and metastases), energy metabolism pathways, oxidative stress response, apoptosis and sirtuins expression. Our experiments are still ongoing. Preliminary results show that growth and dispersion, at the different experimental time intervals, are alternatively up and down-regulated in a similar fashion across the two systems. The modulation of energetic metabolism, estimated in terms of intensity of glycolysis, Krebs cycle and pentose phosphate pathway, also displays a common motive. Moreover, the expression of Sirtuin 1 (SIRT1), a metabolic fine-tuner long studied in mammals, is indeed congruous between cancer and *A. flavus*. Our future plans will involve RNA-seq analysis of the samples collected, oxylipins assay for both systems (oxylipins are products of fatty acid oxidation employed in interspecies communication), and the generation of SIRT1 knock-downs, to verify the extent of the role played by this metabolism modulator in the matter at hand. We also plan to perform a new round of experiments in different culture conditions. Ultimately, our work aims to prove a phylogenetic common ground between two different forms of eukaryotic life: tumors and filamentous fungi. At the same time, it represents an alternative method to tackle cancer as a disease, with the purpose of referring it to a theoretical framework devised in the light of evolution.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T51

Tuesday 5th April
14:00 - 16:00

DIAS Luciana Pereira *(1), ARAÚJO Claudinéia Aparecida Da Silva (2), PUPIN Breno (2), FERREIRA Paulo Cesar (2), PEDRINI Nicolás (3), BRAGA Gilberto Úbida Leite (4), RANGEL Drauzio Eduardo Naretto (2)*
(1) Pós-Graduação em Biotecnologia Industrial, Escola de Engenharia de Lorena, Universidade de São Paulo, Lorena, São Paulo, Brazil
(2) Inbioter, Institute of Biotechnology, São José dos Campos, São Paulo, Brazil
(3) Facultad de Ciencias Médicas, Universidad Nacional de La Plata, La Plata, Argentina
(4) Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil

Outcome of blue, green, red, and visible light during mycelial growth on conidial stress tolerance

The influence of light on Metarhizium robertsii was studied based on conidial tolerance to UV radiation and osmotic stress (KCl). Fungi were grown at 26 °C: 1) on potato dextrose agar medium (PDA) in the dark (control); 2) under nutritive stress (=Czapek medium without sucrose=MM) in the dark; and 3) on PDA under continuous (a) visible light (4.98 W.m-2), (b) blue light 1 (4.8 W.m-2), (c) blue light 2 (9.7 W.m-2), (d) green light (2.2 W.m-2), (e) red light (2.8 W.m-2). The conidial suspensions of each treatment were inoculated on medium and exposed from 100 to 210 min (with increments of 10 min) to an irradiance of 0.60 W.m-2 inside the QSUN® Xenon Test Chamber. After irradiation, the plates were incubated for 48 h in the dark at 26 °C. For the osmotic stress, the conidial suspensions were dropped on PDA medium supplemented with KCl concentrations of 0 (control) 1.0, 1.3, 1.4, 1.5, 1.9, and 2.1 M, and the germination was counted after 24 h. Conidia produced on MM were significantly more tolerant to both UV radiation and osmotic stress. Conidia produced under blue light 1 were the second most tolerant to UV radiation, followed by the UV tolerances of conidia produced under visible light. Conidia produced under red light were the least UV tolerant. For KCl tolerance, conidia produced under visible, blue 1, and blue 2 were the second most tolerant, followed by conidia produced under green light. Again conidia produced under red light were the least tolerant to KCl.

POSTER SESSION ABSTRACTS
Session CS3 Environmental sensing, stress response
CS3T52

Tuesday 5th April
14:00 - 16:00

MENDOZA Ariann *(1), LARA Fernando (1), SÁNCHEZ Olivia (1), AGUIRRE Jesús (1)*
(1) Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Mexico, Mexico

Transcription factors NapA, SrrA and AtfA regulate the antioxidant response and development in *Aspergillus nidulans*

Reactive oxygen species (ROS) are partially reduced oxygen derivatives, produced mainly through respiration and NOX enzymes. ROS can cause cell damage but also play diverse signaling roles and regulate development. *Aspergillus nidulans* transcription factors (TFs) SrrA, NapA and AtfA are individually required to survive oxidative stress. SrrA contains a heat-shock-like DNA-binding domain and is closely related to Prr1 from *S. pombe*. NapA is a peroxide sensor homologous to *S. pombe* Pap1. AtfA is a b-ZIP protein, homologous to mammalian ATF-2, which we have shown to interact with the stress MAPK SakA (also called HogA) under different stress and developmental conditions. We used GFP functional fusions to study the cellular localization these TFs and generated double and triple mutants to test them under different stress conditions. SrrA::GFP and AtfA:.GFP show constitutive nuclear localization, while NapA::GFP accumulates in the nucleus in the presence of oxidative stress, where it interacts with SrrA. Spores and mycelia displayed different patterns of oxidative stress sensitivity: *ΔnapA* mutation conferred the highest sensitivity to H2O2 to the spores, while inactivation of srrA made mycelia more sensitive to this stress. ΔatfA mutants were in general less sensitive to H2O2 and only the lack of NapA made both, spores and mycelia, sensitive to menadione. Our results indicate that although AtfA, NapA and SrrA are all required for a proper antioxidant response, each TF plays differential roles in this response and also show different roles in the regulation of A. nidulans development.

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POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T1

Tuesday 5th April
14:00 - 16:00

ILLANA Adriana *(1), GALHANO Rita (3) (2), RYDER Lauren (2), RODRÍGUEZ-ROMERO Julio (1), BADARUDDIN Muhammad (2), MARTINEZ-ROCHA Ana Lilia (2), SOANES Darren M. (2), STUDHOLME David J. (2), TALBOT Nicholas J. (2), SESMA Ane (3) (1)*

(1) Centre for Plant Biotechnology and Genomics, Universidad Politécnica de Madrid, Campus de Montegancedo, Madrid, Spain
(2) School of Biosciences, University of Exeter, Exeter, UK
(3) Disease & Stress Biology dept. John Innes Centre, , Norwich, UK

The transcriptional regulator Tpc1 is required for NADPH oxidase-dependent polarized growth and virulence in the rice blast fungus

The establishment of polarity is a critical process in pathogenic fungi. Here, we report the identification of TPC1 (Transcription factor for Polarity Control 1), which regulates invasive polarized growth in the rice blast fungus *Magnaporthe oryzae*. TPC1 encodes a putative transcription factor of the fungal Zn(II)2Cys6 family, exclusive to filamentous fungi. TPC1-deficient mutants showed severe defects in conidiogenesis, infection-associated autophagy, glycogen metabolism and plant tissue colonisation. By tracking actin-binding proteins, septin-5 and autophagosome components, we show that TPC1 regulates cytoskeletal dynamics and infection-associated autophagy during appressorium-mediated plant penetration. Importantly, we found that Tpc1 regulates NoxD, the p22phox sub-unit of the fungal NADPH oxidase complex. Tpc1 therefore controls the spatial and temporal regulation of cortical F-actin through regulation of the NADPH oxidase complex during appressorium re-polarisation. We conclude that Tpc1 is a core developmental regulator in filamentous fungi, linking the regulated synthesis of reactive oxygen species with polarity control during host invasion.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T2

Tuesday 5th April
14:00 - 16:00

SCHUMACHER David *(1), TRAEGER Stefanie (1), ALTEGOER Florian (1), NOWROUSIAN Minou (1)*
(1) Department of General and Molecular Botany, Ruhr-University, Bochum, Germany

Regulation of sexual development in filamentous fungi: revealing components of the regulatory network of fruiting body formation

In the last decades knowledge of fungal development has increased, but only little is known about the regulation of fruiting body development. Therefore we investigate the regulatory network of the sexual development of the filamentous ascomycete *S. macrospora*. The GATA-transcription factor (TF) PRO44 and the MYB-Domain containing TF PRO46 are both involved in the regulatory network of fruiting body development in *S. macrospora* as deletion of the genes leads to a sterile phenotype where development stops at the stage of protoperithecia. Fluorescence microscopy revealed a localization of PRO44 and PRO46 in the nucleus, and yeast two hybrid studies show that PRO44 is interacting with itself. We also discovered splicing variants of PRO44 that lead to 3-9 bp extensions of the third exon. Yeast two hybrid screens with PRO44 and PRO46 as baits and a collection of *S. macrospora* cDNA as prey results in the discovery of eight (PRO44) and seven (PRO46) putative interaction partners. The homolog in *Neurospora crassa*, SUB-1, regulates several genes and we analyzed four of these genes via qRT-PCR in *S. macrospora*. Two of the four investigated genes show an increase of expression in the *Δpro44* strain indicating that PRO44 acts as a repressor of these genes. Investigation of the influence of *pro44* on *pro46* and vice versa showed a slight upregulation of PRO46 if PRO44 is absent. Future studies will include ChIP-Seq and RNA-Seq experiments to identify target genes of PRO44 and PRO46.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T3

Tuesday 5th April
14:00 - 16:00

TRAEGER Stefanie (1), SCHUMACHER David (1), GESING Stefan (1), ALTEGOER Florian (1), ***NOWROUSIAN Minou*** *(1)*
(1) Ruhr-University Bochum, Bochum, Germany

Comparative genomics and transcriptomics to study fruiting body development in ascomycetes

Filamentous ascomycetes develop several types of fruiting bodies that share a common ancestor, and a set of common core genes most likely controls this process. One way to identify such genes is to search for conserved genes and expression patterns. In a genome and transcriptome mining approach, we are using data from the Sordariomycete *Sordaria macrospora* and the Pezizomycete *Pyronema confluens* to identify evolutionary trends in fruiting body morphogenesis. Among the genes with conserved expression patterns were the histone chaperone gene *asf1*, the transcription factor gene *pro44*, and the SNARE protein gene *sec22*. *asf1* and *pro44* are essential for fruiting body development in *S. macrospora*, whereas *sec22* is involved in ascospore maturation. Furthermore, the *P. confluens* orthologs of *asf1* and *pro44* can complement corresponding *S. macrospora* mutants, indicating a conserved function. In addition to identifying target genes, comparative studies can be used to determine genome-wide transcription patterns. Based on the genome and development-dependent transcriptomes of *P. confluens*, we analyzed if genes with different levels of evolutionary conservation differ in their expression. 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) while being less than 2 % among conserved genes, consistent with the idea of rapid evolution of sex-associated genes.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T4

Tuesday 5th April
14:00 - 16:00

MARSCHALL Robert *(1), TUDZYNSKI Paul (1)*
(1) Institute of Plant Biology and Biotechnology, WWUM, Muenster, Germany

Living at the crossroads: Bc-Lqg1, a signaling hub in a major plant pathogen scaffolding NADPH oxidase, MAP kinase and calcium signaling

NADPH oxidases (Nox) are major producers of reactive oxygen species (ROS) in multicellular eukaryotic organisms. Apart from triggering defense reactions in phagocytes and plant cells, they are involved in a broad range of differentiation processes. Recent work has shown that fungal Nox complexes also play a central role in most vegetative, sexual and pathogenic developmental processes. However, in contrast to mammalian systems, knowledge is limited about the composition, localization and connection to the major signaling cascades in fungi. We characterized a fungal homolog of the RasGAP protein IQGAP (BsLqg1) which functions as a scaffold linking several major signaling processes, including Nox in mammalian cell lines. We showed that BcLqg1 interacts directly with cytosolic and membrane-associated subunits of a Nox-complex in *Botrytis cinerea* and therefore may be a mediator between the catalytic Nox subunits and its regulator BcNoxR. Perhaps of even greater importance, the protein was shown to also interact with modules of the MAP kinase signaling and calcium-dependent signaling pathways. Functional analysis of BcLqg1 substantiated its central hub function: it mediates the Ca-triggered nuclear translocation of a calcineurin responsive transcription factor (BcCrz1) and the MAP kinase BcBmp1. BcLqg1 is involved in resistance against oxidative, osmotic and membrane stress and in all major developmental processes: the formation of conidial anastomosis tubes, infection cushions and sclerotia.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T5

Tuesday 5th April
14:00 - 16:00

PARDO-MEDINA Javier *(1), RUGER-HERREROS Macarena (1), PARRA-RIVERO Obdulia (1), ROMERO-CAMPERO Francisco J. (1), LIMÓN M. Carmen (1), AVALOS Javier (1)*
(1) University of Seville, Seville, Spain

Effect of light and carS mutation on transcriptome of *Fusarium fujikuroi* and *Fusarium oxysporum*

Some species of the genus *Fusarium* are well-known models for phytopathogenicity and secondary metabolite production. We focus our attention on the molecular mechanism governing the production of carotenoids in *F. fujikuroi* (Ff) and *F. oxysporum* f. sp. *lycopersici* (Fo). Carotenoid biosynthesis in these fungi is controlled by environmental signals, with light playing a major stimulating role. Two key structural genes of the pathway, *carRA* and *carB*, together with a gene for a retinal producing dioxygenase, *carX*, and an opsin gene, *carO*, are organized in a light-regulated car cluster. Transcription of the cluster is repressed by the RING finger protein CarS by an unknown mechanism and the mutation of the *carS* gene results in a carotenoid overproducing phenotype. We have performed RNAseq analysis of Ff and Fo wild types and *carS* mutants grown in the dark or exposed to one-hour illumination. Overall results were similar in both species, although significant differences were found in the corresponding sets of regulated genes, suggesting different regulatory needs in their natural environments. Impact of light was higher than that of the *carS* mutation, with a prevalence of photoinduction over photorepression. Interestingly, a strong correlation was found between the sets of light- and *carS*-regulated genes, suggesting links between the molecular mechanism of the CarS protein and the light regulation machinery that go beyond the control of the car cluster.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T6

Tuesday 5th April
14:00 - 16:00

CASTANERA Raul *(1), LÓPEZ-VARAS Leticia (1), BORGOGNONE Alessandra (1), PISABARRO Antonio. G (1), RAMÍREZ Lucía (1)*
(1) Genetics and Microbiology Research Group, Public University of Navarre, Pamplona, Spain

Production of endogenous small RNAs by transposable elements in *Pleurotus ostreatus*

Transposable elements (TEs) mediate eukaryotic genome evolution causing mutations, chromosomal rearrangements and modulating gene expression. Previous works have shown that TEs are often epigenetically inactivated. Nevertheless, recent insights highlight that these elements are not simply genomic parasites, and play an important role in the epigenetic regulation of the host genome. In the last years the basidiomycete *Pleurotus ostreatus* has become an interesting model for genome studies due to the availability of high quality genomic sequences of the compatible monokaryons PC15 and PC9, parental strains of the dikaryotic strain N001. Using a combination of homology and structure-based bioinformatics approaches, we have carried out an exhaustive annotation of transposable elements in the two genomes. In addition, we have performed RNAseq and small RNAseq profiling of both strains as well of their dikaryotic counterpart in several developmental stages, including primordia and fruit bodies. According to our results TEs accounted for 5 to 10% of the *P. ostreatus* genome and the major fraction was composed by retrotransposons in the Gypsy and Copia families, often arranged clusters. We found that up to 75% of TE copies lacked of transcription, and genes with insertions of TEs in their promoter regions showed lower expression than the average. Interestingly, the lack of expression in TEs was coincident with a high production of small RNAs within element boundaries, suggesting that TEs are subjected to post-transcriptional repression. A deeper analysis showed that 55% of the TE-associated small RNAs originated in double stranded hairpin structures compatible with micro-RNA precursors. Efforts are ongoing to characterize the impact of these mechanisms in *P. ostreatus* development.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T7

Tuesday 5th April
14:00 - 16:00

BORGOGNONE Alessandra *(1), MORSELLI Marco (2), CASTANERA Raúl (1), RUBBI Ludmilla (2), LÓPEZ-VARAS Leticia (1), PISABARRO Antonio Gerardo (1), PELLEGRINI Matteo (2), RAMÍREZ Lucía (1)*
(1) Genetics and Microbiology Research Group, Public University of Navarre, Pamplona, Spain
(2) Department of Molecular, Cell and Developmental Biology, University of California Los Angeles, Los Angeles, CA, USA

DNA methylation analysis highlights epigenetic events in the basidiomycete *Pleurotus ostreatus*

5-methyl cytosine (5mC) is an epigenetic modification observed in a wide range of eukaryotic organisms involved in the regulation of several processes such as development and genome integrity. In higher eukaryotes, gene-bodies are common methylation targets while in fungi it has been reported that methylation is primarily targeted towards repetitive sequences and transposons (TEs), contributing to the silencing of these elements. However, to date little is known about the epigenetics of basidiomycetes, a group of fungi, whose genome contain a variable TE content. TE expression have been recently reported in the monokaryotic genomes (PC9 and PC15) of *Pleurotus ostreatus*, both parental strains of the dikaryotic strain N001. *P.ostreatus* is an edible and lignin degrader basidiomycete with outstanding biotechnological applications. In order to investigate the 5mC content of *P.ostreatus* genome, we performed whole-genome bisulfite sequencing on different developmental stages of *P.ostreatus* monokaryotic and dikaryotic strains grown on straw as substrate. The analysis of this sequencing data showed that methylation is higher at CpG dinucleotides compared to CH. Furthermore, our results showed that genes were generally hypomethylated (<5% methylation), whereas TEs were generally highly methylated. Moreover, a negative correlation between gene body methylation and TEs located at increasing distances from the gene transcription start site was found, even if the majority of genes were hypomethylated. Strikingly, however, we found distinct differences in the methylation patters of the two haplotypes. Overall, significantly higher global methylation levels were found in the PC9 monokaryon. Specifically, Class I transposons showed higher methylation levels than Class II between PC9 and PC15. This data suggests that DNA methylation in *P.ostreatus* differs substantially between haplotypes, and may contribute to understand genome-wide methylation in dikaryotic strains of filamentous fungi.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T8

Tuesday 5th April
14:00 - 16:00

HERZOG Stephanie *(1), LEHMAN-HANO Jessica (1), BRANDT Ulrike (1), FLEIßNER André (1)*
(1) Institut für Genetik; Technische Universität Braunschweig, Braunschweig, Germany

The chitin synthase regulator CSR-3 and the SO protein are involved in cell cell fusion and stress-induced cell wall remodeling in *Neurospora crassa*

Germinating conidia of *Neurospora crassa* undergo cell-cell fusion in order to from a supracellular network, which further develops into the mycelial colony. These fusion events employ an unusual mode of communication, in which the cells appear to switch between signal sending and receiving, indicated by the alternating recruitment of the SO protein and the MAK-2 MAP kinase module to the growing cell tips. To specify the cellular function of SO, we identified potential interaction partners. One factor found in an Y2H screen is the chitin synthase regulator 3 (CSR-3). In fusing germlings, CSR-3 is recruited to the prospective fusion point after cell-cell contact, where it transiently co-localizes with SO and remains until membrane merger is completed. Germling pairs lacking the *csr-3* gene tend to lyse during this process. Based on these observations, we hypothesize that CSR-3 functions in stabilizing the forming fusion pore and that a fine-tuned equilibrium between chitin synthesis and cell wall degradation is required for this process. As additional SO-interacting partners MEK-1 and MIK-1, the two up-stream kinases of the MAK-1 cell wall integrity MAP kinase cascade, were identified. Earlier studies in *S. macrospora* indicated that the SO homologue functions as a scaffold protein for this MAP kinase module. Consistent with this finding, MAK-1 also co-localizes with SO and CSR-3 at the fusion point of *N. crassa* germlings. In addition, SO, MEK-1 and CSR-3 co-aggregate in complexes, which form at the cell periphery in response towards induced cell wall stress. Together, these findings suggest that cell wall reconstruction during cell fusion and during stress share common molecular signaling networks, in which SO mediates the interaction of the MAP kinase module and its targets. In addition, SO homologues are involved in the mutualistic and pathogenic interactions of various fungi and their respective host plants. Recently, *N. crassa* was identified as a potential endophytic symbiont of scots pines. To determine a potential role of SO and other fusion related proteins for the endophytic life style, we are currently establishing experimental test systems in our laboratory using *Pinus silvestris* as the host plant.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T9

Tuesday 5th April
14:00 - 16:00

PARRA RIVERO Obdulia *(1), LIMON MIRON Maria Del Carmen (1), AVALOS CORDERO Javier (1)*
(1) Department of Genetic. Faculty of Biology. University of Seville, Seville, Spain

Participation of a putative lncRNA in the photoregulation of carotenoid biosynthesis in *Fusarium oxysporum*

The *Fusarium* species produce carotenoids, terpenoid pigments that provide a typical orange pigmentation to their surface colonies. Carotenoid biosynthesis is stimulated by light through the transcriptional induction of the structural genes. In addition, the carotenoid pathway is down regulated by RING finger protein CarS. Carotenoid overproducing mutants are usually affected in gene *carS*, but we recently identified T-DNA insertional mutants with a *carS*-like phenotype, carrying sequence alterations in a long upstream *carS* intergenic region. A miRNA detection software identified two possible miRNA precursor sequences in this DNA segment. Their targeted deletion results in a partial loss of photoinduction of carotenogenesis in one case and in total loss in the other, indicating their participation in the regulatory mechanism of the pathway. Interestingly, search of small RNAs through RNAseq experiments failed to identify miRNAS in the whole upstream *carS* intergenic segment, but standard RNAseq analyses revealed the occurrence of a 1.2-kb transcript, covering at least one of the targeted deleted sequences, while the other could be located in its promoter. The transcript lacks putative ORFs and it is absent in the genome annotation of *F. oxysporum*, suggesting that it could be a long non-coding RNA (lncRNA). We currently focus our attention in the molecular characterization of this putative lncRNA, and the reasons of being needed for the induction by light of carotenoid biosynthesis in *F. oxysporum*.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T10

Tuesday 5th April
14:00 - 16:00

JIANG Ping (1), ***LU Ling*** *(1)*
(1) College of Life Sciences, Nanjing Normal University, Nanjing, China

Suppressors of the septum initiation network function on septation and conidiation in *Aspergillus nidulans*

Timely cytokinesis/septation is essential for hyphal growth and conidiation in *Aspergillus nidulans*. Genetic analysis have identified that *A. nidulans* has components of the septum initiation network (SIN) pathway; one of these, SEPH, is a key player for early events during cytokinesis. However, little is known about how the SEPH kinase cascade is regulated by other components. Here, through UV mutagenesis, 116 independent mutants were obtained that could restore cytokinesis in the absence of *sepH*. Among them, the phosphoribosyl pyrophosphate synthetase family acts antagonistically against the SIN so that the downregulation of AnPRS family can bypass the requirements of the SIN for septum formation and conidiation. The transcription defect of the Anprs gene family accompanied with the reduction of AnPRS activity causes the formation of hyper-septation as well as the restoration of septation and conidiation in the absence of SEPH. Moreover, we demonstrated that AnPRS members are able to form the heterodimers for functional interacting entities but they appear to contribute so unequally that deletion of *Anprs1* displays relatively normal septation, but deletion of either *Anprs2* or *Anprs3* is lethal. Interestingly, overexpression of *Anprs1* or *Anprs3* could partially rescue the defect phenotype of *Anprs2*. Transcriptional expression profiles revealed that the mRNA levels of *Anprs1*, *2* and *3* are dynamic during germination, hyphal growth and conidiation stages and all of them showed the most abundant expression during tested hyphal growth time point. However, turn-off the expression of *Anprs2* or *Anprs3* but not *Anprs1* in conditional strains significantly decreased the total PRPP synthetase activity, further indicating *AnPrs2* and *3* have the dominant roles than that of *AnPrs1*. In addition, two regulatory subunits of protein serine/threonine type 2A phosphatases (PP2A)-ParA and PabA, whose orthologos are suppressors of SIN in *Schizosaccharomyces pombe* had been found to be required for conidiation and septation. Deletion of *parA* but not *pabA* caused the hyper-septation in hyphal cells, especially in conidiophore cells. Therefore, SIN, as a major signaling pathway in regulating cytokinesis, might have to work together with multiple other protein complexes.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T11

Tuesday 5th April
14:00 - 16:00

SCHUMANN Marcel (1), HARTUNG Lisa (1), BRANDT Ulrike (1), ***FLEISSNER Andre*** *(1)*
(1) Technische Universität Braunschweig, Institut für Genetik, Braunschweig, Germany

The penta-EF-hand Protein PEF-1 functions in a calcium-mediated response to membrane damage in *Neurospora crassa*

In recent years, *Neurospora crassa* has advanced as a model for studying eukaryotic cell fusion. Germinating spores of this fungus fuse during colony establishment. Fusion pore formation involves cell wall deconstruction and plasma membrane merger. These steps bear the risk of cell lysis and death by membrane rupture. Previous studies indicated that lysis of fusion pairs occurs more frequently in the plasma membrane fusion mutant *ΔPrm1*. This lysis rates increased even further on media with reduced calcium, suggesting the presence of Ca2+-mediated membrane repair mechanisms. We identified the Ca2+-binding penta-EF-hand protein PEF-1 as a potential part of this proposed healing mechanism. Subcellular localization and live-cell imaging revealed that PEF-1 is recruited to the fusion point of lysing germling pairs, and accumulates at the plasma membrane after treatment with the membrane destabilizing drug nystatin. Deletion of *pef-1* in the *ΔPrm1*mutant had a comparable effect to the reduction of calcium and resulted in an increased lysis rate. The mammalian PEF-1 homolog ALG-2 is thought to link calcium-dependent signaling with vesicle trafficking. In addition, vesicles commonly contribute to membrane repair, for example by the formation of wound patches in response to a calcium influx. In contrast to recent publications on Pef-1 functions in the budding yeast *Saccharomyces cerevisiae*, a deletion of *pef-1* in *N. crassa* does not result in reduced stress tolerance. Interestingly, when we tested a deletion of *pef-1* in the human fungal pathogen *Candida albicans*, reduced growth in the presence of cation-chelating agents EGTA and membrane-destabilizing SDS was observed. We hypothesize that in these fungi membrane damage results in the influx of calcium and activation of PEF-1, which mediates vesicle-based plasma membrane repair. Further studies aim to fully characterize this repair mechanism, which is also activated in response to the important anti-fungal drug nystatin.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T12

Tuesday 5th April
14:00 - 16:00

TEICHERT Ines *(1), BEIER Anna (1), RADCHENKO Daria (1), NORDZIEKE Steffen (1), KÜCK Ulrich (1)*
(1) Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr University Bochum, Bochum, Germany

Structure-Function Analysis of the Fungal Striatin-Interacting Phosphatase and Kinase (STRIPAK) Complex

The STRIPAK complex is a conserved eukaryotic multiprotein complex first described in humans. In the filamentous ascomycete *Sordaria macrospora*, STRIPAK consists of protein phosphatase 2A (PP2A) scaffolding subunit PP2AA, catalytic subunit PP2Ac1, striatin homolog PRO11, striatin-interacting protein (STRIP) homolog PRO22, phocein homolog SmMOB3, germinal center kinases SmKIN3/24, and PRO45, homologous to sarcolemmal membrane-associated protein. STRIPAK complexes have been shown to function in regulation of cytokinesis and cell migration, and STRIPAK defects have been associated with various diseases such as diabetes and cancer. *S. macrospora* STRIPAK controls hyphal fusion and fruiting body formation, but single subunits have additional functions, e.g. PRO22 for ascogonial septation. To gain further insight into the mechanism of STRIPAK, we performed functional analysis of subunits PP2Ac1, PRO22, and PRO45 by domain deletion, domain swapping creating fungal-human chimeras, and point mutations defining active sites of catalytic domains. Our results will provide mechanistic insights into STRIPAK functions and interactions in a fungal model and will be relevant for higher eukaryotes including humans.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T13

Tuesday 5th April
14:00 - 16:00

FAN Feiyu (1), MA Guoli (1), LI Jingen (1), LIU Qian (1), BENZ J.philipp (2), ***TIAN Chaoguang*** *(1), MA Yanhe (1)*
(1) Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, China
(2) Holzforschung München, TUM School of Life Sciences Weihenstephan, Technische Universität München, Freising, Germany.

Genome-wide analysis of the endoplasmic reticulum stress response during lignocellulase production in *Neurospora crassa*

Lignocellulolytic fungal cells suffer endoplasmic reticulum (ER) stress during lignocellulase synthesis; however, understanding of this integrated process on a genome-wide scale remains poor. Here, we undertook a systematic investigation of this process in *Neurospora crassa* (*N. crassa*) using transcriptomic analysis coupled with genetic screens. A set of 766 genes was identified as the ER stress response targets (ESRTs) in *N. crassa* under cellulose utilization conditions. Among these, the expression of 223 and 186 genes showed dependence on IRE-1 and HAC-1, respectively. A total of 527 available mutants for ESRT genes were screened, 249 of which exhibited ER stress susceptibility, including 100 genes with unknown function. Disruption of *ire-1* or *hac-1* in *N. crassa* did not affect transcriptional induction of lignocellulase genes by cellulose, but severely affected secretion of the corresponding enzymes. A global investigation of transcription factors (TFs) discovered three novel regulators (RES-1, RES-2, RRG-2) involved in lignocellulase secretion. Production of lignocellulases in *Δres-1* increased by more than 30% in comparison to WT, while secretion decreased by nearly 30% in strains *Δres-2* and *Δrrg-2*. Transcriptional profiling of the three TF mutants suggests they are deeply involved in lignocellulase secretion and ER stress response. Here, we determined the transcriptional scope of the ER stress response during lignocellulase synthesis in the model cellulolytic fungus *N. crassa*. Through genome-wide mutant screening and analysis, dozens of novel genes were discovered to be involved in the process. The findings of this work will be useful for strain improvement to facilitate lignocellulase and biomass-based chemical production.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T14

Tuesday 5th April
14:00 - 16:00

JUN Sang-Cheol (2), JAHNG Kwang-Yeop (2), ***HAN Kap-Hoon*** *(1), KIM Jong-Hwa (1)*
(1) Department of Pharmaceutical Engineering, Woosuk Univeristy, Wanju, South Korea
(2) Department of Life Sciences, Chonbuk National University, Jeonju, South Korea

The loss of MpkB MAPK activity does not affect mycotoxin production in *Aspergillus flavus* and *Aspergillus nidulans*

MAP kinase pathways play important roles in regulation of growth, development, and stress responses in most eukaryotes. *Aspergillus nidulans* mitogen-activated protein kinase (MAPK) encoded by *mpkB* was known to coordinate sexual development as well as secondary metabolism. Also, it had been reported that the *mpkB* gene would regulate sterigmatocystin (ST) gene expression and produce mycotoxin at low levels. However, in the results of our TLC investigation, we found that *mpkB* gene did not effect on the ST production and ST related gene expression. ST production of *ΔmpkB*, *ΔmkkB* and *ΔmpkB ΔmkkB* mutants in the veA+ background were not different with compared to wild type. Furthermore, MpkB constitutively activated mutant and MpkB constitutively inactivated mutant showed no significant effect on the ST production. The biosynthesis genes required for ST production (*aflR*, *stcE* and *stcU*) were constitutively expressed in each mutant of the MAPK module. Similarly, in *Aspergillus flavus*, MpkB ortholog *AflmpkB* mutant could not produce any sclerotia, and it produced normal level of aflatoxin B1. However, ST production of *mpkB* and *mkkB* mutants was remarkably delayed in the veA1 background, suggesting that the ST production is affected by the veA gene rather than *mpkB*. These data indicated that *mpkB* does not affect the expression of genes involved in mycotoxin production such as ST in *A. nidulans* or aflatoxin B1 in *A. flavus*. Our results suggest that the signal of MpkB MAPK and the mycotoxin production pathway were independent.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T15

Tuesday 5th April
14:00 - 16:00

SCHUMACHER Julia *(1)*
(1) IBBP, WWU Münster, Münster, Germany

Impact of light on differentiation and virulence of the gray mold fungus *Botrytis cinerea*

Botrytis cinerea has a worldwide distribution and is the causal agent of gray mold diseases in more than 200 plants species including high-value crops such as grape vine and strawberry. The fungus may reproduce asexually by forming macroconidia for dispersal (summer cycle) and sclerotia for survival (winter cycle); the latter also participate in sexual reproduction by bearing the apothecia. Light induces the differentiation of conidia and apothecia, while sclerotia are exclusively formed in the absence of light. In view of the impact of light on the life cycle of *B. cinerea* and the limited knowledge about light signaling in plant pathogenic fungi in general, we aim to investigate the network of photoreceptors, light-responsive transcription factors (LTFs) and chromatin modulators that regulates the differentiation programs in response to the light, pigment formation and virulence. Apart from targeted approaches i.e. the characterization of orthologs of known light regulators such as the White Collar complex (WCC) (Canessa et al. 2013; PLoS One 8:e84223), we pursue a forward genetics approach to identify further possibly *B. cinerea*-specific components of the regulatory network. Random mutagenesis by *Agrobacterium tumefaciens* is a powerful tool that has led to the identification of several virulence-associated genes including the GATA-type TF BcLTF1 (Schumacher et al. 2014; PLoS Genet 10:e1004040) and the SAGA complex component BcSPT3 (Giesbert et al. 2012; MPMI 25:481-495). Recently we found the gene *bckdm1* (the ortholog of *Aspergillus nidulans kdmA*; Gacek-Matthews et al. 2015, Mol Microbiol 96:839-860) tagged by the T-DNA in the mutant PA2810. BcKDM1 is a jumonji family protein with putative H3K36 demethylase activity. Targeted mutations of *bckdm1* confirmed its role for proper growth on synthetic media, virulence and light-dependent differentiation. The expression of a catalytically deficient protein (BcKDM1H360A) restored the growth but not the differentiation phenotype suggesting that the demethylase activity is specifically required for adequate light responses. Interestingly, KDM1 orthologs from other fungi (*A. nidulans*, *Alternaria alternata*, *Fusarium fujikuroi*) cannot replace BcKDM1 indicating that this protein exerts a specific function in the gray mold fungus.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T16

Tuesday 5th April
14:00 - 16:00

MARCOS Ana T. (1), RAMOS Maria S. (1), MARCOS Jose F. (2), CARMONA Lourdes (2), STRAUSS Joseph (3), ***CANOVAS David*** *(1)*
(1) Dept. Genetics, University of Sevilla, Sevilla, Spain
(2) Dept. Food Sciences, Institute of Agrochemistry and Food Technology (IATA), Valencia, Spain
(3) Dept. Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria

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

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

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T17

Tuesday 5th April
14:00 - 16:00

TILL Petra *(1), PUCHER Marion E. (1), MACH Robert L. (1), MACH-AIGNER Astrid R. (1)*
(1) Technical University of Vienna, Institute of Chemical Engineering, Department for Biotechnology and Microbiology, Gumpendorfer Str. 1a, A-1060, Vienna, Austria

A hitherto unknown hydrolase activator in *Trichoderma reesei*

As a potent producer of hydrolases for the degradation of plant biomass the filamentous fungus *Trichoderma reesei* is of crucial importance for several industrial applications. Hence, studies gaining knowledge about the regulatory mechanisms involved in the production of those plant cell wall-degrading enzymes (PCDE) are in the centre of scientific research. Recently, a hitherto unknown regulator with influence on the PCDE-production has been identified. The disruption of an intergenic region in *T. reesei* QM9414 by insertion of a marker cassette resulted in mutant strains with a reduction in both, cellulase and xylanase activity. At least one used algorithm for in silico analyses of this locus assigned the sequence as encoding for a gene. Hence, this regulator was considered to function as a hydrolase activator. As its impact on PCDE-production reminds of the main transactivator Xyr1 (Xylanase regulator 1) and the genomic organisation of the locus even suggests an interplay with Xyr1, the novel regulator was termed Hax1 (Hypothetical activator of Xyr1). Interestingly, in contrast to other regulators of the PCDE expression, Hax1 seems to lack any homologues even in closely related microorganisms.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T18

Tuesday 5th April
14:00 - 16:00

DOWNES Damien J. (1), ***TODD Richard*** *(1)*
(1) Department of Plant Pathology, Kansas State University, Manhattan KS, USA

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

The *Aspergillus nidulans* Zn(II)2Cys6 transcription factor TamA is widely conserved throughout the Ascomycetes. TamA binds the promoter of the key nitrogen assimilation gene *gdhA* directly via its DNA binding domain, while acting as a coactivator of the global nitrogen GATA transcription factor AreA independent of its DNA-binding motif at two other nitrogen utilization gene promoters. *gdhA* encodes NADP-glutamate dehydrogenase, which catalyzes formation of glutamate from 2-oxoglutarate and ammonium. To determine the physiological role of TamA, and to identify the TamA DNA binding and coactivator genome-wide regulatory networks we used RNA-Seq to compare global gene expression in *tamA*Δ complete loss-of-function and *tamAC90L* loss-of-DNA binding mutants. We show that TamA affects expression at some promoters as a DNA binding transcription factor, at some promoters independent of its DNA binding domain, and at others via both modes of action. Gene ontology networks analysis reveals that TamA regulates directly or indirectly genes for amide metabolism, nitrogen assimilation, carboxylic, oxo- and organic acid metabolism, transport, iron homeostasis, and secondary metabolism, as well as oxidoreductase genes. The TamA nitrogen metabolic and transport regulatory networks are mostly regulated via the coactivator mode of action, whereas the TamA iron homeostasis regulatory network is largely regulated via the TamA DNA-binding motif. The oxidoreductase and nitrogen assimilation pathway targets suggest that TamA may transcriptionally modulate levels of 2-oxoglutarate, which is an oxygen donor or co-substrate for oxidoreductases and the carbon skeleton for nitrogen assimilation to glutamate. In addition, TamA transcriptionally regulates multiple enzymes in glycolysis, gluconeogenesis, and the tricarboxylic acid cycle. These TamA-regulated carbon metabolism genes are involved in metabolism of precursors diverted for biosynthesis of all 20 protein-containing amino acids and ornithine. TamA also regulates anaplerotic enzyme genes for replenishing intermediates used for biosynthesis. Our data suggests that TamA coordinates homeostasis of both carbon skeletons and amino donation from glutamate for biosynthesis of amino acids, and compounds produced from amino acid precursors including iron siderophores and certain secondary metabolites.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T19

Tuesday 5th April
14:00 - 16:00

FENG Xuehuan (1), RAMAMOORTHY Vellaisamy (1), ***CALVO-BYRD Ana M*** *(1)*
(1) Department of Biological Sciences, Northern Illinois University, Dekalb, Illinois, USA

cpsA **regulates mycotoxin production, morphogenesis and cell wall biosynthesis in the fungus** ***Aspergillus nidulans***

The model filamentous fungus *Aspergillus nidulans* synthesizes a variety of secondary metabolites, including the mycotoxin sterigmatocystin (ST). The production of this toxin is positively controlled by veA, a global regulatory gene that also governs sexual and asexual development in *A. nidulans*. In the absence of veA (*ΔveA*), ST biosynthesis is blocked. Previously we performed random mutagenesis in a *ΔveA* strain and identified several revertant mutants that are able to synthesize ST, among them RM1. Complementation of RM1 with a genomic library revealed that the mutation occurred in the coding region of a gene, putatively involved in polysaccharide biosynthesis, designated as *cpsA*. While in the *ΔveA* genetic background deletion of *cpsA* restores ST production, in a veA wild-type background absence of *cpsA* reduces and delays ST biosynthesis by decreasing the expression of ST clustered genes. Furthermore, *cpsA* is also necessary for the production of other secondary metabolites, including penicillin, by affecting the expression of PN biosynthetic genes. Besides its role in secondary metabolism, *cpsA* is necessary for normal asexual and sexual development in this model fungus. Furthermore, chemical and microscopy analyses revealed that *cpsA* is required for normal composition and integrity of the *A. nidulans* cell wall, affecting biofilm formation and sensitivity to oxidative stress. Our studies confirmed that the *cpsA* gene product is the first functional hyaluronan synthase described in Ascomycetes, and its role in maintaining the integrity of the cell wall has a major influence on other fungal biological processes. The conservation of *cpsA* in other Ascomycetes suggests that *cpsA* homologs might have similar roles in other fungal species.

POSTER SESSION ABSTRACTS
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CS4T20

Tuesday 5th April
14:00 - 16:00

YU Yidong (1), GLENN Steven (2), WILL Cornelia (1), AMICH Jorge (3), SZEWCZYK Edyta (3), WANG Clay C. C. (2), ***KRAPPMANN Sven*** *(1)*
(1) Institute of Microbiology, Clinical Microbiology, Immunology and Hygiene, University Hospital Erlangen and Friedrich-Alexander University , Erlangen, Germany
(2) School of Pharmacy, University of Southern California, Los Angeles CA, USA
(3) Research Center for Infectious Diseases, Julius-Maximilians University Würzburg, Würzburg, Germany

In-depth characterization of the *Aspergillus fumigatus* mating-type system

Sexual reproduction of the human fungal pathogen *Aspergillus fumigatus* was assumed to be absent or cryptic until fertile crosses among geographically restricted environmental isolates were described in 2008. The existence of cryptic sexuality in this species had been proposed before, based on genomic and genetic analyses revealing the presence of mating-type idiomorphs (MAT1-1 and MAT1-2) and of several putative genes orthologous to recognized determinants of pheromone signalling, mating, karyogamy, meiosis, or fruiting body formation in the fertile species *Aspergillus nidulans*. Furthermore, the products of *the A. fumigatus MAT1-1* and *MAT1-2* genes had been proven to be functional in *A. nidulans*. We provided evidence for mating, fruiting body development, and ascosporogenesis accompanied by genetic recombination between unrelated clinical isolates of *A. fumigatus*, which revealed the generality and reproducibility of this long-time undisclosed phase in the lifecycle of this heterothallic fungus. We could also demonstrate that successful mating requires the presence of both mating-type idiomorphs MAT1-1 and MAT1-2, as does expression of genes encoding factors presumably involved in this process. Comprehensive transcriptional profiling studies reveal the depth of the MAT1-driven transcriptomes. Functional categorization of genes that are significantly up- or down-regulated in these transcriptomes led us to further investigation of candidate genes and gene clusters that are under control of the bipolar mating-type system, especially those involved in secondary metabolism, which are ideal for validation on the product level. Secondary metabolite profiling of recombinant strains that are deregulated or mis-regulated in sexual development confirms the association. Furthermore, functional analysis of a novel presumed mating-type gene MAT1-2-4 associated with the MAT1-2 idiomorph indicates its necessity for fruiting body formation, assigning the corresponding gene product a functional role in the mating process. By yeast two-hybrid screening we could identify a putative interactor for the product of MAT1-2-4, and further investigation of this interaction is currently ongoing.

POSTER SESSION ABSTRACTS
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CS4T21

Tuesday 5th April
14:00 - 16:00

MELLO-DE-SOUSA Thiago M. (1), MACH Robert L. (1), YAVER Debbie (2), ***MACH-AIGNER Astrid*** *(1)*
(1) TU Wien, Wien, Austria (2) Novozymes Inc., Davis, CA, USA

DNA methylation plays a crucial role in spontaneous degeneration of production strains

Strain degradation is an often-observed phenomenon in eukaryotic microorganisms and it spans from the loss of pathogenicity to the degeneration of production strains that are used in biotechnology. Nonetheless such phenomenon hampers various basic research approaches as well as biotechnological applications, it is only poorly understood. Genetic imprinting was considered as one possible explanation for this phenomenon. We investigated epigenetic effects on the production of cellulases in the industrially used saprobe *Trichoderma reesei*. For our study we used a moderate and a top production strain (and their respective (cel-) descendants) because their degeneration behaviour differs. The strains were analysed with regard to the chromatin status, histone modifications, and the impact of DNA methylation. We will present insights into the molecular mechanisms of the degeneration phenomenon in order to be able to develop strategies for both, the reconversion into the (cel+) phenotype as well as the prevention of the occurrence of the (cel-) phenotype.

POSTER SESSION ABSTRACTS
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CS4T22

Tuesday 5th April
14:00 - 16:00

STUDT Lena *(1), JANEVSKA Slavica (2), BÖDI Stefan (1), ARNDT Birgit (3), SULYOK Michael (4), HUMPF Hans-Ulrich (3), TUDZYNSKI Bettina (2), STRAUSS Joseph (1)*
(1) Department of Applied Genetics and Cell Biology, BOKU, University of Natural Resources and Life Science, Vienna, University and Research Center, Tulln, Donau, Austria
(2) Institute for Plant Biology and Biotechnology, WWUM, Münster, Germany
(3) Institute of Food Chemistry, WWUM, Münster, Germany
(4) Department for Agrobiotechnology, IFA, Tulln, Austria

The COMPASS component Ccl1 balances H3K4me2 at secondary metabolite gene clusters in the plant-pathogenic fungi *Fusarium fujikuroi* and *F. graminearum*

Fusaria are among the most important group of plant-pathogenic fungi infecting various economically important host plants. Two prominent members are the notorious rice pathogen *Fusarium fujikuroi* (teleomorph *Gibberella fujikuroi*) causing the bakanae disease, i.e. hyperelongated and chlorotic internodes, and the cereal crop pathogen *F. graminearum* (teleomorph *G. zeae*) responsible for severe epidemics of head blight on wheat. Both fusaria produce a similar but distinct spectrum of secondary metabolites (SMs) including pigments, mycotoxins as well as the natural growth hormones gibberellic acids (GAs) and the mycotoxin deoxynivalenol (DON) produced by *F. fujikuroi* and *F. graminearum*, respectively, which are both associated with the disease symptoms caused by these two fungi. Here, we investigated the role of Ccl1, a component of the COMPASS complex (Complex associated with Set1) involved in methylation of histone 3 lysine 4 (H3K4), in fungal development and regulation of secondary metabolism in both fusaria. While hyphal growth and asexual development were only slightly affected, production of several SMs was altered upon deletion of CCL1. Cross-complementation experiments indicate functional conservation of Ccl1 as phenotypes of the respective *Δccl1* mutants were rescued in both fungi. Production of the virulence factors, i.e. GA and DON in *F. fujikuroi* and *F. graminearum*, respectively, is significantly decreased in CCL1-deficient mutants. However, both fungi still show a wild type-like infection pattern and cause typical disease symptoms on their respective hosts, an observation that goes in line with the remediation of GA and DON production in planta. Notably, overall H3K4 trimethylation (H3K4me3) was significantly decreased upon deletion of CCL1 in both fungi, while H3K4 mono- and dimethylation levels were not affected. However, no significant changes in H3K4me3 were observed at the respective SM gene loci. Instead we found elevated H3K4me2 levels at some of the SM gene clusters in the CCL1 deletion mutants, suggesting that Ccl1 plays a pivotal role in balancing H3K4me2 levels.

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CS4T23

Tuesday 5th April
14:00 - 16:00

HORTSCHANSKY Peter (2), SCHAFFERER Lukas (1), JÖCHL Christoph (1), BRAKHAGE Axel A. (2), ***HAAS Hubertus*** *(1)*
(1) Division of Molecular Biology/Biocenter, Medical University of Innsbruck, Innsbruck, Austria
(2) Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology (HKI), and Friedrich Schiller University Jena, Jena, Germany

Interaction of the CCAAT-Binding Complex and HapX in Iron Regulation in *Aspergillus fumigatus*

To sustain iron homeostasis, microorganisms evolved fine-tuned mechanisms for uptake and storage of the essential but toxic metal iron. In the opportunistic fungal pathogen *Aspergillus fumigatus*, the bZIP-type transcription factor HapX, the inactivation of which attenuates virulence, mediates adaption to both iron starvation and iron excess. The HapX N-terminal amino acid sequence predicts interaction with the DNA-binding, heterotrimeric CCAAT-binding complex (CBC), which is conserved in all eukaryotes. Here, we characterized the combinatorial CBC/HapX promoter recognition of genes involved in iron regulation of *A. fumigatus*. Whole transcriptome sequencing during iron starvation conditions revealed that CBC-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 either HapX or CBC derepressed genes involved in iron-consuming pathways, but decreased genes involved in siderophore production as well as secondary metabolism, strongly indicating cooperation of the CBC and HapX in regulation of these genes. In agreement, inactivation of the CBC was epistatic to HapX-deficiency. Taken together, these data indicate that the CBC is essential for both the activating and repressing functions of the iron-regulatory transcription factor HapX. Recently, bipartite CBC/HapX DNA-binding motifs were characterised in a limited number of target promoters in *Aspergillus nidulans* and *A. fumigatus*. Here, identification of phylogenetically conserved, CBC/HapX DNA binding motifs by MEME analysis in numerous *A. fumigatus* target promoters combined with in vitro (surface plasmon resonance) and in vivo (ChIP) analysis of the CBC/HapX/DNA interaction revealed an astonishing plasticity of the CBC/HapX DNA recognition mode.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T24

Tuesday 5th April
14:00 - 16:00

NORDZIEKE Steffen *(1), RUGER-HERREROS Macarena (1), LIMÓN M. Carmen (1), AVALOS Javier (1)*
(1) University of Seville, Seville, Spain

Molecular identification of transcriptional regulators of carotenoid biosynthesis in the fungus *Fusarium fujikuroi*

Carotenoids include over 600 different terpenoid pigments widely distributed in nature, produced by bacteria, plants, and fungi. Their diverse individual abilities include coloration, protection against light induced or oxidative damage, or their use as a source for other physiologically active compounds, reflecting their importance for all classes of eukaryotic life. The plant pathogen Fusarium fujikuroi serves as a fungal model system to study carotenoid biosynthesis since the 1980's. Despite the identification of all structural genes needed for the production of carotenoids, the molecular basis of the regulation of these genes has not been sufficiently elucidated. A major up-regulatory signal for carotenoid biosynthesis is light, a response mediated at transcription level by the White Collar protein WcoA. Based on mutant phenotypes, we postulate the occurrence of other specific transcriptional regulators of carotenoid biosynthesis in addition to WcoA. These regulators are expected to interact with the already described negative regulator CarS and/or with the promoters of target carotenogenic genes. Based on predicted characteristics attributed to such proteins, co-Immunoprecipitation, pull-down assays, and heterologous expression in *S. cerevisiae* are currently used for their identification. The obtained results may contribute to a better understanding of the regulatory networks controlling carotenoid biosynthesis in fungi.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T25

Tuesday 5th April
14:00 - 16:00

SCHEVEN Mareike *(1), MISSLINGER Matthias (2), HORTSCHANSKY Peter (1), HAAS Hubertus (2), BRAKHAGE Axel A. (1)*
(1) Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute (HKI) and Friedrich Schiller University, Jena, Germany
(2) Division of Molecular Biology, Biocenter, Innsbruck Medical University, Innsbruck, Austria

Iron regulation in pathogenic fungi: Functional domain analysis of the central regulator HapX in *Aspergillus fumigatus*

Aspergillus fumigatus is a ubiquitous saprophytic mould, which is capable to cause life-threatening diseases in immunocompromised patients. During infection, sufficient iron supply is crucial for fungal growth. Iron is a vital nutrient, but can be harmful in excess by triggering the formation of cell damaging reactive oxygen species. As a result, *A. fumigatus* has evolved fine-tuned mechanisms to maintain iron equilibrium. Adaptation to iron limitation is mediated by the bZIP transcription factor HapX, which represses iron consuming pathways and activates iron uptake. Additionally, HapX contributes to resistance against iron excess by activation of vacuolar iron storage. For gene repression during iron starvation and activation of iron detoxification, the physical interaction of HapX with the heterotrimeric CCAAT-binding complex (CBC) is essential. Currently, it is unclear whether cooperation of HapX with the CBC is also required for gene activation under low-iron conditions. Via surface plasmon resonance interaction analysis using recombinant *A. fumigatus* CBC and HapX proteins that included deletion of the CBC-binding domain or mutation of four amino acids within the DNA-binding domain, we demonstrate here that both the CBC-binding and DNA-binding domains of HapX are mandatory for combinatorial sequence-specific DNA-binding of the CBC and HapX in vitro. In agreement, lack of the HapX CBC-binding domain or mutation of the HapX bZIP domain phenocopied HapX-deficiency in vivo. Interestingly, however, deletion of the CBC-binding domain had a greater impact on siderophore biosynthesis than mutation of the DNA-binding domain. In summary, these data provide first evidence for combinatorial DNA-binding of HapX with the CBC to activate siderophore biosynthesis during iron starvation.

POSTER SESSION ABSTRACTS
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CS4T26

Tuesday 5th April
14:00 - 16:00

MARIAN Ioana (1), SCHULLER Margo (1), PARK Hongjae (2), CHOI In-Geol (2), LUGONES Luis (1), WÖSTEN Han (1), GRIGORIEV Igor (3), ***OHM Robin*** *(1)*
(1) Utrecht University, Utrecht, Netherlands
(2) Korea University, Seoul, Korea
(3) US DOE Joint Genome Institute, Walnut Creek, CA, USA

Leveraging the diversity in the hypervariable species *Schizophyllum commune* to understand mushroom development and lignocellulose degradation

Schizophyllum commune is a wood-decaying fungus and is used as a model system to study mushroom development and lignocellulose degradation. A large number of wild isolates of *S. commune* have been isolated from all over the world. These strains show a large diversity in phenotypes. For example, some strains develop mushrooms in high CO2 concentrations, which usually inhibit mushroom development. This trait shows mendelian inheritance, allowing it to be mapped by genome sequencing and bulk segregant analysis. Two strains with different wood-decaying properties were sequenced, from Tattone (France) and Loenen (The Netherlands). Sequence comparison shows remarkably high sequence diversity between the strains. The overall SNP rate of > 100 SNPs/kb is among the highest rates of within-species polymorphisms in Basidiomycetes. Some well-described proteins like hydrophobins and transcription factors have less than 70% sequence identity among the strains. Gene expression on glucose, cellulose and wood was analyzed in two *S. commune* strains. Overall, gene expression correlated between the two strains, but there were some notable exceptions. Of particular interest are CAZymes (carbohydrate-active enzymes) that are regulated in different ways in the different strains. Moreover, a large number of hypothetical genes were strongly up-regulated on cellulose and/or wood, and these may encode novel enzymes involved in lignocellulose degradation. Proteomics analyses of the secretome of these strains during growth on various carbon sources provided additional insight, as well as additional potentially novel enzymes. In both strains the transcription factor TF1 was strongly up-regulated during growth on cellulose and wood, when compared to glucose. A knock-down of TF1 resulted in an inability to grow on cellulose, as well as decreased cellulase activity, which suggests that TF1 is involved in regulating CAZyme gene expression.

POSTER SESSION ABSTRACTS
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CS4T27

Tuesday 5th April
14:00 - 16:00

VALIANTE Vito *(1), BALDIN Clara (2), HORTSCHANSKY Peter (2), THYWIßEN Andreas (2), STRAßBURGER Maria (3), HEINEKAMP Thorsten (2), BRAKHAGE Axel A. (2)*
(1) LRC Junior Research Group - Biobricks of Microbial Natural Product Syntheses, Leibniz-Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena, Germany
(2) Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena, Germany
(3) Transfer Group Anti-infectives, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena, Germany

The DHN-melanin production in *Aspergillus fumigatus* is regulated by RlmA (MEF2-like) and DevR (bHLH) transcription factors

Aspergillus fumigatus is one of the most prominent human fungal pathogens. This fungus normally proliferates on compost and produces numerous spores that can reach and invade immunocompromised hosts. The ability to easily adapt to the host environmen also relies on the production of metabolites that facilitate the protection of the fungus against the host defence mechanisms. Melanins are molecules that play an important role in protecting organisms against external hazards. In several pathogenic fungi, melanin production was shown to be essential for virulence. *A. fumigatus* produces two different types of melanins. One of them, the dihydroxynaphthalene (DHN)-melanin, is classified as secondary metabolite, and is mainly produced during sporulation. It is responsible for the dark grey-green pigmentation of *A. fumigatus* conidia and was proven to contribute to the fungus" pathogenicity. The DHN-melanin biosynthesis pathway contains six genes grouped in a cluster. The central gene in the cluster is *pksP*, which codes for a polyketide synthase. Promoter analyses identified specific DNA binding sites in the *pksP* promoter region that can be potentially recognised by basic helix-loop-helix (bHLH) and myocyte enhancer factor-2 (MEF2-like) transcriptional regulators. Independent and combined deletion of two genes coding for the transcription factors DevR and RlmA disturbed sporulation and melanisation. Furthermore, both transcription factors were proven to control the expression of the DHN-melanin gene cluster. *In vitro* surface plasmon resonance interaction analysis indicated that the computationally predicted binding sites were recognized by the respective transcriptional regulators with high affinity and specificity. Moreover, *in vivo* experiments with targeted mutations of the *pksP* promoter, combined with either deletion or over-expression of the transcriptional regulators, confirmed that both factors cooperatively regulate melanin biosynthesis genes. Additionally, these experiments revealed that DevR and RlmA both act as repressors and activators, depending on the recognised DNA binding motif, suggesting a highly regulated mechanism for DHN-melanin production. Taken together, the presented results revealed a novel mechanism of gene regulation for general bHLH and MEF2-like transcription factors.

POSTER SESSION ABSTRACTS
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CS4T28

Tuesday 5th April
14:00 - 16:00

GADERER Romana (1), FLIPPHI Michel (2), SEIDL-SEIBOTH Verena (1), ***KAPPEL Lisa*** *(1)*
(1) Research Division Biotechnology and Microbiology, Institute of Chemical Engineering, TU Wien, Vienna, Austria
(2) Department of Biochemical Engineering, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

RON1, an Ndt80-like transcription factor, is a key regulator of the final steps of chitin catabolism in filamentous fungi

Chitin, a homopolymer assembled from N-acetylglucosamine (GlcNAc) units, is present in the inner layer of the fungal cell wall and is the major constituent of insect and crustacean exoskeletons. In scarce environments chitin can serve a vital role as C- and N-source for fungi. Chitin degradation involves chitinases and N-acetylglucosaminidases (NAGs) which release GlcNAc that is further converted to fructose-6-phosphate. The final steps for GlcNAc catabolism had so far only been studied in yeast *Candida albicans*. Recently we could show that in ascomycete filamentous fungi the three GlcNAc catabolic genes are clustered similar to *C. albicans*. Interestingly, the cluster often contains an additional gene encoding an Ndt80-like transcription factor, named RON1 (regulator of N-acetylglucosamine catabolism 1). Moreover, a gene for a glycoside hydrolase 3 protein related to bacterial N-acetylglucosaminidases can be found in the cluster in filamentous fungi. Functional analysis in *Trichoderma reesei* showed that the transcription factor RON1 is a key activator of the GlcNAc genes and essential for GlcNAc catabolism. Furthermore, RON1 induces expression of genes upstream of GlcNAc catabolism, *nag1* and *nag2*, which are important for cleavage of chitobiose, the GlcNAc dimer. Thus, RON1 is an essential player in controlling chitin catabolism. Advancing our understanding of the regulation of chitin metabolism in fungi is not only relevant for investigations of natural chitin turnover in soil and marine habitats, but also crucial for the potential transfer of this knowledge to biotechnological processes to use this highly abundant, underexploited biopolymer in biorefinery processes.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T29

Tuesday 5th April
14:00 - 16:00

IVANOVA Christa *(1), AOUAM Thiziri (1), SEIBOTH Bernhard (2), RAMONI Jonas (2), FRISCHMANN Alexa (2), MARGEOT Antoine (1), BAKER Scott (3), LE CROM Stéphane (4), BIDARD Frédérique (1)*
(1) IFP Energies nouvelles, Paris, France
(2) Research Division Biotechnology and Microbiology, Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria
(3) Environmental Molecular Sciences, Pacific Northwest National Laboratory, Washington, USA
(4) Sorbonne Universités, UPMC Université Paris 06, Institut de Biologie, Paris, France

Genome sequencing and transcriptome analysis of *Trichoderma reesei* QM9978 reveals vib1 to be essential for cellulase induction

The hydrolysis of biomass to simple sugars used for the production of biofuels requires the action of cellulolytic enzyme mixtures. *Trichoderma reesei*, the main source for industrial cellulase and hemicellulase cocktails, has been subjected to several rounds of classical mutagenesis with the aim of higher production levels. These random mutagenesis events produced cellulase-negative strains as a by-product. Sequencing of one of those strains, QM9978, was used for the identification of mutations underlying the cellulase-negative phenotype. A surprisingly low number of mutagenic events in the promoter and coding regions of genes was detected in the strain QM9978, when compared to its progenitor *T. reesei* QM6a. Altogether, we identified 23 single nucleotide variants, nine indels and one translocation. The identified translocation led to a break in the promoter region of the putative transcription factor vib1. Transcriptomic analysis revealed that vib1 expression is abolished, linking the translocation to a null phenotype. Overexpression and introduction of vib1 under the control of the wild-type promoter version in QM9978 restored cellulase expression, thus confirming that vib1 is required for cellulase expression. Gene deletion in the moderate producer *T. reesei* QM9414 rendered this strain cellulase-negative, whereas vib1 deletion in the high producer strain RutC30 had no effect on cellulase expression. Since RutC30 is additionally carbon catabolite derepressed due to a truncation in the main negative regulator cre1 and studies performed on *Neurospora crassa* (Xiong et al., 2014) show vib1 to be involved in carbon sensing we assume that a similar functional relationship between vib1 and cre1 exists in *T. reesei*. Overexpression of vib1 in QM9414 and RutC30 had no effect, most likely because natural vib1 expression is already sustaining maximum cellulase production. We conclude that the translocation event in QM9978 results in abolished vib1 expression and propose that in *T. reesei*, vib1 acts upstream of cre1, alleviating carbon catabolite repression and thus inducing cellulase expression. The data presented here show an example of how the genome sequencing approach linked to transcriptomic studies can lead to an explanation of a specific trait, in this case the QM9978 cellulase-negative phenotype.

Yi Xiong, Jianping Sun, N. Louise Glass (2014) Plos Genetics 10: e1004500

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T30

Tuesday 5th April
14:00 - 16:00

NIU Jing *(1), HOMAN Tim G. (1), ARENTSHORST Mark (1), DE VRIES Ronald P. (2), VISSER Jaap (3), RAM Arthur F.j. (1)*
(1) Leiden University, Institute of Biology Leiden, Molecular Microbiology and Biotechnology, Leiden, Netherlands
(2) Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre & Fungal Molecular Physiology, Utrecht University, Utrecht, Netherlands
(3) Fungal Genetics and Technology Consultancy, Wageningen, Netherlands

The interaction of induction and repression mechanisms in the regulation of galacturonic acid-induced genes in *Aspergillus niger*

Aspergillus niger is an important industrial fungus expressing a broad spectrum of pectinolytic genes. The main constituent of pectin, polygalacturoninc acid (PGA), is degraded into galacturonic acid (GA) by the combined activity of endo- and exo-polygalacturonases some of which are specifically induced by GA. The regulatory mechanisms that controls the expression of genes encoding PGA degrading enzymes is not well understood. Based on genome-wide expression profiles, we selected five genes that are specifically induced by GA. These genes include three exo-polygalacturonases (*pgaX*, *pgxB* and *pgxC*), a GA transporter (*gatA*) and an intracellular enzyme involved in GA metabolism (*gaaB*). These five genes contain a conserved motif (5"-TCCNCCAAT-3") in their promoter regions which we named GARE (galacturonic acid responsive element). Promoter deletion and site-directed mutagenesis studies for the *pgaX* gene showed that the conserved element is required for the induction in response to the presence of GA. A set of promoter reporter strains was constructed by fusing the promoter region of the five above mentioned genes to the amdS reporter gene. Expression of the *amdS* gene is quantitatively correlated with ability to utilize acetamide as an N-source, hence higher expression of amdS improves growth of the strain on acetamide and therefore can be used as an in vivo reporter for gene expression. Growth analysis of the reporter strains indicate that four genes (*pgaX*, *pgxB*, *pgxC* and *gatA*) are specifically induced by GA. The in vivo promoter reporter strains were also used to monitor carbon catabolite repression control (CCR). All promoter-reporter genes analysed, except for *gaaB* were repressed by glucose in a glucose concentration dependent way. Interestingly, the strength of glucose repression was different for the different promoters. CreA is important in mediating the carbon repression as deletion of the creA gene in the reporter strains abolished carbon repression for most promoters as expected. Interestingly, the *pgxC* promoter was still repressed by glucose even in the creA null background, suggesting alternative repression mechanisms. Finally, we showed that low concentrations of GA are required to induce gene expression of pgaX, pgxB and pgxC even under derepressing conditions. The results obtained are consistent with a model in which a GA-specific transcription factor is activated by GA or a GA-derivative which binds to the conserved motif, possibly in combination with the HAP-complex, to drive GA specific gene expression.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T31

Tuesday 5th April
14:00 - 16:00

JANEVSKA Slavica *(1), STUDT Lena (1), TUDZYNSKI Bettina (1)*
(1) Institute of Plant Biology and Biotechnology, University of Münster, Münster, Germany

The H3K4 methylation mark is essential for vegetative growth, sporulation, secondary metabolism and pathogenicity in *Fusarium fujikuroi*

The rice pathogen *Fusarium fujikuroi* is well-studied due to its ability to produce highly bioactive plant hormones, gibberellic acids, that cause the bakanae disease of rice plants. In addition, *F. fujikuroi* possesses the powerful potential to synthesize a vast range of other secondary metabolites (SMs), with 47 SM key enzymes being encoded by the fungal genome [1]. However, the majority of the corresponding products is not known to date, particularly due to gene clusters being silent under standard laboratory conditions. The activation of these «cryptic» gene clusters can be achieved via genetic manipulation of SM biosynthesis on different regulatory levels. The manipulation of chromatin-mediated regulation, the hierarchically highest regulation level, represents an especially powerful tool, as a wide range of different SM gene clusters can be affected both in a positive and negative manner. In the present work, we focused on the histone 3 lysine 4 (H3K4) methylation mark which is generally associated with euchromatin and thus, transcriptional activation. The complex of proteins associated with Set1 (COMPASS) is responsible for conferring H3K4 methylation, being highly conserved from yeast to humans. Among the complex partners, the methyltransferase Set1 catalyzes the H3K4 methylation [2]. We show that the *F. fujikuroi* Set1 homolog, FfSet1, represents the major H3K4 methyltransferase in this fungus. The respective deletion mutant *Δffset1* is viable, however, strongly affected in its vegetative growth on both complex and minimal media. Furthermore, conidia formation is fully abolished in the deletion mutant. Intriguingly, *Δffset1* exhibits a strongly altered SM profile, concerning both the known as well as the unknown SMs. In vitro, production of gibberellic acids is completely downregulated, going in line with a decreased pathogenicity of *Δffset1* in rice infection assays. Finally, as a counterpart of FfSet1, two putative H3K4 demethylases are under investigation, in order to further shed light onto the role of H3K4 methylation in the regulatory network of gene expression.

[1] Wiemann P. et al. (2013) PLoS Pathog., 9, e1003475.
[2] Shilatifard A. (2012) Annu. Rev. Biochem., 81, 65-95.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T32

Tuesday 5th April
14:00 - 16:00

KÜES Ursula *(1), SUBBA Shanta (1), YU Yidong (1), SEN Mandira (1), KHONSUNTIA Weeradej (1), SINGHADAUNG Wassana (1), LANGE Karin (1), VOIGT Oliver (1), LAKKIREDDI Kiran (1)*
(1) University of Göttingen, Büsgen-Institute, Göttingen, Germany

Regulation of fruiting body development in *Coprinopsis cinerea*

Coprinopsis cinerea is an excellent model to study genetics of fruiting body development in the Agaricomycetes. Fruiting follows a conserved scheme defined by day and night phases, with well predictable distinct stages over the time. It starts with primary hyphal knot formation in the dark, followed by aggregation into compact secondary hyphal knots in which stipe and cap tissues differentiate. Primordia development (stages P1 to P5) takes five days to culminate on day 6 of development in karyogamy and meiosis within the basidia and subsequent basidiospore production which parallels fruiting body maturation (stipe elongation and cap expansion). *matA* genes control fruiting body initiation at the stages of primary and secondary hyphal knot formation and the decision to enter the alternate pathway of sclerotia formation. *matA* induced steps in fruiting end at the P5 state. Activation of the *matB* genes is required for karyogamy and fruiting body maturation. *matB* functions as a mediator between light, nutritional signals and matA control of development. Homokaryotic strains with defects in the mating type loci are useful in generation of mutants in fruiting. Mutations do not evenly distribute over the pathway of fruiting body development. High mutant numbers are available from the early developmental stages up to stage P1, comparably few in the subsequent steps from P1 to P5 and again high numbers in the later processes occurring normally on day 6 for fruiting body maturation and sporulation. Mutants with defects in primary and secondary hyphal knot formation helped before by complementation to clone genes *cfs1* and a gene of the NWD2 family. In our studies we focus on genes that act at the first stages in regulation and light control of fruiting.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T33

Tuesday 5th April
14:00 - 16:00

KHONSUNTIA Weeradej (1), DÖRNTE Bastian (1), ***KÜES Ursula*** *(1)*
(1) University of Göttingen, Büsgen-Institute, Göttingen, Germany

Characterization of some developmental regulators in the mushroom *Coprinopsis cinerea*

Putative regulator genes involved in signalling in developmental processes in *Coprinopsis cinerea*, FLU1-II, crg1 and NWD2, are being investigated. In *C. cinerea*, there are two FLU1-II genes related to *Aspergillus* fluG that code for proteins with a glutamine synthase I (GSI)-like domain domain. *C. cinerea* Crg1 possesses two DEP (Dishevelled, Egl-10, and Pleckstrin) domains which function in subcellular localization, and a C-terminal regulator of G-protein signaling (RGS) domain. The *C. cinerea nwd2* gene encodes a signal transduction protein of the NACHT-NTPase family and has been found to suppress a defect in primary hyphal knot formation (*pkn1*) of *C. cinerea* mutant Proto159. We are investigating the functions of the three genes in vegetative growth of mono- and dikaryons, oidiation, mating, and fruiting body formation, facilitated by overexpression and by homologous gene targeting using a *Δku70 C. cinerea* monokaryon that is inactivated in the non-homologous end joining pathway.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T34

Tuesday 5th April
14:00 - 16:00

KÜES Ursula *(1), YU Yidong (2)*
(1) University of Göttingen, Büsgen-Institute, Göttingen, Germany

The A42 and A43 mating type alleles in *Coprinopsis cinerea*

Coprinopsis cinerea has two multi-allelic mating type loci (*matA* and *matB*) that control different steps in sexual development of the fungus. There are an estimated 160 different matA alleles in nature. The matA locus encodes two types of homeodomain transcription factors, HD1 and HD2. For sexual regulation, HD1 and HD2 proteins from different *matA* alleles have to interact with each other to give transcription factor complexes that are transferred into the nucleus. *HD1* and *HD2* genes are divergently transcribed in pairs and there are three or more paralogous gene pairs in the alleles of the *matA* locus. *matA* alleles can differ in the numbers of *HD1* and *HD2* genes present. Here, we present the structures and interactions of two fully sequenced *matA* alleles, i.e. A42 and A43. Gain and loss of genes and gene inactivation by mutations within *matA* are observed.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T35

Tuesday 5th April
14:00 - 16:00

RASSINGER Alice *(1), MELLO-DE-SOUSA Thiago (1), REGNAT Katharina (1), DERNTL Christian (1), MACH Robert (1), MACH-AIGNER Astrid (1)*
(1) Institute of Chemical Engineering/Vienna University of Technology, Vienna, Austria

Impact of inducer molecules on DNA accessibility in cellulase and xylanase gene expression in *Trichoderma reesei*

The ascomycete *Trichoderma reesei* produces industrially applied, plant cell wall-degrading enzymes. The transactivator Xyr1 (encoded by xyr1), the repressor Cre1, and gene-specific transcription factors regulate the expression of two major cellulases (encoded by cbh1 and cbh2) and xylanases (encoded by xyn1 and xyn2) amongst those enzymes. On D-glucose, Cre1 mediates carbon catabolite repression (CCR), which leads to a down-regulation of expression of xyr1 and of both cellulase and xylanase-encoding genes. Inducer substances such as sophorose and/or D-xylose achieve an induction of gene expression of the respective enzymes. Transcription factors do not act solely in the induction mechanism, the chromatin packaging plays also an essential role. Therefore, the chromatin status in two upstream regulatory regions (URRs) of xyr1, cellulase and xylanase-encoding genes was investigated by chromatin accessibility real-time PCR (CHART-PCR) in the wild-type strain and in the CCR-released industrial ancestor strain Rut-C30. The cellulases show interestingly no remarkable changes in chromatin on repressing and inducing conditions in both strains, whereas the xylanases do. However, together with in vivo footprinting analyses we detected differences in protein-DNA interactions particularly for xyn2 depending on the applied inducer in the wild-type. Using sophorose, the protein-DNA interactions on the functional URR of xyn2 were similar in the wild-type strain and Rut-C30.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T37

Tuesday 5th April
14:00 - 16:00

PIDRONI Angelo *(1), BAUER Ingo (1), VERGEINER Stefan (1), GROSS Silke (1), BROSCH Gerald (1), HERMANN Martin (2), GRAESSLE Stefan (1)*
(1) Division of Molecular Biology, Innsbruck Medical University, Innsbruck, Austria
(2) Department of Anesthesiology and Critical Care Medicine, Innsbruck Medical University, Innsbruck, Austria

Class 1-type histone deacetylases in filamentous fungi: Essential enzymes with fungal specific properties

The rapid increase of invasive fungal infections and growing resistance of fungal pathogens to conventional antifungal therapies result in high mortality rates that demand novel strategies to combat systemic mycosis. Due to adverse effects of many of the antifungal substances known, toleration by patients is a major challenge in the discovery of new therapeutic approaches. Detailed knowledge of fungal-specific attributes supporting virulence, germination, invasion, dissemination or drug-resistance is a prerequisite to define novel targets for efficient antifungal drugs with no or only moderate side effects. In recent years, it has become more and more apparent that histone deacetylases (HDACs) play a decisive role in the regulation of genes involved in fungal growth, sporulation, pathogenicity, and production of important secondary metabolites. Moreover, one of these enzymes, the class 1 HDAC RpdA, was identified to be even crucial for growth and development of *Aspergillus nidulans* [1]. Analysis of strains expressing different mutated RpdA variants revealed, that a fungal specific, C-terminal region is required for the biological function of the enzyme. Since RpdA, like most classical HDACs, functions as part of large protein complexes within the nucleus, we expressed tagged RpdA variants in order to analyze their cellular location and catalytic activity.
Interestingly our investigations clearly demonstrate, that a few charged residues within and adjacent to the essential fugal specific motif are required for both, nuclear targeting and catalytic activity of RpdA and thus cannot be deleted or substituted without leading to an atrophic phenotype with a drastic restriction in radial growth of the mutant strains. Considering these results, RpdA with its fungal specific motif represents a promising target for novel HDAC-inhibitors that might, in addition to their growing significance as anti-cancer drugs, become important in the therapy of invasive fungal infections of immuno-compromised patients apart or in combination with drugs, administered within classical antifungal therapy regimes.

[1] Tribus, M. et al. Mol. Biol. Cell 21, 345-353 (2010).

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T38

Tuesday 5th April
14:00 - 16:00

LIM Joo-Yeon (1), KANG Eun-Hye (1), ***PARK Hee-Moon*** *(1)*
(1) Department of Microbiology & Molecular Biology, Chungnam National University, Daejeon, South Korea

VdpA, a homolog of the yeast survival factor Svf1, showed multiple effects on *Aspergillus nidulans* development

In *Aspergillus nidulans*, the VeA controls development of vegetative cells into asexual or sexual stage upon exposure of cells to the light and air. Previously, we had identified a novel developmental protein, VdpA (VeA-dependent protein A), a homolog of the yeast survival factor Svf1 by using proteome analysis. Phenotypic analyses with the vdpA-deletion mutant revealed the profound effects of the vdpA-deletion on the developmental processes. In vegetative stage, deletion mutant showed retarded growth and over-production of pigments both on solid medium. In asexual development, formation of abnormal conidiophores and decrease in conidia production were observed. In sexual stage, small and weak reddish cleistothecia with reduced number of asocospores were produced. The expression of developmental genes such as brlA, vosA and medA was also affected by the deletion of vdpA. We also found that the amino acid sequence identity between the VdpA and AfVdp1, a *Aspergillus fumigatus* homolog, was very high (76%) and that the Afvdp1 was able to complement the vdpA-deletion phenotype. These results suggested that the Afvdp1 might have similar functions with vdpA in *A. fumigatus*. Although further investigation is required, our results presented here suggest a novel VdpA-dependent regulatory pathway for *A. nidulans* development.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T39

Tuesday 5th April
14:00 - 16:00

KWON Soo Jeong (1), SONG Jae Ho (1), ***PARK Hee-Moon*** *(1)*
(1) Department of Microbiology & Molecular Biology, Chungnam National University, Daejeon, South Korea

Activation of the CDK inhibitor Rum1 in G1/S progression of cell-cycle by fission yeast LAMMER kinase

In our previous study, we reported that the fission yeast LAMMER kinase, Lkh1, controls the G1/S cell-cycle progression by phosphorylating Thr110 of Rum1, which acts as an inhibitor of the cyclin-dependent kinase Cdc2. The results from in vivo and in vitro experiments with wild type and T110A mutant form of Rum1 indicated the multiple phosphorylation of Rum1 by Lkh1. Analysis with NetPhosK 1.0 had revealed several putative Lkh1-dependent phosphorylation residues on Rum1, thus we introduced phospho-defective mutations in the putative Lkh1-dependent phosphorylation sites and tested their effects on phenotypic changes; Experiments such as observing cell morphology, in vitro pull down assay and DNA profiling using FACS showed that the amino acid residues, Thr5, Thr16, and Ser212, were not related to Lkh1 unlike Thr110. However, the amino acid residue S129 was identified as an additional Lkh1-dependent phosphorylation site on Rum1 by the phosphoprotein analysis with PMF using Rum1T110A. When the effects of single and double mutation of T110A and/or S129A was tested, The T110A mutation exerted stronger effect on the Lkh1-dependent phosphorylation of and thus the CDK inhibitor activity of Rum1.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T40

Tuesday 5th April
14:00 - 16:00

FISCHER Reinhard *(1), ALI Arin (1), HÜBNER Jennifer (1), YU Zhenzhong (1)*
(1) Karlsruhe Institute of Technology, Institute for Applied Biosciences, Dept. of Microbiology, Karlsruhe, Germany

Fungi use the SakA/HogA pathway for phytochrome-dependent light signaling

Bacteria often use two component systems (TCS) as phosphorylation relays to transmit environmental signals from the cell surface to the inner cell. In comparison, microbial eukaryotes use MAP kinase phosphorylation cascades, although TCS are commonly found in the fungal kingdom1. Interestingly, in the case of stress-sensing, fungi use a composite signaling cascade comprised of a TCS plus a downstream MAP kinase cascade to trigger gene expression. Besides osmolarity or oxidative stress, fungi sense many other environmental factors, one of which is light2,3. Light controls morphogenetic pathways but also the production of secondary metabolites such as penicillin. In the case of light sensing, a signaling cascade appears to be unnecessary, because light does not stop at the cell surface. However, here we show that phytochrome-dependent light signaling in *Aspergillus nidulans* uses the stress-sensing signaling cascade to transmit the signal from the cytoplasm into nuclei4. In a screening for blind mutants, the MAP kinase HogA/SakA was identified by whole-genome sequencing. The phytochrome FphA physically interacted with the histidine-containing phosphotransfer protein YpdA and caused light-dependent phosphorylation of the MAP kinase HogA/SakA and its shuttling into nuclei. In the absence of FphA, HogA/SakA still responded to osmotic stress but not to light. The HogA pathway thus integrates several stress factors and can be considered as a hub for environmental signals. So far, there was only phylogenetic evidence that phytochrome evolved from a bacterial two-component system. Here, we present experimental evidence that the fungal phytochrome indeed feeds its information into the HogA two-component system.

1- Bahn, Y., Xue, C., Idnurm, A., Rutherford, J. & Cardenas, M. E. Nat. Rev. Microbiol. 36, 57-69 (2007).
2- Rodriguez-Romero, J., Hedtke, M., Kastner, C., Müller, S. & Fischer, R. Annu. Rev. Microbiol. 64, 585-510 (2010).
3- Dasgupta, A., Fuller, K. K., Dunlap, J. C. & Loros, J. J. Environ. Microbiol. (2015).
4- Yu, Z., Armant, O. & Fischer, R. Nature Microbiol. In press. (2016)

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T41

Tuesday 5th April
14:00 - 16:00

SOYER Jessica *(1), GRANDAUBERT Jonathan (1), LOWE Rohan G. T. (2), LINGLIN Juliette (1), ROUXEL Thierry (1), FUDAL Isabelle (1)*
(1) BIOGER, INRA, AgroParisTech, Thiverval-Grignon, France
(2) School of Botany, The University of Melbourne, Victoria, Australia

In silico analysis of the transcription factor repertoire in *Leptosphaeria maculans* 'brassicae' to identify putative regulators of pathogenicity

The *Leptosphaeria maculans-Leptosphaeria biglobosa* species complex encompasses species showing variable abilities to infect oilseed rape (Brassica napus). Within this species complex, *L. maculans* 'lepidii' (Lml) and *L. biglobosa* 'thlaspii' (Lbt) are found on cruciferous weeds while *L. maculans* 'brassicae' (Lmb), *L. biglobosa* 'canadensis' (Lbc) and *L. biglobosa* 'brassicae' (Lbb) are specialized on oilseed rape though they show different abilities to cause damage and different lifestyles. Lmb is a hemibiotrophic fungus and the most devastating species towards oilseed rape while Lbb and Lbc show a necrotrophic lifestyle and have a limited impact on the host. This species complex is thus an excellent model to infer the regulatory networks involved in lifestyle specialization on the same host plant. Our study aims at identifying putative regulators involved in the infection strategy and the adaptation of *L. maculans* 'brassicae' towards oilseed rape. For this purpose, we established the repertoire of transcription factor (TF) genes of Lmb and assessed their conservation within the species complex. As Lmb exhibits a complex lifestyle compared to the *L. biglobosa* species adapted towards oilseed rape, the transcriptional behavior of the TF genes in Lmb was analyzed and compared to those of Lbc in order to highlight specifically induced regulators of Lmb compared to those of the necrotrophic species. Thirty-seven TF genes of Lmb were identified as being over-expressed in planta, including two TFs unique to Lmb and one showing a signature of accelerated evolution in Lmb. Within the 37 TF genes over-expressed in planta in Lmb, 23 were conserved in Lbc but showed a different transcriptional behavior. This in silico analysis of the Lmb TF genes now provides us with numerous candidates to analyze the regulatory networks involved in drastic changes in pathogenic programs during the complex life cycle of Lmb on oilseed rape.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T42

Tuesday 5th April
14:00 - 16:00

ZAMBORSZKY Judit (1), MATSUURA Toru (1), KWON Jaesang (1), BAEK Mokryun (1), CSIKASZ-NAGY Attila (2), ***HONG Christian*** *(1)*
(1) University of Cincinnati, Cincinnati, USA
(2) King's College London, London, UK

Interconnected network of circadian rhythms and DNA damage response

The maintenance of genome integrity is essential for organisms. Recent findings indicate bidirectional influence between DNA damage response (DDR) and circadian rhythms. In both *Neurospora crassa* and *Mus musculus*, activated DNA damage response checkpoint kinase, PRD-4 (CHK2), phosphorylates a core clock components (i.e. FRQ in Neurospora and PER1 in mouse) and induces phase advances of circadian rhythms. On the other hand, circadian rhythms modulate ATR-mediated DNA damage response. However, detailed understanding of this network between circadian rhythms and DNA damage response remain elusive. In this report, we demonstrate circadian gene expression of key DDR components, mus-21 (ATM) and prd-4 (CHK2) in *Neurospora crassa*. These oscillations are abolished in circadian arrhythmic mutant, frqko. More importantly, rhythmic expression of mus-21 and prd-4 result in distinct circadian time-dependent DNA damage responses, which transiently disrupts conidiation-banding patterns upon DNA damage. Our findings unravel optimized circadian clock-dependent operations of DNA damage response mechanisms via mus-21 and prd-4.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T43

Tuesday 5th April
14:00 - 16:00

RASCLE Christine *(1), DIERYCKX Cindy (1), GIRARD Vincent (1), POUSSEREAU Nathalie (1)*
(1) Microbiology, Adaptation and Pathogenesis Lab, University Lyon 1, CNRS, Bayer SAS Lyon, France

Adaptation to pH and pH signaling in the phytopathogenic fungus *Botrytis cinerea*

The infection strategy developed by *Botrytis cinerea*, an important fungal plant pathogen, is based partly on the modulation of ambient pH to ensure an optimal environment for virulence factors. *B. cinerea* is known as an « acidic « fungus since the first steps of the colonization process are associated with a pH decrease resulting from an accumulation of several organic acids. Fungi adapt to pH variations via the highly conserved signaling pathway Pal/Pac that leads to the activation of the zinc finger transcription factor PACC. Under alkaline/neutral conditions, this transcriptional regulator acts as an activator of genes expressed in alkaline conditions and a repressor of those expressed in acidic conditions. The homologues of the seven components of the pathway were identified in the genome of *B. cinerea* and a functional analysis of *PacC* gene was performed. The deletion mutant reveals a pleiotropic phenotype and an alteration of the virulence was observed. The role of this regulator in the biology of the fungus was investigated with a focus on the different steps of the infectious cycle of B.cinerea.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T44

Tuesday 5th April
14:00 - 16:00

BENOCCI Tiziano *(1), BENOIT Isabelle (1), SEIBOTH Bernhard (2), DE VRIES1 Ronald P. (1)*
(1) CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands
(2) Vienna University of Technology, Institute of Chemical Engineering, Vienna, Austria

The influence of regulatory mutations on growth of *Trichoderma reesei* on plant biomass related carbon sources

Fungi play a major role in the global carbon cycle by converting plant biomass (polysaccharides, proteins, and lignin) to monomeric components that they use as carbon source. For this, fungi produce a broad range of plant biomass degrading enzymes that are controlled by a network of regulators. These regulators also affect the metabolic pathways needed to metabolize the monomers released from the plant biomass degradation. In biotechnology, manipulating these regulatory systems is one of the possibilities to improve enzyme production and therefore lower overall costs in several applications. Most detailed studies into the function of these regulators were performed on defined substrates and during highly specific condition. The aim of this study is to explore the roles of these regulators in the conversion of crude plant biomass. For this, we deleted eight regulators (xyr1, cre1, bglR, clbR, clr-2, rhaR, ara1 and ace3) in *Trichoderma reesei* (Hypocrea jecorina), a major industrial enzyme producer. Comparison of growth of these eight knockout strains and the wild type on 27 different plant biomass related carbon sources, ranging from monosaccharides to crude substrates, revealed significant differences between them and hinted at a link between their enzyme profile and the substrate composition. Based on these results we compared their enzyme profiles on selected plant biomass substrates to further evaluate the effect of the regulator deletions.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T45

Tuesday 5th April
14:00 - 16:00

ALAZI Ebru *(1), RAM Arthur (1)*
(1) Microbial Biotechnology & Health, Institute of Biology Leiden, Leiden University, Leiden, Netherlands

Regulation of glycerol utilization in *Aspergillus niger*

Glycerol is a major by-product of biodiesel production and represents a cheap carbon source on which the filamentous fungus *Aspergillus niger* can grow and produce industrial enzymes and chemicals1,2. This project aims to understand the transcriptional regulation of glycerol utilization in *A. niger*. Unraveling this regulatory process would be rewarding in the design of bio-refineries where biodiesel production can be integrated with bioprocesses converting glycerol into valuable compounds by fungi, thereby valorizing the waste product glycerol. Transcription factors (TFs) involved in glycerol utilization in *A. niger* have not been identified yet. We made use of our collection of 250 TF deletion mutants in *A. niger*3, to screen for mutants with altered growth on glycerol. We identified several TF deletion mutants with impaired glycerol utilization. Expression of the genes involved in glycerol utilization was found to be severely affected by the deletion of some of these identified TFs.

[1] Abdella, A., El-Sayed Mazeed, T., Yang , S., El-Baz, A.F. 2014, Current Biotechnology, 3(2), 197-206.
[2] Andre, A., Diamantopoulou, P., Philippoussis, A., Sarris, D., Komaitis, M., Papanikolaou, S. 2010, Industrial Crops and Products, 31, 407-416.
[3] Arentshorst, M., Arendsen, Y., Pel, H., van Peij, N., Ram, A.F.J. Manuscript in prep.

POSTER SESSION ABSTRACTS
CS4T46

Tuesday 5th April
14:00 - 16:00

AERTS David *(1), HAEUR E (1), AERNTSHORTS M (2). TEERTSTRA WR (1), PHIPPEN C (3), NIELSEN KF (3), RAM AFJ (2), FRISVAD JC (3), WOSTEN HAB (1)*
(1) Microbiology, Department of Biology, Utrecht University, Utrecht, The Netherlands
(2) Department of Molecular Microbiology and Biotechnology, Institute of Biology Leiden, Leiden University, Leiden, The Netherlands
(3) Department of Systems Biology, Technical University of Denmark, Lyngby, Denmark

FumR of *Aspergillus niger* is involved in production of fumonisin and secreted proteins

The sporulation pathway of *Aspergillus niger* represses protein secretion. Colonies of this filamentous fungus secrete proteins throughout the colony except for the sporulating zone. Inactivation of the sporulation gene flbA results in colonies that are unable to reproduce asexually and that secrete proteins throughout the mycelium. In addition, the Δ*flbA* strain mutant strain shows cell lysis and has thinner cell walls. This pleiotropic phenotype is associated with differential expression of 38 transcription factor genes. Here, one of these regulatory genes, fumR, was inactivated. Whole genome expression analysis revealed that 8 out of 63 downregulated genes in Δ*fumR* are implicated in amino acid metabolism. In addition, 11 out of 15 genes of the fumonisin biosynthetic gene cluster were strongly downregulated in ΔfumR. This was accompanied by absence of fumonisin production in the deletion strain. When grown dispersed in liquid shaken cultures with xylose as a carbon source, the fumR deletion mutant showed reduced protein secretion and a different secretion profile when compared to the wild-type. This phenotype was complemented by adding amino acids to the medium. Taken together, it is concluded that fumR is involved in fumonisin production and amino acid production, the latter facilitating protein secretion. As such, fumR is an interesting lead for improving *A. niger* as a cell factory.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T47

Tuesday 5th April
14:00 - 16:00

KOWALCZYK Joanna (1), *Claire Khosravi (1), Samuel Purvine (2), Alice Dohnalkova(2), William B. Chrisler (2), Galya Orr (2), Errol Robinson (2), Erika Zink (2), Ad Wiebenga (1), Evy Battaglia (1), Isabelle Benoit (1), Scott Baker (2), Ronald P. de Vries (1)*

(1) Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre & Fungal Molecular Physiology, Utrecht University, Utrecht, Netherlands
(2) Environmental Molecular Sciences Laboratory, Department of Energy, Richland, USA

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

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

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T48

Tuesday 5th April
14:00 - 16:00

GAWLIK Joanna *(1), PIŁSYK Sebastian (2), SZCZĘSNY Paweł (2), KOPER Michał (3), WĘGLEŃSKI Piotr (4), DZIKOWSKA Agnieszka (3)*
(1) College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Warsaw, Poland
(2) Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
(3) Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Warsaw, Poland
(4) Centre of New Technologies, University of Warsaw, Warsaw, Poland

KAEA (SUDPRO), a member of the ubiquitous KEOPS/EKC protein complex, regulates the expression of several genes in *Aspergillus nidulans*

The kaeAKAE1 (suDpro) gene, identified in *Aspergillus nidulans* as a suppressor of proline auxotrophic mutations, encodes the orthologue of Saccharomyces cerevisiae Kae1p, a member of the evolutionarily conserved KEOPS/EKC (Kinase, Endopeptidase and Other Proteins of Small size/Endopeptidase-like and Kinase associated to transcribed Chromatin) complex. The complex was proposed to participate in chromatin organization and telomere maintenance (Downey et al., 2006; Bianchi and Shore, 2006), transcription (Kisseleva-Romanova et al., 2006) and tRNA modification (Srinivasan et al., 2010; Daugeron et al., 2011). The main function of KAEA seems to be the N6-threonylcarbamoyladenosine (t6A), modification of A37 in tRNAa decoding ANN codons, which plays a crucial role in translational fidelity through stabilization of the codon-anticodon interaction. The modification is one of the few tRNA modifications present in all organisms (de Crecy-Lagard et al., 2007). However, several effects of mutations in genes encoding subunits of the complex in yeast and human suggest additional function of the KEOPS/EKC. In *A. nidulans*, mutations in kaeA result in several phenotypic effects and changes in the expression levels of several genes, including the ones involved in siderophore and amino acid metabolism, sulfate transport, carbon/energy metabolism, translation, and transcription regulation, such as rcoATUP1, encoding the global transcriptional corepressor. It is possible that changes in the expression of some genes observed in kaeA mutant result from the upregulation of the rcoA gene in this strain. In *S. cerevisiae* Kae1p was shown to be located in the nucleus (Won-Ki Huh et al., 2003), what might suggest its function in transcriptional regulation. Localisation of KAEA in *A. nidulans* is under investigation.

Bianchi and Shore, 2006 Cell 124, 1125-1128; Daugeron et al., 2011 Nucl. Ac. Res. 39, 6148-6159; de Crecy-Lagard et al., 2007 IUBMB Life 59, 634-658; Downey et al., 2006 Cell, 124, 1155-1168; Kisseleva-Romanova et al., 2006 EMBO J. 25, 3576-3585; Srinivasan et al., 2010, EMBO J. 30, 873-881; Won-Ki Huh et al., 2003 Nature 425, 686-691

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T49

Tuesday 5th April
14:00 - 16:00

RODRIGUEZ-ROMERO Julio *(1), MARCONI Marco (1), WILKINSON Mark (1), SESMA Ane (1)*
(1) Centre for Plant Biotechnology and Genomics (CBGP), / Universidad Politécnica de Madrid, Madrid, Spain

Interconnections between 3'-UTR mRNA processing, TOR pathway and plant pathogenesis in the rice blast fungus.

The polyadenylation of mRNAs is a two-step process. Pre-mRNAs are first cleaved at their 3' end and then, the poly (A) tail is added by RNA polymerases during 3" end formation. Presence of multiple 3" end cleavage sites is common in eukaryotic genes, and the selection of a proper cleavage site represents an important step of regulation of gene expression. 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 regulates the length of 3"UTRs of transcripts with developmental and virulence-associated functions. In *M. oryzae*, Rbp35 regulates APA in ~25% of genes, and nearly 75% of then show a preference for proximal poly (A) sites. Significantly, Rbp35 regulates APA predominantly in genes related with signaling. These include regulatory proteins such as 14-3-3 and several genes related with Target of Rapamycin (TOR) pathway. , which is the most severely affected signalling pathway in Δrbp35, with at least eighteen genes of the pathway presenting altered 3"UTRs in carbon depleted cells. The Δrbp35 mutant lacks precision in the cleavage and shows an increase of proximal cut sites in pre-mRNAs. In addition, we have observed that APA is involved in regulating *M. oryzae* gene expression in response to nutritional fluctuations. A prominent feature of the 14-3-3 proteins is their ability to bind a multitude of functionally diverse signaling proteins, including kinases and phosphatases. In yeast, two 14-3-3 proteins have been shown to positively regulate rapamycin-sensitive signaling. *M. oryzae* has two proteins 14-3-3 (MGG_01588 and MGG_13806) and both mRNAs contain several polyadenylation sites. Both genes are required for full infection of leaves and roots. The 3"-UTRs of 14-3-3A and 14-3-3B shows a shortening during infection. In addition, delta mutants of 14-3-3A and 14-3-3B shows altered response under osmotic stress, possibly connected to MoMsn2 and MoOsm1 (Hog1) pathway. The identification of Rbp35/CfI25 as a component of the alternative polyadenylation machinery and its interconnections with protein kinase signaling are important step to unravel post-transcriptional networks that regulate *M. oryzae* plant colonization.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T50

Tuesday 5th April
14:00 - 16:00

KRIZSAN Krisztina *(1), ALMÁSI Éva (1), BÁLINT Balázs (2), KOHLER Annegret (3), NAGY István (2), MARTIN Francis (3), HIBBETT David S. (4), NAGY László G. (1)*
(1) Synthetic and Systems Biology Unit, Institute of Biochemistry, BRC, HAS, Szeged, Hungary
(2) Seqomics Ltd,, Mórahalom, Hungary
(3) INRA, Lorraine University, Laboratory of Excellence ARBRE, Champenoux, France
(4) Biology Department, Clark University, Worcester MA, USA

Identifying genes involved in fruiting body morphogenesis in *Lentinus tigrinus* by RNA sequencing

The genetic bases of fruiting body morphogenesis is an intensively studied question recently. Although pioneering gene expression studies provided important insights into the molecular bases of fruiting body formation in individual species, the general principles of fruiting body development including conserved and lineage-specific mechanisms are yet to be understood. The aim of our study is to identify the evolutionary origins of fruiting body formation in *Lentinus tigrinus* (Polyporales, Agaricomycotina) by transcriptomic and comparative genomic analyses of its development. We performed paired-end RNA sequencing on 6 different developmental stages and 3 tissue types (cap, stipe, lamellae) of *L. tigrinus* RLG 9953-Sp, including hyphal knots, early and late primordia, young and mature fruiting bodies as well as the different tissue types. Preliminary results showed that 548 and 570 genes were significantly up- and downregulated in fruiting bodies vs. vegetative mycelium, respectively. Gene Ontology terms related to gene expression regulation and signal transduction, protein, fatty acid and carbohydrate metabolism were enriched among the upregulated genes. Comparison of these transcriptome profiles with published transcriptomic data on *Schizophyllum commune*, Agaricus bisporus, Laccaria bicolor and Coprinopsis cinerea identified 10 gene families with a conserved role in fruiting body morphogenesis in all species being compared, while 244 were specific to *L. tigrinus*. Phylostratigraphic analysis revealed two major waves of the origins of genes upregulated in Lentinus fruiting bodies, one in the most recent common ancestor (mrca) of Pucciniomycota and Agaricomycotina, and the other in the mrca of Agaricales and Polyporales. We suggest that successive waves of genetic innovations paved the way for the development of complex fruiting body types most commonly known as mushrooms.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T51

Tuesday 5th April
14:00 - 16:00

ALMASI Eva *(1), KRIZSÁN Krisztina (1), BÁLINT Balázs (2), NAGY István (2), NAGY G. László (1)*
(1) Synthetic and Systems Biology Unit, Institute of Biochemistry, BRC, HAS, Szeged, Hungary
(2) SeqOmics Biotechnology LTD, Mórahalom, Hungary

Insights into the evolutionary origins of the regulation of fruiting body development in *Schizophyllum commune*

The evolution of multicellular development is orchestrated by a range of genetic innovations, of which changes to the regulatory repertoire of the organism are generally considered to be the most important. Transcriptome analysis of fruiting body development has been performed for several fungal species, however little is known about the regulatory mechanisms coordinating these events. In this study we perform a transcriptomic and comparative genomic analysis of fruiting body formation of *Schizophyllum commune* with a special emphasis on transcription factors. Paired-end RNA-Seq data were obtained for 3 biological replicates of 5 developmental stages (vegetative mycelium, stage 1 and 2 primordia, young and mature fruiting bodies) to identify differentially regulated genes during fruiting body formation. We infer the age distribution of genes, in particular transcription factors, involved in fruiting body formation, their conservation across larger Agaricomycete clades and their evolutionary origins. Besides *Schizophyllum commune*, we are investigating fruiting body development in Auriculariopsis – its closest relative with a comparatively simpler development. Through comparative genomics and analyses of fruiting body transcriptomes we aim to understand how the regulation of fruiting body morphogenesis evolved in these two species.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T52

Tuesday 5th April
14:00 - 16:00

CISSE Ousmane *(1), NGUYEN Anh (5), HEWITT David (3), NOWROUSIAN Minou (4), GREG Jedd (5), JASON Stajich (2)*
(2) University of California-Riverside, Riverside, USA
(3) Academy of Natural Sciences of Philadelphia, Philadelphia, USA
(4) Ruhr-Universität Bochum, Bochum, Germany
(5) National University of Singapore, Singapore, Malaysia

Two origins of complex multicellular organization in the Ascomycota

In the kingdom fungi, two major clades possess the capacity to produce complex multicellular reproductive structures. These are the Pezizomycotina in the Ascomycota and the Agaricomycotina in the Basidiomycota. Both of these monophyletic groups have undergone significant evolutionary radiation and together account for the majority of described fungal species. Early-diverging groups in the Ascomycota harbour taxa that do not produce macroscopic fruiting bodies. The genus *Neolecta* defines an enigma; early diverging from a phylogenetic perspective, *Neolecta* nevertheless produces complex reproductive structures, and possesses cell-cell channels (septal pores) and associated organelles resembling those found in the Pezizomycotina. Here, we present the *Neolecta* irregularis genome and investigate the origins of its multicellular organization. A group of ancient genes playing key roles in multicellular development and environmental sensing are retained in Neolecta and other multicellular groups, but lost in unicellular yeast. By contrast, Pezizomycotina-specific genes associated with fruiting body development and septal pore gating are absent from *Neolecta*. Together, these data suggest that the emergence of *Neolecta*'s multicellularity depended on retention of key ancient genes, combined with lineage-specific innovations providing for its Pezizomycotina-like attributes.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T54

Tuesday 5th April
14:00 - 16:00

TRAIL Frances (2), WANG Zheng (1), STEFANKO Kayla (2), CUBBA Caitlyn (2), ***TOWNSEND Jeffrey*** *(1), TOWNSEND Jeffrey (1)*
(2) Michigan State University, East Lansing, USA

Developmental origins of fungal fruiting body phenotypes in the evolution of the transcriptome

Changes in gene expression have been hypothesized to play an important role in the evolution of divergent morphologies. Testing this hypothesis requires functional analysis of genes with shifting expression in divergent lines in a common garden environment. We used developmental differences in fruiting body morphology of filamentous fungi that diverged approximately 220 mya as a model, profiling genome-wide gene expression of five species throughout fruiting body development. We reconstructed ancestral levels of gene expression and identified genes with the largest evolved increases in gene expression across development. Functional analyses of these genes in two divergent species typically yielded altered fruiting body development in the species that had evolved increased expression, at a higher rate than did projects targeting gene families or whole genomes: 21 of 26 knockouts performed in *F. graminearum*yielded phenotypes in fruiting body development; 41 of 196 knockout strains in *N. crassa* yielded phenotypes in fruiting body development. Orthologous genes knocked out in both species yielded phenotypes that were distinct to each species, and knockout phenotypes were concentrated in early and late fruiting body development-stages when morphological, niche-based differences between the two species are apparent. Bayesian Network inference of gene interactions based on ancestral gene expression generated models of how genes and their interactions evolved along the evolutionary paths towards different body plans. Combining gene expression measurements with knockout phenotypes facilitated the refinement of models of the gene network underlying fruiting body development, regulation of which is one of the least understood processes of multicellular development.

POSTER SESSION ABSTRACTS
Session CS4 Regulatory networks
CS4T55

Tuesday 5th April
14:00 - 16:00

NAGY Laszlo (1)
(1) Synthetic and Systems Biology Unit, Institute of Biochemistry, BRC, Szeged, Hungary

An overview of the evolution of complex multicellularity in fungi: where are we heading?

How multicellularity evolved is one of the most basic questions in biology. Complex multicellularity evolved in animals, plants, some algae but only in fungi has it evolved multiple times independently. Fruiting bodies - the most conspicuous multicellular structures of fungi - emerged both in the Asco- and Basidiomycota as well as in the early-diverging Mucoromycotina and Mortierellomycotina. Understanding the cellular and molecular processes underlying the emergence and development of fruiting bodies is of central importance in mycology, and much progress has been made in the last decades using phylogenetic, genetic and genomic studies. Yet, many aspects of fruiting body evolution remain obscure. Here, I review the current understanding of the evolutionary origins of fruiting body formation from a phylogenetic perspective and summarize some of the major questions associated with fruiting body evolution. There is a wealth of genetic and expression data available for several Asco- and Basidiomycete model systems and it is expected that comparative genomic data will further facilitate the understanding of the genetic bases of fungal development. At the same time, phylogenetic analyses have contributed intriguing evolutionary hypotheses on the origins of fruiting bodies and the trends in transitions between different morphological types. In the postgenomic era we have the genetic and genomic tools to put these hypotheses to the test and to narrow the gap between phylogenetic and genetic/genomic understanding of fruiting body formation. The availability of a large number of genome sequences will also allow us to establish both new model species and develop a synthesis of the current knowledge on existing models, which will hopefully lead to a general synthesis on the evolutionary and genetic origins of complex multicellularity in fungi.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T2

Tuesday 5th April
14:00 - 16:00

BANKS Alice *(1), SONG Lijiang (2), CHALLIS Gregory (2), BAILEY Andy (1), FOSTER Gary (1)*
(1) School of Bioglogical Sciences, University of Bristol, Bristol
(2) Departement of Chemistry, University of Warwick , Warwick, UK

Investigating terpenoid biosynthesis in basidiomycete fungi

Fungi have long been known to produce a plethora of bioactive secondary metabolites, many being of pharmaceutical or agricultural importance. Historically, the vast majority of these bioactive compounds have been identified from ascomycetes, yet basidiomycetes, as a phylum, have been largely neglected due to practical difficulties. Therefore, basidiomycetes are likely to be a relatively untapped source of novel natural products and, with recent advances in molecular techniques, are becoming more accessible for natural product research. One particular class of compounds produced in abundance by basidiomycetes are the terpenoids which possess a whole host of bioactivities including antimicrobial and anticancer properties.
This work focusses on two basidiomycete species, *Coprinopsis strossmayeri* and *Lepista sordida*, both of which exhibit high antimicrobial activity against the growth of *Bacillus subtilis, Escherichia coli* and *Saccharomyces cerevisiae*. A combination of BLAST searches and antiSMASH analyses were used to screen the in-house generated fungal genome assemblies for genes characteristic of terpenoid production. This revealed seven core terpenoid synthases in *C. strossmayeri* and 14 in *L. sordida*. The genomic contexts of these genes were analysed to determine putative gene clusters, and expression analysis indicated genes which were highly expressed in conditions where antimicrobial agents were produced. Phylogenetic analysis was used to predict the core structure and cyclisation patterns of these gene products, by comparison to genes in characterised fungal terpenoid pathways. Genes of interest have been taken forward to be heterologously expressed in *Aspergillus oryzae* to determine gene function. Chemical analysis has so far elucidated the structures of two novel sesquiterpene compounds from *C. strossmayeri*. Further work is ongoing to determine the genes involved in the synthesis of the complete products, and the bioactivity of these compounds is being tested against a panel of medically relevant bacteria.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T3

Tuesday 5th April
14:00 - 16:00

BATISTA GARCIA Ramon Alberto *(1), CUERVO SOTO Laura Inés (1), HERRERA Catalina (1), MARTÍNEZ Claudia (2), SÁNCHEZ CARBENTE María Del Rayo (1), FOLCH MALLOL Jorge Luis (1)*
(1) Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Mexico
(2) Universidad Nacional Autónoma de México, Cuernavaca, Morelos, Mexico

Characterization of a carbohydrate esterase, a lipase and a xylanase from analyses of genomic libraries of *Bjerkandera adusta* : possible applications

The lignocellulosic biomass as raw material has received much interest. Among the enzymes that degrade lignocellulose, which are produced by different organisms, carbohydrate esterases (CE) lipases (Lip) and xilanases (Xil) are required for complete biomass degradation. Hemicellulose shows modifications (presence of acetyl groups) that may be eliminated by the CE. In addition, Xil catalyse the depolymerisation of hemicellulose, while Lip are useful in the biomass emulsification. *Bjerkandera* is a Bacidiomycetes, and their enzymes have great potential for biotechnology applications. Performing an analysis of sequences from a cDNA library of *Bjerkandera*, sequences with similarity to CE, Lip and Xil were found. Knowing the roles of these proteins, it will be interesting to characterize them. 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-AA vector, for expression in P. pastoris. The complete gene for CE has an ORF of 470 aa and high identity with family 4 CE. The protein 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. Acetic acid release was measured from natural substrates, confirming the deacetylase activity. 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, and surprisingly it has preference for cellulose. Finally, a novel Lip belonging to Hormone Sensitive Lipase family was found, being the first report of these enzymes in bacidiomycete. This enzyme shows an interesting structural and biochemical properties and it has high especific activity in presence of NaCl, metal ions and solvents. We are currently completing some structural studies (affinity for different substrates) about of these three proteins. 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.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T4

Tuesday 5th April
14:00 - 16:00

BATISTA GARCIA Ramon Alberto *(1), SUTTON Thomas (2), JACKSON Stephen A. (2), TOVAR Omar (3), BALCAZAR Edgar (1), SÁNCHEZ CARBENTE María De Rayo (1), SÁNCHEZ Ayixon (1), DOBSON Alan D.w. (2), FOLCH MALLOL Jorge Luis (1)*
(2) University College Cork, Cork, Ireland, Ireland
(3) Universidad Autónoma de Nueva León, Nuevo León, Mexico, Mexico

Characterization of Lignocellulolytic Activities from Deep Sea Sponge Associated Fungi Isolated from Stelletta normani

While bacteria associated with marine sponges have been extensively studied, much less information is available on sponge-derived fungi. Culture dependent approaches were employed to study fungi associated with the deep sea sponge Stelletta normani sampled at a depth of 751 metres. Three halotolerant fungal strains were isolated from the sponge and molecular based taxonomic approaches identified these strains as *Cadophora sp.* TS2, *Emericellopsis sp*. TS11 and *Pseudogymnoascus sp*. TS 12. TS2, TS11 and TS12 displayed psychrotolerance and halotolerant growth on cellulose and xylan as sole carbon sources, with optimal growth rates at 20ºC. They produced CMCase and xylanase activities, which displayed optimal temperature and pH values of between 50-70ºC and pH 5-8 respectively, together with good thermostability and halotolerance. In solid-state fermentations TS2, TS11 and TS12 produced CMCases, xylanases and peroxidase/phenol oxidases when grown on corn stover and wheat straw. This is the first time that CMCase, xylanase and peroxidase/phenol oxidase activities have been reported in these three fungal genera isolated from a marine sponge. Given the biochemical characteristics of these ligninolytic enzymes it is likely that they may prove useful in future biomass conversion strategies involving lignocellulosic materials.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T5

Tuesday 5th April
14:00 - 16:00

TERFEHR Dominik *(1), KÜCK Ulrich (1)*
(1) Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-University, Bochum, Germany

Sulfate assimilation influences cephalosporin C production in *Acremonium chrysogenum*

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

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T6

Tuesday 5th April
14:00 - 16:00

LERICHE GRANDCHAMP Mathilde *(1), COUVREUR Julien (1), BRUNISSEN Fanny (1), ALLAIS Florent (1), DUCHIRON Francis (2)*
(1) AGROPARISTECH, CHAIRE ABI, REIMS, France
(2) FARE, URCA, REIMS, France

Development of a screening method to select strains of fungi allowing the production of phenolic monomers from lignin

The valorisation of lignin is a key component in the economical sustainability of biorefinery of second generation. Besides physical and chemical methods, the biological approach benefits from several advantages : mild conditions, reduced energy consumption, limited waste production and higher molecular purity. In order to design such a biological process, we aimed to build a screening method to select lignolytic strains of fungi that produce phenolic monomers from lignin. We selected strains of white-rot fungi and cultivated them on miscanthus straw with the solid-state fermentation method. Moreover, we developed different analytical methods to monitor the depolymerisation of lignin and the production of phenolic monomers produced during this lysis: chromatographic methods (HPLC, GPC and GC-MS), a spectrophotometric method (based on bathochrome and hyperchrome phenomena). We also used classical methods such as Van Soest assay and enzymatic assays. The complete study of one strain enabled us to conclude that this strain depolymerised 10% of lignin contained in straw but internalised the phenolic monomers produced.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T7

Tuesday 5th April
14:00 - 16:00

LI Wan-Chen *(1), CHUANG Yu-Chien (2), CHEN Chia-Ling (2), WANG Ting-Fang (2)*
(1) Molecular Cell Biology, Taiwan International Graduate Program, Institute of Molecular Biology, Academia Sinica and Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan
(2) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan

Initiation of meiotic DNA recombination in the industrial workhorse *Trichoderma reesei*

Meiosis is a special type of cell division process in which sexually reproductive cells generate genetically divergent gametes or sexual spores. In most sexually reproductive organisms, a prominent hallmark of meiosis is DNA recombination (or crossover) between two non-sister homologous chromosomes but not the sister chromatids. Mechanically, interhomolog crossover results in chiasmata that ensure the monopolar attachment of sister chromatids during the transition from metaphase I to anaphase I and their co-segregation toward the proper pole. The two sister chromatids are segregated during anaphase II. Defects in interhomolog recombination often increase the frequency of meiotic nondisjunction, eventually leading to aneuploid gametes or sexual spores. Meiotic recombination is initiated by genome-wide DNA double-stranded breaks (DSBs) during meiotic prophase. The evolutionarily conserved endonuclease Spo11 is responsible for the initiation of the majority of meiotic DSBs in most studied experimental model organisms. The genome of *Trichoderma reesei* CBS999.97 wild isolate contains a reciprocal translocation. The CBS999.97(1-1) haploid genome contains a reciprocal rearrangement between Scaffold 33 and Scaffold M of the CBS999.97(1-2) genome. The CBS999.97(1-1) haploid contains the two new scaffolds, scaffold F and scaffold X. The meiotic recombination between these «homeologous» chromosomes frequently (~90%) results in the formation four or eight inviable segmentally aneuploid (SAN) ascospores and an equal number of viable SAN ascospores. In this study, we have addressed whether Spo11-induced DSBs are also involved in T. reesei meiotic recombination or the formation of SAN ascospores in CBS999.97 wild isolate.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T8

Tuesday 5th April
14:00 - 16:00

SONNENBERG Anton *(1), GAO Wei (2), LAVRIJSSEN Brian (1), HENDRICKX Patrick (1), SEDAGHAT-TELLGERD Narges (1), FOULONGNE-ORIOL Marie (3), KONG Wong-Sik (4), SCHIJLEN Elio (1), BAARS Johan (1), VISSER Richard (1)*
(2) Institute of Agricultural Resources and Regional Planning of CAAS, Bejing, China
(3) INRA, MycSA, Villenave d'Ornon, France
(4) Mushroom Research Division, National Institute of Horticultural and Herbal Science, Eumsung, Republic of Korea

A detailed analysis of the recombination landscape of the button mushroom *Agaricus bisporus var. bisporus*.

Button mushroom (*Agaricus bisporus*) is one of the world's most cultivated mushroom species, but in spite of its economic importance generation of new cultivars by outbreeding is exceptional. Previous genetic analyses of the white bisporus variety, including all cultivars and most wild isolates revealed that crossing over frequencies are low, which might explain the lack of introducing novel traits into existing cultivars. By generating two high quality whole genome sequence assemblies (one de novo and the other by improving the existing reference genome) of the first commercial white hybrid Horst U1, a detailed study of the crossover (CO) landscape was initiated. Using a set of 626 SNPs in a haploid offspring of 139 single spore isolates and whole genome sequencing on a limited number of homo- and heterokaryotic single spore isolates, we precisely mapped all COs showing that they are almost exclusively restricted to regions of about 100 kb at the chromosome ends. Most basidia of the bisporus variety produce two spores and pair preferentially via non-sister nuclei. Combined with the COs restricted to the chromosome ends, these spores retain most of the heterozygosity of the parent thus explaining how present-day white cultivars are genetically so close to the first hybrid marketed in 1980. To our knowledge this is the first example of an organism which displays such specific CO landscape.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T9

Tuesday 5th April
14:00 - 16:00

MAERTENS Jeroen (1), KOZIOL Magdalena (1), LAZARUS Colin (1)
(1) University of Bristol, School of Biological Sciences, Bristol, UK

Production of natural statins by heterologous gene expression in *Aspergillus oryzae*

Statins are HMG-CoA reductase inhibitors and, as inhibitors of cholesterol biosynthesis, have been prescribed as the world's most valuable pharmaceuticals. Pharmaceutical preparations, such as simvastatin and pravastatin, are produced semi-synthetically following fermentation of producers of the natural products compactin (*Penicillium citrinum*) and lovastatin (*Aspergillus terreus*), while atorvastatin is a fully synthetic product. Heterologous production of lovastatin in *Aspergillus oryzae* could be achieved by transferring 5 genes from *A. terreus* on 2 multigene expression vectors. One vector carried genes encoding a nonaketide synthase, an enoyl reductase and a cytochrome P450, while the other vector carried genes for a diketide synthase and an acyl transferase whose function is to join the polyketide chains. However, lovastatin production was enhanced by the inclusion of a sixth gene encoding a thioesterase required to release the nonaketide from the synthase enzyme. This work has been replicated using genes from a P. citrinum gene cluster to produce compactin in A. oryzae. Since lovastatin and compactin differ only by a single methyl group on the nonaketide, work is currently in progress to assess the specificities of the enzymes that have the methylated or non-methylated nonaketide chain as a substrate, and to investigate the reciprocal conversion of the nonaketide synthases for lovastatin and compactin synthesis by domain swapping.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T10

Tuesday 5th April
14:00 - 16:00

WANKA Franziska *(1), ARENTSHORST Mark (2), JØRGENSEN Thomas (2), RAM Arthur (2), MEYER Vera (1)*
(1) Berlin University of Technology, Institute of Biotechnology, Department of Applied and Molecular Microbiology, Berlin, Germany
(2) Leiden University, Institute of Biology Leiden, Department of Molecular Microbiology and Biotechnology, Leiden, The Netherlands

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

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

POSTER SESSION ABSTRACTS
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CS5T11

Tuesday 5th April
14:00 - 16:00

GRAU Michelle *(1), SUN Weiwen (1), YAEGASHI Junko (1), CHUN-JUN Guo (1), CLAY C. C. Wang (1)*
(1) Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, School of Pharmacy, 1985 Zonal Avenue, Los Angeles, CA, USA

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

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

POSTER SESSION ABSTRACTS
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CS5T12

Tuesday 5th April
14:00 - 16:00

CAI Feng *(1), AKCAPINAR B. Günseli (2), PRZYLUCKA Agnieszka (2), PANG Guan (1), SHEN Qirong (1), DRUZHININA Irina (2)*
(1) Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, Nanjing Agricultural University, Nanjing, China
(2) Research Area Biotechnology and Microbiology, Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria

Hydrophobins of *Trichoderma guizhouense* inhibit tomato defence system for successful colonization of rhizosphere

Hydrophobins (HFBs) and cerato-platanins (CPPs) are the small secreted proteins (SSP) common in fungi. They have a distinct ability to self-assemble into stable amphiphilic layers at hydrophobic-hydrophilic interfaces and/or attach to various surfaces modulating their hydrophobicity. Thus, both HFBs and CCPs are involved in host/substrate recognition and to adhesion. The analysis of the genome of the commercialized plant-beneficial fungus *Trichoderma guizhouense* NJAU 4742 (Harzianum Clade) revealed at least 12 class II hydrophobin and 6 cerato-platanin encoding genes, what is essentially higher compared to other Trichoderma species and to any other ascomycete fungi investigated so far. To explain this HFB and CPP multiplicity, we have investigated their expression in the presence or absence of a host plant. Two genes - *hfb4* and *hfb7*, respectively, are overexpressed in the presence of *Solanum lycopersicum* (tomato) seedlings compared to the no plant control. To test the effect of respective proteins on plants, we used the Pichia pastoris cell factory to produce these and several other Trichoderma SSPs. Purified proteins were added to tomato roots in a hydroponic system at low concentrations under open conditions. HFBs were not involved in tomato growth promotion since plant biomass showed no significant difference between the HFB-treated- and non-treated seedlings. However, better root colonization was detected by RT-qPCR in HFB4- and/or HFB7-treated seedlings compared to the control. In the meanwhile, we also detected the inhibition of plant systemic resistance as eight of tomato defence marker genes including ethylene and salicylic acid signalling markers (TSRF and PR-1b) and (PAL and PR-1a), respectively, oxidative stress markers (POD and FD-1) and basal defence markers (Glu and Chi-II), were significantly down-regulated by HFB treatment, while six of the above genes were up-regulated by the putatively novel CPP EPLb treatment. However the jasmonic acid signalling maker gene Lox A was essentially up-regulated due to HFBs. To test whether individual gene plays a role in root colonization, hfb4- and epl1-deleted mutants of this *Trichoderma* strain were constructed. We conclude that HFBs play a role in the early colonization of roots by *Trichoderma*.

POSTER SESSION ABSTRACTS
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CS5T13

Tuesday 5th April
14:00 - 16:00

VAN MUNSTER Jolanda *(1), DALY Paul (1), BLYTHE Martin (1), IBBETT Roger (1), KOKOLSKI Matt (1), BARRY Kerrie (2), PETZOLD Christopher (3), ARVAS Mikko (4), SIMMONS Blake (3), ARCHER David (1)*
(1) University of Nottingham, Nottingham, UK
(2) DOE Joint Genome Institute, Walnut Creek, USA
(3) Joint BioEnergy Institute, Emeryville, USA (4) VTT Technical Research Centre, Espoo, Finland

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

Second-generation biofuel production requires efficient enzymes for lignocellulose digestion. To aid production of better and cheaper enzymes, we investigated how the biotechnology workhorse *Aspergillus niger* responds to untreated and pre-treated substrates. We applied genome-wide transcriptome analysis to provide a 5 day time-course of lignocellulose degradation and are the first to report the fungal response to ionic liquid pre-treated substrates. We mapped transcript levels to a metabolic model, aiding identification of substrate-independent as well as pre-treatment affected metabolic responses. Early time-points featured time-dependent clustering of transcript abundances, and were hallmarked by increased lipid metabolism. Pre-treatment and substrate dependent responses dominated mid time-points. Induction and repression of genes in cultures with ionic liquid pre-treated substrates was dynamic and correlated with pentose sugar concentrations. Hydrothermal pre-treatment of substrates resulted in reduced levels of transcript CAZy gene transcripts, proteins and enzyme activities, correlating to removal of parts of the lignocellulose. Sporulation and starvation were hallmarks of the later time-points. Secondary metabolism was induced mainly in cultures on ionic liquid pre-treated substrates. We show that pre-treatment, substrate and time each have major influence on the fungal responses to lignocellulose.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T14

Tuesday 5th April
14:00 - 16:00

SILVERIO GOMEZ Maria Del Carmen *(1), REYES-ESTEBANEZ María M. (1), ORTEGA-MORALES Benjamín O. (1), CHAN-BACAB Manuel J. (1), RUIZ-MARÍN Alejandro (2), MAGAÑA-ORTIZ Denis (3)*
(1) Universidad Autónoma de Campeche. San Francisco de Campeche, México
(2) Facultad de ingeniería de la Universidad Autónoma del Carmen. Campeche, México
(3) Instituto Tecnológico de Mérida. Mérida, Yucatán, México

Use of non conventional yeast strains isolated from sugarcane for ethanol production with high yields

In tropical countries, the industry based on sugarcane has a great impact in economics and social aspects. Maybe, the most important co-product in sugarcane industry is ethanol. The ethanol production usually employs the yeast *Saccharomyces cerevisiae* (Mayen). However, the fermentation process have to deal to remarkable problems like stress factors (temperature, high sugar content, inhibition of growth by ethanol) and low yields. One of the most important parameter required to increase the ethanol yields is select an adequate strain. For this reason, the purpose of this work was the isolation and selection of yeast strains of different parts of sugarcane plant in Campeche, Mexico. We isolated 86 yeast strains from stalk, floem and leaves of sugarcane. Later, we performed an alcoholic fermentation of the isolated strains; the range of ethanol produced was from 1 to 39 g/L. The medium with high concentration of (NH4)2SO4 (0.3 g/L), high concentration of ZnSO4 (0.1 g/L) and low concentration of sugar (15 °Brix) produced the maximum ethanol yield (up to 156.57 g/L) using the yeast strain named as TSBPL-90. Using ITS marker we identified the best producer strain as *Pichia guilliermondii*. The process optimized allowed yields of 50% v/v at 35 ºC, 25% v/v at 40 ºC and 13% v/v at 45 ºC of ethanol. We propose *Pichia guilliermondii* as native yeast for the production of ethanol with high yields in sugarcane derived industries.

POSTER SESSION ABSTRACTS
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CS5T15

Tuesday 5th April
14:00 - 16:00

KEMPKEN Frank *(1), KUMAR Abhishek (1), PHULE Pradeep (1)*
(1) Botanical Institute, Christian-Abrechts-University Kiel, Kiel, Germany

Analysis of genome and transcriptome for the exploration of secondary metabolites from marine fungi

We report on different properties of genome assemblies and annotations for three marine fungal isolates, *Scopulariopsis brevicaulis*, *Pestalotiopsis sp.*, *Calcarisporium sp.* and a novel isolate from the Baltic Sea, i.e. *Fusarium sp*. The assembled genome of a marine-sponge isolate of *Scopulariopsis brevicaulis* is ~32 Mb in size containing more than 16,000 genes. We were able to annotate 9340 genes (57.3 %) while 6958 genes (43.7 %) remained non-annotated in *Scopulariopsis brevicaulis* genome [1]. 17 genes encoding for non-ribosomal peptide synthetases (NRPSs), 18 polyketide synthases (PKSs) and one gene encoding a hybrid NRPS-PKS were found. We identified a cluster responsible for anti-cancer drug Scrophularid A and B production [2]. Two marine isolates from the mud flats of the North Sea were sequence. The genome size *for Pestalotiopsis sp*. is ~46 Mb containing about 23,500 genes, which is surprisingly high for an ascomycete and caused by partial genome duplication. 60% genes of Pestalotiopsis genome were annotated with an astonishing number of 44 NRPSs, 62 PKSs and 7 hybrid NRPS-PKS genes. The assembled genome size of *Calcariosporium sp*. is about 35 Mb genome containing about 15,500 genes. Here we annotated 72% genes, while 28% genes remained non-annotated for Calcariosporium genome with 52 NRPSs, 66 PKSs and 7 hybrid NRPS-PKS genes. From the secondary metabolite genes of these fungi only few are expressed und laboratory conditions as deduced by transcriptome analysis. Finally we present data from a Baltic Sea isolate of Fusarium sp. This genome comprises 42 Mb with gene models. A total of 136 putative secondary metabolite gene clusters were predicted using Antismash.

[1] Kumar, A., et al.. (2015) PLoS One 10:e0140398
[2] Lukassen MB, et al. (2015) Mar Drugs 13: 4331-4343

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T16

Tuesday 5th April
14:00 - 16:00

KIESENHOFER Daniel *(1), MACH Robert (1), MACH-AIGNER Astrid (1)*
(1) Vienna University of Technology, Vienna, Austria

GoxA production from wheat straw using an engineered *Trichoderma reesei* strain

Trichoderma reesei is a well studied, saprotrophic fungus which is used due to its high secretory capacity for industry scaled enzyme production. The majority of secreted enzymes degrade lignocellulose and are applied in the paper, feed and pulp industry. The most abundant enzyme in the secreted protein cocktail is the cellobiohydrolase CBHI. Therefore, the promoter of the cbh1 gene is considered as one of the strongest promoters known in *T. reesei*. Glucose oxidase (GoxA) is an enzyme that catalyzes the reaction of Y-D-glucose to gluconic acid. It has a wide range of applications, for example as a sugar sensor for diabetes monitoring or as a prominent additive in food industry. *Aspergillus niger*, another ascomycete, naturally expresses and secretes GoxA. So far glucose oxidase is produced in A. niger from yeast peptone dextrose media or in yeasts from sugars present in high concentrations. GoxA production in T. reesei can combine two promising aspects: a high expression and secretion of the product by a strong promoter in a well characterised host, and the utilization of pretreated wheat straw as an inexpensive and sustainable substrate. During our research we discovered that an engineered variant of the cbh1 promoter led to even higher yield of GoxA than the wild-type promoter did. This could be demonstrated in shaking flask and bioreactor cultivation experiments. The obtained yields between 21 U/ml and 40 U/ml from 2% wheat straw exceed the ones reported for *A. niger*.

POSTER SESSION ABSTRACTS
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CS5T17

Tuesday 5th April
14:00 - 16:00

GRYSHYNA Anna *(1), KAUTTO Liisa (1), SUN Angela (1), TE'O Junior (2), NEVALAINEN Helena (1)*
(1) Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, Australia
(2) School of Earth, Environmental and Biological Sciences, Queensland University of Technology, Brisbane, Australia

Expression of human fucosyltransferase 3 (FUT3) in *Trichoderma reesei*

Carbohydrate structures, or glycans, play a central role in many disorders and diseases which makes them a promising therapeutic and diagnostic target. One of the limitations in the glycobiology research is the non-availability and/or high price of glycan-modifying enzymes, such as fucosyltransferases, due to the imperfections of existing production systems. In the current work, cDNA encoding the C-terminal catalytic domain of the human α-1,3/4 fucosyltransferase (FUT3) was synthesised according to the *T. reesei* codon usage and assembled into an expression vector under the strong cellobiohydrolase 1 (cbh1) promoter. The vector design allowed FUT3 to be produced as a fusion to a Strep-tag, mCherry fluorescent protein and the core-linker fragment of the endogenous CBH1 protein to aid in purification and observe/facilitate expression and secretion. The constructs were transformed into *T. reesei* using particle bombardment. Analysis of the transformants not only demonstrated secretion of a full-sized fusion protein but also its degradation, most probably by *T. reesei* proteases. The time- and pH-dependent degradation of the recombinant FUT3 makes it necessary to optimise the cultivation conditions in order to obtain sufficient amounts of the protein for purification and characterisation of the degradation products.

POSTER SESSION ABSTRACTS
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CS5T18

Tuesday 5th April
14:00 - 16:00

THEOBALD Sebastian *(1), VESTH Tammi C. (1), NYBO Jane L. (1), FRISVAD Jens C. (1), NIELSEN Kristian F. (1), LARSEN Thomas O. (1), GRIGORIEV Igor V. (3), MORTENSEN Uffe H. (1), BAKER Scott E. (2), ANDERSEN Mikael R. (1)*
(1) Department of Systems Biology, Technical University of Denmark, Kgs. Lingby, Denmark
(2) Joint Bioenergy Institute, Berkeley, CA, USA
(3) Joint Genome Institute, Walnut Creek, CA, USA

How Aspergilli rearrange secondary metabolic gene clusters to generate new compounds

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

POSTER SESSION ABSTRACTS
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CS5T19

Tuesday 5th April
14:00 - 16:00

RECORD Eric *(1), MATHIEU Yann (1), DAOU Marianne (1), PATEL Ila (1), ARFI Yonathan (1), PIUMI, François (1), NAVARRO David (1), LEVASSEUR Anthony (1), LOMASCOLO Anne (1), FAULDS Craig (1)*
(1) INRA, Aix-Marseille Université, Marseille, France

Filamentous fungi, an invaluable source of enzymes, for deconstruction of the complex lignocellulosic plant cell walls

Saprophytic filamentous fungi are ubiquitous micro-organisms that form a large group of fungi playing an essential role in carbon recycling. Saprophytic fungi grow on fallen branches and trees, and dead leaves. These organisms are well known to produce an array of enzymes that are capable of modifying and degrading lignocellulose and thus have evolved an enzymatic capacity to degrade biomass in order to access their energy source. Consequently, the filamentous fungi represent a source of original enzymes that is underexploited for the hydrolysis or the deconstruction of plant biomass for biotech applications in the green chemistry and, especially, in the biorefinery field. The wealth of genomic data obtained recently from filamentous fungi has revealed the diversity of lignocellulolytic enzymes they produce and two examples with be illustrated in this poster. (i) The genome of *Pycnoporus cinnabarinus*, a white-rot-fungus known to efficiently degrade lignin, was recently sequenced. Its genome was mined to study the enzymatic lignin degradation network in collaboration with the CAZy team (AFMB), and recent results presenting the enzyme characterization and possible enzyme synergies will be presented. (ii) Mangroves forests are one of the most productive ecosystems and are characterized by intense carbon processing. Mangrove fungi are thought to play a key role in the detritic food webs and nutrients cycling by contributing to the degradation of particular organic matter into dissolved organic matter and lignocellulosic biomass. The Xylariales fungus, *Pestalotiopsis sp.*, and specifically its secretome was studied to determine the enzyme composition when grown on mangrove wood chips with or without salt. Selected enzymes and their enzyme characterizations will be presented and their properties in relation to salt discussed.

POSTER SESSION ABSTRACTS
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CS5T20

Tuesday 5th April
14:00 - 16:00

LI Jingen (1), *XU Jing (1), CAI Pengli (1), WANG Bang (1), MA Yanhe (1), BENZ J.philipp (2), TIAN Chaoguang (1)*
(1) Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences., Tianjin, China.
(2) Holzforschung München, TUM School of Life Sciences Weihenstephan, Technische Universität München, Freising, Germany.

The functional analysis of two L-arabinose transporters from filamentous fungi

Limited uptake is one of the bottlenecks for L-arabinose fermentation from lignocellulosic hydrolysates in engineered *Saccharomyces cerevisiae*. This study characterized two novel L-arabinose transporters, LAT-1 from *Neurospora crassa* and MtLAT-1 from *Myceliophthora thermophila*. Although both proteins share a high identity (about 83%), they display different substrate specificities. Sugar transport assays using the S. cerevisiae strain EBY.VW4000 indicated that LAT-1 accepts a broad substrate spectrum. In contrast, MtLAT-1 appeared much more specific for L-arabinose. Determination of the kinetic properties of both transporters revealed that the Km values of LAT-1 and MtLAT-1 for L-arabinose were 58.12 ± 4.06 mM and 29.39 ± 3.60 mM, respectively, with corresponding Vmax values of 116.7 ± 3.0 mmol/h/g DCW and 10.29 ± 0.35 mmol/h/g DCW, respectively. In addition, both transporters were found to use a proton-coupled symport mechanism and showed only partial inhibition by D-glucose during L-arabinose uptake. Moreover, LAT-1 and MtLAT-1 were expressed in the S. cerevisiae strain BSW2AP containing an L-arabinose metabolic pathway. Both recombinant strains exhibited much faster L-arabinose utilization, greater biomass accumulation and higher ethanol production than the control strain. In conclusion, because of higher maximum velocity rates and reduced inhibition by D-glucose, the two characterized transporters are promising target genes for improved L-arabinose utilization and fermentation in S. cerevisiae.

POSTER SESSION ABSTRACTS
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CS5T21

Tuesday 5th April
14:00 - 16:00

TANAKA Takumi *(1), TAKAHASHI Toru (2), NAKAYAMA Mayumi (2), YAMAGATA Youhei (3), ABE Keietsu (1)*
(1) Graduate School of Agricultural Science, Tohoku University, Sendai, Japan
(2) NICHe, Tohoku University, Sendai, Japan (3) Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, Japan

Ionic interaction between *Aspergillus* hydrophobins and *Aspergillus* cutinases.

Hydrophobins are amphipathic secretory proteins with eight conserved cysteine residues and are ubiquitous among filamentous fungi. Hydrophobin RolA and polyesterase (cutinase) CutL1 are co-expressed when the fungus *Aspergillus oryzae* utilizes biodegradable polyester polybutylene succinate-co-adipate (PBSA) as the sole carbon source. RolA which attaches to PBSA surface and changes its structure, then interacts with CutL1 and promotes the PBSA degradation by concentrating CutL1 on the PBSA surface (1). In our previous studies, we revealed that positively charged residues (His32, Lys34) of RolA and negatively charged residues (Glu31, Asp142, Asp171) of CutL1 are cooperatively play an important role in the ionic interaction between RolA and CutL1. In addition, we revealed that CutC, CutL1 homolog of *A. oryzae* also interacts with RolA (2). Here, we analyzed whether ionic interaction of hydrophobins with cutinases exists in other filamentous fungi. Phylogenetic analysis and alignment analysis of RolA orthologs and CutL1 orthologs revealed that the N-terminal regions of RolA orthologs contain several positively charged residues, and acidic amino acid residues corresponding to CutL1 Glu31, Asp142 and Asp171 were highly conserved in the CutL1 orthologs. PBSA-microparticle degradation assay, pulldown assay using Teflon particles, and kinetic analysis using quartz crystal microbalance revealed that hydrophobin RodA (RolA ortholog) of model filamentous fungus *Aspergillus nidulans* interacted with *A. nidulans* cutinase Cut1 (CutL1 ortholog) via ionic interaction. In addition, RodA also showed ionic interaction with CutL1 of A. oryzae. In conclusion, these results suggested that ionic interaction between hydrophobins and cutinases may be common at least among Aspergillus species, possibly among other filamentous fungi.

(1) Takahashi, T., et al.,Mol. Microbiol. 57:1780-1796 (2005)
(2) Takahashi, T., et al., Mol. Microbiol. 96:14-27 (2015)

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T22

Tuesday 5th April
14:00 - 16:00

LANDOWSKI Christopher (1), WESTERHOLM-PARVINEN Ann (1), HUUSKONEN Anne (1), WAHL Ramon (2), SOMMER Benjamin (2), PENTTILÄ Merja (1), OSTERMEIER Christian (2), HELK Bernhard (2), SAARINEN Juhani (3), ***SALOHEIMO Markku*** *(1)*
(1) VTT Technical Research Centre, Espoo, Finland
(2) Novartis Pharma AG., Basel, Switzerland
(3) Glykos FInland Oy, Helsinki, Finland

Next generation biotherapeutic production system: the filamentous fungus *Trichoderma reesei*

The filamentous fungus *Trichoderma reesei* is an important production organism used by industrial enzyme companies world-wide. It secretes its native enzymes at levels exceeding 100 g/L of culture medium and is amenable to large scale fermentation processes. Several *T. reesei* produced enzymes have obtained the generally recognized as safe status by the U.S. Food and Drug Administration. Furthermore, it is a low cost production system that can be cultivated in inexpensive media with short cultivation times. This work aimed at efficient production of therapeutic proteins in *T. reesei*. After constructing the first expression strains it became clear that the host proteases were a major barrier limiting production. The secreted *T. reesei* proteases were characterized with respect to their biochemical properties, their activity against target proteins, and their abundance at mRNA and protein levels. Thirteen protease genes were deleted to reduce the secreted protease activity. Expression strains for monoclonal antibodies, Fab antibody fragments, interferon alpha-2b, insulin-like growth factor 1, and fibroblast growth factor 21 were constructed, cultivated in bioreactors, and expression levels were measured. Strains were engineered to express enzymes needed for producing human glycoforms. There were at least 34 secreted proteases identified from the culture supernatant. After deleting 13 of the most critical protease genes, the general secreted protease activity was reduced over 30-fold. Monoclonal antibodies could be produced up to 7.6 g/L, Fab antibody fragments up to 5.9 g/L, interferon alpha-2b at 7.9 g/L, and insulin-like growth factor fusion protein at 8 g/L. With protease inhibitor treatment interferon alpha-2b could be produced at over 10 g/L, insulin-like growth factor fusion protein at 19 g/L, and full length fibroblast growth factor 21 at 200 mg/L in addition to a shorter form at 3.5 g/L. Human glycoforms such as G0 and FGO were produced on monoclonal antibodies. Expression levels and product quality improved dramatically after multiple protease deletions and optimization of culture conditions. While the production levels achieved are already relatively high, the strains can be developed further to reach the full potential of the organism. This study demonstrates the excellent prospects of *T. reesei* as a host for therapeutic protein production.

POSTER SESSION ABSTRACTS
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CS5T23

Tuesday 5th April
14:00 - 16:00

OVERKAMP Karin M. (1), RAM Arthur F. J. (2), ***PETER Punt*** *(1)*
(1) Dutch DNA Biotech BV, Zeist, Netherlands
(2) Leiden University, Leiden, Netherlands

Filamentous fungi as cell factories for protein production: genomics and molecular genetic screening approaches for development of improved fungal production host strains

Filamentous fungi have been used as sources of metabolites and enzymes for centuries. In the last few decades molecular genetic tools have enabled us to improve metabolite and protein production in these organisms. The use of gene-transfer systems and the development of efficient and versatile fungal expression and secretion vectors has allowed the generation of protein overproducing host strains. Recent development of improved gene modification approaches has allowed further dedicated strain improvement.
For protein production further improvements of the first generation strains have been obtained by rational strain design in two directions; (i) the development of protease deficient host strains obtained by classical or molecular approaches, (ii) the development of host strains with improved fermentation characteristics related to culture viscosity and oxygen transfer. In several cases these types of strain design in combination with fermentation process development has resulted in achieving commercially relevant quantities of protein. To gain more insight in complex processes such as protein and metabolite production and fermentation performance, we have used a combination of genomics / transcriptomics and novel molecular genetic screening approaches for developing improved filamentous fungal production systems. Examples of these approaches will be presented with a focus on novel protease deficient mutant strains. Our results show that both specific (Zn2Cys6; PrtT) and wide domain regulatory proteins, such as LaeA play a role in fungal protease production.

POSTER SESSION ABSTRACTS
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CS5T24

Tuesday 5th April
14:00 - 16:00

SHARMA Esha *(1), TAYAL Pamil (1), KAPOOR Rupam (1)*
(1) applied mycology laboratory, department of botany, university of delhi, delhi, India

Identification of putative virulence factors in *Botrytis cinerea* via *Agrobacterium tumefaciens* mediated transformation

Botrytis cinerea, an economically important necrotrophic plant pathogen with broad spectrum nature and variability has enthused great interest in researchers. In pursuit of identifying novel virulence factors of this pathogen, Agrobacterium tumefaciens mediated transformation was used to generate insertional mutants. Putative mutants were evaluated for the presence of selectable markers and analyzed by Southern blot. Out of several mutants studied, two mutants that showed single insertion and significant impairment in pathogenicity were characterized in detail. The flanking sequence of T-DNA inserted in mutant genome was cloned and analyzed by using Thermal Asymmetric Interlaced-PCR. Sequences were aligned against B05.10 database and NCBI using BLASTN algorithm to decipher possible gene function. Bioinformatics analysis recognized the tagged gene as Nup-188 and Diacyl glycerol O-acyl transferase 2A. Tagged genes have never been linked to play role in fungal virulence. Morphological analysis of mutants depicted variable growth rate, colony texture, colour and rate of sclerotia formation. The concentration and activity of known pathogenicity determinants such as oxalic acid and cell wall degrading enzymes also showed differences among the mutants and the wild type. Gene rescue via complementation and targeted knock out of genes will further provide insights into the virulence mechanism of the pathogen.

POSTER SESSION ABSTRACTS
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CS5T25

Tuesday 5th April
14:00 - 16:00

MARTINEZ Angel T. *(1), FERNANDEZ-FUEYO Elena (2), PACHECO Remedios (1), BARRASA Jose Maria (3), RUIZ-DUEÑAS Francisco Javier (1)*
(1) CIB, CSIC, Madrid, Spain
(2) Dept. Biotechnology, Delft University of Technology, Delft, The Netherlands
(3) Dept. Life Sciences, University of Alcalá, Madrid, Spain

Enzymatic degradation of lignin: The genomic evidence

Lignin removal from plant biomass is a key issue for implementing a bio-based economy to overcome the exhaustion of petrochemical resources and mitigate the climatic change. Considerable confusion existed for years about the mechanisms ultimately responsible for natural degradation of lignin by basidiomycetes. A variety of enzymes and redox mediators have been associated with the so-called "enzymatic combustion" of lignin. Interest in lignin biodegradation led to sequencing the first basidiomycete genomes. Massive sequencing extended the number of white-rot (ligninolytic) and brown-rot (cellulolytic) basidiomycete genomes (in the JGI SAP and other sequencing projects) enabling us to establish conclusions on the mechanisms involved (1,2). Lignin degradation by typical white-rot fungi appears always associated with the presence of high redox-potential peroxidase genes in their genomes, while such genes are absent from all the brown-rot fungal genomes analyzed. The genomic analyses also outlined the evolutionary history of ligninolytic peroxidases in basidiomycetes, fixing their origin at the end of the Carboniferous period, which coincides with the end of coal formation via accumulation of undecayed plant biomass, and revealed that brown-rot fungi appeared several times by the loss of ligninolytic genes (1,2). The paradigm on the role of peroxidase genes in lignin degradation (resulting in white-rot decay) has been recently challenged adducing their absence in two Jaapia and Botryobasidium species that would cause a "white-rot type" decay of wood (3). However, these fungi are very poor wood rotters and, most probably, correspond to transition forms in the above evolutionary scheme (4). Most wood rotting fungi investigated to date are Polyporales, but a detailed analysis of the genome of Pleurotus ostreatus, one of the first Agaricales to be sequenced, suggests that versatile peroxidases play in these fungi the same (central) role in lignin degradation assigned to lignin peroxidases in the wood-rotting Polyporales. Additional genomic evidence on the lignocellulose-degrading mechanisms by these fungi are being obtained in a JGI 2015-project for sequencing the genomes of Agaricales from different taxonomic and eco-physiological groups.

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2. Ruiz-Dueñas FJ et al . 2013. Mycologia 105:1428-1444
3. Riley R et al . 2014. Proc. Natl. Acad. Sci. USA 111:9923-9928
4. Floudas D et al . 2015. Fungal Genet. Biol. 76:78-92

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T26

Tuesday 5th April
14:00 - 16:00

MATTILA Hans *(1), KUUSKERI Jaana (1), VIROLAINEN Tuulia (1), LUNDELL Taina (1)*
(1) Microbiology and Biotechnology, Department of Food and Environmental Sciences, Viikki Campus, University of Helsinki, Helsinki, Finland, Helsinki, Finland

Single step lignocellulose waste material bioconversion potential of phlebia species

Remarkable amount of lignocellulose waste material originating from municipal and wood industry sources are currently left unexploited. Yet carbohydrates in waste lignocelluloses could be recycled into valuable compounds such as bioethanol. Current issue is to achieve a profitable bioconversion level in an environment-friendly manner. While pre-treatments and enzyme additions for saccharification are effective, they are too expensive. Thus simultaneous saccharification and fermentation (SSF) is one of the promising orientations. White rot Basidiomycota and especially Phlebia genus may hold potential candidates for this method. A potent candidate organism should be capable to ferment and saccharificate lignocellulose in order to suit a SSF process. Thus Polyporales phlebioid isolates were screened for their bioconversion capability which was tested by cultivating fungi on glucose and lignocellulose-based media. Results show that a few candidates hold the ability to produce ethanol from lignocelluloses without pre-treatment or additive enzymes. Also concomitant production of organic acids such as oxalate was remarkable. Phlebia species seem to hold much unexplored potential for SSF applications and should be considered alongside with the commonly tested phlebioid species *Phanerochaete chrysosporium*. In addition to bioethanol production commercial production of organic acids with phlebioid fungi may be reasonable due to the low or negative price of the growth substrate.

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CS5T27

Tuesday 5th April
14:00 - 16:00

HÄKKINEN Mari *(1), KUUSKERI Jaana (1), LAINE Pia (2), SMOLANDER Olli-Pekka (2), PAULIN Lars (2), VARJOSALO Markku (3), AUVINEN Petri (2), LUNDELL Taina (1)*
(1) Department of Food and Environmental Sciences, Microbiology and Biotechnology, University of Helsinki, Helsinki, Finland
(2) Institute of Biotechnology, DNA Sequencing and Genomics Laboratory, University of Helsinki, Helsinki, Finland
(3) Institute of Biotechnology, Proteomics Unit, University of Helsinki, Helsinki, Finland

Wood-decay mechanisms and functional dynamics through genome-wide analysis of the white-rot Basidiomycota species *Phlebia radiata*

Fungi secrete a set of carbohydrate-active enzymes and oxidoreductases in order to degrade plant material mainly composed of cellulose, hemicellulose and lignin. White-rot Agaricomycetes of Basidiomycota are able to depolymerize all the wood lignocellulose components but the exact details of fungal degradation processes and diversity of the participating genetic, biochemical and proteomic factors are not yet elucidated. Our aim was to identify the key genes and proteins for wood-decay in an ecologically important species, presenting high applicability and plant biomass degradation efficiency but no genome sequence yet available. The wood-inhabiting and lignin-degrading *Agaricomycetes Phlebia* radiata was selected for de novo genome and transcriptome sequencing also due to its role as type species of the phlebioid clade of Polyporales, and its ability to degrade efficiently all the main components of plant cell wall. Next-generation sequencing of the heterokaryon isolate 79 resulted first in construction of the mitochondrial DNA, and thereafter in high quality assembly of the nuclear genome. Transcriptome analysis by RNA-sequencing was conducted for P. radiata grown as solid-state culture on Norway spruce wood in order to identify genes that were up-regulated in the course of fungal colonization of wood. Integration of the data with total proteome analyses revealed dynamic changes in the set of genes up-regulated and the corresponding proteins expressed upon wood-degradation.

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Tuesday 5th April
14:00 - 16:00

RAMONI Jonas *(1), SEIDL-SEIBOTH Verena (1), MARCHETTI-DESCHMANN Martina (2), SEIBOTH Bernhard (1)*
(1) Research Division Biotechnology and Microbiology, Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria
(2) Institute of Chemical Technologies and Analytics, Vienna University of Technology, Vienna, Austria

Targeted improvement of *Trichoderma reesei* xylanase activity by the restoration of its full genome capacities

The ascomycete *Trichoderma reesei* is a paradigm for the regulation and production of plant cell wall-degrading enzymes, including xylanases. Five xylanases, including XYN1 and XYN2 of glycoside hydrolase family 11 (GH11), the GH10 XYN3, and the GH30 xylanases XYN4 and XYN6, were already described. By genome mining and transcriptome analysis, a further putative xylanase gene, xyn5, belonging to GH11 was identified. Analysis of the xyn5 gene in *T. reesei* QM6a, the parental strain of all industrial enzyme producers, showed, that different mutations lead to a non-functional truncated version of this xylanase. We therefore sequenced several *T. reesei* wild-type isolates and found a strain harboring a full length xyn5, which was overexpressed in *T. reesei* in a xylanase free background. The pH and temperature optima and the kinetic parameters were determined, which classified XYN5 as the second strongest endoxylanase encoded by *T. reesei*. In silico analysis and 3D modelling showed, that the enzyme reveals significant structural similarities to XYN1 and consists of a signal sequence, a KEX2 protease cleavage site and a glycoside hydrolase typical substrate binding groove with a carboxylate pair in the active site. As QM6a and its derivatives have apparently lost the correct coding region for the xylanase, we were interested if a replacement of the truncated xyn5 with the full length gene leads to an overall improvement of the xylanolytic activity of the QM6a derivative QM9414. We thereby improved the xylanolytic activity of this strain on wheat straw by >40%.

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CS5T29

Tuesday 5th April
14:00 - 16:00

DANIEL Wibberg (2), LOUISE Andersson (3), ***GEORGIOS Tzelepis*** *(1), OLIVER Rupp (4), JOCHEN Blom (4), LUKAS Jelonek (4), JOHAN Fogelqvist (1), MARK Varrelmann (5), ANDREAS Schlüter (2), CHRISTINA Dixelius (1)*
(1) Swedish University of Agricultural Sciences, Department of Plant Biology, Uppsala BioCenter, Linnean Center for Plant Biology Uppsala, Sweden
(2) Institute for Genome Research and Systems Biology, CeBiTec, Bielefeld, Germany
(3) Syngenta Seeds AB, Landskrona , Sweden
(4) Bioinformatics and Systems Biology, Gießen University, Gießen, Germany (5) Institute for sugar beet research, IfZ, Göttingen , Germany

Genome analysis of the sugar beet pathogen *Rhizoctonia solani* AG2-2IIIB revealed high numbers in secreted proteins and cell wall degrading enzymes

Sugar beet (Beta vulgaris) is a crop cultivated for its high content in sugar but is vulnerable to many soil-borne pathogens. One of them is the basidiomycete *Rhizoctonia solani*. This fungal species has a compatibility system regulating hyphal fusions (anastomosis). Consequently, *R. solani* species are categorized in anastomosis groups (AGs). AG2-2IIIB isolates are most aggressive on sugar beet. In the present study we report on the draft genome of *R. solani* AG2-2IIIB using the Illumina technology. Genome analysis, interpretation and comparative genomics of five sequenced *R. solani* isolates were carried out. The draft genome of R. solani AG2-2IIIB has an estimated size of 56.02 Mb. The genome of *R. solani* AG2-2IIIB was predicted to harbor 11,897 gene and 4,908 were found to be isolate-specific. Furthermore, it was predicted to contain 1,230 putatively secreted proteins and 473 of them were found to be unique for this isolate. This genome also encodes an increased number of carbohydrate active enzymes compared to the four other *R. solani* genomes. The highest numbers were observed for the polysaccharide lyases family 1 (PL-1), glycoside hydrolase family 43 (GH-43) and carbohydrate estarase family 12 (CE-12).Transcription analysis of selected genes representing different enzyme clades revealed a mixed pattern of up- and down-regulation six days after infection on sugar beets featuring variable levels of resistance compared to mycelia of the fungus grown in vitro.The established *R. solani* AG2-2IIIB genome provide important information on the gene content, gene structure and transcriptional activity for this sugar beet pathogen. The enriched genomic platform provides an important platform to enhance our understanding of *R. solani* biology.

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CS5T30

Tuesday 5th April
14:00 - 16:00

SALOHEIMO Markku
JOENSUU Jussi (1), VITIKAINEN Marika (1), WESTERHOLM-PARVINEN Ann (1), ILMÉN Marja (1), SALOHEIMO Markku (1), PENTTILÄ Merja (1)
(1) VTT Technical Research Centre of FInland, Espoo, Finland

Production of fusion proteins in *Trichoderma reesei* for engineering of novel hybrid materials

Trichoderma reesei is among the protein production cell factories most widely used by the enzyme industry. The protein production levels obtained from *T. reesei* are not surpassed by any other protein production organism. This fungus has a central role as a producer of enzymes for the second generation biofuel and chemical production from lignocellulosic biomass, as well as for other industrial applications including food, feed, textile and pulp and paper sectors. T. reesei has been used very successfully as a recombinant protein production host for many proteins including foreign enzymes and therapeutic proteins. Construction of novel bio-based materials with the help of proteins and other macromolecules is an emerging field in material science. In this work we aim at production of new type of fusion proteins that would create materials with interesting properties when combined with nanocellulose. The main components of the fusion proteins are cellulose binding modules of cellulase enzymes, providing interaction with nanocellulose, hydrophobins, creating interactions between the fusion proteins and elastic or resilient proteins from animal origin, providing elasticity or other kinetic properties to the created material. We report results on fusion proteins containing either locust resilin that can store and release kinetic energy or elastin-like polypeptide (ELP) that has protein domains able to unfold and fold back, giving elasticity to the material. ELP-containing proteins form aggregates in a reversible manner in correct salt concentration and temperature, and we have used this property for purification of the ELP fusion proteins. Proteolysis by secreted proteases of *T. reesei* has turned out to be a limiting factor for production of these fusion proteins, and therefore our low-protease production strains have been valuable in our expression experiments. Results of our material engineering experiments with the produced fusion proteins will be discussed.

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CS5T31

Tuesday 5th April
14:00 - 16:00

KJÆRBØLLING Inge *(1), VESTH Tammi C. (1), FRISVAD Jens C. (1), NYBO Jane L. (1), THEOBALD Sebastian (1), GRIGORIEV Igor (2), BAKER Scott E. (3), LARSEN Thomas O. (1), ANDERSEN Mikael R. (1)*
(1) Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark
(2) Joint Genome Institute, Walnut Creek, CA, USA
(3) Joint Bioenergy Institute, Berkeley, CA, USA

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

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

Acknowledgements: Martin E. Kogle and Ellen Kirstine Lyhn

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Tuesday 5th April
14:00 - 16:00

COWLEY Gwen *(1), CADDICK Mark (1), DARBY Alistair (1), MICHIELSE Caroline (2), VAN DER BURGT Ate (2), VISSER Jaap (2)*
(1) University of Liverpool, Liverpool, UK
(2) Dyadic Netherlands, Wageningen, Netherlands

Long-read PacBio transcript sequencing applied to the thermophilic fungus *Myceliophthora thermophila* for improved annotation

The thermophilic filamentous fungus *Myceliophthora thermophila* has a high capacity for the degradation of biomass. Consequently, it represents a potential reservoir of novel enzymes for industrial applications, including thermostable enzymes such as proteases, cellulases, and lipases involved in the extracellular degradation of biopolymers. Additionally, *M. thermophila* is of particular interest in the biotechnology field due to its ability to produce extracellular proteins at relatively high levels. While the genome of this fungus is fully sequenced and the organism is amenable to genetic modification, very little is known about its transcriptome. High-throughput RNA sequencing generates a global view of gene expression and provides unprecedented insights into gene structure. Novel sequencing techniques are being used to define gene architecture as well as relative gene expression levels. Of the multiple sequencing technologies that exist, most are incapable of generating reads that span entire transcripts. Pacific Biosciences (PacBio) single-molecule real-time (SMRT) sequencing provides a means of overcoming this limitation. This third generation sequencing platform provides a unique opportunity for the improvement of genome annotations with the potential to sequence full-length transcripts up to 15,000 nucleotides in length. Here we report on our use of this sequencing technology to optimise the functional annotation of the *M. thermophila* genome, uncovering novel genes and improving upon previous gene models. We have compared the utility of PacBio with strand-specific Illumina HiSeq data and different bioinformatic approaches, as well as assessing the benefits of combining the two sequencing approaches. The resulting genome re-annotation will significantly benefit functional genomic studies of *M. thermophila* and can readily be applied to other fungi.

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Tuesday 5th April
14:00 - 16:00

VOS Aurin *(1), JURAK Edita (2), SCHOLTMEIJER Karin (2), LUGONES Luis (1), KABEL Mirjam (2), WÖSTEN Han (1)*
(1) Utrecht University, Utrecht, Netherlands
(2) Wageningen UR, Wageningen, Netherlands

Increasing carbohydrate degradation in compost during commercial mushroom production of *Agaricus bisporus*?

After 2-3 flushes of *Agaricus bisporus* mushroom production, champost still contains about 40% of the carbohydrates and 45% of the lignin originally present in compost. Increased uptake of the unused pool of polysaccharides may increase mushroom yield. Lignin is removed efficiently during the vegetative growth phase of *A. bisporus* (phase III) but not during mushroom formation (phase IV). Here, we overexpressed the mnp1 in *A. bisporus* by placing it under control of the actin promoter to improve the removal of lignin, thereby promoting accessibility of hemicellulose and cellulose. Transformants produced MnP activity in liquid malt extract while the wild type strain did not. MnP activity was 3-4 fold increased in wheat bran medium. During a semi-commercial production cycle, MnP activity per gram wet compost was increased significantly at the end of phase III (30%) while the activity was similar to the wild type strain at the initiation of mushroom formation in phase IV. This indicates that other factors than mRNA accumulation may be limiting in MnP1 production at this stage. After the 1st and 2nd flush, MnP activity was increased 3-4 fold. There was no difference in mushroom yield or biomass formation in compost as measured by chitin release. Finally, preliminary results indicate that enzyme hydrolysis releases more glucose from compost colonized by the mnp1 overexpressor as compared to compost colonized by the A15 wild type at the end of phase III. Together, these data indicated that mnp1 overexpression increases accessibility of carbohydrates but does not increase mushroom yield.

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Tuesday 5th April
14:00 - 16:00

BORGOGNONE Alessandra (1), CASTANERA Raúl (1), PISABARRO Antonio G. (1), ***RAMÍREZ Lucía*** *(1)*
(1) Generics and Microbiology Research Group, Public University of Navarre, Pamplona, Navarre, Spain

Targeted elimination of an active helitron family in *Pleurotus ostreatus* progeny

Helitrons are a superfamily of Class II transposable elements recently discovered in a wide range of organisms. Studies based on computational approaches have revealed that helitrons can capture, amplify and express gene fragments, playing a role in the evolution of many eukaryotic genomes. These elements are characterized by conserved structural features. They have 5" TC and 3" CTRR conserved ends carrying a palindromic hairpin 10-20 nucleotides upstream the 3" end and show a preferential insertion between A and T dinucleotides in the host genome. The presence in putative autonomous elements of a motif similar to the replication initiator (Rep) of plasmid rolling-circle replicons, as well as a DNA helicase (Hel) domain suggest that helitrons could mobilize through a rolling circle mechanism. Nevertheless, this mechanism of mobilization has not been yet experimentally demonstrated. *Pleurotus ostreatus* is an edible basidiomycete of increasing importance due to the potential use of its secreted enzymes in different biotechnological applications. Recent bioinformatics and experimental analysis uncovered the presence of two helitron families (HELPO1 and HELPO2) in PC15 and PC9 genomes, the monokaryotic parentals of the commercial strain N001. Within the two families, HELPO2 was shown to have recently invaded *P. ostreatus* genome, on the basis of the high similarity of its elements. Molecular analyses evidenced a somatic transposition of HELPO2 element in the chromosome I of *P. ostreatus* PC15. An exhaustive screening of this locus in N001 as well as in its monokaryotic progeny (68 individuals) revealed that such insertion was exclusive of PC15. In addition, further analysis of HELPO2 loci in chromosomes VI and VIII uncovered a strongly biased segregation in N001 descendants towards the absence of helitrons. The analysis of flanking sequences of such loci confirmed that regions lacking helitrons were favored during meiotic inheritance. Deeper analysis to understand the basis of this mechanism showed recombination events in the immediate flanks of the helitron locus, although in low frequency. Finally, an excision event was observed in one monokaryotic descendant (MK109) and further confirmed by PCR and sequencing. These results evidence the presence of specific mechanism for TE detection and elimination within the fungal genome defense machinery.

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CS5T35

Tuesday 5th April
14:00 - 16:00

LANGE Claudia *(1), WELD Richard J. (2), COX Murray P. (3), BRADSHAW Rosie E. (3), STEWART Alison (4), MENDOZA-MENDOZA Artemio (1), ROSTÁS Michael (1), STEYAERT Johanna (1)*
(1) Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand
(2) Lincoln Agritech Limited, Lincoln University, Lincoln, New Zealand
(3) Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand
(4) Scion, New Zealand Forest Research Institute Limited, Rotorua, New Zealand

Considering genomic, epigenetic and extra-chromosomal features affecting Trichoderma phenotypes

Genome sequencing technologies are a valuable resource for researchers looking for the genetic basis of specific phenotypic traits in Trichoderma. Technological advances have resulted in massive reductions in sequencing costs and facilitated genome comparisons and the study of gene functions and regulatory pathways on a large scale. Our research into the basis of biocontrol efficiency has shown, however, that the genome alone might not answer all questions. We found that the SNPs between nearly clonal T. cf. atroviride strains did not account for all phenotypic differences, which led us to also investigate epigenetic modifications and extra-chromosomal elements in Trichoderma. Methylation-sensitive AFLP and MiSeq analysis identified differential DNA methylation in the two strains. To study the effect of this epigenetic mark, we analysed the expression and splicing of methylation-associated genes and treated the strains with the methylation inhibitor 5-azacytidine. Evidence for extra-chromosomal elements that may explain phenotype differences, such as plasmids or viruses could not be found in these two strains but single-stranded RNA elements were identified in another T. cf. atroviride isolate. We will present these results and discuss the effect that epigenetic modifications and viruses might have on biocontrol in Trichoderma.

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Tuesday 5th April
14:00 - 16:00

MAGAÑA-ORTIZ Denis *(1), AMÉZQUITA-NOVELO Roberto (1), RAMÓN-SIERRA Jesús (1), ORTIZ-VÁZQUEZ Elizabeth (1)*
(1) División de Estudios de Posgrado e Investigación, Instituto Tecnológico de Mérida, Merida, Mexico

Enzymatic and Molecular Characterization of Isolated Fungi in the Yucatan Peninsula with Peroxidase Profiles.

The Yucatán peninsula presents a great fungal biodiversity due to its climatic, geographical and geological conditions. Although there are reports of isolated fungi in different ecosystems of the Yucatan peninsula; the majority of species of these organisms remain uncharacterized. The objective of this work was the isolation and characterization of lignin-degrading fungi from the region of Tizimin, Yucatan. In this zone the degradation of lignin and cellulose by fungi has allowed the soil regeneration and supported the biogeochemical cycles. A total of twelve strains were collected in the area of interest. After isolation and propagation of fungi, DNA was extracted and the ITS gene region was amplified to identify the species. Peroxidase activity was detected using agar plates Remazol Brilliant Blue R (RBBR) as an anthraquinone dye, 2,6-dimethoxyphenol (DMP) as phenolic substrate, and -N,N-dimethyl-p-phenylenediamine sulfate (DMPPDA) as azo-dye. Laccase activity was detected using ABTS 2,2 -azino-bis (3-ethylbenzothiazoline-6-sulfonic acid). We were able to identify one species with laccase activity as Phlebia floridensis and one unidentified species with high peroxidase activity in all substrates. This research represents a new approach for identifying new enzymes capable of degrading xenobiotics and / or recalcitrant compounds due the mechanisms of lignin degradation remains poorly understood and the percentage of fungal species identified is only close to one percent.

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CS5T37

Tuesday 5th April
14:00 - 16:00

BAYRAM AKCAPINAR Günseli *(1), PRZYLUCKA Agnieszka (1), CAI Feng (3), MELLO DE SOUSA Thiago Machado (4), GROTHE Hinrich (5), REIMHULT Erik (6), MACH-AIGNER Astrid (4), SHEN Qirong (3), DRUZHININA Irina S. (1)*
(2) Austrian Centre of Industrial Biotechnology (ACIB) GmbH c/o Institute of Chemical Engineering, University of Technology of Vienna, Vienna, Austria
(3) Jiangsu Key Lab and Engineering Center for Solid Organic Waste Utilization, Nanjing Agricultural University, Nanjin, China
(4) Gene Technology Group, Research Area Biotechnology and Microbiology, Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria
(5) Research Division Physical Chemistry, Institute of Materials Chemistry, Vienna University of Technology, Vienna, Austria
(6) Department of Nanobiotechnology, Institute for Biologically Inspired Materials, University of Natural Resources and Life Sciences Vienna , Vienna, Austria

Rational design of novel Trichoderma hydrophobins for industrial applications

Hydrophobins (HFBs) are the small amphiphilic proteins secreted by fungi. Although the role of these proteins is not fully elucidated, HFBs are required for fungal development and are involved in interactions with other organisms. The primary sequence of HFBs is characterized by eight conserved cysteines that form four disulphide bridges providing the stability of the 3D structure. High surface activity and a distinct ability to self-assemble into stable amphiphilic layers at hydrophobic-hydrophilic interfaces make HFBs excellent candidates for a diversity of biotechnological, environmental and biomedical applications. The genomes of Trichoderma (Hypocreales, Ascomycota) species possess the highest diversity of HFBs found so far. In this study we have identified a repertoire of 160 orthologous genes encoding a novel HFB4 in different species of Trichoderma. Molecular evolution analysis revealed that HFB4s from some Trichoderma spp. evolve under positive selection pressure what is driven by mutation of several surface amino acid residues that are responsible for modulated properties of HFB4s. In order to understand the diversity-structure-function relationship of the HFB4, we performed in silico homology modeling of selected HFB4 orthologues and used an array of bioinformatic tools to design mutated HFB4s with altered surface activity properties. Molecular Dynamics (MD) simulations of rationally designed HFB4s on PET and SiO2 revealed that in addition to hydrophobicity, charged amino acids are also involved in the interaction of HFBs with these surfaces. Selected 9 HFB4s from a library of orthologous variants and 5 engineered HFB4s were heterologously expressed in Pichia pastoris cell factory and characterized by means of biochemical and biophysical techniques. Dynamic light scattering showed that HFB4s formed aggregates in solution and was used to screen the in-solution properties of the HFB4s. Variable surface activities were evaluated using pull down assays and water contact angle analysis. Circular dichroism spectroscopy demonstrated that all HFB4s are folded and formed the characteristic HFB beta sheet structures. Quartz crystal microbalance methods revealed different adsorption characteristics of HFB4s on glass and PET. A fluorescence assay was developed to determine the affinity of HFB4s against SyproOrange dye which binds to hydrophobic regions of proteins. Such comprehensive analysis allows targeted design of HFBs with required properties.

Session CS5 Applied genomics and biotechnology
CS5T38

Tuesday 5th April
14:00 - 16:00

KIM Kyoung Su *(1), HAN Joon-Hee (1)*
(1) Department of Applied Biology, Kangwon National University, Chuncheon, South Korea

Genome sequence and genome annotation of *Colletotrichum acutatum*

The genus Colletotrichum (teleomorph Glomerella) includes important members of plant pathogens, causing a devastating disease worldwide known as anthracnose on many plants. *C. acutatum* is one of major limiting factors on crop production, especially, by damaging developing and mature fruits of peppers and strawberries. Recently, we reported the full mitochondrial sequence of C. acutatum strain KC05 obtained from diseased pepper plants as a part of genome sequence project. Here, we present the first genome sequence of the *C. acutatum* strain KC05, which was revealed by PacBio sequencing. The acquired sequence reads were assembled into 27 scaffolds (N50, 4,416,304 bp; Nmax, 6,776,476 bp), totaling 52,190,760 bp. A total of 13,559 genes were found, which covered 20,550,771 bp of the genome sequence. Detailed information on sequencing, assembly, gene prediction and gene category will be presented.

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CS5T39

Tuesday 5th April
14:00 - 16:00

LÜBECK Mette *(1), ZOGLOWEK Marta (1), AHRING Birgitte K. (2), LÜBECK Peter S. (1)*
(1) Section for Sustainable Biotechnology, Department of Chemistry and Bioscience, Aalborg University., Copenhagen, Denmark
(2) Bioproducts, Sciences and Engineering Laboratory, Washington State University , Tricities/WA, USA

Consolidated bioprocessing using fungi for conversion of lignocellulose into products in biorefineries

The most important challenges for conversion of lignocellulosic plant biomass into bioproducts are to overcome the recalcitrance barriers to biodegradation and to reduce steps needed for its biorefining. Conventionally, conversion is carried out using pretreatment followed by hydrolysis of biomass to monomer sugars that are fermented into different bioproducts. Among the recent improvements in biorefineries are ‘on site" enzyme production and consolidated bioprocessing (CBP), which has been suggested as an efficient and economical method of manufacturing bioproducts from lignocellulose. The main idea in CBP is that hydrolysis and fermentation are integrated into a single process, thereby significantly reducing the amount of steps in a biorefinery. Fungi are able to efficiently degrade biomass by secreting enzymes as well as they are capable of producing a wide range of compounds of commercial interest such as organic acids. ‘On-site' enzyme production and CBP might benefit from application of fungal cell factories, either as single genetically modified strains or as mixed cultures. Black Aspergilli are potential candidates for such processes, since they are known to be excellent enzyme producers enabling the degradation of the complex lignocellulosic plant biomass, and they are capable of producing organic acids in high amounts. Moreover, Aspergilli are able to tolerate harsh conditions, including low pH, as well as they in general have high growth rates. An additional advantage is that both biomass degradation and sugar fermentation for production of some of the organic acids, e.g. citric acid, are aerobic processes in contrast to bioethanol fermentation. Enzymes from mixed cultures of *Trichoderma reesei* RutC30, with *Aspergillus saccharolyticus*, *Aspergillus carbonarius* or *Aspergillus niger* cultivated in solid-state fermentation have been successfully applied for hydrolysis of pretreated wheat straw. Metabolic engineering of the central carbon metabolism of A. saccharolyticus and *A. carbonarius* strains for increased production of organic acids are also being carried out with the long term goal of integrating the bioproduction with biomass degradation in a consolidated process.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T40

Tuesday 5th April
14:00 - 16:00

LÜBECK Peter S. *(1), YANG Lei (1), LINDE Tore (1), AHRING Birgitte K (2), LÜBECK Mette (1)*
(1) Section for Sustainable Biotechnology, Department of Chemistry and Bioscience, Aalborg University, Copenhagen SV, Denmark
(2) Bioproducts, Sciences and Engineering Laboratory, Washington State University, Tricities/WA, USA

Metabolic engineering of two black *Aspergilli* with completely different organic acid profiles for the production of biochemicals in biorefineries

In future there will be a growing demand for sustainable production of biochemicals that substitute fossil based chemicals. Many filamentous fungi are interesting as potential biocatalysts in biorefineries as they naturally produce and secrete a variety of hydrolytic enzymes and different organic acids that can be used as building blocks in the chemical industry. The black Aspergilli *A. carbonarius* and *A. niger* naturally produce citric acid and gluconic acid in high amounts, while the novel black *A. saccharolyticus* naturally produces malic acid and succinic acid. These fungi have all an excellent cell wall degrading apparatus, and have high tolerance to stress conditions which make them potential biocatalysts for use in lignocellulosic biorefineries. Ideally, by utilizing their potential for secretion of hydrolytic enzymes and of organic acids, the fungi could be considered in a consolidated approach where they hydrolyze the plant biomasses and ferment the resulting sugars into different organic acids. However, for developing the fungi into efficient biocatalysts for biochemical production, it is necessary to include metabolic engineering of biochemical pathways for increasing the glucose and xylose uptake and flux, and directing the carbon towards production of selected organic acids. Deletion of the gluconic acid pathway and engineering of selected genes in the glycolytic pathway and in the pentose phosphate pathway have led to increased citric acid production in A. carbonarius. With the further aim to reroute production of citric acid towards other organic acids, several putative malate/citrate antiporters and other pathways genes in *A. carbonarius* are being studied. Also pathway engineering in *A. saccharolyticus* is being carried out, and insertion of a fumarate reductase resulted in increased succinic acid production. The impact of these genetic modifications on organic acid production will be presented.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T41

Tuesday 5th April
14:00 - 16:00

SOUSA Thiago *(1), DERNTL Christian (1), GORSCHE Rita (1), REGNAT Katharina (1), JOVANOVIĆ Birgit (1), MARCHETTI-DESCHMANN Martina (1), POÇAS-FONSECA Marcio (2), MACH Robert (1), MACH-AIGNER Astrid (1)*
(1) Vienna University of Technology, Vienna, Austria
(2) University of Brasilia, Brasilia, Brazil

The transactivator Xyr1 of *Trichoderma reesei* functions in a nuclear receptor-like way

Engineering transcription factors is an interesting research target that gains increasing attention in case of industrially used organisms. With respect to sustainability, biomass-degrading saprophytic fungi such as *Trichoderma reesei* are promising industrial work horses because they exhibit a high secretory capacity of native and heterologously expressed enzymes and compounds. A single point mutation in the main transactivator of xylanase and cellulase expression in *T. reesei*, Xyr1, led to a strongly enhanced xylanase expression. Circular dichroism spectroscopy revealed changes in the protein"s secondary structure caused by this mutation. According to electrophoretic mobility shift assays and determination of the equilibrium binding constants, the DNA-binding affinity of the mutated Xyr1 was considerably reduced, but not abolished. Investigation of the promoter architecture of the target genes xyn1 and xyn2 did also not point to increased DNA accessibility. Finally, the allosteric response to carbohydrates signalling repression or induction to Xyr1 target genes was investigated. In contrast to the wild-type Xyr1, the mutated protein no longer exhibited a conformational change in response to the corresponding carbohydrates. Altogether, we postulate that Xyr1 functions in a nuclear receptor-like way by receiving carbohydrate signals, which influence the Xyr1 secondary structure and subsequently its transactivating property.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T42

Tuesday 5th April
14:00 - 16:00

KWON Min Jin *(1), SCHÄPE Paul (1), NITSCHE Benjamin (1), MEYER Vera (1)*
(1) Department Applied and Molecular Microbiology, Institue of Biotechnology, TU Berlin, Berlin, Germany

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

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

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

POSTER SESSION ABSTRACTS
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CS5T43

Tuesday 5th April
14:00 - 16:00

TANAKA Mizuki (1), YOSHIMURA Midori (1), OGAWA Masahiro (2), KOYAMA Yasuji (2), SHINTANI Takahiro (1), ***GOMI Katsuya*** *(1)*
(1) Graduate School of Agricultural Science, Tohoku University, Sendai, Japan
(2) Noda Institute for Scientific Research, Noda, Japan

FlbC is involved in transcriptional regulation of *Aspergillus oryzae* glucoamylase and protease genes specifically expressed in solid-state culture

Aspergillus oryzae produces a large amount of secreted proteins in solid-state culture, and some proteins such as glucoamylase (GlaB) and acid protease (PepA) are specifically produced in solid-state culture, but rarely in submerged culture. From the disruption mutant library of A. oryzae transcriptional regulators, we successfully screened a disruption mutant showing an extremely low production level of GlaB but a normal level for A-amylase production. This strain was a disruption mutant of the C2H2-type transcription factor, FlbC, which is reported to be involved in regulation of conidiospore development. The disruption mutants of other upstream regulators comprising a conidiation regulatory network had no apparent effect on GlaB production in solid-state culture. In addition to GlaB, the production of acid protease in solid-state culture was also markedly decreased by flbC disruption. Northern blot analysis revealed that transcription products of glaB and pepA were definitely decreased in the flbC disruption strain. These results suggested that FlbC is involved in transcriptional regulation of genes specifically expressed under solid-state cultivation conditions, possibly independently of the conidiation regulatory network.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T44

Tuesday 5th April
14:00 - 16:00

GREENFIELD Bethany P.j. *(1), ARMITAGE Andrew D. (1), TAYLOR Andrew (2), JACKSON Alison (2), OTT Sascha (3), BAXTER Laura (3), CLARKSON John P. (2), HARRISON Richard J. (1)*
(1) East Malling Research, West Malling, UK
(2) Warwick Crop Centre, University of Warwick, Warwick, UK
(3) Systems Biology, University of Warwick, Warwick, UK

Characterisation of lineage specific regions in onion basal rot pathogen *Fusarium oxysporum f.sp. cepae*

Pathogenic isolates of Fusarium oxysporum formae speciales (f.spp.) are of great socio-economic importance, causing crown rot, root rot and vascular wilt in several important crops worldwide. *F. oxysporum f.sp. cepae* (FOC) infects the roots and basal plate of onions, resulting in an estimated £10-11 million worth of losses a year, in the UK alone. Pathogenic (FOC) and non-pathogenic *F. oxysporum* isolates from onion were subjected to whole genome sequencing and compared to publicly available sequence data from *F. oxysporum f.sp. lycospersici*, revealing regions of the genome that are lineage specific to FOC. Comparative genomics has also revealed differences in gene compliments between pathogenic and non-pathogenic isolates from onion. Multiple genes in lineage-specific regions contain protein domains implicated in self recognition and incompatibility. Work is currently underway to characterize the function of candidate lineage-specific genes though gene knockout studies.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T45

Tuesday 5th April
14:00 - 16:00

HOSSAIN Abeer *(1)*
(1) Dutch DNA Biotech B.V., Zeist, Netherlands

Rewiring secondary metabolite pathway towards itaconic acid production in *Aspergillus niger*

Growing concerns about global carbon emissions are forcing industries to look for alternative processing and production methods. Biotechnologically produced organic acids promise to be an attractive alternative for the chemical industry to replace petrochemicals. Itaconic acid has been identified as one of the top twelve building block chemicals which have high potential to be produced by biotechnological means. Here we report the successful integration of the itaconic acid biosynthesis cluster (cadA, mttA and mfsA) from *Aspergillus terreus* in *Aspergillus niger* AB1.13. We have previously showed that expression of cadA from A. terreus results in itaconic acid production in *A. niger*, albeit at low levels. This low-level production is boosted five-fold by the overexpression of mttA and mfsA in itaconic acid producing AB1.13 CAD background strains. Controlled batch cultivations with AB1.13 CAD+MFS+MTT strains showed higher productivity, titer and yield of itaconic acid compared with AB1.13 CAD strain. Moreover, preliminary RNA-Seq analysis of an itaconic acid producing *A. niger* strain has led to the identification of the putative cytosolic citrate synthase citB, which appears to be part of a secondary metabolite cluster in A. niger. We have overexpressed citB in a AB1.13 CAD+MFS+MTT strain and by doing so presume to have targeted itaconic acid production to the cytosolic compartment. Targeting the pathway to the cytosol would render shuttling of intermediate compounds during itaconic acid production obsolete and thereby make the pathway more efficient. Indeed, by overexpressing citB in AB1.13 CAD+MFS+MTT strains we have achieved titers of up to 26.2 g/l IA with a productivity of 0.35 g/l/h in controlled batch cultivations, which is about 2 fold higher than in a strain without citB overexpression.

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CS5T46

Tuesday 5th April
14:00 - 16:00

NYBO Jane *(1), VESTH Tammi C. (1), THEOBALD Sebastian (1), KJÆRBØLLING Inge (1), FRISVAD Jens C. (1), LARSEN Thomas O. (1), GRIGORIEV Igor V. (2), BAKER Scott E. (3), ANDERSEN Mikael R. (1)*
(1) Department of Systems Biology, Technical University of Denmark, Lyngby, Denmark
(2) Joint Genome Institute, Walnut Creek, California, USA (3) Joint Bioenergy Institute, Berkeley, California, USA

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

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

Acknowledgements: Martin E. Kogle (1) and Ellen K. Lyhne (1)

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T47

Tuesday 5th April
14:00 - 16:00

DUTRA ALBUQUERQUE Erica (1), VIJGEBOOM Erik (1), ***PETER Punt*** *(1)*
(1) Leiden University, Leiden, The Netherlands

INcrement of Commercial cellulase by REcombination of *Aspergillus* and *Streptomyces* Enzymes (INCREASE)

Cellulosic ethanol production is the largest promise source of biofuel in the near future. Agricultural residues are an important environmental issue, for example, thousands tons of lignocellulosic biomass are discarded every day in tropical regions. Thus, producing ethanol from lignocellulose seems a solution to tackle the pollution problem. Lignocellulose is characterized by its structural hardness promoted by lignin. Therefore unpacking cellulose, hemicelluloses and lignin and finding the right hydrolytic enzymes are two key elements to achieve an efficient hydrolysis for bioethanol production. Another problem is the large amount of inhibitors (*e.g.* reducing sugars and polyphenols) present in pretreated lignocellulosic biomass decreasing its hydrolysis. We applied two approaches to improve the lignocellulosic hydrolysis for bioethanol production. First, genes encoding interesting enzymes from lignocellulolytic soil bacteria, such as *Streptomyces* were identified and successfully expressed in *Aspergillus niger*. Secondly, genes encoding fungal specific activities were expressed in *Streptomyces*. The secreted enzymes were mixed with a commercial cellulase cocktail showing increased saccharification of pretreated lignocellulosic biomass such as wheat straw.

Acknowledgements: Science Without Borders (CAPES/Brazil).

POSTER SESSION ABSTRACTS
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CS5T49

Tuesday 5th April
14:00 - 16:00

BRANDL Julian (1), WORKMAN Mhairi (1), ARVAS Mikko (2), MEYER Vera (3), RAM Arthur Fj (4), ***ANDERSEN Mikael Rørdam*** *(1)*
(1) Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark
(2) Synthetic Biology Team, VTT Technical Research Centre of Finland, Esbo, Finland
(3) Technical University of Berlin, Berlin, Germany
(4) Institute of Biology Leiden, Leiden University, Leiden, The Netherlands

The development of a community consensus model for *Aspergillus niger*

Fungal primary metabolism is an essential part of fungal physiology and affects all phenotypic traits of the organism as well as carrying the biotechnological potential for the fungal host. While the study of individual pathways have gained essential knowledge and important scientific breakthroughs, a genome-scale view of metabolism is required to gain a holistic understanding of the cell. Mathematical models based on the stoichiometry of known enzymatic reactions have been developed in order to facilitate this approach and proven useful for guiding metabolic engineering in well characterized model organisms like *S. cerevisiae* and *E. coli*. With the sustained interest in *Aspergillus niger* as a potent host organism for citric acid and enzyme production, it was timely to improve on previous genome-scale modeling efforts. Here we have updated the genome-scale model by a combination of modeling, experimental work, and integration of the most recent literature information. In this new version of the model, the gene assignments have been improved by utilizing the genomes of the section *Nigri* being sequenced and analyzed in a community effort. Using these genomes in a comparative approach, we were able to identify shared isoenzymes and gene groups involved in primary metabolism between these closely related species in the section *Nigri*. In order to improve the coverage of our model in terms of nitrogen and carbon sources, we used Biolog plates for the screening of more than 270 carbon and nitrogen sources. Using this information we could identify missing substrates that have been subsequently added to the model as well as validated the presence of pathways already included in the model. In order to being able to simulate protein production we have included all enzymes that have been reported to be secreted in the literature thereby accounting for the natural catabolic activity of this fungus. Additionally we added all known pathways for the synthesis of secondary metabolites in order to enable the usage of the model to guide engineering of secondary metabolite cell factories. Here we present results from the modeling performance of said model and show an improvement over previous work. In conclusion this project has generated an experimentally validated community-consensus model of the *A. niger* metabolism being able to describe and predict beneficial modifications to the metabolic network in order to improve protein production on a variety of different substrates.

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CS5T50

Tuesday 5th April
14:00 - 16:00

LINKE Rita (2), THALLINGER Gerhard G. (3), HAARMANN Thomas (4), EIDNER Jasmin (4), SCHREITER Martina (4), LORENZ Patrick (4), KUBICEK Christian P. (1), ***SEIBOTH Bernhard*** *(1)*
(1) Research Division Biotechnology and Microbiology, Institute of Chemical Engineering, Technische Universität Wien, Vienna, Austria
(2) ACIB GmbH, c/o Institute of Chemical Engineering, Technische Universität Wien, Graz, Austria
(3) Bioinformatics, Institute for Knowledge Discovery, Graz University of Technology, Darmstadt, Germany
(4) AB Enzymes GmbH, Vienna, Austria

Introduction of a functional ham5 into *Trichoderma reesei* QM6a restores female fertility and provides the basis for classical strain breeding.

Many filamentous fungi used as production platforms in industrial biotechnology reproduce asexually thereby preventing the application of conventional strain breeding techniques. *T. reesei*, an industrial producer of enzymes for food, feed and biorefinery industries, is heterothallic and all industrially utilized species are derived from the single MAT1-2 isolate QM6a. Simply switching the mating type in QM6a to MAT1-1 did not result in sexual reproducing strains. We therefore used a systems biology approach to identify genes that are able to restore sexual reproduction in this strain line. *T. reesei* QM6a was crossed with the MAT1-1 wild-type strain CBS999.97 and the progenies of this cross were backcrossed 8-times in two lineages with QM6a to obtain mating competent MAT1-1 strains with a minimal set of CBS999.97 specific genes. Comparative genome analysis identified a total of 73 genes of which two encoding an unknown C2H2/ankyrin protein and a homolog of the WD-protein HAM5 were identified to be essential for fruiting body formation. The introduction of a functional ham5 allele in a mating type switched *T. reesei* QM6a allowed sexual crossing with the parental strain QM6a. This discovery provides the basis to establish sexual crossing in this fungus and herald a new era of strain improvement in *T. reesei*.

POSTER SESSION ABSTRACTS
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CS5T51

Tuesday 5th April
14:00 - 16:00

SØRENSEN Jens Laurids *(1), ROMANS-FUERTES Patricia (2), SONDERGAARD Teis Esben (3), NIELSEN Kristian Fog (4), HANSEN Frederik T (1), GIESE Henriette (3), BRODERSEN Ditlev Egeskov (2)*
(1) Aalborg University, Esbjerg, Denmark
(2) Aarhus University, Aarhus, Denmark
(3) Aalborg University, Aalborg, Denmark
(4) Technical University of Denmark, Kgs. Lyngby, Denmark

Identification of the sansalvamide non-ribosomal peptide synthetase in *Fusarium solani*

Members of the *Fusarium* genus have a huge genetic potential for production of secondary metabolites. One of the most interesting compound classes is the non-ribosomal peptides (NRPs), which are synthesized by huge multi-domain synthetases (NRPSs). In a comparative analysis of the genomes sequences from ten different *Fusarium* species we have previously identified 52 NRPS orthology groups of which only 6 produce a known compound [1]. To fill the missing pieces we set out to identify the biosynthetic pathway responsible for production of the NRP sansalvamide. This cyclic pentadepsipeptide was originally isolated from an unidentified *Fusarium* species [2] and subsequently several strains belonging to the *Fusarium solani* species complex [3]. Sansalvamide contains an A-hydroxyisocaproic acid (HICA) unit, which is also found in the cyclic hexadepsipeptide destruxin produced by *Metarhizium* species. The gene cluster responsible for destruxin biosynthesis has been identified in *M. robertsii*, which consists of non-ribosomal peptide synthetase (NRPS; DtxS1), an aldo-keto reductase (DtxS2), a cytochrome P450 monooxygenase and a decarboxylase (DtxS4) [4]. A BlastP analysis of the synthetase DtxS1 against *F. solani* sequences resulted in NRPS30 as the best hit (total score 18001; identity: 45%). An orthologue of DtxS3, which provides the HICA unit from reduction of A-ketoisocaproic acid, was furthermore identified directly downstream of NRPS30. To verify that NRPS30 is responsible for biosynthesis of sansalvamide in *F. solani* we applied an *Agrobacterium tumefaciens*-mediated transformation (ATMT) approach to generate knock-out mutants. Comparative studies of secondary metabolites in the resulting deletion mutants and wild type confirmed the absence of sansalvamide in the NRPS30 deletion mutant, implicating this synthetase in the biosynthetic pathway for sansalvamide.

[1] Hansen, F. T., Gardiner, D. M., Lysøe, E., Fuertes, P. R., Tudzynski, B., Wiemann, P., Sondergaard, T. E., Giese, H., Brodersen, D. E.and Sørensen, J. L. Fungal Genet. Biol. 2015 75, 20-29.
[2] Belofsky, G. N., Jensen, P. R.and Fenical, W. Tetrahedron Lett. 1999 40, 2913-2916.
[3] Short, D. P. G., O'Donnell, K., Thrane, U., Nielsen, K. F., Zhang, N., Juba, J. H.and Geiser, D. M. Fungal Genet Biol 2013 53, 59-70.
[4] Donzelli, B. G. G., Krasnoff, S. B., Sun-Moon, Y., Churchill, A. C. L.and Gibson, D. M. Curr. Genet. 2012 58, 105-116.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T52

Tuesday 5th April
14:00 - 16:00

VAN DIJK Johannes *(1), GUO Chun-Jun (1), WANG Clay C. C. (1)*
(1) University of Southern California, School of Pharmacy, Los Angeles, USA

Thioesterase domain in non-ribosomal peptide synthetase-like enzymes can determine product formation

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

POSTER SESSION ABSTRACTS
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CS5T53

Tuesday 5th April
14:00 - 16:00

SANCHEZ-CARBENTE Maria Del Rayo *(1), BATISTA-GARCÍA Ramón Alberto (2), BALCÁZAR-LÓPEZ Edgar (3), MIRANDA-MIRANDA Estefan (4), SÁNCHEZ-REYES Ayixon (2), CUERVO-SOTO Laura (5), ATRIZTÁN-HERNÁNDEZ Karina (3), MORALES-HERRERA Catalina (1), RODRÍGUEZ-HERNÁNDEZ*
(1) Centro de investigacion en biotecnologia/universidad autonoma del estado de morelos, cuernavaca/morelos, Mexico (2) Centro de investigación en dinamica celular/universidad autonoma del estado de morelos, cuernavaca/morelos, Mexico
(3) Cinvestav/langebio, irapuato/guanajuato, Mexico
(4) Centro Nacional de Investigación Disciplinaria en Parasitología Veterinaria/Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, cuernavaca/morelos, Mexico
(5) Departamento de Biología/Universidad Antonio Nariño, bogota/cundinamarca, Colombia

Aspergillus caesiellus, a moderate halophilic fungi isolated from sugarcane bagasse : a characterization of its lignocellulolytic activities

The use of plant biomass as feedstock for biomaterial and biofuel production is relevant in the current biobased economy scenario of valorizing renewable resources. Fungi, which degrade complex and recalcitrant plant polymers, secrete different enzymes that hydrolyze plant cell wall polysaccharides. The ability of extremophiles to produce cell wall hydrolases that can withstand extreme physico-chemical conditions has been much studied for its possible applications in industries. Mostly halophilic hydrolases such as amylases, cellulases, lipases, xylanases, and proteases have been reported from halophilic bacteria. Except for few preliminary studies there have not been many investigations on the extremozymes from halophilic or halotholerant fungi. The goal of this study was to isolate a microorganism from a sugarcane bagasse fermentation in the presence of high NaCl concentrations. We isolated a moderate halophile and thermotolerant fungal strain that by polyphasic criteria has been identified as *Aspergillus caesiellus*. The fungus showed an optimal growth rate in media containing 1M NaCl at 28°C and could grow in media added with up to 2 M NaCl. This strain was able to grow at 37 and 42°C, with or without NaCl. *A. caesiellus* produced cellulases, xylanases, manganese peroxidase (MnP) and esterases. No laccase activity was detected in the conditions we tested. The cellulase activity was thermostable, halostable, and no differential expression of cellulases was observed in media with different salt concentrations when carboxy-methyl-cellulose was used as carbon source. However, differential band patterns for cellulase and xylanase activities were detected in zymograms when the fungus was grown in different lignocellulosic substrates such as wheat straw, maize stover, agave fibres, sugarcane bagasse and sawdust. These results point to the potential of this fungus to degrade lignocellulosic materials and its possible use in biotechnological applications.

POSTER SESSION ABSTRACTS
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CS5T54

Tuesday 5th April
14:00 - 16:00

DE JONGE Ronnie *(1), EBERT Malaika K. (2), SUTTLE Jeffrey C. (2), JURICK II Wayne M. (3), SECOR Gary S. (4), THOMMA Bart Phj (5), VAN DE PEER Yves (1), BOLTON Melvin D. (2)*
(1) VIB Department of Plant Systems Biology - Bioinformatics and Evolutionary Genomics, Ghent, Belgium
(2) Northern Crop Science Laboratory, United States Department of Agriculture, Fargo, USA
(3) Food Quality Laboratory, United States Department of Agriculture, Beltsville, USA
(4) Department of Plant Pathology, North Dakota State University, Fargo, USA
(5) Wageningen University, Laboratory of Phytopathology, Wageningen, The Nerthelands

Ancient horizontal transfer of the cercosporin biosynthesis cluster reveals auto-resistance mechanisms in the plant pathogen *Cercospora beticola*

The fungus *Cercospora beticola* causes *Cercospora* leaf spot, an economically devastating disease of sugar beet worldwide. Here we report the 37.1 Mbp genome sequence of *C. beticola* that encodes 63 secondary metabolism biosynthetic gene clusters, including 15 type I polyketide synthase (PKS) clusters representing a significant expansion compared to sister plant-pathogenic fungi. Among the identified clusters was the cercosporin toxin biosynthesis (CTB) cluster, the canonical eight-gene PKS cluster instrumental for cercosporin biosynthesis. Cercosporin is a photo-activated secondary metabolite toxic to a wide array of organisms including bacteria, mice, and plants but not to cercosporin-producing *Cercospora* species. Using phylogenomics we show that the CTB cluster is not limited to *Cercospora* species and has experienced an unprecedented number of duplications, losses, and horizontal transfers across a spectrum of plant pathogenic fungi during evolution. Since comparative genomic analysis revealed extensive gene collinearity adjacent to the established CTB cluster in all CTB-harboring species, we confirmed that CTB is larger than previously recognized and includes at least five additional genes, two of which are absolutely required for cercosporin biosynthesis. Of the six genes, a gene encoding a major facilitator superfamily transporter previously shown to be involved with cercosporin resistance and a candidate desaturase contribute to cercosporin auto-resistance in *C. beticola*.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T55

Tuesday 5th April
14:00 - 16:00

LOVETT Brian *(1), BILGO Etienne (2), DIABATE Abdoulaye (2), ST. LEGER Raymond (1)*
(1) Department of Entomology, University of Maryland, College Park, USA
(2) Centre Muraz, Bobo-Dioulasso, Burkina Faso

Applying transgenic fungi in a malaria sphere

Here we report on a semi field trial testing the efficacy of a mosquitocidal *Metarhizium* strain (Mr-hybrid) engineered to express an insect-specific spider neurotoxin (hybrid) and GFP. The experiments are being conducted in a multi-chambered Malaria sphere (a contained near-natural environment), constructed for this purpose in a region of Burkina Faso where malaria is endemic. We used the sphere to test a variety of low technology treatment protocols that could be used routinely by householders and found that suspending *Metarhizium* in locally produced sesame oil and spreading that on netting or black sheets achieves a long term effect in the sphere. Compared to a strain of wild-type virulence expressing RFP (Mr-RFP), Mr-Hybrid killed anopheline mosquitoes in half the time and at much lower spore doses, which increased the percent of lethally infected mosquitoes and the effective persistence of the pathogen. We also demonstrated that Mr-hybrid had important pre-lethal effects that included reduced feeding by infected mosquitoes and improved control of insecticide resistant mosquitoes. This NIH funded, international effort represents an important step in the progression of transgenic mosquito control technologies into field application. We are currently working on community engagement and policy for an eventual open field release to test epidemiological and clinical impact of transgenic *Metarhizium*. Our results will have broad implications for any project proposing to scale up new, complex, and potentially controversial technologies for malaria eradication.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T56

Tuesday 5th April
14:00 - 16:00

*ROMAGNOLO Alice (1), **SPINA Federica** (1), CATUCCI Gianluca (1), POLI Anna (1), SERITO Bianca (1), BRENNA Elisabetta (2), BELMONDO Simone (1), DI NARDO Giovanna (1), LANFRANCO Luisa (1), VARESE Giovanna Cristina (1)*
(1) Department of Life Sciences and Systems Biology, University of Torino, Torino, Italy
(2) Department of Chemistry, Materials and Chemical Engineering, Politecnico di Milano, Milano, Italy

Discovering fungal ene-reductases : transcripts and *in-silico* analysis

The reduction of C=C double bonds and carboxylic acids and esters is often a crucial step in organic chemistry, currently performed by highly polluting and expensive metal catalysts. A viable alternative is given by ene-reductase (ERs) activity which is able to reduce C=C conjugated with different electron-withdrawing groups as carbonyl, nitro and ester and carboxylic acid reductases. To date, most of the information about this enzymatic class comes from bacteria and yeasts. Even though filamentous fungi are good biocatalysts due to their natural biodiversity and their broad heterogeneous enzymatic pattern, they have been poorly investigated. This research aimed to develop fungal whole-cell processes to provide new sustainable synthetic tools for organic chemistry, producing enantiopure chiral compounds. Filamentous fungi belonging to Ascomycota, Basidiomycota and Zygomycota were investigated and most of them were capable of expressing ERs activity. Among them, *Mucor circinelloides*, *M. plumbeus* and *Syncephalastrum racemosum* were the most versatile and effective reducing all the substrates already within the first 1-3 days. *M. circinelloides* was used to assess the dynamics of the bioconversion of three target analytes through a time-course assay. The biotransformation correlated with the expression profile of the putative ERs genes found in *M. circinelloides* genome : the maximal peak of expression always occurred just before the beginning of C=C reduction, and sharply decreased as soon as the reaction started. Eight out of 10 genes have been expressed, among which ER1 and ER2 reached the highest expression levels. A homology modeling approach was adopted to study the 3-D features of the putative ERs and identify peculiarities between them. The overall structure of these enzymes, the FMN-binding site and the catalytic residues were mostly conserved. The models displayed peculiar features, mainly regarding a specific loop, the size of the active site and the surface charge. The substrate-enzyme interaction was studied by a molecular docking approach, revealing high variability in the binding mode, partially justifying the differences observed in the biotransformation assay. Further study will be aimed at the production of ERs of *Mucor circinelloides* by heterologous expression in order to purify and catalytically characterize these isoenzymes.

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CS5T57

Tuesday 5th April
14:00 - 16:00

FERNANDEZ BUNSTER Guillermo *(1), LAZARUS Colin (1)*
(1) University of Bristol, Bristol, UK

Characterization, analysis and heterologous expression of torrubiellone A gene cluster from *Torrubiella sp.* BCC2165 in *A. oryzae*.

Torrubiellones A-D, extracted from *Torrubiella sp.* BCC2165, are structurally similar to 2-pyridone compounds produced in related arthropod-pathogenic fungi, such as tenellin and desmethylbassianin in *Beauveria bassiana* and militarinone in *Cordyceps militaris*. Torrubiellone A is particularly interesting because it presents antimalarial activity (IC50 value of 8.1 µM), with very weak accompanying cytotoxicity. By comparing the gene clusters responsible for the biosynthesis of the structurally similar compounds, we predicted that *Torrubiella* genome sequencing, together with in-silico analysis should lead to the identification of the torrubiellone A biosynthetic gene cluster. *Torrubiella* BCC2165 DNA was extracted, sequenced and analysed using antiSMASH software to reveal a putative torrubiellone A gene cluster, encoding a hybrid PKS-NRPS (named torS), two P450 cytochromes (torA and torB) and an enoyl reductase (torC). Also, by comparison to the tenellin and desmethylbassianin gene clusters, two additional genes (named torD and torE), were also identified within the cluster, which could be responsible for structural differences between torrubiellone and desmethylbassianin. The PKS-NRPS gene (torS fused to eGFP) was assembled without introns by yeast recombination and put on a multigene expression vector with other biosynthetic genes either from the putative torrubiellone cluster (torA, torB and torC), or from the desmethylbassianin gene cluster (dmbA, dmbB and dmbC). The assembled plasmids were transferred into the filamentous fungus *Aspergillus oryzae* NSAR1, and combinations between the synthase and tailoring enzymes yielded strongly yellow-pigmented transformants, whose organic extracts were analysed by liquid chromatography-mass spectroscopy (LC-MS), achieving the production of torrubiellone related compounds. At the same time, torD and torE gene functions were investigated by coexpressing these genes in a tenellin-producing *A. oryzae* transformant. Simultaneously, the overexpression of two transcription factors flanking the torrubiellone gene cluster was achieved in the *Torrubiella* strain, being obtained an overproduction of torrubiellone compounds. Analysis of the promoters within the cluster was analysed for torD and torE because of their proximity and the results shows that one of the promoters is encoded in one of the other genes.

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CS5T59

Tuesday 5th April
14:00 - 16:00

BAHKALI Ali *(1), ABDEL-WAHAB Mohamed (2), EL-GORBAN Abdallah (1), HODHOD Mohamed (1)*
(1) Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia
(2) Department of Botany and Microbiology, Faculty of Science, Sohag University, Sohag, Egypt

Metagenomics study of endophytic fungi of *Avicennia marina* using next generation technology

Endophytic fungi are an excellent source of new pharmaceutical chemicals and may be potential sources of bioactive medicinal compounds. Metogenomics study of environmental samples enables rapid analysis of the composition and diversity of microbial communities at various habitats. We have assessed the endophytic fungi of *Avicennia marina* using next generation generation technology (NGS) by assessing its PCR amplicon of the partial LSU rDNA sequences using LROR and LR3 primers with Illumina metagenomics technique used to generate a total of 35,886 reads from four healthy looking samples of leaves of *A. marina*. Seventy-two OTUs were recorded from the four samples, of which 30 OTUs were single read. Yeast species dominating the fungal of the samples. The Basidiomycetous yeast genus *Malassezia* dominated the fungal community of the samples representing 98.6 % of the reads. Other common yeast genera were: *Hortaea*, *Candida* and *Schwanniomyces*. Common filamentous fungal genera were: *Trichoderma*, *Neocallimastix*, *Geranomyces*, *Ramaria*, *Laetiporus*, *Mucor* and *Gonapodya*.

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CS5T60

Tuesday 5th April
14:00 - 16:00

MANN Ross *(1), KRILL Christian (1), AUER Desmond (1), ROCHFORT Simone (1), PORTER Ian (1), EDWARDS Jacky (1), SAWBRIDGE Tim (1), SPANGENBERG German (1)*
(1) AgriBio, Centre for AgriBioscience, Melbourne, Australia

Genetics underpinning the production of volatile biocidal compounds from endophytic fungi

Endophytic fungi have been identified as a unique source of novel volatile biocides, and as such represent an exploitable resource for the discovery of new fumigants. This is timely given the global phase out of the ozone-depleting soil fumigant methyl bromide, and evidence of insect resistance with the fumigant of stored grain phosphine. For effective fumigation these volatile biocides from endophytes must exhibit broad spectrum bioactivity against a range of pests and pathogens, and possess the physical and chemical properties of a fumigant - high volatility, low residues, synergism. Research has been undertaken to identify novel volatile biocides from endophytic and determine their suitability as fumigants. Investigations have focused on a range of endophytic fungi from cool temperate and tropical Australia, including species of *Nodulisporium*, *Muscodor* and *Daldina* species. *In vitro* bioassays established the spectrum of activity of endophytes against agricultural pathogens (fungi and bacteria), pests (insects) and weeds. Metabolomic analyses indicated that the endophytes produced a diverse range of volatile metabolites, including mono- and sesqui- terpenes, many of which were common to plant defence (*e.g.* eucalyptol). Genomic and transcriptomic studies have complemented metabolomics analyses, to identify putative genes regulating the production of key volatiles. It is envisaged that these volatile metabolites will be used in combination (*e.g.* synergists) with currently available fumigants (*i.e.* not a direct replacement).

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CS5T61

Tuesday 5th April
14:00 - 16:00

WANG Ting-Fang *(1), LI Wan-Chen (1), CHUANG Yu-Chien (1), CHEN Chia-Ling (1)*
(1) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan

Hybrid infertility: the dilemma or opportunity of applying sexual development to improve *Trichoderma reesei* industrial strains

Trichoderma reesei (Teleomorph *Hypocrea jecorina*) RUT-C30 and QM9414 strains are the principal industrial producers of enzymes that hydrolyze lignocellulosic biomass into simple sugars, such as glucose and xylose. These fungi were generated from the wild isolate QM6a *via* multiple rounds of random mutagenesis for enhanced cellulase production and/or catabolite derepression. Accumulating evidence indicates that their genomes have acquired multiple chromosomal alternations (including nucleotide mutations, segmental deletions and rearrangements) compared with that of QM6a. We found that RUT-C30 and QM9414, such as QM6a, efficiently mated with the *H. jecorina* CBS999.97 mating partner, including completed sexual development and meiosis. However, they generated more non-viable segmental aneuploidy (SAN) ascospores than the sexual crossing of CBS999.97 with QM6a. Our results indicate that extensive mutagenesis during strain improvements resulted in speciation, *i.e.*, RUT-C30 and QM9414 are no longer the same species as QM6a and CBS999.97. Our finding is consistent with the classic chromosomal speciation model stating that chromosome rearrangements contribute to heterozygous hybrid infertility and serve as a genetic barrier between recently diverged species. We suggest that RUT-C30 and QM9414 are ideal models for deciphering molecular mechanisms by which new genetic barriers impeding reproduction arise. Hybridization between the industrial strains and the CBS999.97 wild isolates may generate SAN progeny that harbor existing or new beneficial phenotypes for economic applications.

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Tuesday 5th April
14:00 - 16:00

BIDARD Frederique *(1), POGGI PARODI Dante (1), JOURDIER Etienne (1), MARBOUTY Martial (2), MARGEOT Antoine (1), KOSKUL Romain (2), MARIE-NELLY Herve (2)*
(1) IFP Energies Nouvelles, Rueil malmaison, France
(2) Institut Pasteur, Paris, France

Evidence of massive chromosomal rearrangements in *Trichoderma reesei* RutC30 strain using high-quality genome (re)assembly.

The Sordariomycete *Trichoderma reesei* is a mesophilic filamentous fungus originally isolated from the Solomon Islands during World War II. *T. reesei* is currently used to produce enzymes hydrolysing cellulose-rich biomass to glucose then fermented to ethanol. The genome of the wild type strain QM6a was sequenced by the US DOE Joint Genome Institute (Martinez *et al.*, 2008) and recently re-scaffolded and improved through a new experimental and computational approach in only seven well-defined superscaffolds ranging in size from 3.6 to 6.6 Mb and comprising 99.8% of the original 33.3-Mb genome assembly (GRAAL; Marie Nelly *et al.*, Nature communications, 2014). In order to increase the cellulase production, *T. reesei* derivatives of QM6a have been exposed over the years to repeated rounds of random mutagenesis and selection procedures in many research institutes and companies around the world. One of the best producing strains available in the public domain is the *T. reesei* RUT C30. This mutagenized strain was obtained through three mutagenesis steps and its DNA structure was analyzed. In addition to numerous SNVs (130) and InDel (3), large chromosomal rearrangements were predicted by electrophoretic karyotyping, then, confirmed by array comparative genome hybridization method and massively parallel sequencing analysis (Mantyla, Curr. Genet., 1992, Vitikainen *et al.*, BMC Genomics, 2010, Le Crom *et al.*, PNAS, 2009, Koike *et al.*, Industrial Biotechnology, 2013). The GRAAL approach was implemented to assemble the RutC30 strain and to map the chromosomal rearrangements. As a consequence of the stringent mutagenesis processes, the assembly showed massive chromosomal rearrangements. Main rearrangements were located in chromosomes I, V and VII. In chromosome I, a 376-kb fragment of the right end of chromosome I was exchanged with 1.6-Mb fragment of the end of chromosome VII. In chromosome V, the middle region was highly rearranged, with the insertion of a 1.1-Mb fragment of chromosome VII, which was itself internally rearranged. This study highlighted the importance to get a better knowledge of the mutagenesis impact on the chromosomal structure. We are now investigating whether specific rearrangements are associated with the changes in cellulase production. As large structural variations have also been previously described in other industrial strains, achieving their proper assembly would help assessing which role such structural variations played in cellulase production improvements.

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Tuesday 5th April
14:00 - 16:00

WOLTERS Pieter *(1), BIJSTERBOSCH Gerard (1), VISSER Richard (1), VAN DER LINDEN Gerard (1), VLEESHOUWERS Vivianne (1)*
(1) Wageningen UR Plant Breeding, Wageningen, The Netherlands

Resistance to early blight in potato

The fungal necrotroph *Alternaria solani* causes early blight in potato. Early blight is most severe on early maturing cultivars, senescing plants, and plants that are stressed, for example by poor nutrition or drought. The disease is an increasing problem in the US, and *A. solani* strains are emerging that are resistant to most fungicides. We have sequenced the genome of *A. solani* using PacBio technology, resulting in a complete, gapless assembly of all 10 chromosomes. This high-quality genome will be used to make a comparison with genomes of other *Alternaria* species. By combining this data with the analysis of the transcriptome of *A. solani in vitro* and in infected plant tissue, we will predict putative effectors of *A. solani*. These effectors will be subjected to functional tests in potato plants and their role in susceptibility or resistance to early blight will be studied. As no potato cultivars have been identified that are fully resistant to early blight, we screened a large collection of wild potato species (*Solanum* section *Petota*) to identify sources of resistance to early blight. This resulted in the identification of several genotypes that have high levels of resistance to *A. solani*. We are currently crossing susceptible and resistant genotypes to generate progeny populations that can be used for genetic mapping studies. Knowledge of the effectors of *A. solani* will help to distinguish between different sources of resistance and could facilitate genetic mapping of genes that are responsible for the resistance against early blight. Ultimately, the results from this study will be used to develop potato cultivars that are resistant to early blight.

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CS5T64

Tuesday 5th April
14:00 - 16:00

JESENICNIK Taja *(1), STAJNER Natasa (1), RADISEK Sebastjan (2), JAVORNIK Branka (1), JAKSE Jernej (1)*
(1) University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, Ljubljana, Slovenia
(2) Plant Protection Department, Slovenian Institute for Hop Research and Brewing, Cesta Zalskega tabora 2, Zalec, Slovenia

Novel microRNAs discovered in phyto-pathogenic fungi *Vertcillium nonalfalfae* using high-throughput ion torrent sequencing and bioinformatic approaches

MicroRNAs (miRNAs) are endogenous small non-coding RNAs containing about 23 nt. They are capable of pairing with mRNAs and direct post-transcriptional repression or mRNA degradation. They are therefore considered to be negative regulators of gene expression. Several classes of small RNAs (sRNAs), including miRNA-like sRNAs (mil-RNAs), have been recently shown to exist in filamentous fungi. Moreover, some plant pathogens use them during the infection process as effector molecules. However, to date no mil-RNAs have been reported in the phyto-pathogenic fungus *Verticillium nonalfalfae*, a soil borne plant pathogen causing vascular wilt in many important crops worldwide. Two pathotypes of *V. nonalfalfae*, mild strain and lethal strain, have been isolated from Slovenian hop fields, with the lethal strain causing severe symptoms in hop plants, resulting in rapid and intense withering and complete dieback of the plant. With the accessible genome sequence and detailed transcriptome of the two strains, we aim to identify and characterize mil-RNAs in the two pathotypes of *V. nonalfalfae* and to elucidate their possible involvement in the pathogenesis process. Two Slovenian isolates, Rec (mild) and T2 (lethal), were acquired from the fungal genebank of the Slovenian Institute for Hop Research and Brewing. Small and total RNA fractions were isolated from four different sources: spores, mycelia, mycelia grown on simulated xylem fluid medium (SXM) and resting mycelia. Small RNAs were used for small RNA library construction and sequenced using the Ion Proton sequencing platform. Adaptor cleaned and length trimmed sequences were delivered in unaligned BAM format and subjected to quality control analysis. Using the RFAM 12.0 database, the quantity of rRNA, tRNA, snRNA and snoRNA species was determined. Fungal mil-RNA precursors were predicted using MIReNA software and the results were further inspected with the aid of the CLC Genomic Workbench package. Validation and selection of predicted precursors was performed manually using criteria for plant miRNA candidates proposed by Mishra *et al.* (2015). In addition, several candidate mil-RNA precursors were selected and have now been validated with stem-loop RT-PCR using mil-RNA-specific primers. Using this NGS and bioinformatic based approach, the existence of mil-RNA structures of *V. nonalfalfae* was confirmed. Studies to determine their endogenous targets and to investigate their role in the infection are planned for the future.

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CS5T65

Tuesday 5th April
14:00 - 16:00

DRULA - NADOR Elodie *(1), HAON Mireille (1), FAVEL Anne (1**)**, RUIZ-DUENAS Franciso J (3), RILEY Robert (4), HENRISSAT Bernard (**2**), BERRIN Jean-Guy (1), RECORD Eric (1), ROSSO Marie-Noëlle (1)*
(1) Biodiversité et Biotechnologie Fongiques, INRA, Aix Marseille Université, Polytech'Marseille, Marseille, France
(2) CNRS, Aix Marseille Université, Marseille, France
(3) Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, Madrid, Espagne
(4) US Department of Energy Joint Genome Institute (JGI), California, USA

Comparative genomics and transcriptomics highlight functional diversity for lignocellulose breakdown within the genus *Pycnoporus*, Basidiomycetes.

Among saprotrophs growing on lignocellulosic material, wood decay fungi produce and secrete a plethora of enzymes able to degrade the three main polymers in plant biomass : cellulose, hemicellulose and lignin by diverse hydrolytic and oxidative mechanisms. Those fungi are thereby a source of enzymes of interest to the modern human industries. The Basidiomycetes fungi from the genus *Pycnoporus*, order *Polyporales*, have been studied for their efficiency in numerous biotechnological applications related to their capacity to degrade lignin and to secrete laccases. The recently sequenced genome of *P. cinnabarinus* strain CIRM-BRFM137 (Levasseur *et al.*, BMC Genomics, 2014) contained the classical families involved in cellulose and hemicellulose degradation and a complete enzymatic arsenal for lignin breakdown including a set of laccases and class II peroxidases. To deepen the analysis of functional diversity within the genus *Pycnoporus*, we compared the genome of *P. cinnabarinus* with the genome of *Pycnoporus* strains from two other *Pycnoporus* species that originate from different geoclimatic regions. *Pycnoporus cinnabarinus* is widely distributed, especially in the Northern hemisphere. *P. coccineus* is found in countries bordering the Indian and Pacific Oceans and *P. sanguineus* is found in the tropics and subtropics of both hemispheres. All three species are white-rotters mainly found on hardwoods. Our analyses show that the three genomes share highly conserved genome features and gene repertoires coding for lignin- and cellulose-active enzymes. However, their responses to lignocellulosic substrates of various composition are different and involve different enzymatic machineries for lignocellulose degradation. In particular, the regulation of genes coding for oxido-reductases active on lignin is different in each strain despite conserved microsynteny. This study highlights the importance of exploring beyond genome repertoires the functional diversity of wood decay fungi.

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CS5T66

Tuesday 5th April
14:00 - 16:00

NOGUEIRA Karoline *(1), SAVOLDI Marcela (2), DOS REIS Thaila Fernanda (2), GOLDMAN Gustavo Henrique (2), SILVA Roberto Nascimento (1)*
(1) Department of Biochemistry and Immunology, University of São Paulo, Ribeirão Preto, Brazil
(2) Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

Characterization of new sugar transporter involved in the regulation of lignocellulosic biomass degradation in *Trichoderma reesei*

Trichoderma reesei has an astonishing ability to produce and secrete cellulases, and is the most important industrial fungus in the production of these enzymes that are used, among other purposes, in the biofuel industry, such as bioethanol. This work aims to contribute to the understanding of the molecular mechanisms involved in the process of deconstruction of biomass in *T. reesei* through the identification of new sugar transporters associated with this process. The strain QM9414 was grown in sugarcane bagasse 1% (pre-growth in glycerol 1% for 24 hours) for 24, 48 and 72 hours and glucose 2% for 24 and 48 hours, and the absolute expression of transporters, previously identified by *in silico* analysis of RNA-seq, was analyzed by RT- qPCR. A mutant strain carrying a deletion of tra3 was constructed by homologous recombination using pyrG as selection marker. The strains TU6_Δku70 (parental) and Δtra3 were grown on minimum medium and potato dextrose agar, respectively, during 8 days. The expression of genes cel7a and cel6a was evaluated by RT- qPCR in the mutant and parental strains after grown in sugarcane bagasse 1% (pre-growth in glycerol 1% for 24 hours) for 8, 12 and 24 hours. We also evaluated the ability of Tr3A to functionally complement the *Saccharomyces cerevisiae* EBY.VW4000 strain. In *S. cerevisiae* Tr3A were targeted to the plasma membrane by immunofluorescence localization. Deletion of the transporter affected fungal growth especially in presence of lactose and maltose, however, the complementation of the gene in a knockout strain of *S. cerevisiae* to EBY.VW4000 was able to restore growth on glucose, mannose and fructose as a substrate. In addition, the deletion of this transporter affected gene expression of two celobihiodralases (cel7a and cel6a) about three time less assessed during the fungus growing in sugarcane bagasse.
Despite these results, additional experiments should be conducted to confirm its function. With the data obtained, a model related to the role of the transporter in the metabolism of different carbon sources will be built, enabling a better understanding of the behavior of cellulolytic enzymes produced by *T. reesei* and contributing to the implementation of this fungus in the bioethanol industry.

Acknowledgment: FAPESP.

POSTER SESSION ABSTRACTS
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CS5T67

Tuesday 5th April
14:00 - 16:00

ANTONIETO Amanda *(1), DA SILVA Thiago Aparecido (2), SILVA Roberto Nascimento (1)*
(1) Department of Biochemistry and Immunology, University of São Paulo, Ribeirão Preto, Brazil
(2) Cellular and Molecular Biology, University of Sao Paulo, Ribeirão Preto, Brazil

The transcription factor Tr_a1 is involved in sporulation and cellulase production in *Trichoderma reesei*

The fungus *Trichoderma reesei* is known for its high capacity of cellulolytic enzymes production that act degrading cellulose polymer in molecules of glucose, which is a key point in the production of second generation ethanol. In this paper we analyze the role of the transcription factor Tr_a1 in sporulation and cellulase production by *T. reesei*. The strain QM9414 was grown in sugarcane bagasse 1% (pre-growth in glycerol 1% for 24 hours) for 24, 48, 72 and 96 hours and glucose 2% for 24 and 48 hours, and the absolute expression of tr_a1 was analyzed by RT-qPCR. A mutant strain carrying a deletion of tr_a1 was constructed by homologous recombination using hygromycin as selection marker. The strains TU6_Δku70 (parental) and Δtr_a1 were grown on MEX medium (malt extract 3% and agar 2%) and characterization of spore was assessed by flow cytometry and race tubes, during 14 days. The expression of genes cbh1, bgl1 and egl1 was evaluated by RT-qPCR in the mutant and parental strains after growth in glycerol 1% (24 hours) and cellulose 1% (8, 12, and 24 hours - after pre-growth in glycerol). During the growth of the strain QM9414 in sugarcane bagasse, an increase in the absolute expression of the gene tr_a1 was observed when compared to glucose, which expression was almost nil. The growth of TU6_Δku70 and Δtr_a1 in race tubes for 14 days was similar, however, they showed a different sporulation profile, once the mutant sporulated less than the parental strain. The influence of Tr_a1 in sporulation was confirmed by flow cytometry, since the spores of the mutant strain are smaller than the parental strain (11.5% of small spores in Δtr_a1 and 4.7% of small spores in TU6_Δku70). Regarding the cellulase production, it was observed that the deletion of tr_a1 promotes a decreased expression of cbh1, egl1 and bgl1 in almost two times compared to the parental strain in the presence of cellulose.
The transcription factor Tr_a1 is involved in the mechanisms of sporulation and cellulase production in *T. reesei*, becoming an important subject of study for bioethanol production.

Acknowledgment: FAPESP.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T68

Tuesday 5th April
14:00 - 16:00

NIELSEN Maria Lund *(1), PETERSEN Thomas Isbrandt (1), MORTENSEN Uffe Hasbro (1), ANDERSEN Mikael Rørdam (1), HOOF Jakob Blæsbjerg (1), LARSEN Thomas Ostenfeld (1)*
(1) Section for Eukaryotic Biotechnology, Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark

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

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

[1] Qiao, K.; Chooi, Y.-H.; Tang, Y.I. (2011). Metabolic Engineering, 13, 723-732.

POSTER SESSION ABSTRACTS
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CS5T69

Tuesday 5th April
14:00 - 16:00

MÄKELÄ Miia *(1), DILOKPIMOL Adiphol (2), BRÁS Joana L. A. (3), FONTES Carlos M. G. A. (3), GIDIJALA Loknath (4), JÜTTEN Peter (5), PIECHOT Alexander (5), VERHAERT Raymond M. D. (4), DE VRIES Ronald P. (2), HILDÉN Kristiina (1)*
(1) Division of Microbiology and Biotechnology, Department of Food and Environmental Sciences, University of Helsinki, Helsinki, Finland
(2) Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre & Fungal Molecular Physiology, Utrecht University, Utrecht, The Nerthelands
(3) Nzytech genes & enzymes, Campus Do Lumiar Ed. Lisbon, Portugal
(4) ProteoNic BV, Leiden, The Nerthelands
(5) Taros Chemicals GmbH & Co. KG, Dortmund, Germany

Characterization of putative feruloyl esterases of *Aspergillus niger*

Plant biomass consists mainly of the polymeric compounds cellulose, hemicelluloses and lignin. Ferulic acid is covalently attached to both hemicelluloses and lignin and, therefore, significantly contributes to the recalcitrance of cell wall against microbial attack. Ferulic acid esterases (FAEs, EC 3.1.1.73) are hydrolytic enzymes which participate in plant biomass degradation by cleaving ferulic acid polysaccharide linkages within the cell wall. 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. *Aspergillus* species are one of the best studied fungi, largely due to their applicability in biotechnology. The genome sequence of *Aspergillus niger* has revealed five FAE encoding genes. FaeA and FaeB of *A. niger* have been previously well-characterized and we have recently studied the three other FAE enzymes (FaeC, FaeD and FaeE, manuscript in preparation). By using phylogenetic gene prediction strategy we have also detected three more putative FAE gene models which have been cloned and codon optimized for heterologous production in *Pichia pastoris*. In this work, three novel FAEs of *A. niger* have been characterized for substrate profile, thermotolerance and solvent tolerance.

Acknowledgement
This research was supported by OPTIBIOCAT ("Optimized esterase biocatalysts for cost-effective industrial production», EU-FP7, Grant agreement no 613868).

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T70

Tuesday 5th April
14:00 - 16:00

DUNNE Keith *(1), O`DONOGHUE Martin (2), GROGAN Helen (2), BURTON Kerry (3), HENEGHAN Mary (1)*
(1) Institute of technology Sligo, Sligo, Ireland
(2) Teagasc, Dublin, Ireland
(3) East Malling Research (EMR), Kent, England

A genomic approach to understanding and improving mushroom compost utilisation.

Agaricus bisporus (common button mushroom) has an annual global value of over $4.7 billion with a domestic farmgate value of €120 million, making it Ireland's largest horticultural sector. However, significant profits are lost due to *A. bisprous* failure to fully utilise the compost it is grown on. This limits the compost use to just three flushes, after which point the spent mushroom compost has to be changed due to sub-optimal growth of the mushrooms. The overall objective of this research is to identify potential means of improving mushroom yields, particularly addressing the issues at the third flush through improved compost utilisation and strain selection. The ability of *A. bisporus* to colonise compost is due to the specific adaptation in terms of the enzymes it produces to utilise the substrate. Many genes and enzymes have been identified to have critical roles in this utilisation in order to facilitate the development of the mushroom fruit body. However, it is not known which of these are critical in controlling yields and these enzymes are likely to change dynamically in phase with the flush. While ultimately dependent on such enzymes, the yield is also affected by other factors. By examining the genes and enzymes associated with nutrition using microarray analysis, it will be possible to identify gene transcripts which are differentially expressed at specific time points in the cropping cycle. Genes identified to be possibly involved in yield control or flushing patterns will be further studied by promoter analysis. The genes with the most promising expression profiles will be characterised by heterologous/homologous expression systems.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T71

Tuesday 5th April
14:00 - 16:00

GOODWIN Stephen *(1), MCCORISON Cassandra B. (2), GRIMWOOD Jane (3), GRIGORIEV Igor V. (4), KEMA Gert H. J. (5)*
(1) USDA-Agricultural Research Service, Crop Production and Pest Control Research Unit, Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, USA
(2) Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, USA
(3) HudsonAlpha Institute for Biotechnology, Huntsville, Alabama, USA
(4) U.S. Department of Energy Joint Genome Institute, Walnut Creek, California, USA
(5) Plant Research International B.V., Wageningen University and Research Centre, Wageningen, The Netherlands

The mitochondrial genome of the banana pathogen *Mycosphaerella fijiensis* : partial duplications and multiple changes of direction

Pseudocercospora (previously *Mycosphaerella*) *fijiensis* causes black Sigatoka (aka black leaf streak) disease of banana. This disease was first discovered on the Fiji Islands during the 1960s but has spread since to most banana-producing areas worldwide, where it has rapidly displaced the less-aggressive *P. musicola*, the cause of yellow Sigatoka. Black Sigatoka causes huge losses to banana production and can only be managed with multiple sprays of fungicides. This leads to rapid evolution of resistance and loss of efficacy. One class of fungicides, the quinone outside inhibitors, targets the cytochrome b gene (cob) coded in the mitochondrial (mt) genome. However, so far the mt genome of *P. fijiensis* has not been published. To fill this void, the mt genome of *P. fijiensis* was assembled from shotgun-sequencing reads generated with Sanger technology. The mt genome, at 74,095 bases, is the largest yet reported in the class Dothideomycetes. The GC content was very low at 26%, which is typical for fungal mt genomes. This genome contained the usual 14 protein-coding genes seen in fungal mt genomes (atp6, atp8-9, cob, cox1-3, nad1-6, nad4L) plus the large and small ribosomal RNA genes (rnl, rns). The expanded size of the genome relative to other fungi was due to 41 tRNA genes, almost twice as many as seen in other fungi, plus partial duplications of several genes including rns, cox1 and nad2 and 18 open reading frames, potentially coding for genes of unknown function. Two genes (cob and nad5) had introns containing LAGLIDADG-type homing endonucleases. The intron in the cob gene was inserted at position F169, not at the G143 amino acid which is associated with fungicide sensitivity. This is consistent with the rapid development of resistance in field populations. A very unusual feature of the *P. fijiensis* mt genome is that it has at least five changes of direction. Genes on fungal mt genomes usually are coded on a single strand, but previously sequenced mt genomes of species in the Dothideomycetes all had a single change of direction. The *P. fijiensis* mt genome is the largest yet reported in the Dothideomycetes. The partial duplications and multiple changes of coding direction make it unique so far among fungi and continue the high diversity of mt genome structure and content seen among the species in this class.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T72

Tuesday 5th April
14:00 - 16:00

JARCZYNSKA Zofia Dorota *(1), HOOF Jakob Blæsbjerg (1), AASTED Freja (1), HOLM Dorte Koefoed (2), PATIL Kiran Raosaheb (3), NIELSEN Kristian Fog (1), MORTENSEN Uffe Hasbro (1)*
(1) Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark
(2) Novozymes A/S, Bagsværd, Denmark
(3) European Molecular Biology Laboratory, Heidelberg, Germany

Heterologous production of immunosuppressant mycophenolic acid in *Aspergillus nidulans*

Filamentous fungi are well-known producers of a wide range of valuable secondary metabolites, which can be advantageously exploited *e.g.* in the pharmaceutical industry. One of the most prominent examples is mycophenolic acid (MPA). MPA inhibits inosine-5'-monophosphate dehydrogenase (IMPDH), which catalyzes the rate limiting step in the guanine nucleotide synthesis. Since B- and T-lymphocytes rely entirely on de novo purine synthesis, MPA is used as an immunosuppressant during organ transplants. We have recently identified the mpa gene cluster in *Penicillium brevicompactum* [1] and have subsequently verified several steps in the MPA biosynthetic pathway [2,3,4]. However, the role of four genes remained to be characterized. We have therefore heterologously expressed the mpa cluster in a stepwise manner in *Aspergillus nidulans* and established a cell factory for MPA production. Using this strategy, we have demonstrated that MpaA possesses prenyl transferase activity and catalyzes the conversion from 5,7-dihydroxy-4-methylphtalide to 6-farnesyl-5,7-dihydroxy-4-methylphtalide (FDHMP). We have also shown that MpaG catalyzes the last enzymatic step in the biosynthesis of MPA *in vivo*, resulting in the production of MPA. Interestingly, one of the intermediates (demethyl-MPA) can be formed from FDHMP *via* an unknown enzymatic activity present in *A. nidulans*. Lastly, we also found exciting examples of cross chemistry in *A. nidulans*, which resulted in the production of MPA variants, In conclusion, we have successfully characterized the biosynthetic pathway of the top-selling drug, MPA and we have demonstrated that *A. nidulans* is a suitable cell factory for its heterologous production.

[1] T.B. Regueira *et al.* (2011) App. Environ. Microbiol., 77, 3035-3043.
[2] B.G. Hansen *et al.* (2011) App. Environ. Microbiol., 77, 3044-3051.
[3] B.G. Hansen *et al.* (2012) App. Environ. Microbiol., 78, 4908-4913.
[4] B.G. Hansen *et al.* (2011) BMC Microbiol., 11, 202

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T73

Tuesday 5th April
14:00 - 16:00

ISBRANDT Thomas *(1), LUND NIELSEN Maria (1), HOECK Casper (3), J. N. FRANDSEN Rasmus (2), O. LARSEN Thomas (1)*
(1) Natural Product Discovery, Department of Systems Biology, Technical University of Denmark, Søltofts Plads, Lyngby, Denmark
(2) Eucaryotic Molecular Cell Biology, Department of Systems Biology, Technical University of Denmark, Lyngby, Denmark (3) Department of Chemistry, Technical University of Denmark, Kemitorvet, Lyngby, Denmark

Prediction of secondary metabolite encoding genes based on chemical structure analysis

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

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T74

Tuesday 5th April
14:00 - 16:00

SATO Atsushi *(1), MATSUSHIMA Kenichiro (1), ITO Kotaro (1), HATTORI Masahira (2), HATTORI Masahira (3), MITUYAMA Toutai (4)*
(1) Kikkoman Corporation, Chiba, Japan
(2) The University of Tokyo, Chiba, Japan
(3) Waseda University, Tokyo, Japan
(4) National Institute of Advanced Industrial Science and Technology (AIST), Tokyo, Japan

Genome-wide comparative analysis among *Aspergillus* section *Flavi*.

Aspergillus sojae (*As*) and *A. oryzae* (*Ao*) are industrially important filamentous fungi of the genus *Aspergillus*, which are widely used for brewing and fermentation. These two species are taxonomically classified in section *Flavi*, which includes aflatoxigenic fungi : *A. parasiticus* (*Ap*) and *A. flavus* (*Af*). There has been controversy concerning whether these four species represent a single species or distinct taxa. It is difficult to clarify the relationship among these four species because these fungi exhibit considerable morphological and physiological variation. In order to clarify the relationship of these fungi at single base resolution, we performed re-sequencing and re-assembling of *As* NBRC 4239 upon our previous sequencing [1] and obtained high quality 39.4-Mb chromosomal sequences with a 29-kb mitochondrial genome, which is close to the size of *Ap* genome (39 Mb) [2] while larger than *Ao* (37.2 Mb) [3] and *Af* (36.8 Mb) [3]. We conducted genome comparison for these fungi and found that *As* is more homologous to *Ap* than to *Ao* and *Af*. Although *As* and *Ap* genomes are longer than *Ao* and *Af*, we did not find any genomic blocks to infer that *As* and *Ap* gained extra DNA by genome duplication. Results of our sequence analysis indicate that *As* and *Ap* genomes are more closer to their ancestral genome than *Ao* and *Af*. We measured homology of all predicted genes using alignment score of Scipio program [4]. Alignment score averaged over all predicted genes between *As* and *Ap* is 0.93 while that between *As* and *Ao* is 0.85. These results suggest that these fungi can be classified into two groups. We compared *As* and *Ap* genomes in terms of genomic regions of putative gene clusters for secondary metabolites. We found that *As* is deficient in some backbone genes which are essential for the secondary metabolite synthesis. This result suggests that *As* has lost abilities to generate some mycotoxins in the course of evolution.

[1] A. Sato *et al.* (2011) DNA Research, 18, 165-176.
[2] J.E. Linz *et al.* (2014) Eukaryot Cell, 13,1113-1123.
[3] J.G. Gibbons and A. Rokas. (2013) Trends Microbiol., 21, 14-22.
[4] O. Keller *et al.* (2008) BMC Bioinformatics, 9, 278.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T75

Tuesday 5th April
14:00 - 16:00

MAJCHERCZYK Andrzej (1), ***KÜES Ursula*** *(1)*

Analysis of response of heterobasidion irregulare on *Pinus sylvestris* and *Picea abies* wood

Heterobasidion irregulare is a very common pathogenic fungus that causes severe damage in forests in Canada and the United States. The fungus is one of the most harmful fungi to the modern forest industry, attacks pine trees and occasionally also other wood species, and causes root and butt rot disease. It is a typical white rot, degrading simultaneously or selectively lignin. In advanced decay stages, the rot raises several meters up the stem of infested trees and makes the wood useless for the wood industry. Genome sequencing of *H. irregulare* by the JGI in 2009 provided the genetic information on this fungus required for efficient studies of the fungal proteome. To study the largely unknown proteomic response of this basidiomycete to pine and spruce, the fungus was grown in liquid glucose medium with addition of *Pinus sylvestris* and *Picea abies* wood. Liquid cultures with freely secreted proteins and fungal mycelia were analyzed by modern shotgun proteomics. We developed sophisticated methods for effective isolation of fungal secretomes from complex media and fungal mycelia allowing quantitative comparison of the samples. Shotgun analysis of the proteomes by modern ESI-UPLC-MS/MS allowed identification and quantification of the proteomes of *H. irregulare* cultivated with and without wood supplement. Growth of the fungus was dramatically influenced by addition of wood with strongly increased growth. Using the annotated *H. irregulare* genome, hundreds of secreted proteins have been identified from culture media and a few thousands from fungal mycelia. Supplementation with wood suppressed only a minor number of proteins but has a strong inducing effect on proteins with functions related to wood degradation as well as on many uncharacterized proteins with unknown function.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T76

Tuesday 5th April
14:00 - 16:00

HETTIARACHCHIGE Inoka (1,2,3), SAWBRIDGE Timothy (1,2), MANN Ross (1,2), ***GUTHRIDGE Kathryn*** *(1,2), FORSTER John (1,2,3), SPANGENBERG German (1,2,3)*
(1) AgriBio, Centre for AgriBioscience, La Trobe University, Bundoora 3086, Victoria, Australia
(2) Dairy Futures Co-operative Research Centre, Australia
(3) School of Applied Systems Biology, La Trobe University, Bundoora 3086, Victoria, Australia

Transcriptome analysis of perennial ryegrass - asexual *Epichloë* symbioses during seedling growth and maturation

Perennial ryegrass (*Lolium perenne L.*) is one of the most important species utilised for temperate pastoral agriculture, forming mutualistic associations with a genetically diverse group of *Epichloë spp.* fungal endophytes. The advent of "next-generation" sequencing has enabled genome sequencing of both the host grass and the fungal endophytes. Genome sequence analysis of isolated asexual *Epichloë* fungal endophytes has confirmed the identities of 3 distinct taxa that colonise perennial ryegrass : *E. festucae var. lolii* (LpTG-1; *Lolium perenne* taxonomic group), LpTG-2 and LpTG-3. Transcriptome analysis was performed to study the major changes that occur in host and endophyte transcriptomes during seedling growth and maturation, from post imbibition to 10 day old seedlings. Analysis of a time-course of developing symbiota was undertaken for 3 different perennial ryegrass (*cv. Alto*) - endophyte (strains representing *E. festucae var. lolii*, LpTG-2 and LpTG-3) symbioses compared to endophyte free (Alto-E-) hosts. This has been achieved through mapping RNA derived reads to genes from the reference genomes of the perennial ryegrass host grass and the fungal endophytes simultaneously. The analysis revealed that the transcriptome of the *E. festucae var. lolii* symbiosis is somewhat different to that of the LpTG-2 and LpTG-3 symbioses. Furthermore, studying host-endophyte interactions at the early stages of the germination reveals endophyte presence affects plant genes involved in de-etiolation responses.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T77

Tuesday 5th April
14:00 - 16:00

HETTIARACHCHIGE Inoka (1,2,3), LUDLOW Emma (1,2), ***GUTHRIDGE Kathryn*** *(1,2), FORSTER John (1,2,3), SPANGENBERG German (1,2,3)*
(1) AgriBio, Centre for AgriBioscience, La Trobe University, Victoria, Australia
(2) Dairy Futures Co-operative Research Centre, Australia
(3) School of Applied Systems Biology, La Trobe University, Victoria, Australia

Generation and characterisation of transgenic endophytes containing the peramine biosynthesis gene

Endophytic fungi (*Epichloë spp.*) of pasture grass plants produce four classes of bioactive alkaloids that confer resistance to herbivory by a range of organisms. Peramine and lolines are highly active against invertebrate pests, but non-toxic to livestock. Development of grass-endophyte associations with minimal or no undesirable effects in combination with desirable characteristics is important for pastoral agriculture. A single gene (perA) has been identified as solely responsible for peramine biosynthesis. Though the perA gene is distributed widely across *Epichloë* taxa, several mutations have been identified that result in endophyte strains that do not produce the metabolite. In the current study, the perA gene was characterised for 21 distinct ryegrass endophyte strains representing *E. festucae var. lolii* (LpTG-1; *Lolium perenne* taxonomic group), LpTG-2, LpTG-3 and LpTG-4. Intact perA genes were identified for peramine-producing strains, while non-functional versions characterised by partial deletions associated with miniature inverted-repeat transposable elements (MITE) insertions and premature stop codons were observed for non-producers. A functional copy of the perA gene was introduced into three recipient endophyte genomes by *Agrobacterium tumefaciens*-mediated transformation. Target strains included those that lack perA and otherwise fail to produce anti-mammalian toxins, and those characterised by different perA gene copies. Transformants were characterised in order to confirm transgene insertion and mitotic stability. Substantial *in vitro* production of peramine was observed for transformants of all three endophyte strains at variable levels. Following inoculation into perennial ryegrass, in planta peramine production of transgenic endophytes was confirmed for novel host-endophyte associations.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T78

Tuesday 5th April
14:00 - 16:00

EKANAYAKE Piyumi (1,2), HETTIARACHCHIGE Inoka (1,2), MANN Ross (1,2), KAUR Jatinder (1,2), SAWBRIDGE Timothy (1,2), GOMES-CARDOSO Patricia (1), ***GUTHRIDGE Kathryn*** *(1,2), SPANGENBERG German (1,2)*
(1) AgriBio, Centre for AgriBioscience, La Trobe University, Bundoora 3086, Victoria, Australia
(2) School of Applied Systems Biology, La Trobe University, Bundoora 3086, Victoria, Australia

Novel endophyte discovery and pan-genome analysis of endophytes of *Brachiaria* species

Brachiaria is a pan-tropical grass genus comprising 100 species, of which several are economically important forage pasture crops. Although widely used for pasture-based agriculture in tropics, *Brachiaria* exhibits a number of shortcomings that constrain both its use and genetic improvement. Endophytes provide an excellent mechanism for novel trait delivery in *Brachiaria* improvement. Fungal endophytes were isolated from 11 *Brachiaria* species. Phylogenetic analyses based on nuclear ribosomal DNA variation indicated that the isolates represented at least seven distinct taxonomic groups. Endophyte isolates from each group were subjected to genome sequencing to evaluate genomic diversity and taxonomic status across the fungal endophyte population. Assembly and annotation of the sequences indicated a genome size of 29 - 36 MB, which contained approximately 9950 - 12000 genes. Gene sequences, including translation elongation factor 1-a (tefA) and Y-tubulin (tub2) "house-keeping" genes, were identified for each *Brachiaria*-derived endophyte taxon from whole genome sequence data. The assembled gene sequences were used to reconstruct evolutionary relationships between the novel *Brachiaria*-derived endophytes and their closely related fungal species available in the GenBank public database. Results from the present study confirmed the distinct taxonomic status of the newly identified *Brachiaria* endophytes as well as provided an enhanced understanding of the genomic variation amongst fungal endophytes of *Brachiaria*.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T79

Tuesday 5th April
14:00 - 16:00

MARINOVIC Mila *(1), MÄKELÄ Miia (1), DILOKPIMOL Adiphol (2), DE VRIES Ronald (2), HILDÉN Kristiina (1)*
(1) Department of Food and Environmental Sciences, Division of Microbiology and Biotechnology, University of Helsinki, Helsinki, Finland
(2) Fungal Physiology, CBS-KNAW & Fungal Molecular Physiology, Utrecht University, Utrecht, The Nerthelands

Glutathione S-transferases of the white-rot fungus *Dichomitus squalens* : expression of genes and production of enzymes

Glutathione S-transferases (GSTs; E.C. 2.5.1.18) constitute a complex and diverse superfamily of multifunctional enzymes with essential roles in cellular detoxification. These proteins are widespread among all members of animals, plants, bacteria and fungi. However, only few fungal proteins have been reported so far, and little is known about these enzymes. Some of the GSTs possess Y-etherase activity, which is responsible for selective cleavage of Y-O-4 aryl ether linkages present in lignin. Classical lignin-modifying oxidoreductases (laccases and peroxidases) catalyze non-selective cleavage of different types of bonds present in lignin. In order to unlock the potential of lignin to become major renewable source of bulky and fine chemicals, novel enzymes catalyzing aromatic conversions are needed for lignin valorization. In this study, putative GST encoding genes from the white-rot basidiomycete *Dichomitus squalens* were cloned and heterologously expressed in *E.coli*. Biochemical properties of *D. squalens* GSTs will be presented, and their potential for selective Y-O-4 linkage cleavage will be evaluated.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T80

Tuesday 5th April
14:00 - 16:00

PIETRUSIŃSKA Aleksandra *(1), CZEMBOR Jerzy Henryk (1)*
(1) IHAR - PIB National Centre for Plant Genetic Resources, Radzików, 05-870 Błonie, Poland

Applied biotechnology to combat the leaf rust caused by *Puccinia triticina* and powdery mildew caused by *Blumeria graminis* in polish wheat cultivars

The main purpose of crop production is to achieve the highest possible yield while minimizing use of pesticides. Growing varieties with beneficial traits, also with a high potential of yield is closely connected with their resistance to fungal and viral diseases. The important role is playing breeding for resistance. At present, many tools of molecular genetics and biotechnology exist which can be successfully used to obtain plant disease resistance. Leaf rust and powdery mildew are occurring in all wheat growing regions of Poland and every year contribute to losses in cereal production. The main reason for these losses is the cultivation of varieties that are susceptible to these diseases.
The aim of presented research is:
- pyramiding of leaf rust and powdery mildew resistance genes in a one genotype;
- to clarify the location Lr55 gene in wheat genome;
- screening for resistance leaf rust and powdery mildew.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T81

Tuesday 5th April
14:00 - 16:00

VERWAAL René *(1), KROES Wouter (1), PEL Herman (1), HENSING Marco (1)*
(1) DSM Biotechnology Center, Delft, The Netherlands

Optimizing enzyme cocktails and process conditions for production of cellulosic ethanol

DSM's origin and biotechnology roots date back to 1869 when Jacques C. van Marken, an innovative businessman believing in science, founded NG&SF (Dutch Yeast & Spirits Factory) to produce baker's yeast and potable alcohol. More than a century (145 years) later, DSM *via* its joint venture POET-DSM Advanced Biofuels, is again involved in ethanol production, but now *via* licensing advanced yeast and enzyme technologies to an emerging lignocellulosic bioethanol industry based on corn crop residue. Operation of the joint venture's commercial scale demonstration plant "Project LIBERTY" in Emmetsburg, Iowa has started up in 2014. The role of DSM's advanced yeasts and cellulosic enzyme cocktails is to enable the industry to diversify from starch crops to lignocellulosic agricultural residues, unlocking the full industrial potential of this abundantly available, sustainable type of feedstock. While this opportunity has been recognized since many years, enzyme costs were still prohibitive for commercial operations and had to be reduced by a factor of more than 10. DSM took different approaches to reach this ambitious cost reduction target and developed robust thermostable enzyme cocktails which will a/o be offered as an element of the POET-DSM technology package to parties interested in building their own commercial plants. We will highlight how combined efforts in enzyme discovery and development, fungal strain construction and high throughput fungal strain development, enzyme cocktail optimization, and fermentation as well as application developments were used to make lignocellulosic bioethanol a commercial reality NOW.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T82

Tuesday 5th April
14:00 - 16:00

KRAŠEVEC Nada *(1), BELC Sabina (1), NOVAK Maruša (2), SEPČIĆ Kristina (2), MAČEK Peter (2), ANDERLUH Gregor (1)*
(1) National Institute of Chemistry, Laboratory for Molecular Biology and Nanobiotechnology, Ljubljana, Slovenia
(2) University of Ljubljana, Biotechnical Faculty, Department of Biology, Ljubljana, Slovenia

Fungal actinoporin-like proteins: analysis in *Aspergillus niger*

Actinoporins (20 kDa) are potent cytolytic toxins isolated from sea anemones, structurally defined by a rigid beta-sandwich flanked by two alfa-helices (PF06369). Superfamily of actinoporin-like proteins (ALPs) comprises diverse protein families sharing structural similarity: fungal fruit body lectins (FB lectin PF07367), necrosis inducing proteins (NPP1 PF05630), pathogenesis-related proteins (Thaumatin PF00314), Vibrio thermostable direct haemolysin (TDH PF03347) and aegerolysins (PF06355). Combination of bioinformatics and molecular biology tools will be applied to determine the occurrence and imply the possible function of ALPs in fungi. Genome mining for ALPs in fungal kingdom and comparison of lifestyles, analysis of genome loci, promoters, transcription patterns, secretion and protein signatures, literature search for described function and application were performed. ALPs from *Aspergillus niger* were chosen for cloning, expression, deletion and GFP fusion studies. We determined the distribution for fungal fruit body lectins, necrosis inducing proteins and aegerolysins. The occurrence was highly heterogeneous, with aegerolysins and necrosis inducing proteins overrepresented, while fungal fruit body lectins were rarer and we observed no obvious correlation to taxonomy or pathogenous lifestyle. At least part of ALPs may be considered as small secreted proteins, often without recognizable signal peptide. Some potential biotechnological applications of aegerolysins are already evident, despite the still limited knowledge at present (Novak M. *et al.*, Appl. Microbiol. & Biotechnol. 99:601, 2015). Aegerolysins can be used as markers to detect and label specific membrane lipids, or as biomarkers of fungal exposure and progression of infectious diseases, or as a species determination tool for closely related phytopathogen species. Strong promoters that regulate aegerolysin genes can promote secretion of heterologous proteins from fungi.

POSTER SESSION ABSTRACTS
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CS5T83

Tuesday 5th April
14:00 - 16:00

SOLTYS Julie (1), PEYRARD Stéphane (2), GAUTHIER Pierrick (2), DAROCHA Martine (1), VILLALBA François (2), ***GOURGUES Mathieu*** *(1)*
(1) UMR Institut Sophia Agrobiotech, INRA, CNRS, Université de Nice Sophia Antipolis, 400 route des chappes, BP 167, 06903 Sophia Antipolis, Sophia Antipolis, France
(2) Bayer Cropsciences, La Dargoire Research Center, Disease Control Biology Lyon, 14-20 Avenue Pierre Baizet, 69263 Lyon Cedex 09, Lyon, France

Towards a pipeline for rapid identification of the molecular targets of fungicides

Identification of the mode of action of fungicides is an essential process. It brings together small molecules and the corresponding targets in filamentous plant pathogens and is required for the development of active ingredients together with their optimization. Moreover, detecting new targets is a key driver to develop innovative and sustainable strategies against plant diseases. The present work aimed at developing a combination of genetic and bioinformatics approaches dedicated to the identification of the molecular target of fungicides Strains with an improved resistance pattern to a selected fungicide are first obtained by mutagenesis. Mutations involved in the reduction of susceptibility to the molecule are identified in a second step, by comparing the transcriptome of resistant strains together with that of the wild type strain. The soil borne oomycete *Phytophthora parasitica* (INRA 310 strain) was selected as the model oomycete organism. Oxathiapiprolin was chosen as the active compound since its mode of action was previously reported. This molecule shows selectivity against different oomycetes species and targets an oxysterol-like binding protein (OBP) as reported in *Phytophthora capsici*. An EMS mutagenesis protocol was developed and twelve mutants with an enhanced tolerance to oxathiapiprolin were obtained in a first instance. Five showed only a limited resistance. On the contrary, the remaining seven mutants exhibited a significant improvement of their tolerance to Oxathiapiprolin since a resistance factor from 79 to 3500 was calculated for these strains. Transcripts accumulated in mycelium were sequenced from the wild type INRA 310 strain, from two mutants with a low tolerance to oxathiapiprolin and from the seven mutants with high level of resistance. Around 5000 to 6000 mutations per mutant were detected in the nine mutant strains. The OBP gene was mutated in all but two mutants with reduced tolerance. Comparison of relevant SNP observed in the seven mutants ended up with a list of 40 candidate genes with a putative OBP encoding gene (PPTG08785-1) among them. Three alleles (G686V, N753I and I793F) of the OBP were observed and corresponded to mutations previously described as leading to oxathiapiprolin resistance. Altogether these results proved that the combination of genetics and SNP Discovery is a promising method to quickly reduce the number of target candidate to be further investigated.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T84

Tuesday 5th April
14:00 - 16:00

KHONSUNTIA Weeradej *(1), SEN Mandira (1), SINGHADUANG Wassana (2), KÜES Ursula (1)*
(1) University of Göttingen, Büsgen-Institute, Göttingen, Germany
(2) Rajamangala University of Technology Lanna , Phitsanulok, Thailand

Genetic stability of commercial *Coprinopsis cinerea* strains

Coprinopsis cinerea is an Agaric that is easy to grow in artificial media and on natural substrates (horse dung). Upon fast mycelial growth, within a few days the fungus easily forms mushroom in the laboratory. Although it has been long used as a model species for studies in genetics and development of higher basidiomycetes, commercial production of edible *C. cinerea* has thus far mostly been overlooked. In Thailand however, coprini are grown by small farmers on agricultural wastes for sale as specialty mushrooms on local markets. The species identity however was so far not clear. Mushroom spawn was bought from providers in Thailand. ITS sequencing of the dikaryotic mycelium proofed the fungi to be *C. cinerea*. Although produced in cultures plentiful in numbers, *C. cinerea* fruiting bodies are relatively small and very delicate. Additionally, the mushrooms once matured easily autolyze within a few hours. Accordingly, the mushroom shelf-lives are remarkably short. *C. cinerea* mushrooms need thus be consumed immediately after picking or they have to be cleaned and blanched and pickled in saline for longer storage. In Thailand *C. cinerea* mushrooms are therefore harvested as young primordia in which spore production has not been started. Mature fruiting bodies are black in appearance due to the masses of colored basidiospores on the gills. We coincidently obtained a sporeless mutant from progenies of a *C. cinerea* fruiting body formed on commercial spawn bought in Thailand. The strain has white caps as a result of very few basidiospores produced on the surfaces of the gills and fruiting bodies did not autolyze. Such sporeless strain can thus be favorable for mushroom cultivation. However, the strain lost the ability to produce fruiting bodies and the originally strong fluffy-growing mycelium turned into a thin slow growth type lacking much aerial mycelium, indicating some type of genetic instabilities to occur in the strain. We also observed with time alterations in the fruiting behavior of the parental strain with black mushrooms. To learn more about these instabilities, monokaryons were isolated from germinating basidiospores from a black fruiting body of the original commercial strain as well as monokaryons from uninucleate oidia the dikaryotic strain that formerly gave rise to white mushrooms. We use these monokaryons in studies to address the origin of the genetic instabilities in the commercial strains from Thailand.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T85

Tuesday 5th April
14:00 - 16:00

*YANG Dongqing (1), **MIAO Youzhi** (1), KUBICEK Christian (2), GRIGORIEV Igor (3), KOPCHINSKIY Alexey (2), ZHANG Jian (1), ATANASOVA Lea (2), ZHANG Ruifu (1), SHEN Qirong (1), DRUZHININA Irina (2)*
(1) Jiangsu Key Lab for Organic Waste Utilization and National Engineering Research Center for Organic-based Fertilizers, Nanjing Agricultural University, NanJing, China
(2) Microbiology Group, Research Area Biotechnology and Microbiology, Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria
(3) JGI, Walnut Creek, CA, USA

trichoCODE : a modal genome annotation pipeline for *Trichoderma*

Trichoderma (Hypocreales, Ascomycota) - a large and relatively well studied genus of mycotrophic fungi - is mainly known because of several «good» species that are used as agents of biological control of plant pathogenic fungi (biofungicides) and/or as biofertilizers because they promote plant's growth and development. Moreover, one other species, *T. reesei*, is used in biotechnology for production of cellulases, hemi-cellulases and as a cell factory for protein expression and biosynthesis of secondary metabolites. The genomics of *Trichoderma* began almost a decade ago with first genomes sequenced and annotated in JGI. However in recent years numerous research institutions published genomes of *Trichoderma* strains making the total number of whole genome projects approaching 15 (2015). However the comparative analysis shows considerable incongruence in genomic properties of even closely related species or strains that have been sequenced and annotated using different gene prediction pipelines. In this work we present trichoCODE, the modular genome annotation pipeline that includes a high quality training set for ab initio gene calling in *Trichoderma*. The pipeline is based on the fungal genome annotation protocol of the Broad Institute. It includes four stages for gene structure annotation: (i) preparation of the training set; (ii) training and prediction; (iii) combination and fusion of multiple outputs; (iv) updating of gene structures with UTR (if EST/RNA-Seq data provided). The novelty of the pipeline comes from the fact that it is semi-automated, *i.e.* it allows the user to set up the best possible training set and to evaluate the annotation at several checkpoints. The comparative analysis of genome annotations of the standard *Trichoderma* genomes, *T. reesei*, *T. virens* and *T. atroviride* made using JGI, MAKER2 and trichoCODE pipelines revealed that JGI's pipeline gave the most possible gene models while MAKER2's were very conservative and trichoCODE's stay in the middle. Nevertheless, trichoCODE allowed the calling of 607 new gene models in *T. reesei*, 506 for *T. virens* and 468 in *T. atroviride*, respectively. Our work also demonstrates the need to unify the annotations of previously published *Trichoderma* genomes, such as *T. longibrachiatum* and *T. hamatum* annotated with different pipelines and with no use of a *Trichoderma*-specific training sets. Although prepared for *Trichoderma*, trichoCODE pipeline is open for any other organism as it is training-set dependent.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T86

Tuesday 5th April
14:00 - 16:00

O'CONNOR Eoin *(1), FITZPATRICK David (1), BURTON Kerry (3), GROGAN Helen (2)*
(1) Maynooth University, Kildare, Ireland
(2) Teagasc Food Research Centre, Ashtown, Dublin, Ireland
(3) East Malling Research, Kent, UK

Determination of the antiviral genetic attributes of *Agaricus bisporus* strains

The edible mushroom *Agaricus bisporus* is the most important horticultural food crop produced in Ireland. Approximately 20 strains of *A. bisporus* are cultivated commercially around the world, which are almost genetically identical. High usage of this monoculture and the suceptibility of this strain to mushroom virus X (MVX) has resulted in outbreaks of this complex disease in many European countries, causing moderate to severe crop loss. MVX is associated with multiple mycoviruses. *Agaricus bisporus* virus 16 (Abv16) has been isolated as the cause of brown cap mushroom disease. The cap browning symptom is the most prominent feature of MVX infection and it is an undesirable trait as it reduces the quality of mushrooms to an unacceptable level. Research involving MVX challenge studies has shown some virus tolerance by selected strains. Understanding the genetic and molecular mechanisms associated with this phenomenon may contribute to future breeding research. Sequencing of entire genomes and genome assembly is being carried out for selected *A. bisporus* strains. Genome assembly of high-throughput data, derived initially as raw sequence data, allows for confidence in high coverage of a given genome. This approach can expose principles of potential resistance to MVX at the genome level. Challenges arise when assessing assemblies of eukaryotic genomes for multiple reasons, a common example is the presence of highly repetitve sequences in many eukaryotic chromosomes. Using high-tech computational software such as the Velvet algorithms, erroneous data can be carefully circumvented. Sequencing of the transcriptome is being carried out for selected strains and will elucidate changes in expression profiles across strains following viral challenge studies. Comparisons of bespoke expression profiles will provide information on characteristics indicative of viral suceptibillity and viral resistance. Analyses involving LC-MS for observation of protein expression will further advance our knowledge of transcriptome data. This work will result in new insights on the response of selected *Agaricus bisporus* strains to viral challenge.

POSTER SESSION ABSTRACTS
Session CS5 Applied genomics and biotechnology
CS5T87

Tuesday 5th April
14:00 - 16:00

VARGA Torda *(1), KRIZSÁN Krisztina (1), SZARKÁNDI János Gergő (3), DIMA Bálint (2), KISS Brigitta (1), VÁGVÖLGYI Csaba (3), PAPP Tamás (3), NAGY G. László (1)*
(1) Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary
(2) Department of Plant Anatomy, Eötvös Loránd University, Budapest, Hungary
(3) Department of Microbiology, University of Szeged, Szeged, Hungary

Does enclosed development of young fruiting bodies confer an evolutionary advantage in the Agaricomycetes?

Early morphogenesis is one of the most critical steps in the life of an organism. Both higher plants and animals evolved strategies for enclosed development of early embryos, which might increase the chance of survival of the organism. Fungal fruiting body initials undergo a highly integrated developmental program, including distinct types based on the position of the initiation of young fruiting body (epinodular, endonodular) and the presence or absence of protective veil layers, yet, whether enclosed development confers any evolutionary benefit is unknown. Here we test if there is a trend towards enclosed primordium development in the evolution of Agaricomycetes. We assembled the biggest multilocus dataset of the Agaricomycetes to date, comprising 5541 species with three loci (5034 nrLSU; 1295 RPB2 and 751 ef1-a sequences), including 1386 newly sequenced taxa. We collected literature data on developmental types across all families for which such data were available. We then inferred maximum likelihood phylogenies for the 5541-taxon dataset using a phylogenomic dataset of 103 species as a backbone constraint and analyzed the transition probabilities between developmental types using likelihood based comparative methods to test whether there is a trend towards enclosed primordium development. We discuss the results in the context of fruiting body evolution and phylogenetics of Agaricomycetes.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W1

Wednesday 6th April
14:00 - 16:00

MOR Mariana (1), ELAD Yigal (2), ***HAREL Arye*** *(1)*
(1) Department of Vegetable Research, Institute of Plant Sciences, ARO, The Volcani Center., Bet Dagan, Israel
(2) Department of Plant Pathology and Weed Research, ARO, The Volcani Center., Bet Dagan, Israel

Scanning diversity of *Botrytis cinerea* strains

Botrytis cinerea (teleomorph: *Botryotinia fuckeliana*) is a broad host-range necrotroph, inflicting extensive economic burden worldwide [1, 2]. A dominant factor hindering its control is a remarkable genetic variability resulting from mating capability and other sources of variation (*e.g.*, transposons, variable chromosome number, and vegetative compatibility), enabling broad adaptation capability. To characterize the variability of *B. cinerea*, we tested (30) isolates from different locations and crops in Israel. Isolates were characterized by traits associated with growth parameters and pathogenicity on tomato (*i.e.*, growth rate on PDA, rate of development of necrotic lesion on leaves and stems, and rate of conidia germination on leaves). Genetic variability was further analyzed using polymorphic microsatellite markers [3]. Over all the variable isolates collection may : i) facilitate breeding programs developing sustainable resistance in crops, based on a "strains-mix" that will represent diverse pathogenicity mechanisms; ii) serve as a base for future research of the underline genetic mechanisms based on differential virulent-phenotypes.

[1] Dean R, Van Kan JA, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, *et al.* (2012) Mol Plant Pathol. 13(4):414-30.
[2] Droby A, and Lichter A. (2004) In Botrytis: Biology, Pathology and Control. Elad Y, Williamson B, Tudzynski P, and Delen N, eds. (Dordrecht, The Netherlands: Kluwer Academic Press), pp. 349–367.
[3] Fournier E, Giraud T, Loiseau A, Vautrin D, Estoup A, Solignac M, *et al.* (2002) Mol Ecol Notes. 2:253-255.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W2

Wednesday 6th April
14:00 - 16:00

VILLANI Alessandra (1), PROCTOR Robert H. (2), MCCORMICK Susan P. (2), BROWN Daren W. (2), MORETTI Antonio (1), LOGRIECO Antonio (1), ***SUSCA Antonia*** *(1)*
(1) Institute of Sciences of Food Production, CNR, Bari, Italy
(2) National Center for Agricultural Utilization Research, U.S. Department of Agriculture, Peoria, Illinois, USA

Genetic variability in cereal isolates of the *Fusarium incarnatum-equiseti* species complex

The *F. incarnatum-equiseti* species complex (FIESC) includes fungi associated with diseases of multiple agricultural crops. Although members of FIESC are considered moderately aggressive, they produce diverse mycotoxins, including trichothecenes. Because FIESC exhibits cryptic speciation, DNA-based phylogenetic analyses are required to identify some species. Here, we used EF-1a, RPB2, CaM and TUB2 gene sequences to examine phylogenetic relationships of 69 isolates with FIESC-like morphology recovered from cereals in the EU and North America. In the resulting phylogeny, the majority of isolates were resolved into 4 previously described phylogenetic species of FIESC, and 7 isolates were resolved into a clade that likely constitutes a novel species (FIESC 31). Comparisons of 12 genomes, representing FIESC 31 and 11 previously described FIESC lineages, revealed marked variation in distribution of mycotoxin biosynthetic gene clusters. The trichothecene cluster was present in all 12 genomes, but differed in presence, absence and arrangement of genes relative to the cluster in the *F. sambucinum* species complex (FSAMSC), the other major lineage of trichothecene-producing fusaria. Notably, the FIESC clusters includes a transcription factor gene that is absent in the FSAMSC cluster. These results indicate that at least 5 FIESC species occur on cereals, and although the species vary in genetic potential to produce several mycotoxins, their potential to produce trichothecenes is uniform.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W3

Wednesday 6th April
14:00 - 16:00

LO Ying-Chu *(1), LE PRIEUR Stéphanie (1), BRANCA Antoine (1), GIRAUD Tatiana (1)*
(1) Université de Paris-Sud, Laboratoire Ecologie Systématique et Evolution (ESE), Bâtiment 360, 91400 Orsay , France

Adaptive sausagenomics

An important goal in evolutionary biology is to unravel the genetic and genomic changes underlying adaptation in different environments, and fungi are ideal research models for understanding the genomic mechanisms of adaptation. Fungi have several advantages, such as short generation times, small genomes, and they are experimentally tractable. According to previous studies, horizontal gene transfers (HGT) have been frequent among *Penicillium* species. In particular, there have been multiple, recent HGTs among fungi used for cheese-making, allowing them to better grow on cheese. HGT events can therefore be key for adaptation in *Penicillium*. In this study, I focus on the *Penicillium* species growing onto salami. Salami are fermented meat product, and molds are characterized by high lipolytic and proteolytic activity but also increase the dehydration by creating microspores and prevent lipids oxidation by covering the salami surface. Common species found on dry sausages and salamis are the inoculated *Penicillium nalgiovense* and the naturally occurring *Penicillium salamii,* this latter being a recently described species. These *Penicillium* form the whitish to greyish crusts on salamis, providing protection against contaminations by other fungi or bacteria. These *Penicillium* have thus adapted to a particular environment with high concentration of salt, animal proteins and fatty acids during ripening. The two species *P. nalgiovense and P. salamii* are distantly related, providing an interesting case of parallel adaptation to the same extreme environment. In order to unravel the genomic processes involved in adaptation, we have isolated *Penicillium* strains from salami and have sequenced their genomes, for comparisons with other *Penicillium* fungi adapted to other environments. We have in particular investigated HGTs and gene gains/losses, and whether convergent adaptation to the same environment occurred by the same mechanisms. We have also investigated the genetic diversity within these two species, to assess whether the genetic structure, if any, corresponds to different types of salami, or different regions, as what was found for *P. roqueforti* in the case of blue cheeses.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W4

Wednesday 6th April
14:00 - 16:00

BÜKER Britta *(1), BEGEROW Dominik (1)*
(1) Ruhr-University Bochum, Bochum, Germany

Evolutionary constrains of host specificity in the smut fungus *Microbotryum*

In fungal pathogens, the occurrence of interspecific hybridization is often linked to host species' distribution and characteristics. Therefore, analysis of the genetics underlying host specialization is a crucial factor for understanding pathogen's evolution and the involved mechanisms. In the current study we use the basidiomycetes smut fungus *Microbotryum* - a species complex with independent evolutionary lineages that typically specialize to a given host plant species - to study genetic determinants of hybridization and host specialization. To do so, we are analyzing and comparing the genomes of the two *Microbotryum* species, *M. lychnidis-dioicae* and *M. silenes-acaulis* - both species that are well adapted to distinct host environments. The use of hybrids and selective infection experiments allows us to assess genomic constraints on hybrid viability and to isolate the effect of the mating type chromosomes in relation to host adaptation. The results suggest that loci involved in the disease interactions may be associated with the mating type chromosomes. Furthermore, by focusing on the occurrence of genes underlying positive selection in F1 hybrids and selected backcrosses, potential candidate genes that play a crucial role in infection and virulence can be described. Thus, the application of selective infection experiments in combination with genomic analysis is a feasible approach to elucidate the evolutionary forces of host specificity in the *Microbotryum* pathogen complex.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W5

Wednesday 6th April
14:00 - 16:00

AMENT-VELÁSQUEZ Sandra Lorena *(1), BASTIAANS Eric (1), SVEDBERG Jesper (1), MOLNAR Ruxandra (1), DEBETS Alfons J. M. (2), JOHANNESSON Hanna (1)*
(1) Systematic Biology, Department of Organismal Biology, Uppsala University, Uppsala, Sweden
(2) Laboratory of Genetics, Wageningen University, Wageningen, The Nerthelands

Evolutionary genomics of spore killing in *Podospora anserina*

The fair transmission of alleles during meiotic segregation is a fundamental aspect of sexual reproduction and its evolution. Selfish genetic elements that «cheat» and enhance their own transmission to the expense of others are called meiotic drivers. Meiotic drive, or segregation distortion, introduces intragenomic conflict that has potential implications for the evolution of genome architecture. In ascomycete fungi, meiotic drive is accomplished by «spore killing»: only the spores containing the genetic element (the killer) survive during ascus development. The sibling spores without such element get killed. The coprophilus pseudohomothallic fungus *Podospora anserina* exhibit a particularly complex case of spore killing, where up to six different coexisting spore killers (Psk) have been described in a natural population of the Netherlands. When confronted in matings, these Dutch Psk can either kill or not other Psk in a hierarchical pattern. Although a novel type of gene (spok) conferring spore-killing abilities has been described in French strains, nothing is known on the genetic basis of Dutch Psk or their effects on the organism fitness. We have performed whole genome re-sequencing of 30 monokaryotic isolates of the Dutch population using Illumina Hi-Seq (paired-end) at a 70x depth of coverage, including all known Psk and sensitive strains. Around 100 more isolates will be sequenced with the same methodology. These samples will be used to describe the genetic diversity and population structure of the Dutch *P. anserina*. Additionally, seven isolates representing each Psk will be re-sequenced using the PacBio technology to get insights into possible genomic rearrangements. Ultimately, we will attempt to uncover the selfish genetic elements behind *P. anserina* spore killing, while exploring the dynamics of birth and death of new killers over evolutionary time.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W6

Wednesday 6th April
14:00 - 16:00

CHEN Eric *(1), BEAUDET Denis (1), DALPÉ Yolande (2), CORRADI Nicolas (1)*
(1) University of Ottawa, Department of Biology, 30 Marie Curie, Ottawa, Ontario, Canada
(2) Agriculture and Agrifood Canada, 960 Carling avenue, Ottawa, Ontario, Canada

Single-cell transcriptomics as a novel approach to expose the genomic content of understudied glomeromycetes

Arbuscular mycorrhizal fungi (AMF) are a group of organisms that form symbioses with the roots of more than 80% of known plants. As such, the ecological and economical importance of these fungi cannot be understated. Yet much is still unknown about the AMF due to difficulties in studying them. Their obligate biotrophic nature prevents culturing of most AMF species in typical *in vitro* conditions, so most knowledge of their biology is based on the few model species that can grow *in vitro* through a surrogate root, in the absence of bacterial diversity. To date, most of the available AMF strains are maintained in pot cultures, whose inherent background contaminations limits the effectiveness of traditional bioinformatic analyses. Here, we aim to offset these difficulties by analyzing the transcriptome of nine AMF species (*Rhizophagus irregularis*, *Funneliformis mosseae*, *Claroideo claroideum*, *Diversispora versiforme*, *Acaulospora morrowiae*, *Scutellospora calospora*, *Racocetra castanea*, *Ambispora leptoticha*, and *Archaeospora trappei*) using a modified protocol typically based on single cell transcriptomics. We present differences and similarities between the transcriptomes of these nine AMF species, and explore the variations in transcriptome isolated from pot-grown and the Petri dish grown *R. irregularis*. Overall, the single-cell based transcriptomics approach we show represents a promising new methodology for studying the genetics of many AMF that have long been understudied.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W7

Wednesday 6th April
14:00 - 16:00

GIRLANDA M. (1), VOYRON S. (1), SALVIOLI A. (1), DAGHINO S. (1), ERCOLE E. (1), CHIALVA M. (1), POLI A. (1), SPADARO D. (1), BONFANTE P. (1), ***PEROTTO Silvia*** *(1,2)*
(1) University of Turin, Turin, Italy,
(2) IPSP-CNR, National Research Council (CNR) Turin, Italy

Reciprocal influence of tomato plants and their root associated fungal microbiota

Plants can influence the composition of the microbiota associated with their underground and aerial organs but, at the same time, they can be affected by the presence and activities of these associated microbes. In the framework of an interdisciplinary project (Mycoplant) that combines meta-barcoding and systems biology to investigate the interactions between different tomato cultivars and their root mycobiota, we focused in particular on the relative impact of soil type and plant cultivar on the recruitment of the fungal microbiota. The responses of tomato roots to different soil types and fungal microbiota were investigated by transcriptomics and proteomics. Tomato genotypes either susceptible (Moneymaker, Cuore di Bue) or resistant (Heinz, Battito) to *Fusarium oxysporum* f. sp. *lycopersici* (FOL) were grown in two different soils under greenhouse conditions. The two resistant cultivars were also inoculated with the pathogen (10^4 FOL conidia/ml). Metabarcoding of the fungal ITS2 gene yielded approx. 900,000 amplicons, assigned to 1128 OTUs. Both soil type and plant genotype significantly affected the structure of the root mycobiota. Generally, the effect of soil type on the structure of the fungal communities was higher than the effect of the plant genotype. However, in the presence of the pathogen, the magnitude of the impact of the two factors was comparable. This finding indicates greater plant control over the fungal microbiota in a modified biological environment. Several differentially expressed genes and proteins were identified in susceptible tomato plants exposed to the different soil types and microbiota, including components of the defense response.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W9

Wednesday 6th April
14:00 - 16:00

SILLO Fabiano *(1), GARBELOTTO Matteo (2), GONTHIER Paolo (1)*
(1) University of Torino, Department of Agricultural, Forest and Food Sciences (DISAFA), Grugliasco, Italy
(2) University of California-Berkeley, Department of Environmental Science, Policy and Management, Berkeley, CA, USA

Comparative genomics between two allopatric species and their hybrids provide insights on the mechanisms of invasion of fungal plant pathogens

Heterobasidion irregulare is a major fungal pathogen of pines in North America and was accidentally introduced into Italy within the natural range of its allopatrically diverged sibling species *H. annosum*, becoming invasive. Phenotypic observations have shown that the invasive species is outcompeting the native one, especially in saprobic and sporulation potential. Moreover, the two species retained high levels of interfertility, leading to massive hybridization between the two and the generation of hybrid swarms in the sympatric area. Here, we report on a comparative genomic study aimed at 1) elucidating the genomic structure of sibling taxa, with similar biology and host preference, that underwent speciation in allopatry, 2) identifying the genomic traits providing the advantage the invasive species *H. irregulare* has over the native one and 3) determining the mechanisms underlying the current massive hybridization between them. A whole-genome sequencing of three pure genotypes of *H. irregulare*, three of H. annosum and nine genotypes previously identified as hybrids through Amplified Fragment Length Polymorphism (AFLP) analysis was performed. Comparative genomics analysis among the pure genotypes identified a significant macrosynteny between the genomes of the two species. A large fraction of genes under divergent positive selection was described as involved in transcriptional functions and mitochondrial factors. In addition to these two categories, genes in interspecific structural variations were found to be related to transposable element activity. Genes involved in pathogenicity appeared to significantly harbour a lower number of non-synonymous mutations between the two species compared to genes involved in saprobic growth and sporulation. This finding provided genomic evidence that differences in fitness are more likely to be determined by these two last functions, as previously documented by in vitro experiments. Furthermore, analyses on natural hybrids allowed detecting genomic regions harbouring introgressed genes from one species into the other. Linking genomic to ecological traits, this study showed that factors related to transmission rather than those related to pathogenicity might explain the invasiveness of exotic plant pathogens.

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POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W10

Wednesday 6th April
14:00 - 16:00

HARTMANN Fanny *(1), SÁNCHEZ-VALLET Andrea (1), CROLL Daniel (1)*
(1) Plant Pathology, Institute of Integrative Biology, ETH Zürich, Zürich, Switzerland

A segregating gene deletion polymorphism is linked to pathogenicity in populations of the fungal wheat pathogen *Zymoseptoria tritici*

The fungus *Zymoseptoria tritici* is the causal agent of Septoria tritici Blotch (STB) of wheat causing major economic losses. However, the genetic architecture of virulence is poorly understood in natural populations. We performed a genome-wide association study (GWAS) to identify genetic variation linked to virulence in natural populations. We analysed 106 isolates originating from four geographical locations: Australia, Israel, Switzerland and the United States. The isolates showed significant variation in pycnidia production, a key indicator of virulence in *Z. tritici*, among populations and depending on the identity of the wheat cultivar. Using Illumina whole-genome sequencing, we genotyped 779"178 high-quality single nucleotide polymorphisms (SNPs) segregating in multiple populations. GWAS analyses identified multiple chromosomal regions associated with virulence, showing that virulence is based on a complex genetic architecture. We found little overlap in associated regions identified for each cultivar separately, suggesting that many virulence loci may be cultivar-specific. Significant associations localized to genes encoding for proteins of diverse functions, including transporters, nutrient degradation and general metabolism. The most significantly associated SNP was in near complete linkage disequilibrium with a deletion polymorphism of a gene encoding a small secreted protein. The gene is highly transcribed during leaf infection and belongs to an important class of virulence gene candidates. In order to characterize the chromosomal region harboring the gene deletion polymorphism, we used Illumina read coverage. We found high levels of deletion polymorphisms segregating among the resequenced isolates. The gene was located at the boundary between a transposable element-rich region showing large-scale deletion polymorphisms within the species and a conserved, gene-rich region. Isolates lacking the gene showed higher virulence, suggesting that the gene plays a role in plant defense activation or recognition. The finding that a gene deletion polymorphism was strongly associated with virulence shows that chromosomal rearrangements in pathogen populations can be a major driver of virulence evolution.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W11

Wednesday 6th April
14:00 - 16:00

DUSSERT Yann *(1), GOUZY Jérôme (2), RICHART-CERVERA Sylvie (1), DEMEAUX Isabelle (1), LEGRAND Ludovic (2), CARRÈRE Sébastien (2), MESTRE Pere (3), DELMOTTE François (1)*
(1) INRA, ISVV, Villenave d'Ornon, France
(2) INRA, LIPM, Castanet-Tolosan, France
(3) Santé de la Vigne et Qualité du Vin, INRA, Colmar, France

De novo sequencing and population genomics of the grapevine downy mildew

Invasions by plant pathogens are responsible for tremendous damage in crops and are increasing in frequency, notably due to human-mediated dispersal. Consequently, population genetics studies of pathogen introductions are of great interest to understand invasive population dynamics and the processes of adaptation to new hosts and environments. *Plasmopara viticola* is an oomycete responsible for grapevine downy mildew, a major and costly disease worldwide. It has been introduced very recently (around 150 years ago) from North America in Europe and has subsequently invaded European vineyards in a few years. To study the genetic consequences of the introduction of *P. viticola* on its genome, we carried out a population genomics approach. The genome of *P. viticola* has been sequenced using three Illumina libraries (paired-end and mate-pair reads) with different insert sizes. This assembly covered 70% of the estimated genome size (CEGMA pipeline: 95% completeness). Around 30% of the assembly was made up of repetitive elements, and 17,131 genes were annotated. A total of 18 strains from Europe and 2 strains from North America, *P. viticola* native area, have been resequenced, producing 1.08 million SNPs. The genetic diversity of the European strains was not structured geographically. Inference of the demographic history of the species using Approximate Bayesian Computation and detection of selection footprints along the genome are currently on-going.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W12

Wednesday 6th April
14:00 - 16:00

MARYANI MARTAWI Nani *(1), LOMBARD Lorenzo (2), POERBA Yuyu (3), SUBANDIYAH Siti (4), CROUS Pedro (2), KEMA Gert (1)*
(1) Wageningen University and Research Center (WUR), Plant Research International, Wageningen, The Netherlands (2) CBS-KNAW Fungal Biodiversity Center, Uppsalalaan 8, 3584CT, Utrecht, the Netherlands
(3) Research Centre for Biology, Indonesian Institute of Sciences (LIPI), Cibinong, Indonesia
(4) Dept. Of Plant Protection, Gajah Mada University, Yogyakarta, Indonesia

Phylogenetic diversity of *Fusarium oxysporum f.sp. cubense* on a variety of Indonesian banana cultivars

Fusarium wilt or Panama disease is one of the major constraints in banana cultivation. A genetic lineage of *Fusarium oxysporum f.sp. cubense* (Foc), named vegetative compatibility group 01213 (VCG01213) commonly known as Tropical Race 4 (TR4), was first detected in Southeast Asia in the 1990s. Since then, it has spread in the region and recently it was reported in Jordan, Pakistan and Lebanon and most likely also in Mozambique and Oman. Indonesia is the center of origin for wild and cultivated bananas that likely co-evolved with Foc. Hence, we hypothesize a large Foc diversity in this country. A sampling collection in Sumatera, Java and Kalimantan comprised 29 locations in 12 provinces and resulted in a comprehensive collection of 114 Foc isolates from over 35 different local Indonesian banana cultivars. The phylogenetic diversity of this collection was assessed by sequence analysis of the translation elongation factor 1a (TEF1a), RNA polymerase II largest subunit (RPB1) and RNA polymerase II second largest subunit (RPB2). Approximately 85% of the isolates were positive for a molecular TR4 diagnostic, but showed high and unknown genetic diversity beyond VCG01213. Ongoing research is aimed at defining the Foc landscape in Indonesia by further enlargement of the Foc collection followed by high-throughput genotyping and phenotyping.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W13

Wednesday 6th April
14:00 - 16:00

ROPARS Jeanne *(1), BOUGNOUX Marie-Elisabeth (2), MAUFRAIS Corinne (3), LAVAL Guillaume (4), SERTOUR Natacha (1), SCHWARTZ Katja (5), VOELZ Kerstin (6), BENNETT Richard (7), SHERLOCK Gavin (5), D'ENFERT Christophe (1)*
(1) Institut Pasteur, Unité Biologie et Pathogénicité Fongiques, Département Génomes et Génétique, Paris, France
(2) Université Paris Descartes, Laboratoire Microbiologie, Hôpital Necker-Enfants-Malades, AP-HP, Paris, France
(3) Institut Pasteur, Centre d'Informatique en Biologie, Paris, France
(4) Institut Pasteur, Unité de Génétique Evolutive Humaine, Département Génomes et Génétique, Paris, France
(5) Stanford University, Department of Genetics, School of Medicine, Stanford, California, USA
(6) University of Birmingham, Institute of Microbiology & Infection and the School of Biosciences, Birmingham, UK

Population genomics of the most common human fungal pathogen *Candida albicans*

Discovering the genetic and genomic processes behind adaptation is a long-standing question in evolutionary genetics. *Candida albicans* is a part of the normal human intestinal flora, but it is also the most common opportunistic human fungal pathogen that causes mucosal diseases in healthy individuals as well as deep-seated infections in hosts with decreased defenses. Previous population studies using Multi-Locus Sequence Typing (MLST) have revealed a strong genetic differentiation between strains, showing at least 18 well-differentiated clusters. Each clade comprises strains that have evolved independently from those in other clades, possibly through past association to a geographic locale. In order to investigate genomic processes of adaptive divergence in *C. albicans* isolates, our team sequenced 144 diploid genomes of commensal/clinical isolates originating from four continents and showing significant diversity in terms of growth rates, ability to form hyphae, as well as in virulence in a non-mammalian model of *C. albicans*. Here, we will present results of this population genomic analysis, based on single nucleotide polymorphisms (SNPs), that aimed at addressing a fundamental evolutionary question, i.e. how do fungal species adapt, particularly with respect to human selection pressure.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W14

Wednesday 6th April
14:00 - 16:00

ORDONEZ ROMAN Nadia Ivonne *(1), GARCIA BASTIDAS Fernando (1), PAPAGIANNAKI Evangelina (1), SEIDL Michael (1), WAALWIJK Cees (1), DRENTH Andre (2), THOMMA Bart (1), PLOETZ Randy (4), MEIJER Harold (1), KEMA Gert (1)*
(1) Wageningen University and Research, Wageningen, Netherlands
(2) Centre for Plant Science, Brisbane, AustraliaA
(3) Diversity Arrays Technology, Canberra, Australia
(4) Tropical Research & Education Center, Institute of Food & Agricultural Sciences, University of Florida, Homestead, USA.

Unveiling the genetic and pathogenic diversity of the banana pathogen *Fusarium oxysporum f.sp. cubense*.

The soil-borne fungi *Fusarium oxysporum f.sp. cubense* (Foc) is the causal agent of *Fusarium* wilt or Panama disease in bananas (*Musa sp.*). Foc devastated «Gros Michel» banana plantations in Latin America, leading to the conversion of the banana industry to Cavendish banana plantations in the 1960s. Initially, Foc genetic diversity was assessed by the vegetative compatibility group (VCG) method that divides Foc strains into 24 unique VCGs (VCG0120 through VCG0126 and VCG0128 through VCG01224). Later, DNA markers revealed the polyphyletic origin of described VCGs, showing a remarkable dichotomy, referred to as types or clades. Here, we used high-resolution genotyping-by-sequencing analyses to validate and extend these findings through genome-wide DArTseq analyses. Foc strains representing all VCGs split into two groups, confirming the aforementioned clades and previously unclassified VCGs (01221 to 01224) clearly sort into clade 2. Additionally, we showed that the mating type genes MAT1-1 and MAT1-2 occurred in each clade, but that each VCG represents only one ideomorph, regardless of their spatial or temporal distribution, thereby confirming their clonal structure. Furthermore, we showed VCG cross-compatibility between VCG0120/15 and VCG0124/5/8/20, and new cross-compatibility events were observed between VCG01222 and the VCG0124/5/8/20 complex as well as VCG0123 and VCG01211 to the VCG0120/15 complex. Such cross compatible events only occurred between VCGs with a Jaccard genetic distance below 0.2. Isolates associated to VCG 0120/11/15 and 0124/5/8/20/22 complexes as well as VCG01213, the latest colloquially known as Tropical Race 4, were able to colonize and produce discoloration in «Grand Naine» Cavendish corms under greenhouse conditions. These data further elaborate on the genetic make-up of the currently known Foc VCG diversity and pathogenicity tests provide novel insights in «Grand Naine» susceptibility.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W15

Wednesday 6th April
14:00 - 16:00

HOSSEINI Sara *(1), MEUNIER Cécile (1), HEIDARI Nahid (1), HILTUNEN Markus (1), JOHANNESSON Hanna (1)*
(1) Systematic Biology, Evolutionary Biology Center, Uppsala University, Uppsala, Sweden

Nuclear interactions in fungal heterokaryon *Neurospora tetrasperma*

A heterokaryon is a tissue type composed of cells containing genetically different nuclei. Although heterokaryosis is commonly found in nature, an understanding of the evolutionary implications of this phenomenon is largely lacking. The ascomycete model species *Neurospora tetrasperma* is naturally heterokaryotic throughout its life cycle. It contains nuclei of two opposite mating types (mat A and mat a) in the same cell and thereby is a self-fertile species. Recently it has been shown that the nuclei of a heterokaryon (when grown separately as homokaryons) show different expression profiles. Furthermore, a tissue-specific interaction between nuclei in a heterokaryon at the expression level was found. These data suggest an optimization of the heterokaryon by the alteration of nuclear and expression ratio at different stages of the life cycle, but the mechanism by which this interaction is mediated is still obscure. The aim of the present study is to investigate the involvement of epigenetic processes in this interaction. Three heterokaryons of *N. tetrasperma* (containing different nuclear ratio) from three lineages, and their homokaryons, are grown and tissue samples harvested at sexual and asexual developmental stages. For each tissue we will perform mRNA sequencing, small RNA sequencing and bisulfite genome sequencing. This data will allow us to study the correlation between nuclei DNA ratio and transcriptome ratio in the heterokaryons, the gene expression ratio for each gene, the targets of small RNA and the degree of genome methylation of each nucleus. The data will unravel whether epigenetic marks are different between the nuclei and can explain differences in gene expression, and thereby nuclear cooperation, over the course of the life cycle.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W16

Wednesday 6th April
14:00 - 16:00

CARLIER Jean *(1), ZAPATER Marie-Françoise (1), BIEYSSE Daniel (1), MONTERO Yanetsy (2), ROUSSEL Véronique (1), HABAS Rémi (1), PEREZ-VICENTE Luis (2), ABADIE Catherine (3), WRIGHT Steven (4)*
(1) CIRAD, BGPI, Montpellier, France
(2) INISAV, Havana, Cuba
(3) CIRAD, BGPI, Guadeloupe, France
(4) University of Toronto, Toronto, Canada

Detecting host-selected genes in the plant pathogenic fungus *Mycosphaerella fijiensis*

Plant pathogenic fungi are able to erode quantitative host resistance through changes in aggressiveness, thereby threatening the durability of host resistance. Such erosions are suspected in some areas in the fungus *Mycosphaerella fijiensis*, responsible for a recent and devastating banana pandemic, Black Leaf Streak Disease (BLSD). This study aims to test for the action of host-specific adaptation and to detect host-selected genes in *M. fijiensis*. We collected six samples in Cuba in three locations distributed throughout the banana production zones where resistant cultivars have been used for about 15 years. For each location, about 40 isolates were collected from two banana plots containing either a resistant variety or a susceptible variety located two to 10 km apart. We also included in the study three samples from Honduras where the disease was first introduced in the Latin America- Caribbean area. A significant host effect was detected in some locations for some aggressiveness traits evaluated under controlled conditions. A genome scan approach was conducted from whole-genome sequencing of pools of individuals (pool-seq). Differentiated genomic regions were detected between pathogen populations from the two cultivars in some locations. Genes putatively under positive selection were identified within these regions using different statistics. Annotation analysis and biological data suggested that some of these genes could play a role in aggressiveness and host adaptation.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W17

Wednesday 6th April
14:00 - 16:00

DUTECH Cyril *(1), DEMENE Arthur (2), SHERMAN David James (4), DURRENS Pascal (5), FIEVET Virgil (2), ROBIN Cécile (1), FABREGUETTES Olivier (1), SAINT-JEAN Gilles (1), LEGRAND Ludovic (3), GOUZY Jérôme (3)*
(1) INRA, BIOGECO, Cestas, France
(2) Univ. Bordeaux, BIOGECO, Pessac, France
(3) INRA, CNRS, LIPM, Castanet-Tolosan, France
(4) INRIA Bordeaux Sud-Ouest, Talence, France
(5) LaBRI CNRS, Talence, France

Genome evolution of clonal lineages after an introduction of the fungal pathogen (*Cryphonectria parasitica*) in Europe.

The chestnut blight fungus (*Cryphonectria parasitica*), an Ascomycete infecting bark and cambial tissues of its host, is a prominent example of an invasive plant fungal pathogen that has had a dramatic impact in its introduced range, North America and Europe (Anagnostakis 1987). Previous genetic studies have clearly identified two major introductions in Europe, one directly from Asia, the native area of the species, and the other from North America where the species was previously introduced (Dutech et al. 2012). In each case, a limited number of clonal lineages have successfully spread from southern to northern part of Europe questioning the importance of recombination events as a major driver for adaptation during the invasion (Dutech et al. 2010). In order to identify the main genetic changes which have occurred during the introduction step and the expansion step of these clonage lineages, we analyzed the genomes of several strains sampled from seven clonal lineages widely spread in France using NGS. We identified some variations indicating that few recombination events occurred along the genome within each clonal lineage. This genomic study allowed us to identify the origin of major genetic changes in clonal lineages, and to explore their putative effects on the adaptation of the fungal pathogen for its new environment.

Anagnostakis SL (1987) Mycologia: 79 23-37
Dutech C., Fabreguettes O., Capdevielle X., Robin C. (2010) Heredity 105: 220-228
Dutech C, Barres B, Bridier J, Robin C, Milgroom MG, Ravigne V (2012) Mol Ecol 21: 3931-3946

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W18

Wednesday 6th April
14:00 - 16:00

GILLOT Guillaume (1), JANY Jean-Luc (1), POIRIER Elisabeth (1), MAILLARD Marie-Bernadette (2), DEBAETS Stella (1), THIERRY Anne (2), COTON Emmanuel (1), ***COTON Monika*** *(1)*
(1) Université de Brest, Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, Plouzané, France
(2) INRA, Science et Technologie du Lait et de l'Œuf, AGROCAMPUS OUEST, Science et Technologie du Lait et de l'Œuf, Rennes, France

Genetic and functional diversity within *Penicillium roqueforti*

Penicillium roqueforti is used as a ripening culture for blue-veined cheeses and is greatly responsible for their organoleptic quality and typicity. These features are largely due to different manufacturing methods but also to the specific *P. roqueforti* strains used. Indeed, *P. roqueforti* inoculated strains, via their proteolytic and lipolytic activities, have an effect both on texture and flavor of blue-veined cheeses but may also be involved in producing other secondary metabolites such as mycotoxins. This study investigated an unprecedented large worldwide *P. roqueforti* collection from 120 blue-veined cheeses and 21 other substrates in order to test: (i) whether *P. roqueforti* phenotypic diversity corresponds to a complex of species, (ii) what is the *P. roqueforti* intraspecific diversity and population structure, and (iii) whether the assessed differentiated populations correspond to distinct functional traits (proteolytic activities, aroma production and mycotoxin production). A Genealogical Concordance Phylogenetic Species Recognition analysis confirmed the existence of a single species. Overall, 28 haplotypes were identified among 164 isolates and three highly differentiated populations were revealed linking isolates to blue-cheese types. Then, 55 representative strains were screened for different metabolic properties including proteolytic activity, aroma compounds (using HS-Trap GC-MS) and mycotoxin production (via LC-MS/Q-TOF). Mini model cheeses were used for aroma production and proteolysis analysis while Yeast Extract Sucrose (YES) agar medium was used for mycotoxin production. This study highlighted key differences in functional traits. Noteworthy, when *P. roqueforti* strains isolated from Protected Designation of Origin (PDO) or Protected Geographical Indication (PGI) blue-veined cheeses were only considered, a clear relationship was demonstrated between genetic diversity, population structure and the assessed functional properties.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W19

Wednesday 6th April
14:00 - 16:00

PLISSONNEAU Clémence (1), OLLIVIER Bénédicte (1), FUDAL Isabelle (1), ROUXEL Thierry (1), ***BALESDENT Marie-Hélène*** *(1)*
(1) -BIOGER INRA, AgroParisTech, Thiverval-Grignon, France

Unusual evolutionary mechanisms to escape Effector-Triggered-Immunity in the fungal phytopathogen *Leptosphaeria maculans*

Leptosphaeria maculans is responsible for the stem canker (phoma) disease of oilseed rape (*Brassica napus*). AvrLm3 and AvrLm4-7, two avirulence effector genes of *L. maculans*, are involved in an unusual relationship: the presence of AvrLm4-7 induces the Rlm7-mediated resistance but suppresses the Rlm3-mediated recognition (Plissonneau et al., 2015; doi: 10.1111/nph.13736). To investigate this relationship, we assessed the sequence diversity of AvrLm3 in *L. maculans* populations. The analysis of more than 200 isolates revealed a high level of allelic polymorphism for this gene, but no deletion event nor inactivating mutations were found. This observation contrasts with the presence/absence or RIP-(Repeat-induced-point-mutation) inactivation polymorphisms usually found for other *L. maculans* avirulence genes and questioned the role of AvrLm3 in fungal fitness. Two distinct mechanisms responsible for the «double virulent» phenotype (i.e. virulent toward both Rlm3 and Rlm7, «a3a7») were identified. In isolates displaying an inactivation of AvrLm4-7, amino acid changes in AvrLm3 were responsible for the virulent phenotype towards Rlm3. However, 56% of the a3a7 isolates displayed an avirulent allele of AvrLm3, combined with point mutations in AvrLm4-7. Such situations allow the fungus to escape Rlm7-mediated resistance while maintaining the suppression of the AvrLm3 phenotype effective. They also allow the fungus to escape to the recognition by two resistance genes while keeping the secretion of the two corresponding effector proteins. The complex evolutionary mechanisms displayed by *L. maculans* to escape Rlm3-mediated resistance while preserving AvrLm3 sequence integrity, along with the reduced virulence in isolates silenced for AvrLm3 confirmed the importance of this effector in pathogenicity towards *B. napus*.

This work was funded by the INRA SMaCH metaprogram «K-masstec» and the CTPS project «ICOSCOP»

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W20

Wednesday 6th April
14:00 - 16:00

RAZZAQ Asad *(1), WHEELER David (1), SCHMID Jan (1)*
(1) Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand

Role of protein-coding DNA in yeast

Tandem repeats present in the open reading frames (TR-ORFs) mutate more frequently than other parts of the genome, mostly through indels and this may assist in rapid or long-term adaptation. The availability of complete genome sequences of *Saccharomyces cerevisiae* strains from different niches provided an opportunity to investigate functions of TR-ORF mutability in this species. A survey of 30 yeast genomes showed that twenty percent (±3.45% SD) of ORFs in these genomes contain tandem repeats. In the reference strain S288 ~60% of these TR-ORFs encoded amino acid repeats (AARs) that may be important for protein function. We explored the distribution of homologues of 70 randomly selected TR-ORFs (encoding cell surface, nuclear, vacuolar, cytoplasmic proteins plus proteins of unknown function with repeat unit lengths ranging from 1 to 51 amino acids) across 30 strains of *Saccharomyces cerevisiae* isolated from different niches (14 from alcoholic beverages and 16 from other niches, including trees, bakeries and tree litter). None of the TR-ORFs was present in all 30 strains. Homologues of a given TR-ORF were present on average in 74±6.38% of strains and their median frequency was 83.3%. (an additional 3±1.6 % strains had a TR-less homologue, but in almost all cases these encoded truncated and thus probably dysfunctional proteins). AARs appeared more conserved than DNA repeats, whose purity was diminished by synonymous mutations. Ratios of nonsynonymous to synonymous mutations indicated that most TRs encode parts of proteins relevant to their function. There was usually little or no difference in the number of repeat units of a given TR-ORF in different strains. Around 14.5% of TRs showed pronounced length variation, but neither genetic background nor ecological niche occupied by a strain appeared to be determinants of TR-region length. The latter, as well as the limited length variation of many TR-ORFs, suggest that different niches do not select for different lengths, arguing against a role of TR hypermutability in short-term adaptation. Our data indicates that while many TR-ORFs are not part of the core genome, the proteins, including their TR encoded parts, are functionally significant. Rather than short-term adaptation the main biological function of TR-hypermutabilty may be to assist in survival in the Red Queen"s race, by increasing the rate of long-term protein evolution.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W21

Wednesday 6th April
14:00 - 16:00

LAURENT Benoit *(1), MOINARD Magalie (1), SPATARO Cathy (1), BLANC Richard (2), LASSERRE-ZUBER Pauline (2), PALAIOKOSTAS Christos (3), HOUSTON Ross (3), FOULONGNE-ORIOL Marie (1)*
(1) INRA, MycSA, Bordeaux, France
(2) INRA, GDEC, Clermont-Ferrand, France
(3) The Roslin Institute, University of Edinburgh, Edinburgh, Scotland

How the genomic era is putting quantitative genetics back under the spotlight: a case study of *Fusarium graminearum*.

Quantitative genetics is the study of population variation in complex traits, including identification of polymorphic loci underlying this variation (quantitative trait loci or QTL). In fungal species, numerous economically important traits show quantitative variation, e.g. aggressiveness in phytopathogenic fungi, yield in edible mushroom or industrial production of drugs and other fungal metabolites of interest. Thus, understanding its genetic architectures is essential to limit or to improve fungal activities of interest. Advantageously, most of the fungal species have short life cycles and small genomes, which simplify identification of QTL. However in non-model fungi species, this approach has received moderate attention because of -among other reasons- genotyping limitations. Nevertheless, the genomic tools and large-scale genotyping facilitated by next generation sequencing (NGS) have boosted the field and opened new opportunities. We present a summary of quantitative genetic concepts in fungi by integrating new tools available from NGS. These concepts will be illustrated by the genetic dissection of quantitative traits related to the pathogenicity of *Fusarium graminearum* in wheat from an experimental population. This work highlights, in particular, the use of NGS i) to increase the genotyping output in a cost and time effective way and ii) the new possibilities to identify the potential causal mutation responsible for variation in quantitative traits, by intersecting QTL analysis and effect prediction of genetic variants within the genomic region of interest.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W22

Wednesday 6th April
14:00 - 16:00

BARONCELLI Riccardo *(1), ZAPPARATA Antonio (2), SUKNO Serenella (3), LE FLOCH Gaetan (1), THON Michael (3)*
(1) Université de Bretagne Occidentale, LUBEM, Plouzané, France
(2) Università di Pisa, DiSAAA-A, Pisa, Italy
(3) Universidad de Salamanca, CIALE, Salamanca, Spain

Comparative analysis of mitochondrial genomes from *Colletotrichum* species

Mitochondrial (mt) genomes are gaining interest due to their genetic variability in terms of size, non-coding regions, gene order and gene content. High-throughput sequencing is contributing to the development of 'mitogenomics' (mitochondrial genomics), leading to the discovery of recombination events in fungal mitochondrial genomes. Here, we report the mt genomes of four closely related species belonging to *Colletotrichum acutatum sensu lato* (Ascomycetes), a fungal species complex causing anthracnose on many plant species worldwide. The mt genomes range from 29,868 to 36,621 bp in size and encode 14 to 17 genes. Comparing the newly sequenced and annotated mt genomes, with three other *Colletotrichum* species available (*C. graminicola, C. lindemuthianum and C. acutatum sp.*), we investigated mitochondrial synteny and variation in gene content, horizontal gene transfer (HGT) and transfer RNAs (tRNAs) distribution. Remarkably, we found intra-generic and also intra-complex variation in terms of gene content, with at least 2 species encoding lineage specific genes. The *C. nymphaeae* mt genome encodes two GIY-YIG endonucleases and *C. graminicola* encodes a DNA polymerase protein not present in closely related mt genomes. Overall, the comparative analysis of mt genomes of revealed an unexpectedly large variation of gene content in closely related fungal species.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W23

Wednesday 6th April
14:00 - 16:00

***BASTIAANS Eric* (1)**
(1) Uppsala University, Uppsala, Sweden

Evolutionary dynamics of Spore Killer elements in the fungus *Podospora anserina*

Natural selection may favour selfish genetic elements in the genome, in spite of them causing harm to the individuals carrying them. One class of such selfish genetic elements is meiotic drivers, also referred to as segregation distorters. Although meiotic drive is widespread in nature and has been identified in a wide range of eukaryotes, there is a profound lack of empirical insight into the evolutionary causes and consequences of this phenomenon. We study spore killing in the ascomycete fungus *Podospora anserina* to gain insight in the evolutionary dynamics of meiotic drive. In *P. anserina* several meiotic drive elements that cause spore killing are present at high frequencies in natural populations. During sexual development, spores carrying the Spore killer allele inhibit the development of spores not carrying the allele. We are using Spore killers found in a large collection of natural isolates from the Netherlands to study the evolutionary dynamics of spore killing. About a quarter of the collection shows killing in crosses with the sensitive labstrain. Six different Spore Killers that act in hierarchy in their ability to kill when crossed with each other have been described in this collection. The coexistence of these different killers implies there is a complex dynamics of coevolution between the killers and the sensitive strains in the population. A model on the evolution of Spore Killer in *Neurospora* predicts that the Spore Killer requires additional benefits in order to be successfully selected. We are experimentally investigating the population biology of *Podospora* Spore Killers, i.e., under which conditions they may invade a population. For this we analyse the effects of spore killing on a range of potential fitness parameters. We compare crosses between a killer and a sensitive strain with crosses between two sensitive strains and two identical killer strains. We compare both the efficiency of the cross and the quality of the spores that come out of this cross. The ultimate aim is to provide a model predicting what factors cause meiotic drivers to invade and go to extinction in natural fungal populations.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W24

Wednesday 6th April
14:00 - 16:00

HESS Jaqueline *(1), BALASUNDARAM Sudhagar V. (1), EASTWOOD Daniel (2), BRANDSTRÖM DURLING Mikael (3), FOSSDAL Carl Gunnar (4), HÖGBERG Nils (3), KAUSERUD Håvard (1), SKREDE Inger (1)*
(1) Department of Biosciences, University of Oslo, Oslo, NORWAY
(2) Department of Biosciences, University of Swansea, Swansea, UK
(3) Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, SWEDEN (4) Norwegian Institute of Bioeconomy Research, Ås, NORWAY

Exploring specialization in wood decay mechanisms in the harmful house-invader *Serpula lacrymans* using an evolutionary transcriptomics approach

Serpula lacrymans is a common house inhabiting fungus in Europe. Although other fungi are also present in the indoor environment, *S. lacrymans* is by far the most aggressive species. It can cause rapid decay of timber construction, and an individual may grow to a size of several meters across, internally connected by thick hyphal cords. Exactly what makes *S. lacrymans* so successful compared to other species found in the built environment remains elusive, but there are good indications that its ability to transport water and nutrients across large distances and effective use of substrate contribute to its competitive advantage in this specialized environment. The order *Serpulaceae* houses several species of wood-decaying fungi, including the globally-distributed *Serpula himantioides* which may also be found in houses, but more commonly in the wild, *Serpula lacrymans var. shastensis* which inhabits a narrow niche, growing under fallen trees on Mt. Shasta in the Cascade mountains in North America, and *Serpula lacrymans var. lacrymans* which is found almost exclusively indoors in Europe but is thought to derive from a wild, native population in the Himalayas. In order to characterize how different *Serpula* species utilize different substrates and how this may relate to their respective niches, we investigated decomposition rates of four strains when grown on spruce, pine and abies wood blocks. We chose to work with one strain each of *Serpula himantioides* and *Serpula lacrymans var. shastensis*, and two strains of *Serpula lacrymans var. lacrymans*, one from the indoor-only European population, and one from a Japanese indoor population that is descendant from a different invasion event. Our results show significant differences in the decomposition rates of different types of wood by different strains, suggesting that strains with more specialized niches also show greater substrate specialization. To further our understanding of the genetic component of specialization and investigate the evolution of wood decay mechanisms within the *Serpulaceae*, we have undertaken RNA-seq experiments of the four target strains growing on the different types of wood. We will present the evolutionary analysis of our gene expression data and discuss the role of substrate specialization with respect to the invasive behavior of *Serpula lacrymans var. lacrymans* in Europe.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W25

Wednesday 6th April
14:00 - 16:00

LOONEY Brian (2), MATHENY Brandon (1), ***JESSY Labbé*** *(1)*
(1) Oak Ridge National Laboratory, Oak Ridge/TN, USA
(2) Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, Knoxville/TN, USA

Dense genome sampling of the ectomycorrhizal *Russulaceae* (*Russulales*) associated to *Populus*

Recent evidence suggests that some ectomycorrhizal (ECM) fungi have retained the capability to degrade complex plant biopolymers, thus contributing to carbon cycling in new and unexpected ways. The family *Russulaceae*, including the genera *Russula*, *Lactarius, Lactifluus, and Multifuraca*, is one of the most widespread and species rich ECM lineages, and preliminary evidence suggests that members of this family have retained genes responsible for the degradation of the most recalcitrant class of plant biopolymers, lignin. This group has also been implicated as being the dominant producer of laccases in leaf litter of hardwood temperate forests and certain nitrophilic species are highlighted as vital for the transport of nitrates to their plant partners. Here I will present an overview of a project that seeks to utilize dense genome sampling within the family to investigate to what extent genes involved in plant biopolymer degradation have been maintained within a single, diverse, ECM lineage. I will present progress and issues involved in sample preparation for genome sequencing, which is considered a major bottleneck towards genome-enabled mycology. Plans for utilizing genomic information in an experimental system investigating the ectomycorrhizome of *Populus* will also be discussed.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W26

Wednesday 6th April
14:00 - 16:00

SKREDE Inger *(1), HESS Jaqueline (1), BALASUNDARAM Sudhagar V. (1), KAUSERUD Håvard (1), EASTWOOD Daniel (3), HÖGBERG Nils (4), KOHLER Annegret (2), MURAT Claude (2), DURLING Mikael Brandstöm (4), MARTIN Francis (2)*
(1) University of Oslo, Oslo, Norway
(2) Interactions Arbres-Microorganismes, INRA, Lorraine University, Champenoux, France
(3) University of Swansea, Swansea, UK
(4) Swedish University of Agricultural Sciences, Uppsala, Sweden

Invasiveness of the harmful house-invader *Serpula lacrymans* – population genomics of the Japanese and European populations

The dry rot fungus, *Serpula lacrymans*, is the most efficient decomposer of buildings in temperate regions worldwide. Population genetic data indicate that the species invaded Europe and Japan independently from its native range in central Asia. The European population has further dispersed to the Americas and Australia, and both the Japanese and European populations have reached New Zealand and there apparently admixed. In this study we are investigating the genetic and physiological basis for the success of the fungus as an invader of human-made wood constructions. Whole genome sequences are obtained from 39 strains from the two initial founder populations (Europe and Japan) and the admixed New Zealand population. Our preliminary results (of about 450 K SNPs based 32 genomes from Japan, Europe and New Zealand) show that the genetic diversity is higher in the Japanese population, as there is a tenfold difference in number of SNP detected within populations. This is also supported by previous studies using microsatellites and mating type linked markers. We also find that the Japanese strains compete better for resources when being confronted with other brown rot species than the European strains and resembles more the competition ability seen in the wild *Serpula himantioides*. We suggest that the better performance among the Japanese isolates may be linked to a different demographic history and a closer relationship to a wild population.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W27

Wednesday 6th April
14:00 - 16:00

MAURICE Sundy *(1), SKREDE Inger (1), KAUSERUD Håvard (1)*
(1) University of Oslo, Department of Biosciences, Genetics and Evolutionary Biology , OSLO, Norway

Genetic diversity of polypores in boreal forest

Forest loss and fragmentation is recognized as one of the main global threats to biodiversity. The boreal forests of Fennoscandia have undergone dramatic changes during the last centuries, where intensive forest management and short rotation times have led to the loss and fragmentation of natural forests. This has in turn resulted in biodiversity decline. However, very little is known about how genetic variation within species, the basic biodiversity component, has been affected by habitat fragmentation. In this project we assess the level of genetic variation in common and rare fungi, how this relates to various species traits, and whether habitat fragmentation is affecting the level of genetic variation. We focus on wood-decomposing polypores, a group of fungi known to be negatively influenced by forest fragmentation. Here we report from a first analysis of ten polypore species growing in the same forest area in eastern Finland. To genotype each individual (20-24 for each species) we applied restriction-site-associated DNA sequencing (RADseq). Depending on the polypore species, between 2 500 and 13 000 polymorphic loci containing between 5 000 and 15 000 single nucleotide polymorphisms (SNPs) were identified. Different trends in the level of genetic variation were observed between the ten species. Allelic frequencies were inferred from high sequencing coverage data and population genetic statistics calculated and compared between species. Our first analyses bring insights about genetic variation of fungal populations at a finer geographic scale. Further knowledge about the effect of forest fragmentation (versus other factors) in shaping the genetic structure in common and rare fungi is critical for predicting future population trends for red-listed species and for developing evidence-based conservation policies.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W28

Wednesday 6th April
14:00 - 16:00

SCHARDL Christopher *(1), MOORE Neil (1), KANG Qiwen (1), HAWS David (2), JAROMCZYK Jerzy (1), YOSHIDA Ruriko (1)*
(1) University of Kentucky, Lexington, USA
(2) International Business Machines Corp. (IBM), New York, New York, USA

Genome-wide phylogenomics of plant-associated *Clavicipitaceae* and investigation of alkaloid gene evolution

The fungal family *Clavicipitaceae* includes a large clade of plant associates that range from sterilizing or floral-replacement pathogens to systemic and vertically transmitted mutualistic symbionts (endophytes), and are renowned for their diversity of specialized metabolites including a variety of neurotoxic alkaloids. Their alkaloids serve apparent defensive roles against invertebrate or vertebrate animals, and alkaloid biosynthesis and diversity are determined by the presence and variation of gene clusters for: ergot alkaloids (EAS gene cluster), indole-diterpenes (IDT), aminopyrrolizidines such as the lolines (LOL), and the pyrrolopyrazine alkaloid, peramine (PER). We devised a genome-wide phylogenetic study in order to determine if non-housekeeping genes such as alkaloid genes tended to differ from housekeeping genes in evolutionary history. Genomes were sequenced for 25 strains in 20 species from five genera, *Aciculosporium, Atkinsonella, Balansia, Claviceps* and *Epichloë.* Genes were then inferred or predicted for these and two published *Metarhizium spp.* genomes. Using OrthoMCL, MAFFT and COCO-CL we then inferred 8170 clusters of orthologous genes («genes» hereafter) and their protein sequence alignments. Filtering out those present in fewer than five of the genomes, and those with one or more questionable annotations or alignments, gave 5024 genes. Of these, 3443 were putative housekeeping genes and 1581 were putative non-housekeeping genes, 12 of which were EAS, IDT or LOL genes. (The other alkaloid genes, including the sole PER gene, did not pass the filters.) For each of the filtered 5024 genes we inferred maximum likelihood (ML) trees constrained or unconstrained to the 70% consensus of housekeeping gene trees, and then we determined ratios of constrained:unconstrained tree lengths. Ratios ranged from 0.95011 to 6.76272. Whereas ratios for housekeeping genes averaged 1.02413 ± 0.00076 (SE), ratios for the alkaloid genes ranged from 1.05209 to 1.42300. Ranking the ratios placed the alkaloid genes into the following percentiles: 87th (lolD), 90th (lolU), 93rd (idtP), 96th (idtQ), 97th (lolF), 98th (lolC, lolA, easB, easC and easG) and 99th (easA and idtB). Phylogenetic analyses indicated that alkaloid genes in *Clavicipitaceae* undergo duplications and losses, and also exhibit trans-species polymorphisms suggesting that they are under balancing selection. Such evolutionary processes may be selectively favored because of defensive roles of the alkaloids.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W29

Wednesday 6th April
14:00 - 16:00

NIELSEN Jens Christian *(1), SPRIGENT Sylvain (1), NIELSEN Jens (1)*
(1) Chalmers University of Technology, Gothenburg, Sweden

Genomics based analysis of the secondary metabolism in the *Penicillium* genus

Production of pharmaceutically relevant secondary metabolites (SMs) is one important characteristic of *Penicillium* species, which has formed the foundation for a lot of research within the genus. In order to uncover the full biosynthetic potential of SMs in *Penicillium* species, and to elucidate the evolutionary hallmarks of the secondary metabolism, whole genome sequencing was conducted for 10 selected *Penicillium* species known to produce a diverse array of SMs. Together with additional 12 previously published genomes of *Penicillium* species, a comparative analysis was performed to reveal the diversity of the secondary metabolism in these species. The results have shown that secondary metabolite biosynthesis is the most diverse part of the metabolism among *Penicillium* species. Moreover, we defined conserved families of biosynthetic gene clusters of polyketides and non-ribosomal peptides and inferred horizontal gene transfer events within the genus. The diverse distributions and evolutionary histories of these biosynthetic gene clusters provide insights into the biochemical mechanisms that produce SMs, and guided discovery of novel gene clusters.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W30

Wednesday 6th April
14:00 - 16:00

SVEDBERG Jesper *(1), HAMMOND Thomas M. (2), JOHANNESSON Hanna (1)*
(1) Department of Organismal Biology, Uppsala University, Uppsala, Sweden
(2) School of Biological Sciences, Illinois State University, Normal, Illinois, USA

The effects of meiotic drive on genome architecture in *Neurospora*

Spore killer is a type of meiotic drive elements found in natural populations of *Neurospora intermedia* and *Neurospora sitophila*. In crosses between strains carrying a Spore killer genetic element and strains that are sensitive to Spore killer, half of the spores will die and the surviving spores will all carry the Spore killer element. Using PacBio long-read sequencing (to >50x coverage) together with short-read Illumina data we have created high quality, full chromosome genome assemblies of fifteen strains of *Neurospora* which are representative of all known Spore killer types as well as of sensitive and resistant strains. This data has revealed that Spore killer strains of N. intermedia carry complex patterns of tandem inversions, interspersed with large clusters of repetitive DNA, that covers an approximately 3 Mbp large region surrounding the Spore killer genetic element where recombination with sensitive strains is suppressed. These structural differences may be instrumental in maintaining linkage between the genes causing the killer phenotype. The two different Spore killer types found in *N. intermedia* both show similar patterns of structural variation compared to sensitive strains, but they also differ from each other, suggesting they have evolved separately and accumulated structural variation independently, even though they are found within the same species and exhibit very similar phenotypes. Here we present a comparative analysis of the genomic architectures of the Spore killer strains and make the case that the need for linkage between the necessary genes is the primary driver of the evolution of genome architecture, but that relaxed selection due to sheltering by the meiotic drive and degeneration due to reduced recombination may also play important roles. The Spore killer genomes shows signatures of evolution similar that of other situations where the selective environment has shifted strongly and suddenly, such as when an organism turns pathogenic or gains the ability to colonize a new host.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W31

Wednesday 6th April
14:00 - 16:00

MEUNIER Cécile *(1), MARIUSH Zaywa (1), HEIDARI Nahid (1), JOHANNESSON Hanna (1)*
(1) Uppsala university, Evolutionary Biology Centre, Systematic Biology, Uppsala, Sweden

Neither diploid nor haploid: control and fitness consequences of heterokaryosis in *N. tetrasperma*

Heterokaryosis, the association of genetically different haploid nuclei in the same organism, is a feature of numerous fungi. Heterokaryons can be viewed as functional diploids, enjoying masking of deleterious recessive mutations. Nevertheless, heterokaryosis is also distinct from diploidy: the divergent haploid nuclei remain independent, prone to haploid selection. Heterokaryosis may thus lead to conflicts between selection levels. Conversely, heterokaryosis is also believed to confer adaptive phenotypic plasticity to the organism as a whole. Additional flexibility may also arise from imbalanced ratios among genetically different nuclei: controlling the nuclear ratio might be adaptive under varying environmental conditions. We used *Neurospora tetrasperma* as a model to investigate into the adaptive significance of heterokaryosis. In this species, heterokaryosis is maintained throughout the life cycle. Different nuclei of opposite mating-types (A or a) coexist in the mycelium, and are packaged together in asexual and sexual spores. *N. tetrasperma* is thus self-fertile. Heterokaryosis may, though, occasionally break down during asexual or sexual reproduction, thus restoring haploid homokaryons. Is there any adaptive advantage of heterokaryosis per se in *N. tetrasperma*, besides conferring self-fertility? Previous studies in our group have shown that nuclear A:a ratios may be imbalanced, and that opposite mating-type nuclei might assume different roles throughout the life cycle. We first investigated into the control of nuclear ratios, surveying heterokaryons under different growth conditions. Nuclear ratios, even when imbalanced, seem fairly constant over time and conditions: mitosis rates seem thus under tight control. This might possibly help to control against cheating nuclear lineages. We then investigated into the fitness consequences of heterokaryosis, as compared to homokaryosis. We conducted fitness assays to compare the performances of homokaryons and of balanced and imbalanced heterokaryons, both under vegetative growth and sexual reproduction. This allows assessing for any adaptive advantage of heterokaryosis, and also for potentially distinct roles of opposite mating-type nuclei throughout the life cycle.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W32

Wednesday 6th April
14:00 - 16:00

TUOVINEN Veera *(1), BERGSTRÖM Linnea (2), AMENT Sandra Lorena (2), SPRIBILLE Toby (3), VANDERPOOL Dan (4), NASCIMBENE Juri (5), YAMAMOTO Yoshikazu (6), THOR Göran (1), JOHANNESSON Hanna (2)*
(1) Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden
(2) Department of Systematic Biology, Uppsala University, Uppsala, Sweden
(3) Institute of Plant Sciences, University of Graz, Graz, Austria
(4) Division of Biological Sciences, The University of Montana, Missoula, MT, USA
(5) Department of Agronomy, Food, Natural resources, Animals and Environment, University of Padova, Padova, Italy (6) Department of Biological Production, Akita Prefectural University, Akitashi, Japan

Reproductive system in *Letharia* – implications for conservation in Scandinavia

Letharia is a lichen genus with high species diversity in western North America and low in Europe, Caucasus and North Africa. The genus consists of at least six putative species, of which four commonly reproduce by apothecia (i.e., sexual structures; *Letharia columbiana s. str., L. lucida, L. rugosa, L. gracilis*) and two mainly by asexual propagules (*L. vulpina s. str. and L. lupina*). Only the latter occur in Europe. *L. vulpina* is red-listed in Scandinavia, where it produces apothecia extremely rarely. Decline of the species in Scandinavia has been connected to decline in suitable habitats, as the species prefers pine snags in open forests with long continuity. These habitats are today fragmented due to intensive forest management practises. We identified and characterized the mating-type locus from whole genome data of four *Letharia* species (*L. lucida, L. rugosa, L. vulpina, and L. lupina*). In all studied species we found only one of the allelic variants of the mating-type locus, suggesting they are self-incompatible (heterothallic). We found only one mating-type of *L. vulpina* present in Swedish populations, suggesting that there is currently no potential for sexual reproduction. In the Italian Alps, by contrast, our data indicate that both mating-types are present. We suggest that the poor success of the species in Scandinavia is due to the combination of lack of suitable, connected habitats and reproductive limitations caused by the distribution of different mating types. We suggest that only one mating type arrived to Sweden after last glaciation or that one mating-type got lost by chance during the habitat decline.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W33

Wednesday 6th April
14:00 - 16:00

ROSLING Anna *(1), HOUSE Geoffrey (2), KAONONGBUA Wittaya (3), SCHUETTE Ursel (2), BEVER James (4)*
(1) Evolutionsbiologiskt Centrum, Uppsala University, Sweden
(2) Biology department, Indiana University, Bloomington/ IN, USA
(3) Department of Microbiology, King Mongkut's University of Technology, Thon Buri, Thailand
(4) Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence/KS, USA

Exploring the hybrid origin of *Claroideoglomus candidum*

Arbuscular mycorrhizal fungi have a unique genomic and reproductive organization. They reproduce asexually by forming large multinucleate spores and thus have no single nucleate stage in their life-cycle. Within organismal rDNA gene polymorphism is high. Hyphal fusion and nuclear mixing in genetically identical strains are common and can also occur between genetically dissimilar strains and result in heterokaryosis. Arbuscular mycorrhizal fungi thus have the mechanism for hybridization between species but until now there is no evidence that such hybrids form or that they can be stable in nature. Here we use different techniques to sequence rDNA genes from single spore extracts and find evidence consistent with a hybrid origin of *Claroideoglomus candidum*. From replicate single spore extracts, 11-21 unique genotypes were recovered using cloning and Sanger sequencing of 1500 bp rDNA fragments. Variation detected in the LSU was recovered with both 454 and Illumina sequencing. In *C. candidum* the majority of rDNA sequences show high homology with other *Clarioideoglomus* sequences. However, 10 15% of the recovered sequences are of *Rhizophagus intraradices* origin. This study is part of a larger one where over fifty single spore extracts, representing species across the *Glomeromycota* were sequences. Additional analysis using PacBio and nuclei sorting are underway. No cultures of *R. intraradices* were in the lab at the time of the sequencing and sequences from this species were not detected from any other isolates examined in the larger study. Together these results support the idea that *C. candidum* is a species formed by hybridization of distantly related taxa.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W34

Wednesday 6th April
14:00 - 16:00

CROLL Daniel *(1), HARTMANN Fanny (1), ZALA Marcello (1)*
(1) Plant Pathology, ETH Zurich, Zurich, Switzerland

The population genomics of chromosomal plasticity in a fungal wheat pathogen

Plant pathogens show extraordinary adaptive potential to overcome host resistance and evolve tolerance to fungicides. Frequent sexual reproduction, the capacity to disperse and large population sizes are thought to be the main drivers of rapid evolution. However, adaptive evolution to resistant host genotypes may require complex mutations including gene deletion polymorphisms or chromosomal rearrangements. How such complex genetic variation is generated in the genome and how this variation contributes to virulence evolution is poorly understood. To this end, we aimed to identify the basis for genomic plasticity in populations of *Zymoseptoria tritici*, the causal agent of Septoria tritici blotch (STB) on wheat. We generated a complete genome assembly using long-read technology and high-density genetic maps of an isolate from a Swiss wheat field. Comparative genomics analyses showed that core chromosomes of the newly assembled genome harbored orphan regions not found in the reference genome. Orphan regions were most likely generated by non-homologous recombination among copies of transposable elements (TE). Genes in orphan regions were more likely to show evidence for degeneration, however a subset of orphan genes had sequence signatures and transcriptional profiles indicative of a role in virulence. As TE likely played a key role in generating chromosomal polymorphisms, we aimed to characterize the TE dynamics using population genomics analyses of Illumina sequenced isolates. We found that a family of TEs showed evidence for population-specific invasions and, hence, may have led to population-level differences in the propensity to undergo chromosomal rearrangements. In conclusion, chromosomal plasticity enables highly divers pathogen populations to rapidly gain or lose virulence loci in response to selection pressure imposed by the host.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W35

Wednesday 6th April
14:00 - 16:00

KOHLER Annegret *(1), NAGY Laszlo G (2), MORIN Emmanuelle (1), KUO Alan (3), MURAT Claude (1), VENEAULT-FOURREY Claire (1), LINDQUIST Erika A (3), GRIGORIEV Igor (3), HIBBETT David S (4), MARTIN Francis (1)*
(1) Interactions Arbres/Microorganismes, INRA, Laboratory of Excellence ARBRE) Université de Lorraine, Champenoux, France
(2) Synthetic and Systems Biology Unit, Institute of Biochemistry, Szeged, Hungary
(3) US Department of Energy, Joint Genome Institute (JGI), Walnut Creek, California, USA
(4) Department of Biology, Clark University, Worcester, Massachusetts, USA

Exploring the genome diversity of mycorrhizal fungi to understand the evolution and functioning of symbiosis

To advance in our understanding of the evolutionary origin of mycorrhizal symbiosis and to elucidate the molecular mechanisms involved, a large genome sequencing project of species from different taxa, phylogenetic clades and symbiotic lifestyles (ectomycorrhiza, ericoid and orchid mycorrhiza) was started in 2011 by the Joint Genome Institute (JGI) and the mycorrhizal genome initiative (MGI). In 2013 followed a large-scale transcriptome sequencing project of mycelium and mycorrhizal roots from these fungi in order to identify and to compare symbiosis-regulated genes. To date about 50 mycorrhizal genomes and a dozen of mycorrhizal transcriptomes are available for comparative analyses. All ectomycorrhizal fungi sequenced so far have a reduced set of genes encoding plant cell wall degrading enzymes (PCWDE) compared to their ancestral wood decayers. Nevertheless, they possess diverse abilities to decompose lignocellulose. There seems to be a clear cut in the number of PCW degrading glycoside hydrolases between ECM fungi on one hand and ericoid and orchid mycorrhizal fungi on the other hand, while the situation for lignin decomposition enzymes is less clear. The analysis of mycorrhizal transcriptomes revealed the involvement of both conserved AND clade-specific genes. Induced are genes coding for the same functions but without orthology, like (often clade-specific) small-secreted proteins, transporters, redox metabolism or carbohydrate active enzymes, suggesting a convergent evolution.

Kohler et al., 2015, Nature Genetics 47, 410-415

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W36

Wednesday 6th April
14:00 - 16:00

BAKKEMO Renee Isabel *(1), HESS Jaqueline (1), SKREDE Inger (1)*
(1) Department of Biosciences, University of Oslo, Oslo, Norway

Comparative genomics of dry rot fungus *Serpula lacrymans* to reveal nutrient transport mechanisms

Serpula lacrymans (the dry rot fungus) is of great concern for homeowners. This fungus is an effective brown-rot decomposer of wooden houses in the Northern Hemisphere. *Serpula lacrymans* causes severe destruction to houses in this region and results in great economical losses for the homeowners. There are two main linages of the species, *Serpula lacrymans var. shastensis* found in natural habitats in North America and *Serpula lacrymans var. lacrymans* colonizing indoor environments in temperate regions worldwide. Their closest sister species is the wild living *Serpula himantioides*. Our aim for this project is to compare the differences in gene expression related to nutrient transport mechanisms. The house inhabiting *var. lacrymans* is known to make thick hyphal cords and transport water and nutrients for long distances in houses. We therefore hypothesize that the in-house strains have more effective transcriptional response encountering nutrients than the wild types. In this project we cultured four variants of *Serpula (var. lacrymans* from Japan and Europe, *var. shastensis and S. himantioides*) on split-plates containing media with different nitrogen content. This altered the carbon-nitrogen ratio on the different sides on the plate and we expect to find higher levels of gene expression on the nitrogen-positive side. In addition, we expect the gene expression to vary between the different strains of *Serpula*.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W37

Wednesday 6th April
14:00 - 16:00

SGHYER Hind *(1), TELLIER Aurélien (2), HÜCKELHOVEN Ralph (1), MÜNSTERKÖTTER Martin (3), GÜLDENER Ulrich (3), HESS Michael (1)*
(1) Phytopathology/Center of Life and Food Sciences Weihenstephan, Technische Universität München, Freising, Germany
(2) Section of Population Genetics/Center of Life and Food Sciences Weihenstephan, Technische Universität München, Freising, Germany
(3) Institute of Bioinformatics and Systems Biology, Helmholtz Center Munich, Munich, Germany

Investigating the genetic structure and diversity of the barley pathogen *Ramularia collo-cygni*

Ramularia collo-cygni (Rcc) is the biotic factor responsible for the disease Ramularia leaf spot (RLS) of barley (*Hordeum vulgare*). The fungus is attracting interest in the scientific community as a result of the increasing number of economically damaging disease epidemics. To understand more about its epidemiology, the knowledge of its genetic structure and diversity is essential. To address its genetic structure, the genome of Rcc (urug2 isolate) was de-novo sequenced (Illumina HiSeq, paired-end of 500 and 8k libraries) and assembled using Allpaths-LG assembler. The finished assembled genome of Rcc is about 32 Mb and is currently to be found in 78 scaffold (N50 scaffold size 1.9 Mb). The fungal RNA from 6 different conditions, especially one that mimics the plant environment (Barley Straw Agar BSagar) was also sequenced to help in one hand the annotation and in other hand to uncover putative genes of interest that might be involved in the pathogenicity or the fungicide resistances for example. Finally, the complete annotation was performed using gene predictors (Genmark, Fgenesh, Augustus) and was manually, gene by gene, corrected using the RNA-seq information. The overall annotation enabled the prediction of 12346 genes. As for the RNA-seq data, we had a first look into the functional distribution of the genes differentially expressed among the different conditions. Among the highly distributed functional categories ($p <= 0,05$), categories such as «disease, virulence factors», «secondary metabolites», «polysaccharide metabolism», or even «phenylalanine metabolism» drew our attention. These categories are known to be involved in plant-pathogen interactions. The search and study of genes of interest in these categories are still underway. To evaluate the true genetic diversity of this fungus, whole genome sequencing of 19 Rcc isolates from multiple geographic locations and non-barley hosts was performed and mapped to the Rcc reference genome. The analysis of these data is still in progress. However, preliminary analysis of the sequence data from four Rcc housekeeping genes indicated substantial genetic diversity between the isolates and a possible Rcc population size expansion, which might help explain the recent emergence of this fungus. We hope by this approach to provide valuable insights in to the genetic diversity of this organism and to address how this diversity has influences on the evolution of the fungus.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W38

Wednesday 6th April
14:00 - 16:00

GENISSEL Anne *(1), GOUT Lilian (2)*
(1) BIOGER, INRA, , AgroParisTech, Thiverval-Grignon, France
(2) BIOGER, AgroParisTech, INRA, Thiverval-Grignon, France

Detecting molecular footprint of local adaptation in *Zymoseptoria tritici*

Zymoseptoria tritici (Zt) is one of the most common pathogen of wheat and yet the genetic basis of adaptation in this species is largely unknown. Population genetics studies using neutral markers suggest for the most part that there is a low level of genetic differentiation between natural population samples in Zt. Using new generation sequencing data, now we can provide an in depth analysis of the genetic variation segregating in natural populations, and better understand the mode and tempo of genome evolution at the intraspecific level. The aim of this work was to identify the genes / genome regions that are under extreme genetic differentiation between two French population samples that differ in environment, collected in the North and South of France. To reach this goal, we used new generation sequencing data from 30 isolates. We found a large genetic diversity for both single nucleotide polymorphisms and structural variants within and between population samples. Many polymorphic sites throughout the genome were highly differentiated between the population samples, especially we discovered a large inversion on chromosome 7. We detailed the nature of genetic variants in this specific region. We also described the pattern of linkage disequilibrium and quantify the skew in allele frequency spectrum across inverted regions to understand the nature of the selection in action. Last, we discuss the potential role of inversions in local adaptation.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W40

Wednesday 6th April
14:00 - 16:00

BARANYI Nikolett *(1), KOCSUBÉ Sándor (1), SZEKERES András (1), BENCSIK Ottó (1), VÁGVÖLGYI Csaba (1), VARGA János (1)*
(1) Department of Microbiology,Faculty of Science and Informatics,University of Szeged, Szeged, Hungary

Examination of genetic variability of *Aspergillus flavus* species with UP-PCR and microsatellite typing methods

Aspergillus flavus is one of the most important pathogens of various economically important crops including maize, cotton and peanut, and causes serious yield losses throughout the world. In addition, *A. flavus* is among the most common organisms causing fungal keratitis in subtropical and tropical areas of the world. *Aspergilli* are able to produce a range of mycotoxins which can be harmful to animals or humans. Aflatoxins are produced by at least 20 species of the genus *Aspergillus*, the most important one is *A. flavus*. In this study, 59 *Aspergillus flavus* isolates from four various populations (clinical and environmental isolates from India, isolates from indoor air from Croatia and Hungary, and isolates from maize from Serbia) and 1 A. minisclerotigenes isolate from India were examined. The isolates were determined at the species level using sequencing of part of their calmodulin gene. The genetic variability of the isolates were investigated with the combination of partial calmodulin gene sequences, UP-PCR and microsatellite data. The four populations formed different well-defined clades in the phylogenetic tree. Phylogenetic analysis showed that the isolates deriving from indoor air and from maize formed well-defined clusters with high support values, while those originating from environmental and clinical samples came from India were separated from the European isolates. According to HPLC analysis none of the examined isolates collected from indoor air or from maize in Central Europe were able to produce aflatoxins, nevertheless about half of the isolates derived from India produced aflatoxins. Further studies of clarification of the molecular background of the aflatoxin non-producing strains are in progress.

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POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W41

Wednesday 6th April
14:00 - 16:00

VETROVSKY Tomas *(1), BALDRIAN Petr (1)*
(1) Institute of Microbiology of the ASCR, Prague, Czech RepublicC

Fast and easy processing of fungal amplicons: SEED - a GUI based user friendly sequence editor and pipeline for high-throughput amplicon processing

The SEED (http://www.biomed.cas.cz/mbu/lbwrf/seed/) is a free-to-use GUI-based sequence editor and pipeline for Windows platforms providing access to internal functions as well as those performed by external software that is installed for full functionality (1). SEED was created to provide an intuitive interface for fast bioinformatic analysis of PCR amplicons on desktop computers according to the suggested workflow. The most recent 64-bit version allows comfortable work with up to 8 million sequences (~4 GB of data) on a standard personal computer with 8 GB RAM. SEED is especially suitable for sequential analysis of the sequences of PCR amplicons obtained by Illumina or 454-pyrosequencing, such as sequences of fungal ITS regions, bacterial 16S rDNA or other target genes. The program has a wide array of functions including editing of sequences and their titles, sorting, quality trimming, pair-end joining, grouping of sequences based on sequence motifs or sequence titles, batch processing of sequence groups, denoising, chimera removal, ITS extraction, sequence alignments and clustering, OTU table construction, construction of consensus sequences, creation of local databases for BLAST and searching either them or the whole NCBI, retrieval of taxonomical classification from the NCBI, calculation of diversity parameters, construction of phylogenetic trees and many more.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W42

Wednesday 6th April
14:00 - 16:00

MOORE Geromy *(1), BELTZ Shannon (1)*
(1) Southern Regional Research Center, USDA-ARS, New Orleans, USA

Exploring recombination breakpoints across the aflatoxin gene clusters of *A. flavus* F1 progeny

At the population level, evidence of recombination within the *A. flavus* aflatoxin biosynthesis pathway has been reported-- a finding based solely on sequence breakpoints that existed within the intergenic regions of potentially unrelated isolates. A complete mapping of recombination breakpoints spanning the entire aflatoxin gene cluster has yet to be performed. In an effort to correlate recombination breakpoints with sibling diversity, we paired two sexually-compatible *A. flavus* parent strains (MAT1-1 aflatoxigenic + MAT1-2 non-aflatoxigenic) and isolated 15 F1 progeny via single-spore method. Our goal is to ascertain whether or not sequence breakpoints are consistently observed for F1 progenies sharing the same parents. This ongoing project involves amplification, sequencing and assembly of a total of 17 aflatoxin gene clusters, identification of polymorphisms that indicate the presence of a recombination breakpoint within the aflatoxin cluster of each F1 progeny, and extrapolating the amount of diversity that can manifest from a single generation of recombination. Additionally, traditional vegetative compatibility testing concomitant with multi-locus sequence type (MLST) analysis are being implemented as a means to determine individuality within a single generation of F1 progeny.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W43

Wednesday 6th April
14:00 - 16:00

SUMIKOVA Tatana *(1), PALICOVÁ Jana (1), CHRPOVÁ Jana (1), ŠÍP Václav (1)*
(1) Crop Research Institute, Prague-Ruzyně, Prague, CzechRepublic

Fusarium **species causing head blight in the Czech Republic and genetic variability of their chemotypes**

Survey of a random collection of naturally infected wheat ears (1606 ear samples) from various localities in the Czech Republic was carried out in the years 2004 - 2015. Mycological identification of the samples from 2004 - 2009 revealed that the dominant species was *Fusarium graminearum* (average frequency 56.6%), followed by *F. poae* (19.2%), *F. culmorum* (16.9%) and *F. avenaceum* (12.8%). *F. equiseti*, *F. sporotrichioides*, *F. tricintum*, *F. acuminatum* and *F. semitectum* were detected in minor frequencies. 233 *F. graminearum* and 66 *F. culmorum* isolates were further analyzed by 7 molecular markers based on trichothecene biosynthesis genes Tri7, Tri13 and Tri3 to determine their genetic variability in the ability of trichothecene production. Most of the *F. graminearum* isolates belonged to 15-ADON chemotype, only one isolate was determined as 3-ADON producer and one isolate as a NIV producer. Similarly most of *F. culmorum* isolates belonged to DON producing chemotype and only two isolates represented NIV chemotype. *Fusarium* species in samples from 2011-2015 were identified using PCR assays with published species specific primers for the most common species causing head blight. In 2011 *F. graminearum* dominated (53.1%), followed by *F. poae* (30.8%) and *F. culmorum* (24.6%). During 2012 - 2015 the frequency of occurrence considerably changed and *F. poae* became dominant species causing head blight, followed by *F. graminearum* and *F. avenaceum*. *F. culmorum*, *F. equiseti*, *F. sporotrichioides* and *F. langsethiae* were detected in minor frequencies. Since the changes in *Fusarium* species composition the growing importance of other "emerging" mycotoxins (beauvericin and enniatins) than only currently the major "traditional" *Fusarium* mycotoxins DON and NIV can be expected.

Supported by Ministry of Agriculture of the Czech Republic, Projects No. MZeRO0416, QJ1210189.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W44

Wednesday 6th April
14:00 - 16:00

KOBMOO Noppol *(1), WICHADAKUL Duangdao (2), INGSRISWANG Supawadee (2), SITHICHOKE Tangphatsornruang (2), CHANTASIGH Duriya (2), LUANGSA-ARD Janet Jennife (2)*
(1) ESE; Université Paris-Sud, CNRS, AgroPArisTech, Orsay, France
(2) National Center for Genetic Engineering and Biotechnology, Pathum Thani, Thailand

Comparative genomics between hypocrealean entomopathogenic fungi of different host ranges shed light on the evolution of host specificity

The evolution of entomopathogenic fungi (or insect fungi) is associated with specialization to different groups of insect hosts. Many species complexes are composed of closely related species being specific to different hosts. How different groups of insect fungi get specialised to different hosts and which ecological strategies for exploitation of hosts they opt for are major questions in the study of entomopathogenic fungi. These questions have furthermore potential applications for biological control in agriculture. In this study, we have conducted comparative analyses on genes involved in pathogenicity and virulence between Hypocrealean entomopathogenic fungi with different host ranges, from *M. robertsii* and *B. bassiana* which have very broad host ranges infecting several insect orders, to *O. unilateralis* which infects only a tribe (*Camponotini*) in a sub-family of ants (*Formicinae*). Our analyses showed a tendency toward contractions of various gene families for narrow host-range species, including cuticle-degrading genes (proteases, carbohydrate esterases) and some families of pathogen-host interaction (PHI) genes, arguing for reduced capacity to exploit broad host ranges. For many families of genes, *O. unilateralis* had the least number of genes found; some genes commonly found in other insect fungi are even absent. However, there are expansions of genes involved in 1) the production of bacterial-like toxins in *O. polyrhachis-furcata*, compared with other entomopathogenic fungi, and 2) retrotransposable elements, suggesting unexpected strategies and biology.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W45

Wednesday 6th April
14:00 - 16:00

CONFAIS Johann *(1), DUCASSE Aurelie (1), GENISSEL Anne (1), GOUT Lilian (2)*
(1) BIOGER, INRA, AgroParisTech, Thiverval Grignon, France
(2) BIOGER, AgroParisTech, INRA, Thiverval Grignon, France

Resolution provided by microsatellite markers in detecting population structure of the wheat pathogen *Zymoseptoria tritici*

Zymoseptoria tritici (*Zt*) causes Septoria leaf blotch, one of the most important worldwide diseases of wheat. Yield losses can reach 50% in disease-conducive climates and epidemics occur regularly on bread wheat (*Triticum aestivum*). A set of 614 isolates was sampled from naturally infected fields of main French regions during two years, and genotyped using 12 microsatellite markers. The data were analyzed with population genetic statistics and population structure analyses. The genetic structure of *Zt* in France is characterized by high genetic diversity (95% of unique genotypes), regular recombination (all populations were in gametic equilibrium), and gene flow. No significant genetic differentiation was found among the sampled populations. To further investigate the extent of genetic structure in *Zt*, we re-sequenced using Illumina technology the whole genome of 30 isolates from two geographically distant populations (sampled in the north and the southwestern regions of France). The NGS data were used to identify more than 4000 microsatellite markers and to analyze their variation across the 30 isolates. In this study, we compared the resolution of these new markers spread across the genome with the 12 microsatellites previously used in detecting genetic patterns in *Zt*.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W46

Wednesday 6th April
14:00 - 16:00

VESELSKÁ Tereza *(1), KOLAŘÍK Miroslav (1)*
(1) Institute of Microbiology of the ASCR, v. v. i., Prague, Czech Republic

Role of genome size in evolution of *Geosmithia* fungi living in association with bark beetles

Geosmithia belongs among fungi living in symbiosis with phloem-feeding bark beetles. Some of them are considered to be generalists, living in association with insects on a variety of plant hosts as well as on alternative substrates. The others are specialists restricted to limited range of vectors in Europe, living on the single plant family *Pinaceae*, which suggest their long-term co-evolution with vectors. Several species, including *G. microcorthyli, G. eupagioceri* and likely *G. cnesini*, changed their ecology to that of obligatory symbiosis with ambrosia beetles, which has led to a shift in their phenotype and caused formation of large spherical spores. The genome size is often linked with cell size and serves as a tool for adaptation to new environment. We designed new flow cytometry protocol for genome size estimation in fungi and we introduce new suitable fungal standard *Aspergillus fumigatus* CEA10 for genome size calculation. We revealed a positive and strong correlation between genome size and spore volume in *Geosmithia*. We also found that species more narrowly associated with the vector tend to have a larger genome and spore size than less narrowly associated species. Ambrosia fungi achieved the biggest genome size (mass) and spore volume compared to other species. *G. eupagioceri* was unique among *Geosmithia spp.* in that it produced multinucleated spores. We believe that polyploidisation and multiplication of nuclei per spore occurred during the evolution of ambrosia species in the genus *Geosmithia*.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W47

Wednesday 6th April
14:00 - 16:00

TASCHEN Elisa *(1), ROUSSET François (2), SAUVE Mathieu (1), BENOIT Laure (1), DUBOIS Marie-Pierre (1), RICHARD Franck (1), SELOSSE Marc-André (3)*
(1) CEFE UMR 5175, CNRS - Université de Montpellier - Université Paul-Valéry Montpellier - EPHE, Montpellier, France (2) Institut des Sciences de l'Evolution, Université de Montpellier, CNRS, IRD, EPHE, Montpellier, France
(3) Institut de Systématique, Évolution, Biodiversité (ISYEB - UMR 7205 – CNRS, MNHN, UPMC, EPHE), Muséum national d'Histoire naturelle, Sorbonne Universités, Paris, France

How the truffle got its mate: insights from genetic structure in spontaneous and planted Mediterranean populations of *Tuber melanosporum*.

Tuber melanosporum, a haploid *Ascomycota* forming ectomycorrhizae, is a self-sterile hermaphrodite: its meiospores mature in ascocarps supported by one (maternal) parent after fertilization by another (paternal) parent. Whereas the maternal parent is known to be vegetatively present as ectomycorrhizae, the biology and the origin of the paternal gamete remains unknown. In this study, we investigate pending issues on *T. melanosporum* population biology: [1] genetic spatial structure, to assess gene dispersal; [2] parental relatedness from ascocarps, to assess male gametic gene flow; and, given the development of plantations of inoculated trees, [3] the differentiation of planted versus spontaneous, uninoculated compartments. We analysed SSR polymorphism in 1453 samples collected in South-East France plantations and spontaneous truffle-grounds. We confirm the previously observed strong genetic isolation by distance on brûlés, probably due to massive co-deposition of spores from same ascocarp(s) by animal dispersers, and to an important soil spore bank. Diploid ascocarps were highly inbred, supporting very limited spatial dispersal of paternal gametes. We confirm statistically the previous suspicion that maternal mycelia form patches with identical mating types. Since paternal mycelia were not found on ectomycorrhizal and given the results of our study, we hypothesize that they may be germlings from the spore bank. Finally, spontaneous sites and plantations revealed no genetic differentiation, except a higher paternal genetic diversity and lower heterozygote deficit on planted brûlés, perhaps due to the dispersal of ascocarp fragments by truffle growers in plantation. Thus, the on-going domestication poorly impacted *T. melanosporum* populations in South-East France.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W48

Wednesday 6th April
14:00 - 16:00

KOLAŘÍK Miroslav *(1), KOSTOVČÍK Martin (1), VĚTROVSKÝ Tomáš (1), HUBKA Vít (1)*
(1) Institute of Microbiology, Czech Academy of Sciences, Videnska 1083, Prague, Czech Republic

Paralogous genes in fungal taxonomy: ITS rDNA and β-tubulin as examples

Paralogous genes represent well-known problem in taxonomy that uses molecular features as the indispensable tool for delineation of taxa. Incongruent phylogenetic trees and incorrect taxonomic conclusions can result from mixing of paralogous genes in taxonomic studies. Beta-tubulin (benA), ITS rDNA both represent important loci in fungal taxonomy and the examples of incorrect use of their paralogues are presented. Beta-tubulin paralogue tubC was amplified and mistaken with benA gene in approximately 20 studies dealing with taxonomy of *Aspergillus* section Nigri (Hubka and Kolařík 2012). It was previously shown that both paralogues can be successfully distinguished using codon usage analysis. Similar analysis was applied on fungal beta-tubulin sequences deposited in GenBank and the results suggest that similar problem with beta-tubulin paralogues is probably not only restricted to *Aspergillus*. Multicopy ITS rDNA is another fungal barcode gene, with many time documented paralogues. Intragenomic sequence variability is typicaly low (<3%), but more divergent copies were also found. Some of them possess deviations in GC content, length and secondary structure stability, suggesting their lower functionality. Such deep paralogues, representing possible pseudogenes found in *Geosmithia* and *Hyphoderma sp.* are documented here.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W49

Wednesday 6th April
14:00 - 16:00

CMOKOVA Adela *(1), KOLARIK Miroslav (2), HUBKA Vit (1)*
(1) Department of Botany, Charles University in Prague, Prague, Czech Republic
(2) Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Molecular typization of *Arthroderma benhamiae*, a zoonotic agent of epidemic dermatophytosis in Central Europe

This study focuses on the newly spreading fungal pathogen, *Arthroderma benhamiae*, which has become common cause of superficial infections in Central Europe during last decade. A few years ago, this pathogen was almost unknown for human medicine, but nowaday it is the most common zoonotic agent of dermatomycoses in Czech patients. Similar situation is also reported in other Central and Western European countries. Here we propose a new typing scheme for purposes of enhanced surveillance and outbreaks detection (10 microsatellite markers and two genetic loci). The population structure of the pathogen was investigated on a set of 329 isolates from Europe, Asia and North America by using mentioned scheme. Analysis revealed presence of 37 genotype clustering into four major subpopulation, three of them are present in Europe. Approximately 80% of infections in Europe were caused by a clonally spreading virulent subpopulation. This subpopulation was unisexual (MAT-1), showed characteristic yellow phenotype and probably spread from North America (absent in Europe until 2003), where it occurs in natural hosts. Infections caused by strains of this subpopulation are common in children (median age 12 years), especially in girls (72.5%). Our data confirmed that infections in the Czech Republic are mostly transmitted to humans from rodents (mainly guinea pigs).

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W50

Wednesday 6th April
14:00 - 16:00

CARROLL Emily (1), SEO Jeong-Ah *(1)*
(1) School of Systems Biomedical Science, Soongsil University, Seoul, South Korea

Diversity and Enzyme Activity of Fungal Species Found in Korean Traditional Fermenting Starter, Nuruk

The traditional Korean fermentation starter, Nuruk, is made throughout South Korea, both commercially and at home, in the production of the Korean rice wine, makgeolli. Between 2013 and 2014 over sixty samples of nuruk were collected and the filamentous fungi isolated. The fungal species were identified by sequencing of the 5.8rDNA region along with physiological and morphological observations. Amongst the commercially made nuruk the most predominate species were yeast whereas home-made nuruk had more filamentous fungi. The source of the raw materials appeared to be important as nuruk made with imported wheat showed a different fungal profile than nuruk made with domestic wheat. Major filamentous fungal genera of fungus in nuruk included *Aspergillus, Lichtheimia*, and *Mucor* while *Saccharomycopsis* was the major yeast. Approximately, 132 species isolated from nuruk collected in 2014 were tested for alpha-amylase, glucoamylase, and protease activity. Most of the species with high alpha amylase and glucoamylase enzyme activity were *Ascomycetes* with many *Aspergillus* and *Sacccharomycopsis* species. Species with high protease activity were *Ascomycetes* including *Penicillium* and *Syncephalastrum* species, as well as *Zygomycetes*. Among these strains with high enzyme activity 10 were selected for use in making makgeolli. Future genomics and metabolomics analysis of these fungal strains will help further our understanding of the role of filamentous fungi and yeast strains in the makgeolli making process.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W51

Wednesday 6th April
14:00 - 16:00

BONITO Gregory *(1), HAMEED Khalid (2), BUSBY Posy (2), BENUCCI Gian Nm (1), TUSKAN Gerald (3), SCHADT Christopher (3), VILGALYS Rytas (2)*
(1) Michigan State University, East Lansing, USA
(2) Duke University, Durham NC, USA
(3) Oak Ridge National Laboratory, Oak Ridge TN, USA

Using common gardens to study effects of soil origin and host genotype on the *Populus* and *Pinus* root mycobiome

Populus is a model woody plant species important ecologically as a pioneer and economically as a biofuel and fiber crop. Nutrition and aboveground biomass of *Populus* is largely dependent upon belowground root growth and interactions with ectomycorrhizal, arbuscular mycorrhizal and endorrhizal fungi, as well as prokaryotic organisms and other soil fauna. Our research utilizes trap-plant experiments and characterizes fungal communities through next generation amplicon sequencing. In this experiment we chose a panel of *Populus trichocarpa* DOE-BESC reference genotypes, selected on account of their productivity, lignin type, lignin content, and geographic origin. Cuttings were grown in triplicate in soils collected from four common garden sites in the Pacific Northwest, a natural *Populus* soil from NC and in sterile sand as control group. Plants were grown for 5 months and then roots were harvested, washed, and fungal communities were characterized by MiSeq amplicon sequencing of the ITS & LSU rDNA. Y-diversity of fungal communities on BESC reference genotypes shows strong biogeographic structuring. Uninoculated control plants were significantly shorter in height ($P<0.01$) and their microbiome consisted of fewer taxa. Fungal community similarity on *Populus* roots grown in different soils follows a decay by distance model, although some root-assocated fungal taxa are widespread geographically. Host species and genotypes may influence fungal and bacterial community structure in a context dependent manner, and although particular host-genotype x fungal species interactions were documented, these were most pronounced in soils harboring higher fungal biodiversity and were not related to any particular preselected host character.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W52

Wednesday 6th April
14:00 - 16:00

CIAMPI-GUILLARDI Maisa *(1), ROGÉRIO Flávia (1), MORAES Sylvia Raquel Gomes (2), BARBIERI Marina Coan Goldoni (1), MASSOLA JR Nelson Sidnei (1)*
(1) University of São Paulo, ESALQ, Department of Plant Pathology and Nematology, Piracicaba, SP, Brazil
(2) Federal University of Mato Grosso, Sinop, MT, Brazil

Temporal genetic diversity of the causal agent of soybean anthracnose, *Colletotrichum truncatum*, in Brazil

Colletotrichum truncatum is an ascomycete associated with anthracnose on soybean and other hosts. We compared haplotypes of soybean-infecting isolates sampled in ten states within 22 years in Brazil to examine the influence of the introduction of new soybean genotypes in the genetic diversity of this pathogen. A network of haplotypes constructed for combined multilocus sequences (ITS-5.8S rDNA, glyceraldehyde-3-phosphate dehydrogenase and histone 3) by statistical parsimony, revealed a high level of diversity amongst the fungal isolates, the occurrence of recombination events, and lack of structure by geography or by host species. In 69 fungal isolates from six countries and five host species, 29 haplotypes were identified. Of them, only four haplotypes were shared among fungal isolates, suggesting a low frequency of clones in the sample. Three genetic clusters were identified, which could reflect independent introductions of the pathogen in Brazil. The first clade grouped *C. truncatum* isolates from Indonesia, India, Denmark, United States (USA) and all but one Brazilian state, including the most frequent haplotype (H1) found in 36 isolates. This widely distributed haplotype was shared by soybean-infecting isolates sampled previously (1992 to 2007) and recently (2014) in Brazil and also by isolates from the United States, suggesting a possible movement of infected plant material. The two other clades grouped fungal haplotypes from soybean with common beans from Brazil and the United States as well as with cowpea and sedge weed. Taking to account earlier reports that indicated the role of weeds as alternative inoculum sources of *Colletotrichum* in other pathosystems, this could also be occurring in soybean anthracnose. Three out of ten Brazilian haplotypes sampled in 2014 occupied tips of the network, reflecting their most recent origin, and the closely relatedness to previously sampled *C. truncatum* isolates whereas the remaining isolates shared the widespread H1 haplotype. Considering the number of soybean genotypes introduced in soybean fields in the last 20 years, and the increase of both cultivated host area and incidence of the disease, we expected to find higher genetic diversity in the *C. truncatum* strains recently sampled. The selective pressure imposed by large scale uses of fungicides in soybean crops could have influenced the levels of genetic diversity of the pathogen, with the selection of few fungal haplotypes able to overcome such pressure.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W53

Wednesday 6th April
14:00 - 16:00

MCDONALD Megan *(1), MCGINNESS Lachlan (1), HANE James (2), MILGATE Andrew (3), SOLOMON Peter (1)*
(1) The Australian National University, Canberra, ACT, Australia
(2) Centre for Crop and Disease Management Curtin University, Perth, WA, Australia
(3) NSW Department of Primary Industries, Wagga Wagga, NSW, Australia

Utilizing gene tree variation to identify candidate effector genes in *Zymoseptoria tritici*

Zymoseptoria tritici is a host-specific, necrotrophic pathogen of wheat. Infection by *Z. tritici* is characterized by its extended latent period, which typically lasts two weeks, and is followed by extensive host cell death and rapid proliferation of fungal biomass. This work characterizes the level of genomic variation in 13 isolates for which we have measured virulence on 11 wheat cultivars with differential resistance genes. Between the reference isolate, IPO323, and the 13 Australian isolates we identified over 800,000 single nucleotide polymorphisms, of which ~10% had an effect on the coding regions of the genome. Furthermore we identified over 1700 probable presence/absence polymorphisms in genes across the Australian isolates using de novo assembly. Finally, we developed a gene tree sorting method that quickly identifies groups of isolates within a single gene alignment whose sequence haplotypes correspond with virulence scores on a single wheat cultivar. Using this method we have identified <100 candidate effector genes whose gene sequence correlates with virulence towards a wheat cultivar carrying a major resistance gene.

POSTER SESSION ABSTRACTS
CS6W54

Wednesday 6th April
14:00 - 16:00

WONNEBERGER Ronja *(1), FICKE Andrea (2), VIVIAN-SMITH Adam (2), FRIESEN Tim (3), LILLEMO Morten (1)*
(1) Department of Plant Sciences, Norwegian University of Life Sciences (NMBU), Ås, Norway
(2) Norwegian Institute of Bioeconomy Research (NIBIO), Ås, Norway
(3) USDA-ARS, Northern Crop Science Laboratory, Fargo, USA

Identification of molecular mechanisms in the *Drechslera teres*, barley pathosystem and population structure of a Norwegian *D. teres* population

The necrotrophic fungus *Drechslera teres* causes net blotch disease in barley by secreting necrotrophic effectors (NEs) which, in the presence of corresponding host susceptibility factors (SF), act as virulence factors in order to enable host colonization. At present the resistance within most Norwegian cultivars is insufficient. This study aims at detecting QTL associated with resistance and susceptibility in the Nordic barley breeding material and at discovering new NE SF interactions. This knowledge together with an understanding of the genetic background of the Norwegian net blotch population will be utilized to speed up resistance breeding. Resistance of a segregating mapping population of a cross between the closely related Norwegian varieties Arve and Lavrans to three Norwegian *D. teres* isolates was assessed at seedling stage in the greenhouse and in adult plants in the field. QTL mapping revealed four major QTL on chromosomes 4H, 5H, 6H and 7H. The resistance source for the QTL on 5H, 6H and 7H are contributed by the resistant parent Lavrans, while the resistance on 4H stems from the susceptible parent Arve, The 5H and 6H QTL accounted for up to 47% and 14,1% of the genetic variance, respectively, and were found both in seedlings and adult plants with the latter QTL being an isolate-specific association. The high correlation of seedling and adult resistance (R2=0,49) suggests that components of adult plant resistance can be predicted already at the seedling stage. Selected isolates and their culture filtrates will be screened on selected barley lines to characterize novel NE - SF interactions and to map the corresponding sensitivity loci. Effector protein candidates will be purified and further analysed to verify their effect on disease development. Additionally, 365 Norwegian *D. teres* isolates and a selection of globally collected isolates are currently being ddRAD genotyped in order to obtain SNP markers to study the genetic diversity and population structure of the current Norwegian fungal population. This data will also allow us to perform Genome Wide Association Studies (GWAS) to identify potential novel NE genes.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W55

Wednesday 6th April
14:00 - 16:00

KRISHNAN Parvathy *(1), MCDONALD Bruce (1), BRUNNER Patrick (1)*
(1) ETH, Zurich, Switzerland

Widespread signatures of selection at secreted peptidases in a fungal plant pathogen

Secreted peptidases have been extensively studied for their role as virulence factors. Pathogens may deliver peptidases into their host cells to derive nutrients or to modify protein components of the host defense machinery and ultimately suppress defense responses. In this study we combined transcriptomics, comparative genomics and evolutionary analysis to investigate the significance of 39 secreted peptidases in the fungal wheat pathogen *Zymoseptoria tritici* and its close relatives on wild grasses *Z. pseudotritici* and *Z. ardabiliae*. RNA-seq data revealed that a majority of the secreted peptidases displayed differential transcription during the course of *Z. tritici* infection, indicative of life cycle specialization. Genetic analyses detected widespread evidence of diversifying selection acting on most of the secreted peptidases. The aspartic endopeptidases emerged as a particularly interesting group, suggesting a key role in host pathogen co-evolution, host adaptation and pathogenicity. For example, aspartic endopeptidases showed lineage specific rates of molecular evolution, suggesting altered selection pressure in *Z. tritici* after host-specialization on domesticated wheat. Furthermore, sister genes of aspartic peptidases evolve at different rates, possibly as a result of sub-, or neofunctionalization after gene duplications. This detailed study of secreted peptidases in *Zymoseptoria tritici* is a further step in determining suitable targets for controlling this important plant pathogen.

POSTER SESSION ABSTRACTS
Session CS6 Ecological and population genomics
CS6W56

Wednesday 6th April
14:00 - 16:00

CLERGEOT Pierre-Henri (1), BRANDSTRÖM DURLING Mikael (1), ***OLSON Åke*** *(1)*
(1) Dept. of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden

Does genetic distance between nuclei in heterokaryons effect fitness? Study with the root-rot fungus *Heterobasidion parviporum*.

The heterokaryotic (n+n) life form of fungi is characterized by the coexistence of two types of haploid and genetically distinct nuclei in each cell of the same mycelium. The existence of this life form has a number of important functional and evolutionary implications, such as potentially increased adaptability (through the control of the number of each type of nuclei per cell, and through allele-dependent regulation of gene expression), and opportunity for additional genetic variation (through the re-shuffling of nuclei from different heterokaryons). It also challenges the concept of the individualistic mycelium, central to the theory of evolution and population genetics in fungi, by suggesting that suppression of competition among nuclei and trans-acting regulation of gene expression across nuclear compartments might occur in heterokaryons. Tension between conflict and cooperation is a universal feature of genetic interactions at the nuclear level, and fungi have self/non-self recognition systems that mediate reproductive and somatic interactions between conspecifics as well. Physical contact between related individuals is promoted, but controlled by the somatic incompatibility (SI) system, which prevents free exchange of nuclei and cytoplasm between vegetative individuals of the same species in order to maintain their integrity. However the question how genetic distance between conspecifics overall intervenes in this tension between genetic conflict and cooperation has, to our knowledge, never been addressed experimentally. In this study, we have addressed the questions whether, and to which extent, genetic distance between haploid components of heterokaryotic mycelia influences fitness traits in the basidiomycete *Heterobasidion parviporum*. For this purpose, pairwise genetic distances between 32 homokaryotic isolates sampled in Eurasia were compared with growth rates of the heterokaryons obtained by pairing these isolates. Genetic distances were based on single nucleotide polymorphisms called in parallel from the genome sequences of all homokaryotic isolates. Surprisingly no significant correlation between genetic distance and fitness were found.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W1

Wednesday 6th April
14:00 - 16:00

GLASENAPP Anika *(1), MALZ Sascha (1), FRANDSEN Rasmus J. N. (2), SCHÄFER Wilhelm (1)*
(1) University of Hamburg, Hamburg, GERMANY
(2) Technical University of Denmark, Lyngby, Denmark

Secondary Metabolites of *Fusarium graminearum*: Toxic to microbes and effectors in virulence

Fusarium graminearum forms complex infection cushions (IC) to infect its host plant wheat. Secondary metabolite gene clusters are upregulated in IC compared to epiphytically growing hyphae. We evaluated the impact of deoxynivalenol (DON), butenolides (BUT), and aurofusarin (AUR) on the early infection process of this fungus. Aur-deficient mutants show wild type like infection. However, a wild type extract with AUR is able to inhibit bacterial and fungal growth while the Aur-deficient mutant cannot. Therefore AUR may suppress competitors prior to colonization of the host plant. Don-deficient mutants are known to infect the spikelet, but cannot cross the rachis node and fail to colonize it. We show that even the early infection phase following the formation of IC is affected. During the first days of infection, Don-deficient mutants infect slower and with less mycelium compared to the DON producing wild type. DON is an effector molecule in the early infection phase. But-deficient mutants exhibit wild type like virulence. We detected an even increased virulence during the early infection phase compared to the wild type and are currently further evaluating this phenotype. Don/But/Aur triple knock out mutants show a similar reduced virulence as the Don-deficient single mutant. The increased virulence of the But-deficient phenotype cannot compensate for the loss of DON and the reduced virulence.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W2

Wednesday 6th April
14:00 - 16:00

SUN Weiwen *(1), GUO Chun-Jun (1), BRUNO Kenneth (2), WANG Clay (1)*
(1) University of Southern California, Los Angeles, USA
(2) Pacific Northwest National Laboratory, Richland, USA

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

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

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W3

Wednesday 6th April
14:00 - 16:00

HADAR Yitzhak (1), *KNOP Doriv (1), YARDEN Oded (1)*
(1) Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel

Versatile Peroxidase 1 is a key component of the *Pleurotus ostreatus* ligninolytic system under Mn2+ deficiency

The *Pleurotus ostreatus* ligninolytic system is comprised of versatile peroxidases (VPs) and manganese peroxidases (MnPs). Repression of vp1 occurred in Mn2+- sufficient medium, where a number of mnps are induced, and the enzymes react in a Mn2+- mediated mode. Under Mn2+-deficiency, vp1 is the predominantly-expressed gene and its relative abundance is dependent on the age of the culture. In addition, the culture filtrate of a ∆vp1 strain showed negligible values of oxidation of four typical substrates, compared to the wild type. To explore the oxidation mechanisms of aromatic compounds by VP1 and its function under Mn2+- deficiency we purified the native VP1 and characterized the enzyme using Mn2+, Orange II (OII) and Reactive Black 5 (RB5) as substrates. While the pH optimum for Mn2+ oxidation was 5, the optimum pH for direct oxidation of the both dyes was found to be 3. Under the acidic condition Mn2+ also inhibited the direct oxidation of aromatic substances. Moreover, effective in vivo decolorization of these dyes under Mn2+ deficiency occurred only under acidic conditions. We concluded that the direct oxidation of aromatic compounds by P. ostreatus VP1 is controlled by Mn2+ and pH levels both in the growth medium and in the reaction mixture. VP1 activity was detected only extracellularly, while a non-active form of the protein was found in the hyphae. We hypothesize that activation of the enzyme post-translationally is a mechanism that can protect the cell from damage caused by non-specific oxidative activity of VP1.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W4

Wednesday 6th April
14:00 - 16:00

BLACHOWICZ Adriana *(1), KNOX Benjamin P. (3), ROMSDAHL Jillian (1), PALMER Jonathan M. (4), HUTTENLOCHER Anna (3), WANG Clay C. C. (1), KELLER Nancy P. (3), VENAKESWARAN Kasthuri (2)*
(1) Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA; Biotechnology and Planetary Protection Group, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA, Los Angeles, USA
(2) Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI, Madison, USA (3) Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, Los Angeles, USA
(4) Center for Forest Mycology Research, US Forest Service, Madison, WI, Madison, USA

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

Fungi are a great source of natural products that are of use for humankind. A wide range of bioactive compounds produced by fungi is used in pharmaceutical and food industries, agriculture, and beyond. On the other hand, some fungi are plant, animal, and human pathogens. Fungal pathogens may cause serious health complications in immunocompromised human populations. Pathogenic fungi produce a range of secondary metabolites (SMs) that influence their virulence (melanins, siderophores, species-specific toxins) and immunologic potential.*Aspergillus fumigatus* is a saprophytic, filamentous fungus that is ubiquitous outdoors (soil, decaying vegetation) and indoors (hospitals, simulated closed habitats, etc.). *Aspergillus fumigatus* can adapt to various environmental conditions and form airborne conidia that are the inoculum for a variety of diseases (e.g. non- and invasive pulmonary infections, allergic bronchopulmonary aspergillosis, etc.) in immunocompromised hosts.In an on-going Microbial Observatory Experiments on the International Space Station (ISS) molecular phylogeny and radiation resistance of several fungal isolates were characterized. Two strains, ISSF 21 and IF1SW-F4, were isolated from the HEPA filter and the surface of the Cupola of the ISS, respectively. Using primers targeting the internal transcribed spacers ITS1 and 2, both isolates were identified as A. fumigatus. The whole genome sequence analysis of ISSF 21 revealed increased number of single nucleotide polymorphisms (SNPs) when compared to the reference A. fumigatus 293. SM profiles of both ISS isolates were compared to the reference (Af293) on 11 different media but no significant differences were observed. Finally, knowing that A. fumigatus is an opportunistic pathogen and microgravity highly influences the antibiotic susceptibility and pathogenicity of microorganisms, we examined pathogenicity of both ISS isolates using the zebrafish larval model. The ISSF 21 was more virulent than two clinical strains (Af293 and CEA10) the virulence of the second isolate IF1SW-F4 is being tested.Proteomics and transcriptomics of these ISS isolates might reveal the molecular mechanisms of the increased virulence. Subsequently, if the enhanced virulence is attributed to the microgravity, NASA needs to develop countermeasures to protect the astronaut’s health whose immune system is reported to be compromised under microgravity.

Session CS7 Metabolism and physiology
CS7W5

Wednesday 6th April
14:00 - 16:00

KHATEB Aiah *(1), PEREZ Inigo (1), CARR Paul (1), BROMLEY Michael (1), BOWYER Paul (1)*
(1) Manchester Fungal Infection Group, Centre for Respiratory Medicine and Allergy, Institute of Inflammation and Repair, Manchester Academic Health Science Centre, The University of Manchester and University Hospital of South Manchester, NHS Foundation Trust, Manchester, UK

Translational mechanisms of azole resistance in *Aspergillus fumigatus* tRNA-Dihydrouridine synthase (DUS) mediated

Fungal diseases cause approximately 2 million deaths each year. There are few effective antifungal agents, and their widespread use has led to the development of drug resistance increasing the associated mortality and healthcare costs. Azoles are the « gold standard » for treatment of the most common airborne fungal pathogen Aspergillus fumigatus, however azole resistance rates of up to 20% have been reported. Most resistant isolates in the UK carry unknown resistance mechanisms. Moreover, mortality exceeds 85% in individuals who are infected with resistant isolates. This study aims to understand a new mechanism of antifungal drug resistance based on tRNA and tRNA modification enzymes as translation regulatory elements in *A. fumigatus*. We previously identified 8 tRNA genes and several tRNA modification enzymes as well as General Control of amino-acid synthesis 2 (GCN2) and Eukaryotic Initiation Factor 2 (eIF2) in a screen for azole resistance in *A. fumigatus*.An *A. fumigatus* strain with a tRNA modification enzyme (tRNA-dihydrouridine synthase) deletion was generated using A1160 (ΔKu80 pyrG+) as parental strain. Growth rate on solid media containing inhibitory levels of itraconazole (ITZ) and MICs in liquid media (EUCAST protocol) were tested. The strain had a radial growth rate 30% higher than the wild type on 1mg/L ITZ. In addition, the knockout strain demonstrated an MICITZ 4 fold higher than the parental strain. We hypothesise that resistance is linked to altered tRNA regulation in stress response in the *A. fumigatus* mutant. This regulation may influence the translation process in two ways. The first involves alteration of tRNA and tRNA modification enzymes creating the wobble phenomenon, which may cause mis-regulation of genes involved in azole resistance. Alternatively, the tRNA mediates control of the initiation of the translational process via GCN2 and eIF2. Modification of tRNA-dihydrouridine synthase is involved in antifungal resistance mechanism and may provide a new therapeutic or diagnostic target.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W6

Wednesday 6th April
14:00 - 16:00

PROCTOR Robert (1)
(1) National Center for Agricultural Utilization Research, US Department of Agriculture, Peoria, USA
(2) National Academy of Agricultural Science, Rural Development Administration, Wanju, Korea

Dynamic regions within and horizontal transfer of an otherwise stable gene cluster responsible for synthesis of the *Fusarium* mycotoxin fusaric acid

The Fusarium mycotoxin fusaric acid is toxic to plants as well as animals, but its function in the biology of the fungus is not known. Here, we used genome sequencing to survey multiple species in 18 lineages (species complexes) of Fusarium for the presence of the fusaric acid biosynthetic gene (FUB) cluster. We detected the cluster in all members of the closely related *F. babinda*, *F. fujikuroi*, *F. nisikadoi*, *F. oxysporum* and *F. redolens* species complexes, and in the distantly related species *F. aywerte*, but not in other fusaria. Phylogenetic analyses of FUB genes suggest that the presence of the cluster in F. aywerte resulted from horizontal transfer from an unidentified, close relative of the *F. fujikuroi* species complex (FFSC). Two regions within the FUB cluster include insertions of one or more genes that are not considered FUB genes because their presence is variable, they are not co-regulated with FUB genes, and they are absent in some species that produce fusaric acid. Analysis of gene and pseudogene content of these regions indicates that they are highly dynamic with respect to gene acquisition and loss. The largest of the insertions consisted of 21 genes that were likely translocated from another region of the genome. Phylogenetic analysis indicates that the translocation occurred early in the divergence of one FFSC lineage, i.e., the American clade. A second, four-gene insertion occurred later in the divergence of another FFSC lineage (the African clade) and is associated with rearrangement within the FUB cluster. Comparisons of multiple biosynthetic gene clusters in Fusarium and other fungi indicate that regions within clusters that are susceptible to multi-gene insertions are uncommon. The presence of the FUB cluster in all members of five species complexes suggests strong selection to maintain fusaric acid production in certain lineages of Fusarium, which in turn suggests that the mycotoxin has an important biological function in these lineages.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W7

Wednesday 6th April
14:00 - 16:00

OAKLEY Berl *(1), CHIANG Yi-Ming (2), AHUJA Manmeet (1), ENTWISTLE Ruth (1), AKASHI Tomohiro (3), CERQUEIRA Gustavo (4), WORTMAN Jennifer (4), WANG Clay (2), OAKLEY Christine (1)*
(1) University of Kansas, Lawrence/Kansas, USA
(2) University of Southern California, Los Angeles/California, USA
(3) Nagoya University School of Medicine, Nagoya, Japan
(4) Broad Institute of MIT and Harvard, Cambridge/Massachusetts, USA

Identification of a new master regulator of secondary metabolism in *Aspergillus nidulans*

Fungal secondary metabolites (SMs) include many medically useful compounds as well as toxins that contribute to pathogenesis and/or contaminate the food chain. Most fungal SMs are produced by biosynthetic pathways encoded by biosynthetic gene clusters (BGCs) in which the genes are co-regulated. Genome projects have revealed that the number of fungal BGCs far exceeds the number of known SMs, thus revealing that we have not found conditions to activate most fungal BGCs. In order to fully mine fungi for useful SMs and to prevent unwanted production of toxic SMs, it is of importance to understand fungal SM regulation. A great deal has been learned about the laeA, veA, velB SM regulatory network, but many SM BGCs are apparently not regulated by this network and this indicates that there are additional important regulatory systems. We have developed a genetic scheme in *Aspergillus nidulans* that allows us to select for mutations in negative regulators of target BGCs. We have tested the scheme by selecting mutations that upregulate expression of the non-ribosome peptide synthetase required for nidulanin A biosynthesis. Our scheme allowed us to determine rapidly that many of the mutations are trans-acting and likely allelic. Our scheme also allows us to identify the genes in which the mutations occur in a single sequencing run. We identified the relevant mutations in two allelic trans-acting mutants and they were at different positions in the same gene, an uncharacterized transcription factor that we designate mcrA. Deletion of mcrA activates expression of the nidulanin A cluster revealing that mcrA is a transacting negative regulator of the nidulanin A cluster. Metabolite profiles of mcrA deletion and mcrA overexpression strains reveal that mcrA is involved in the regulation of at least 10 SM gene clusters. Initial transcription studies reveal that mcrA is involved in the regulation of transcription of many genes including many secondary metabolite biosynthetic genes. Deletion of mcrA in a genetic dereplication strain we have constructed, has allowed us to discover two novel compounds and an antibiotic not previously shown to be produced by *A. nidulans.* mcrA is conserved in ascomycetes and manipulation of mcrA homologs is likely to be a useful tool in fungal SM discovery.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W8

Wednesday 6th April
14:00 - 16:00

BECCACCIOLI Marzia *(1), GROTTOLI Alessandro (1), MAGALI Delia (1), FANELLI Corrado (1), REVERBERI Massimo (1), LUDOVICI Matteo (1), SCALA Valeria (1)*
(1) Department of Environmental Biology, University of Rome "Sapienza", Rome, Italy

Fatty acid metabolism in Fusarium verticillioides wild type and mutant strains deleted for lds1 oxylipin gene

Fusarium verticillioides is a fungal pathogen of maize producer of fumonisins, secondary metabolites harmful to humans and animals (classified as IARC2B). As shown in previous studies, the production of some mycotoxin (e.g. fumonisins) is related to fatty acids metabolism and oxylipins signaling. Moreover, fungal lipids play a crucial role in regulating the fungal growth and the interaction with the host. Scala et al. in 2014 studied the role of the linoleate diol synthase coding gene, lds1, in F. verticillioides. LDS1 is involved in the synthesis of oxylipin (e.g. 8-HPODE) and its inactivation strongly influenced the interaction with the host, the secondary metabolism and, intriguingly, the polyunsaturated fatty acid (PUFA) amount in the fungal cell.The aim of this work is to study the expression of some genes encoding for enzymes involved in the fatty acid metabolism and involved in the synthesis of oxylipin in F. verticillioides grown under mycotoxins inducing and non-inductive condition. We focused our attention on the expression of different fatty acid desaturases involved in the synthesis of oleic, linoleic and linolenic acid. Gene expression profile was compared with the PUFA content in WT as well as in lds1-deleted strains of *F. verticillioides*. It emerges a close link between fatty acids desaturation and oxylipin synthesis.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W9

Wednesday 6th April
14:00 - 16:00

KARAFFA Levente *(1), KULCSÁR László (1), KOVÁCS Anita (1), KAVALECZ Napsugár (1), FLIPPHI Michel (1), FEKETE Erzsébet (1)*
(1) Department of Biochemical Engineering, University of Debrecen, Debrecen, Hungary

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

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

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W10

Wednesday 6th April
14:00 - 16:00

FREITAG Michael *(1), ADPRESSA Donovon (2), CONNOLLY Lanelle (1), HUSK Jacob (1), SMITH Kristina (1), GAUTSCHI Jeff (2), LOESGEN Sandra (2)*
(1) Dept. of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon, USA
(2) Dept. of Chemistry, Oregon State University, Corvallis, Oregon, USA

From genes to molecules: progress towards assigning natural products to gene clusters

Fungi are widely known as producers of a large variety of natural products, many of which have been developed into pharmaceuticals. Pathogenic fungi on plants, animals and humans use their arsenal of secondary metabolites to aid in infection, establishment and maintenance of disease. Aided by high-quality draft genome sequences, much progress has been made over the past decade to sort specific metabolites to corresponding gene clusters, but in many cases annotated clusters remain transcriptionally silent even when strains are grown on a wide variety of media. In previous work, we found that several Fusarium species maintain a repressive chromatin mark, histone H3 lysine 27 methylation (H3K27me3) on ~33% of their genome. Deleting genes responsible for H3K27 methylation resulted in overexpression of more than 25% of all genes in F. graminearum. This discovery gives us new opportunities to express the "cryptic genome". We have generated additional mutants that are defective in H3K9me3-dependent gene silencing and are analyzing them for secondary metabolite profiles. While some previously undetected metabolites emerge from the mutant, we found that it is not always the end product that is overproduced. In several cases intermediates of well-studied pathways accumulate or new shunts result in accumulation of compounds previously not known from F. graminearum.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W11

Wednesday 6th April
14:00 - 16:00

KEMPKEN Frank *(1), REGULIN Annika (1), KELLER Nancy (2)*
(1) Institute of Botany, Christian-Albrechts-University, Kiel, Germany
(2) Dept Medical Microbiology and Immunology, Dept of Bacteriology, Madison, USA

Survival rates of insects upon competition is dependent on *A. nidulans* ability to produce secondary metabolits

Fungi produce an astonishing variety of secondary metabolites, some of which belong to the most toxic compounds in the living world. It is likely that the fungal ability to regulate secondary metabolism reflects an evolutionary adaptation to ensure efficient exploitation of environmental resources and to synthesize secondary metabolite only when the ecological conditions demand it against natural enemies and competitors (1,2). However, it should be noted that secondary metabolites are not the sole defense mechanisms of fungi (3). In our current research the vinegar fly *Drosophila melanogaster* and its natural antagonist *Aspergillus nidulans* are being used as an ecological model system. Microarrays of *Aspergillus nidulans* have been utilized in order to identify the respective fungal up- or down regulated target genes in the interaction with Drosophila larvae. Quantitative RT-PCR was employed to analyze secondary metabolite gene expression upon confrontation in the veA+ and veA1 background with very different results for both backgrounds. We also tested the survival rate of larvae in the veA+ and veA1 background and in several different secondary metabolite knock-out lines. Survival in the veA1 background is significantly higher than in veA+. RNA-seq data are currently analyzed to identify other up- or down regulated genes.

(1) Rohlfs, M., Albert, M., Keller, N. P., and Kempken, F. (2007) Biol Lett 3, 523-25.
(2) Kempken, F., and Rohlfs, M. (2010) Fungal Ecol 3, 107-14.
(3) Kempken, F. (2011) Mol Ecol 20, 2876-77.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W12

Wednesday 6th April
14:00 - 16:00

WIERCKX Nick *(1), GEISER Elena (1), PRZYBILLA Sandra (1), BÖLKER Michael (2), BLANK Lars (1)*
(1) Institute of Applied Microbiology, RWTH Aachen University, Aachen, GERMANY
(2) Department of Biology. Philipps-University Marburg., Marburg, GERMANY

New insights into the itaconic acid production pathway of *Ustilago maydis*

Itaconic acid is a promising bio-based platform chemical for the production of pharmaceuticals, adhesives and polymers. *Ustilago* is a promising fungal host for the production of itaconic acid. Contrary to established filamentous itaconate producers, *Ustilago* grows unicellulary. This has distinct process advantages relating to morphology control, viscosity and aeration. However, the itaconate production yield, titer and rate of *Ustilago* are thusfar below that of the commercial production hosts. In order to optimize itaconate production in *Ustilago*, detailed biochemical knowledge is needed. An itaconate gene cluster was identified in *Ustilago*. The function of the cluster genes and their encoded proteins was characterized by knockout and overexpression. The activity of two essential catalytic proteins was determined in vivo by permeabilized cell assays and in vitro by E. coli expression and purification. The genes tad1 (trans-aconitate decarboxylase), itp1 (Major Facilitator Superfamily extracellular itaconate transporter), adi1 (aconitate-Δ-isomerase), mtt1 (mitochondrial tricarboxylate transporter), and ria1 (transcriptional itaconate regulator) are involved in the itaconate biosynthesis. In contrast to the known itaconate biosynthesis pathway of Aspergillus terreus, *Ustilago*'s itaconate production proceeds first via an isomerization from cis- to trans-aconitate, followed by decarboxylation of the trans-aconitate (1). Two additional genes in the cluster, cyp3 (cytochrome P450 monooxygenase) and rdo1 (ring-cleaving dioxygenase) are not directly involved in itaconate production. Instead, they appear to have a role in the further conversion of itaconate, likely into the more oxidized products 2-hydroxyparaconate and itatartarate. Although these products have been identified previously (2), the associated genes and pathway was until now unclear.

(1) E. Geiser, S.K. Przybilla, A. Friedrich, W. Buckel, N. Wierckx, L.M. Blank and M. Bölker. (2015) Microb. Biotechnol.
(2) Guevarra, E.D. and Tabuchi, T. (1990) Agric. Biol. Chem. 54:2353

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W13

Wednesday 6th April
14:00 - 16:00

GEISER Elena *(1), MUELLER Markus (3), SCHLEPÜTZ Tino (3), SCHIPPER Kerstin (2), FELDBRÜGGE Michael (2), BÜCHS Jochen (3), WIERCKX Nick (1), BLANK Lars M. (1)*
(1) iAMB – Institute of Applied Microbiology, ABBt – Aachen Biology and Biotechnology, RWTH Aachen University, Aachen, Germany
(2) Heinrich Heine University Düsseldorf, Cluster of Excellence in Plant Sciences, Institute for Microbiology, Düsseldorf, Germany
(3) AVT – Biochemical Engineering Department, ABBt – Aachen Biology and Biotechnology, RWTH Aachen University, Aachen, Germany

Activation of intrinsic enzymes for degradation of plant biomass side-streams

The generation of value-added products by microbial conversion of non-food plant biomass is considered essential to achieve a sustainable bioeconomy and could greatly increase the cost-efficiency of bio-based processes. One strategy is to valorize industrial residuals and side-streams derived from plant biomass. Here, we aim to apply a microbial bioprocess to generate platform chemicals from biomass components. More specifically, pectin-rich biomass side-streams should be degraded to fermentable sugars by enzymatic conversion. Concurrently, these sugars should be converted to the platform chemical itaconate. We will extend our previous research in exploiting *U. maydis* for the degradation of lignocellulosic plant biomass. This microorganism produces a set of hydrolytic enzymes that is required for the infection and colonization of its host plant maize. Besides, this fungus naturally produces value-added platform chemicals, such as itaconic acid or biosurfactants. We were successful in generating *U. maydis* strains in which the expression of specific intrinsic CAZymes (carbohydrate activating enzymes) was synthetically activated using constitutive promoters. This way the conversion of xylan, cellobiose and carboxymethylcellulose to fermentable sugars could already be shown, enabling the modified organisms to produce itaconic and malic acid from these complex bio-based substrates. In order to enhance the efficiency and to be able to switch to more complex substrates, such as pectin-rich sugar beet pulp, further improvements are necessary. This comprises the activation of intrinsic pectinolytic enzymes, the deregulation and increase of itaconate production and in parallel, the establishment of a cultivation methodology to enable growth on viscous and complex substrates and subsequent up-scaling to the fermenter level. With the itaconate production from pectin-rich industrial waste, we aim a consolidated process using the plant pathogenic fungus U. maydis as a whole-cell biocatalyst.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W14

Wednesday 6th April
14:00 - 16:00

FRANCK Panabieres *(1), KUHN Marie-Line (1), LE BERRE Jo-Yanne (1)*
(1) INRA, Univ. Nice Sophia Antipolis, CNRS, UMR 1355-7254 Institut Sophia Agrobiotech, 06900, Sophia Antipolis, FRANCE

The organization and evolution of glycolysis among oomycetes: duplications and signatures of functional diversification within *Phytophthora* spp.

Oomycetes are part of the Stramenopiles and the controversial supergroup Chromalveolates. They include the genus *Phytophthora* which is considered one of the most destructive plant pathogens responsible of huge losses in agriculture and environment worldwide. The genus *Phytophthora* encompasses > 130 species that greatly vary in ecology, biology, pathogenicity and host range. *Phytophthora spp.* are hemibiotrophic pathogens: they first establish an intimate contact with living host cells before inducing host cell death and development in dead tissues. To ensure plant invasion, the pathogen needs to adapt to the nutrient availability in the host, and to nutritional changes occurring during the course of infection. In addition, *Phytophthora spp.* can be easily cultured on a range of artificial media. It implies robust adaptive mechanisms to optimize nutrient acquisition from either living or dead plant cells, as well as sophisticated metabolic adjustments to fulfill the nutritional requirements in a wide range of environmental conditions. We intended to investigate the molecular bases of adaptation of *Phytophthora spp*. to nutrient availability with an initial analysis of genes associated to glycolysis. Exploration of genome sequences and comparative genomics revealed that *Phytophthora*, and to a wider extent pathogenic oomycetes, possess an alternative, glycolytic pathway similar to that encountered in several amitochondriate, parasitic protists. Nearly all steps are committed by enzymes encoded by multigene families. Sequence divergence led to a possible diversification of subcellular location of these enzymes. In addition, alternate, redundant enzymatic pathways were encountered in *Phytophthora spp.* We further focused our analysis to *P. parasitica*, a soilborne pathogen able to infect hundreds of woody and herbaceous plants, and to survive in a variety of environmental conditions. Biochemical and transcriptome analyses reveal possible cases of functional divergence in multigene families associated to glycolysis in this species. Together, our results suggest that the combination of gene duplication followed by sequence divergence, diversification of expression patterns and possible acquisition of novel functions may shape the metabolic flexibility of *Phytopthora spp.* and participate to the adaptation to their environment.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W15

Wednesday 6th April
14:00 - 16:00

SCHÄFER Wilhelm *(1), BÖNNIGHAUSEN Jakob (1), GEBHARD Daniel (2), KRÖGER Cathrin (1), HADELER Birgit (1), LIEBEREI Reinhard (3), TUMFORDE Thomas (3), BERGEMANN Jörg (2), BORMANN Jörg (1)*
(1) University of Hamburg, Biocenter Klein Flottbek, Department of Molecular Phytopathology and Genetics, Hamburg, Germany
(2) Albstadt-Sigmaringen University of Applied Sciences, Department of Life Sciences, Sigmaringen, Germany
(3) University of Hamburg, Biocenter Klein Flottbek, Department of Applied Plant Ecology and Biodiversity of Useful Plants, Hamburg, Germany

Disruption of the GABA shunt affects mitochondrial respiration and virulence in the cereal pathogen *Fusarium graminearum*

The cereal pathogen *Fusarium graminearum* threatens food and feed production worldwide. It reduces the yield and poisons the remaining kernels with mycotoxins, notably deoxynivalenol (DON). We analyzed the importance of gamma-aminobutanoic acid (GABA) metabolism for the life cycle of this fungal pathogen. GABA metabolism in *F. graminearum* is partially regulated by the global nitrogen regulator AreA. Genetic disruption of the GABA shunt renders the pathogen unable to utilize the plant stress metabolites GABA and putrescine and let to the identification of two independent DON induction pathways. The mutants showed increased sensitivity against oxidative stress, GABA accumulation in the mycelium, down regulation of two key enzymes of the TCA cycle, disturbed potential gradient in the mitochondrial membrane, and lower mitochondrial oxygen consumption. In contrast, addition of GABA to the wild type resulted in its rapid turnover and increased mitochondrial steady state oxygen consumption. GABA concentrations are highly upregulated in infected wheat tissues. We conclude that GABA is metabolized by the pathogen during infection increasing its energy production, whereas the mutants accumulate GABA intracellularly resulting in decreased energy production. Consequently, the GABA mutants are strongly reduced in virulence but, because of their DON production, are able to cross the rachis node.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W16

Wednesday 6th April
14:00 - 16:00

MARMEISSE Roland (1), *BARBI Florian (1), VALLON Laurent (1), FRAISSINET-TACHET Laurence (1), , LUIS Patricia (1)*
(1) CNRS-Université Lyon 1, Ecologie Microbienne, Villeurbanne, France

Exploring the functional diversity of sugar transporters in active soil fungal communities

«Sugars» (mono-, di-, oligosaccharides) certainly represent the main primary carbon and energy sources for fungi in the environment. In soils, most sugars originate from the enzymatic hydrolysis of plant-derived polysaccharides such as cellulose, hemicelluloses or starch composed of hexoses (essentially D-glucose, D-mannose and D-galactose) and pentoses (D-xylose and L-arabinose) linked together by different types of chemical bonds. Prior to entering specific catabolic pathways, soluble sugars cross the plasma-membrane using sugar porters whose substrate range and specificity certainly contribute to fungal metabolic versatility. The sugar porter (SP) family is highly diversified with frequently more than 10-40 gene copies present in a single fungal genome. Despite the essential role played by this protein family in cell metabolism, with very few exceptions (e.g. *S. cerevisiae*), no comprehensive analysis of this family has been reported in terms of evolution of substrate range and specificities. To address the complexity of this transporter family we implemented an environmental genomics approach based on the functional complementation of sugar transport-deficient *S. cerevisiae* mutants by cDNA libraries prepared from soil-extracted poly-A mRNA. These soil cDNA libraries are not only likely to contain sugar porters originating from numerous, phylogenetically different, fungal species but we also hypothesized that they may contain sugar porters of different specificities owing to the diversity and complexity of sugar sources present in the studied forest soils. Following this approach using libraries from Beech (*Fagus sylvatica*, a «xylan-rich, mannan-poor» tree species) and Spruce (*Picea abies*, «xylan-poor, mannan-rich») forest soil cDNA libraries, we selected phylogenetically distinct SPs with different specificities for monosaccharides, including putative mannose-specific ones not reported previously.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W17

Wednesday 6th April
14:00 - 16:00

PRIGENT Sylvain *(1), NIELSEN Jens Christian (1), NIELSEN Jens (1)*
(1) Department of Biology and Biological Engineering, Chalmers University of Technology, Göteborg, Sweden

Metabolic reconstructions of *Penicillium* species with emphasis on secondary metabolism

Since a lot of years antibiotic discovery is declining while antibiotic resistance increase a lot, leading to a major crisis of antibiotic resistance. This pose a major threat to the public health and efforts into research for novel antibiotics should be renew. It has been previously shown that filamentous fungi produce natural products with strong bioactivity. The most known of those products is probably the penicillin produced by *Penicillium chrysogenum*. An emphasis on seconday metabolites production by Penicillium species could them enable the discovery of new antibiotics or new way to produce them in a large scale. We have sequenced the genome of several *Penicillium* species. The obtained sequences coupled with metabolomic studies will enable us to identify gene clusters responsible for the production of interesting secondary metabolites. Nevertheless knowing which species possess the required gene cluster to produce an interesting biochemical compound is not enough. Quite often filamentus fungis only express interesting gene clusters under specific environments. By reconstructing the metabolic networks of interesting *Penicillium* species we aim at having a better understanding of the global metabolism of filamentous fungi with a particular emphasis on secondary metabolism. We will reconstruct around 20 metabolic networks of *Penicillium* species based on newly sequenced genomes and previously published ones. The metabolic networks reconstructions will be based on the previously reconstructed networks of *Penicillium chrysogenum*, *Aspergillus niger* and *Aspergillus nidulans*. New genomic annotations will also be used to identify the presence of reactions in the metabolic networks. A manual curation will finally be done on the pathways responsible for newly discovered secondary metabolites biosynthesis. Once the reconstruction of the metabolic networks will be done, we will be able to run simulations to predict genomic changes that should be done to improve the production of secondary metabolites. We will for example be able to predict gene knock out that enable redirection of molecule fluxes threw the production of interesting compounds while maintaining enough growth. These metabolic models could also be used in order to predict potential gene transfer from one species to another and thus improve the production of the interesting compounds.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W18

Wednesday 6th April
14:00 - 16:00

LUNDELL Taina *(1), VIROLAINEN Tuulia (1), KUUSKERI Jaana (1), MATTILA Hans (1)*
(1) University of Helsinki, Helsinki, Finland

Saprotrophic fungal interactions and their effect on wood-decay enzyme activities

Fungal communities are in a constant change in nature, and different fungal species and isolates may be strong or weak competitors, or even strong combatants upon interactions. The influence of *Fomitopsis pinicola*, a common brown-rot polypore species, on the growth and enzyme production of five wood-decaying white-rot species of *Polyporales Basidiomycota* was studied. Activities of CAZy enzymes (laccase, manganese peroxidase, xylanase, beta-glucosidase) were studied for individual species, and in interactive co-cultures of various combinations of the species on coniferous wood-supplemented defined medium. Hyphal extension rate as a measure of fungal growth was quantified on malt-extract agar for each species and their combinations. *F. pinicola* proved to be a supreme colonizer on agar and its hyphae were advancing over the mycelia of the white-rot species studied. *Phlebia radiata* formed mycelial blocks against the other white-rot fungi but not against F. pinicola. In the wood cultures and most cases, acidification of the liquid medium -; due to fungal production of organic acids - was in correlation to the increase of fungal biomass. Number of fungal species had no clear effect on the production of enzymes in the co-cultivations, yet *P. radiata* caused an increase in the oxidoreductase activities. In conclusion, a few key species in the Polyporales co-cultivations on coniferous wood had an impact on fungal interactive growth and increment of production of wood-decay enzymes.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W19

Wednesday 6th April
14:00 - 16:00

BENOIT GELBER Isabelle *(1), ZHOU Miaomiao (1), VIVAS DUARTE Alexandra (1), DOWNES Damien J. (2), TODD Richard B. (2), POST Harm (3), HECK Albert J. R. (3), ALTELAAR A. F. Maarten (3), DE VRIES Ronald P. (1)*
(1) Fungal Physiology CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands
(2) Department of Plant Pathology, Kansas State University, Manhattan, KS , USA
(3) Biomolecular Mass Spectrometry and Proteomics, Utrecht University, Utrecht, Netherlands

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

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

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W20

Wednesday 6th April
14:00 - 16:00

DERRNTL Christian *(1), KLUGER Bernhard (2), BÜSCHL Christoph (2), SCHUHMACHER Rainer (2), MACH Robert (1), MACH-AIGNER Astrid (1)*
(1) TU Wien, Institute of Chemical Engineering, Department for Biotechnology and Microbiology, Vienna, Austria
(2) University of Natural Resources and Life Sciences, Department of Agrobiotechnology, Center for Analytical Chemistry, Tulln an der Donau, Austria

The transcription factor Xpp1 is a repressor of the fungal secondary metabolism

Fungi produce a vast number of different chemical compounds via their secondary metabolism that are of great interest due to their applicability in medicine, pharmacy, and biotechnology. However, under standard cultivation conditions the fungal secondary metabolism remains widely inactive. This is an obstacle for the production of known metabolites and discovery of new secondary metabolites (SM). Here we report on the regulatory role of the transcription factor Xpp1 on the secondary metabolism. Xpp1 was previously described as a repressor of xylanases expression in *Trichoderma reesei*. However, orthologues of Xpp1 are found in a broad range of other fungi including the biocontrol agent *T. atroviride*, the plant pathogen *Fusarium graminearum*, the model organism *Neurospora crassa*, and the ergot fungus *Claviceps purpurea*. We observed an earlier and stronger secretion of a typical yellow pigment by *T. reesei* in the absence of Xpp1. RNAseq analysis demonstrated that the secondary metabolism is upregulated in the absence of Xpp1, which was supported by expression analyses of genes encoding for enzymes involved in SM. An untargeted metabolomics approach showed that the deletion of Xpp1 led to an enhanced secretion of SM concerning quantity and types. These findings lay the foundation for the discovery of novel SM and suggest a strategy for fungi that are (in)famous for their SM.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W21

Wednesday 6th April
14:00 - 16:00

HUANG Ying *(1), YAN Jianhua (1), WAN Yirong (1), ZHANG Qingzhen (1), MA Xuting (1), ZHANG Juan (1)*
(1) Jiangsu Key Laboratory for Microbes and Genomics, School of Life Sciences, Nanjing Normal University, Nanjing, China

The *Schizosaccharomyces* pombe PPR protein PprX interacts with a novel putative PPR protein Mpa1 and plays a general role in mitochondrial translation

The pentatricopeptide repeat (PPR) proteins characterized by tandem repeats of a degenerate 35-amino-acid motif play critical roles in all aspects of organellar RNA metabolism. Here we report the characterization of two putative fission yeast *Schizosaccharomyces pombe* PPR proteins PprX and Mpa1. The pprX deletion mutant exhibits growth defects in respiratory media, and is dramatically impaired for viability during the late-stationary phase. The predicted PPR motifs in PprX are required for respiratory growth of *S. pombe*. Deletion of pprX does not seem to significantly affect steady-state levels of mtDNA-encoded transcripts, but severely impairs mitochondrial protein synthesis. PprX associates with a novel putative PPR-motif-containing protein that we called Mpa1 in vivo and directly interacts with it in vitro. PprX colocalizes with Mpa1 in the mitochondrial matrix. One of Mpa1's functions is to maintain the normal protein levels of PprX, most likely by preventing it from degradation. Consistent with this, we observed that the phenotypes of the mpa1 deletion mutant were very similar to those of the pprX deletion mutant. These findings demonstrate that PprX functions as a general translational activator for mitochondrial translation. Our study also reveals that the function of PprX requires its association with Mpa1.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W22

Wednesday 6th April
14:00 - 16:00

KIM Yangseon *(1), LEE Hyunjung (1), BANG Wooyoung (1), YEO Joo-Hong (1), KIM Soonok (1)*
(1) National Institute of Biological Resources, Incheon, South Korea

Antimicrobial effect of plant endophytic fungi isolated from *Morus alba L*.

Endophytic fungi are microorganisms inhabiting living plant tissues without causing apparent symptoms on the host. They are drawing increasing attention due to their ability to produce various bioactive compounds as well as their effects on host growth and resistance to biotic and abiotic stresses. In this study, antimicrobial effects of endophytic fungi isolated from *Morus alba L.* were evaluated. The crude ethyl acetate extract of five strains showed antimicrobial activity in a dose dependent manner against human pathogenic microorganisms including *Candida albicans, Candida glabrata, Cryptococcus neoformans, Staphylococcus aureus, and Streptococcus mutans*. The effects were dose dependent and each fungal strain showed specific activity against different pathogens. These strains are identified as *Fusarium solani* (JS-169, JS-170, JS-171) and *Colletotrichum sp.* (JS-361, JS-367) by ITS sequencing. These data will serve as a valuable resources to develop novel antifungal or antibiotic materials.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W23

Wednesday 6th April
14:00 - 16:00

COTON Monika *(1), GILLOT Guillaume (1), JANY Jean-Luc (1), POIRIER Elisabeth (1), SANTOS Rebecca (2), HIDALGO Pedro (1), ULLAN Ricardo (2), COTON Emmanuel (1)*
(1) Université de Brest, EA 3882 Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, Plouzané, France
(2) Instituto de Biotecnología de León (INBIOTEC), Léon, Spain

Genetic basis for mycophenolic acid production and strain-dependent production variability in Penicillium roqueforti

Mycophenolic acid (MPA) is a well-known mycotoxin produced by various Penicillium species including *Penicillium roqueforti*. The MPA biosynthetic pathway gene cluster was recently described in *Penicillium brevicompactum*. In the present study, a 23.5-kb putative MPA cluster was localized for the first time in the recently available *P. roqueforti* FM164 genome sequence via an in silico analysis. The cluster is composed of seven genes putatively coding for seven proteins (MpaA, MpaB, MpaC, MpaDE, MpaF, MpaG, MpaH) and is highly similar in terms of gene synteny and sequence homology to the cluster described in *P. brevicompactum.* In order to confirm the involvement of this gene cluster in MPA biosynthesis, a gene silencing approach, using RNA interference targeting mpaC (coding for a putative polyketide synthase), was performed in a high MPA-producing *P. roqueforti* strain (F43-1). Transformants exhibited contrasted mpaC gene expression and MPA production. In parallel, mycotoxin quantification on multiple *P. roqueforti* strains in our collection suggested strain-dependent MPA-production. The entire MPA gene cluster was therefore sequenced for *P. roqueforti* strains with contrasted MPA production and a 174 bp deletion in mpaC was observed in low MPA-producers. PCRs targeting the identified deleted region were carried out on 55 strains and showed an excellent correlation with MPA quantification indicating the clear involvement of mpaC gene in MPA biosynthesis as well as the full functionality of the 7-gene MPA biosynthesis cluster in *P. roqueforti*. The developed molecular tools could be useful for *P. roqueforti* ripening culture selection.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W24

Wednesday 6th April
14:00 - 16:00

BERRY Daniel *(1), GRAGE Katrin (1), MACE Wade (2), YOUNG Carolyn (3), SCHARDL Chris (4), DIJKWEL Paul (1), SCOTT Barry (1)*
(1) Massey Univeristy, Palmerston North, New Zealand
(2) AgResearch, Palmerston North, New Zealand
(3) The Samuel Roberts Noble Foundation, Ardmore, OK, USA
(4) University of Kentucky, Lexington, KY, USA

Evolution at work: transposon-driven development of a novel NRPS

Peramine synthetase (PerA), encoded by the gene perA, is a two-module non-ribosomal peptide synthetase (NRPS) responsible for the production of the di-peptide peramine. To date, perA has been found exclusively within the Epichloë grass endophytes, a symbiosis for which peramine provides deterrence against insect herbivory. The perA gene is widespread throughout Epichloë, but due to a number of independent mutations, the ability of any given strain to produce peramine is much more discontinuous. The mutations responsible for these peramine-negative alleles are almost always confined to a closely related group of isolates within a single Epichloë spp. The sole exception is the perA-ΔR allele, which is widely distributed across Epichloë in a classic example of trans-species polymorphism. The 3" region of perA that encodes the reductase (R) domain, usually responsible for product release and cyclisation, has been replaced in perA-ΔR via integration of a miniature inverted-repeat transposable element. Through generation and analysis of a number of perA, perA-ΔR and hybrid perA / perA-ΔR expression constructs using the heterologous host Penicillium paxilli we show that perA-ΔR-unique mutations altering predicted substrate binding residues have changed the substrate specificity of the first module of PerA-ΔR relative to normal PerA. Furthermore, we detail the impact that perA-ΔR-unique mutations have had on the function of each of the other domains within this NRPS, and provide insight into the release mechanism utilised by PerA-ΔR in the absence of a C-terminal R-domain. Finally, we demonstrate that many modern perA-ΔR alleles are the result of recombination between an ancestral perA-ΔR allele and a canonical perA allele, perhaps as a repair mechanism for a perA-ΔR null allele in response to changing environmental pressures.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W25

Wednesday 6th April
14:00 - 16:00

DALLERY Jean-Felix *(1), ADELIN Emilie (2), PIGNE Sandrine (1), LESPINET Olivier (3), OUAZZANI Jamal (2), KOMBRINK Erich (4), LEBRUN Marc-Henri (1), O'CONNELL Richard (1)*
(1) BIOGER, INRA, AgroParisTech, Thiverval-Grignon, France
(2) Institut de Chimie des Substances Naturelles, CNRS, Gif-sur-Yvette, France
(3) Institut de Génétique et Microbiologie, CNRS, Université Paris Sud, Orsay, France
(4) Chemical Biology Laboratory, Max Planck Institute for Plant Breeding Research, Cologne, Germany

Regulation of secondary metabolism in the anthracnose fungus *Colletotrichum higginsianum*

Species of the genus *Colletotrichum* cause devastating anthracnose or blight diseases on numerous crop plants worldwide. *C. higginsianum* uses a hemibiotrophic strategy to infect Arabidopsis and other Brassicaceae. Its genome contains a large number of genes (87) encoding secondary metabolism (SM) key enzymes. As in other fungi, these key genes are organized into clusters that may also contain genes encoding accessory enzymes of the same biosynthetic pathway, efflux transporters and pathway-specific transcription factors. A remarkable finding from RNA-Seq transcriptome profiling was that 27 SM clusters are specifically expressed only in planta by appressoria and/or biotrophic hyphae. Since each cluster potentially synthesizes one final metabolite, this suggests appressoria and biotrophic hyphae deliver a cocktail of different metabolites to the first infected host cell. To identify and characterise these metabolites we need to mass-produce them from in vitro cultures. We therefore deleted histone-modifying enzymes controlling chromatin status and over-expressed global transcriptional regulators (e.g. Dim5, Hp1, Kmt6, CclA, Sge1). This lead to the discovery of metabolites which are not produced by wild type mycelia in vitro. Bioassays are ongoing to evaluate their biological activities against plants, bacteria and fungi.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W26

Wednesday 6th April
14:00 - 16:00

PRZYBILLA Sandra *(1), ZAMBANINI Thiemo (1), STOCKDREHER Yvonne (2), GEISER Elena (1), MEYER Andreas J. (2), BLANK Lars M. (1), SCHWARZLÄNDER Markus (2), WIERCKX Nick (1)*
(1) RWTH Aachen University, Institute of Applied Microbiology, Aachen, Germany
(2) University of Bonn, INRES - Chemical Signalling, Bonn, Germany

Novel expression tools for *Ustilago maydis* derived from the itaconic acid gene cluster

Itaconic acid is a dicarboxylic acid widely used for the production of plastics, paints and cosmetics. Itaconic acid production has also been observed in the basidiomycetous fungus *Ustilago maydis*. Previous cultivation studies showed a clear separation between a growth phase and an itaconate production phase, which is induced upon nitrogen limitation. In addition, from the stoichiometry of the metabolic pathway from glucose to itaconate a surplus production of NADH can be expected. However, in itaconate producing fungi, high NADH/NAD ratios inhibit central metabolic enzymes, thereby lowering production.Here we report the detailed characterization of the promoter activities of the *U. maydis* itaconic acid gene cluster, which have recently been shown to be activated under nitrogen limiting conditions. With this study we were able to establish two promoters exhibiting different expression levels during itaconate production phase and therefore suitable for the expression of genes targeting metabolic redox balance in *U. maydis*. Furthermore we were able to determine the exact point of induction of these promoters. Existing promoter systems, such as the inducible nar1 and crg1 promoters, cannot be used to express genes during itaconate production because of their interference with the fundamental metabolic and/or regulatory basis of itaconic acid production. The findings of our study were subsequently applied to express the water-forming NADH-oxidase NOX for the tuning of NADH/NAD balance, aiming towards an increased itaconate production rate of U. maydis. Therefore, these inducible promoter systems represent new genetic tools for timed gene expression during itaconate production, allowing us to express genes that target metabolic redox balance without disturbing growth phase.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W27

Wednesday 6th April
14:00 - 16:00

COTON Monika (1), *HIDALGO Pedro (1), POIRIER Elisabeth (1), VICENTE ULLAN Ricardo (2), MESLET-CLADIÈRE Laurence (1), COTON Emmanuel (1)*
(1) Université de Brest, EA 3882 Laboratoire Universitaire de Biodiversité et d'Ecologie Microbienne, Plouzané, France (2) INBIOTEC, Instituto de Biotecnología de León, Léon, Spain

PR-toxin gene cluster determination in *Penicillium roqueforti*

Penicillium roqueforti is a well-known filamentous fungi found as contaminant in various products, especially in silage, or used for its technological abilities for blue-veined cheese ripening worldwide. In the latter context, this species gives the typical organoleptic properties and characteristic colour to blue cheeses. On the other hand, *P. roqueforti* can potentially produce secondary metabolites (mycotoxins) including PR-toxin. However, in the cheese context, this mycotoxin is not an issue as it is degraded. To date, the PR-toxin pathway has not yet been completely elucidated although a 4-gene cluster including the ari1 gene encoding an aristolochene synthase (TPS family) from *P. roqueforti* was recently characterized and gene silencing resulted in a drastic reduction in PR-toxin production (Hildago et al., 2014). In this study, an in silico analysis of the *P. roqueforti* FM164 genome was carried out and the potential biosynthetic PR-toxin cluster was fully determined. This cluster contains 11 opening reading frames spanning an approximately 30 kb region. Three genes, potentially encoding for an acetyltransferase and two P450 monooxygenases, were selected for gene silencing studies. Gene silencing was carried out using the pJL43RNAi plasmid that relies on the expression of an exonic gene fragment by two convergent promoters (namely gpdA and pcbC) that produces dsRNA molecules. Several transformants obtained for each target gene were analyzed to confirm proper integration of the silencing cassette in the *P. roqueforti* ATCC48936 genome by PCR and Southern blot experiments. Positive *P. roqueforti* transformants were investigated by LC-Q-ToF to determine the actual involvement of each gene in PR-toxin production. Results showed consistent reductions in PR-toxin production as well as eremefortins A and B (pathway intermediates) in comparison to the parental strain thus allowing us to propose a detailed and complete PR-toxin biosynthesis pathway. Further investigations would be of interest to better understand the impact of abiotic factors on both the growth and metabolite expression by the *P. roqueforti* species.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W28

Wednesday 6th April
14:00 - 16:00

MARCONI Marco *(1), SESMA Ane (1), WILKINSON Mark (1)*
(1) Centro de Biotecnología y Genómica de Plantas (CBGP)/Universidad Politécnica de Madrid, Madrid, Spain

Evolutionary patterns of protein complexes involved in mRNA metabolism in the fungal kingdom

Multiple levels of regulation tightly control and coordinate eukaryotic gene expression. Studies on yeast and filamentous fungi have revealed that some of the cellular processes that regulate gene expression are not present or simply have evolved in a different way in these organisms compared to other eukaryotes. Consequently, regardless the onset of events that initiate the flow of genetic information are highly conserved, i.e. transcription, splicing, polyadenylation and translation, there is room for a wide diversity of proteins and regulatory mechanisms that fine-tune gene expression in eukaryotic cells. We performed a bioinformatic analysis and characterized the evolutionary history of the major protein complexes that participate in fungal mRNA metabolism. For this purpose, we analysed the protein repertoire of more than 300 proteins that participate in mRNA metabolism i.e. transcription, polyadenylation, splicing and translation at the major branches of the fungal kingdom. Interestingly, we discovered variable conservation criteria based on the phylogenetic classification of fungi. In some cases, the protein architecture indicated a species-specific conservation of certain domains. Differences were also found at the genome and transcriptome levels regarding introns and UTRs architecture, polyadenylation site selection and protein-binding motifs. Our results suggest that the knowledge of these processes acquired from model species such as yeasts and human alone is not sufficient to fully represent the outstanding diversity of the fungal kingdom.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W29

Wednesday 6th April
14:00 - 16:00

NIEHAUS Eva-Maria *(1), STUDT Lena (1), VON BARGEN Katharina (2), RÖSLER Sarah (1), KUMMER Wiebke (3), REUTER Gunter (3), TUDZYNSKI Bettina (1)*
(1) Institut für Biologie und Biotechnologie der Pflanzen, Westfälische Wilhelms-Universität Münster, Münster, Germany (2) Institut für Lebensmittelchemie, Westfälische Wilhelms-Universität Münster, Münster, Germany (3) Institut für Genetik, Martin Luther Universität Halle-Wittenberg, Halle, Germany

Activation of the beauvericin cluster through deletion of the histone deacetylase Ffhda1 in *Fusarium fujikuroi*

Recent genome sequencing of the rice pathogen *Fusarium fujikuroi* revealed the presence of 45 putative secondary metabolite (SM) gene clusters of which most are cryptic and not expressed under laboratory conditions. Gene regulation by chromatin modifications is a promising mechanism to activate those silent fungal SM gene clusters and to identify their products. Previously, we have shown that several of the SM clusters with known products are controlled by the histone deacetylases Hda1 and Hda2. In this work, we detected a strong new signal in the HDA1 deletion mutant using high performance liquid chromatography coupled to a diode array detector (HPLC-DAD). Further analyses identified this unknown compound as the cyclic peptide beauvericin (BEA). The respective non-ribosomal peptide synthetase (NRPS) gene and adjacent genes were significant up-regulated and the BEA yield has increased 1000-fold in the ΔHDA1 mutant compared to the wild type. Furthermore, we discovered the first fungal homolog of a global mammalian transcription factor which acts as repressor of BEA biosynthesis. Mutation of its proposed DNA-binding motif resulted in significant elevation of product yields. In addition, the BEA cluster is enriched of trimethylated histone H3 lysine 27 (H3K27me3) modification and seems to be one of the reasons for gene silencing of this cluster.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W30

Wednesday 6th April
14:00 - 16:00

GSALLER Fabio *(1), HORTSCHANSKY Peter (2), FURUKAWA Takanori (1), CAPILLA Javier (3), MUELLER Christoph (4), BRACHER Franz (4), BOWYER Paul (1), HAAS Hubertus (5), BRAKHAGE Axel A (2), BROMLEY Michael J (1)*
(1) Manchester Fungal Infection Group, Institute of In[lammation and Repair, University of Manchester, Manchester, UK (2) Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany
(3) Microbiology Unit, Medical School, Universitat Rovira i Virgili, Reus, Spain
(4) Department of Pharmacy, Center for Drug Research, Ludwig-Maximilians University of Munich, Munich, Germany
(5) Division of Molecular Biology, Biocentre, Innsbruck Medical University, Innsbruck, Austria

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

Antifungal azole drugs have been the cornerstone of systemic antifungal therapy for the last 30 years. However, resistance to the azoles, particularly in the major human mould pathogen *Aspergillus fumigatus*, is emerging and reaching levels that have prompted some centres to move away from azoles as a sole first line therapeutic. One particular family of pan-azole resistant isolates dominates. Strains with TR34/L98H, a combination of a 34 base pair tandem repeat in the cyp51A 5" non-translated region with a mutation of the coding sequence resulting in the amino acid alteration L98H, have been found globally. Isolates harboring the TR34 mutant allele display increased cyp51A expression levels however, the precise mechanism underlying upregulation of the gene remained unclear. Two transcriptional regulators have been associated with modified azole tolerance in A. fumigatus, the sterol regulatory element SrbA, and the CCAAT binding complex (CBC). SrbA acts a positive regulator of ergosterol biosynthesis and promotes azole tolerance. Hence loss of SrbA activity results in an increase in azole susceptibility. Modification of the HapE subunit of the CBC (to HapEP88L) has been shown promote azole resistance. Here we demonstrate that the 34 mer duplicated in TR34 is bound by both SrbA and the CBC. We show that CBC acts complementary to SrbA as a negative regulator of ergosterol biosynthesis and show that lack of CBC activity results in increased sterol levels via transcriptional derepression of multiple ergosterol biosynthetic genes including those coding for HMG-CoA-synthase, HMG-CoA-reductase and sterol 14-alpha demethylase. We reveal that the P88L substitution within HapE significantly impairs the binding affinity of the CBC to its target site resulting in derepression of cyp51A and other genes in the ergosterol biosynthetic pathway. We identify that the mechanism underpinning TR34 driven overexpression of cyp51A results from duplication of SrbA but not CBC binding sites and show that deletion of the 34 mer results in lack of cyp51A expression and increased azole susceptibility similar to a cyp51A null mutant. Finally we show that strains lacking a functional CBC are severely attenuated for pathogenicity in a pulmonary systemic model of aspergillosis indicating that, although null mutants are resistant to azoles, they would be unlikely to be able to sustain an infection.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W31

Wednesday 6th April
14:00 - 16:00

KHOSRAVI Claire *(1), DALHUIJSEN Sacha (1), AGUILAR-PONTES Maria-Victoria (1), ZHOU Miaomiao (1), HEYMAN Heino (2), KIM Young-Mo (2), BATTAGLIA Evy (1), DE VRIES Ronald (1)*
(1) CBS KNAW Fungal Biodiversity Centre, Utrecht, Netherlands
(2) Pacific Northwest National Laboratory, Richland,WA, USA

Re-routing central metabolism in *Aspergillus nidulans*

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

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W32

Wednesday 6th April
14:00 - 16:00

KUMAR Anil *(1), GHOSH Sumit (2), BHATT Dharmendra Nath (1), NARULA Alka (3), DATTA Asis (1)*
(1) National Institute of Plant Genome Research, New Delhi, India
(2) Central Institute of Medicinal and Aromatic Plants, Lucknow, India
(3) Hamdard University, New Delhi, India

Magnaporthe oryzae aminosugar metabolism is essential for successful host colonization

Pathogens encounter and metabolize a range of host-derived metabolites while proliferating inside the host. Therefore, understanding of pathogen's metabolic pathways operational during in planta colonization is of great importance for developing disease control strategies. However, our existing knowledge on nature of these metabolites and metabolic processes has remained largely incomplete. Here, we investigated functional role of the aminosugar N-acetylglucosamine (GlcNAc) catabolic pathway of the rice blast fungus *Magnaporthe oryzae* during host colonization. The GlcNAc catabolic pathway is composed of GlcNAc transporter (MoNgt1), GlcNAc kinase(s), GlcNAc-6-phosphate deacetylase (MoDac) and GlcN-6-phosphate deaminase (MoDeam). By developing and characterizing *M. oryzae* knockouts for MoNgt1, MoDac and MoDeam genes, we have shown that impaired GlcNAc catabolism significantly reduce its virulence towards the susceptible rice cultivar due to the failure to develop invasive hyphal growth within the host tissue. Interestingly, under oxidative stress, wild type strain proliferated efficiently in GlcNAc-containing media, compared to other sugars and transcript expression of the antioxidant genes such as superoxide dismutase and catalases was induced following GlcNAc treatment; suggesting a beneficial effect of the GlcNAc to overcome oxidative stress. However, to our surprise, GlcNAc inhibited growth of the Δmodac and Δmodeam mutants and the growth inhibitory effect was further pronounced under oxidative stress. Thus, reduced virulence of the mutants could be due to its inability to counteract the host defence response marked by ROS (Reactive Oxygen Species) burst; this was further validated by our result that these mutants were unable to neutralize ROS at the infection sites. These results suggest that GlcNAc helps fungus to overcome oxidative stress inside the host, perhaps by activating anti-oxidant defense, and in absence of a functional catabolic pathway, GlcNAc becomes toxic to the cells.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W33

Wednesday 6th April
14:00 - 16:00

BOYNTON Primrose *(1), KOWALLIK Vienna (1), GREIG Duncan (1)*
(1) Max Planck Institute for Evolutionary Biology, Plön, Germany

Why do yeasts ferment?

The wine yeast Saccharomyces cerevisiae ferments glucose even when oxygen is abundant. Evolutionary theory predicts that fermentation gives yeast a competitive advantage over respiring competitors when growth rate, but not yield, is selected. Experimental tests in defined laboratory media support the theoretical prediction: fermenting *S. cerevisiae* is more fit than respiring *S. cerevisiae*. Yeast ecologists hypothesize that *Saccharomyces* evolved in high-sugar fruit environments where high growth rates are advantageous. Under realistic fruit conditions, however, fermentation does not give a competitive advantage. In grape and apple juice, a wild-type fermenting *S. cerevisiae* strain is less fit than a mutant respirer. Unlike standard laboratory media, grape juice is nitrogen-limiting; respiring yeasts may use the poor-quality nitrogen sources found in juice more effectively than fermenting yeasts. When grown in media with proline, a low-quality nitrogen source, fermenting yeasts have low fitness under high carbon conditions and high fitness under carbon limiting conditions. We propose an updated hypothesis and suggest that *Saccharomyces* yeasts evolved in sugar limited environments such as soils or plant surfaces.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W34

Wednesday 6th April
14:00 - 16:00

GALUSZKA Petr (1), *VRABKA Josef (1), HRADILOVÁ Michaela (1), MAJESKÁ ČUDEJKOVÁ Mária (1), HINSCH Janine (2), TUDZYNSKI Paul (2)*
(1) Palacký University, Olomouc, CZECH REPUBLIC
(2) Westfälische Wilhelms-Universität, Münster, Germany

Do phytopatogenic fungi perceive a phytohormonal signal by their own cells?

Some phytopatogenic fungi are able to produce plant hormones by their own metabolic pathways. For instance, *Fusarium fujikuroi* is a potent producer of gibberellins, which are causative agents of bakanae disease symptoms. Recently, we have revealed unique metabolic pathway for de novo cytokinin production in *Claviceps purpurea* (Hinsch et al., 2015, Environ Microbiol 17: 2935-2951). Auxins were shown to be produced by fungi from different classes. Nevertheless, it is still not clear whether fungal cells are able to perceive phytohormonal signal and induce expression of responsive genes similarly as plants. Axenic cultures of *Claviceps purpurea* have been treated by different phytohormones and sequencing libraries were prepared from RNA to conduct differential gene expression analysis with mock-treated controls. Statistical evaluation of RNA-seq experiment will be presented. An orthologous gene to plant auxin efflux carriers, which are important for auxin transport and polarity of plant cells, has been found in *Claviceps purpurea* genome. Localization and functional analysis of the unknown protein in fungal as well as plant cells will be presented.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W35

Wednesday 6th April
14:00 - 16:00

TRDÁ Lucie *(1), BAREŠOVÁ Monika (1), DOBREV Petre (2), MOTYKA Václav (1), BURKETOVÁ Lenka (1)*
(1) Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Rozvojová 263, 165 02 Prague 6, Czech Republic, Praha
(2) Department of Biochemistry and Microbiology, Institute of Chemical Technology, Technická 5, 165 21 Prague 6, Czech Republic, Praha

Cytokinin metabolism in the hemi-biotrophic pathogenic fungus *Leptosphaeria maculans*

Plant infection with some microorganisms can be linked with modifications in the cytokinin (CK) content. This was investigated in the interaction of oilseed rape (*Brassica napus*) with a hemi-biotrophic fungus *Leptosphaeria maculans* that causes blackleg (or phoma stem canker), the most damaging disease of this crop. This study shows that the infection with *L. maculans* alters CK profile of the host leaf tissue, with the whole spectrum of cis-zeatin derivatives being induced. Different fungi were reported to synthesize CKs. Indeed, here we show that *L. maculans* produces a wide range of cytokinins in the axenic culture, with the cis-zeatin being the main derivative. We report on the isopentenyltransferase (IPT) and adenosine kinase (AK) genes of *L. maculans* and prove their function in the CK metabolism using a silencing strategy. L. maculans expresses a single copy of plant IPTs, highly similar to plant tRNA-IPTs. We show that LmIPT is implicated in the cis-zeatin production. Further, AK present in *L. maculans* regulates, like in some plants, the conversion of ribosides into riboside 5''monophosphates and contributes to the CK homeostasis. Several models of CK metabolism pathway are suggested. Together, this study reports on the CK metabolism gene involved in CK recycling in fungi and brings a first insight on the CK biosynthesis and metabolism genes in a Dothideomycetes fungus.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W36

Wednesday 6th April
14:00 - 16:00

HUININK Henk *(1), KAMP-PEETERS Loes (1), ABASSI Negar (1), ADAN Olaf (2)*
(1) Eindhoven University of Technology, Eindhoven, Netherlands
(2) TNO, Delft, Netherlands

Growth of *Aureobasidium* on vegetable oils

When wood is impregnated with vegetable oil and left outside, a black biofilm forms. This biofilm, with *Aureobasidium* as the main fungus, is self-healing and has low maintenance costs. For large-scale industrial production, understanding of the growth mechanisms of *Aureobasidium* on oil is crucial. So far, the main observation in outdoor experiments is that the biofilm formation is faster on wood impregnated with olive oil compared to linseed oil. A possible reason could be that linseed oil cross-links under influence of oxygen. Here, the use of oil as a carbon source by *Aureobasidium melanogenum* is further investigated. Firstly, the difference in use of different oil types is quantified. Secondly, impact of cross-linking is investigated. Growth of *A. melanogenum* on linseed and olive oil is investigated under controlled temperature and relative humidity. Growth is compared visually and by measuring metabolic activity via MTT assay. Degradation of oil into fatty acids and glycerol is monitored with Fourier transform infrared spectroscopy (FT-IR).It is found that *A. melanogenum* shows no significant difference in metabolic activity on or degradation of fresh linseed and olive oil, although the colour of the colonies is different. This is in contrast with outdoor experiments, were faster growth was seen on olive oil compared to linseed oil. We hypothesize that this is due to different rates of cross-linking of the oil. Linseed oil cross-links faster than olive oil, which makes it more difficult to degrade. In contrast with outdoor experiments, indoor experiments have a relatively short time scale, in which cross-linking does not play a role. To study the effect of cross-linking, linseed oil was heat-treated before inoculation, until a rigid, cross-linked layer was obtained. There was no visible growth observed, no metabolic activity and no degradation of the oil. From this study we can conclude that *A. melanogenum* can use oil as a carbon source. Secondly, we can conclude that cross-linking of oil leads to slower growth. Olive oil contains mostly mono-unsaturated fatty acids, linseed oil mostly poly-unsaturated, therefore a third oil with high degree of saturated fatty acids will be tested, to further investigate the influence of oil type. Secondly, the effect of cross-linking will be further investigated by growth experiments on linseed oil with different degrees of cross-linking, quantified with NMR (nuclear magnetic resonance).

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W37

Wednesday 6th April
14:00 - 16:00

SCHÜLLER Christina *(1), WIESENBERGER Gerlinde (1), PERUCI Michaela (1), PARICH Alexandra (2), MALACHOVA Alexandra (2), BERTHILLER Franz (2), SCHUHMACHER Rainer (2), ADAM Gerhard (1)*
(1) Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Tulln, Austria
(2) Center for Analytical Chemistry, Department of Agrobiotechnology, IFA Tulln, University of Natural Resources and Life Sciences, Tulln, Austria

Lysine biosynthesis and secondary metabolite production in *F. graminearum*

The phytopathogen *Fusarium graminearum* contains multiple polyketide synthases (PKS) and non-ribosomal peptide synthetases (NPS) in secondary metabolite biosynthesis gene clusters. Post translational modification of PKS and NPS apo-proteins by phosphopantetheinyl transferase (PPT) is required for functional enzymes. Disruption of the PPT1 gene leads to simultaneous inactivation of all PKSs and NPSs. Loss of PPT1 function in addition causes lysine auxotrophy, as the lysine biosynthesis enzyme Lys2 also requires phosphopantetheinylation. Since fungal lysine auxotrophy alone affects virulence on host plants we tried to uncouple the effect on primary and (PKS and NPS dependent) secondary metabolism. The yeast *Saccharomyces cerevisiae* is devoid of secondary metabolite biosynthetic genes, but contains the specialized gene pair ScLYS2-ScLYS5. In yeast expression of FgLYS2-FgPPT1 cDNAs can complement auxotrophy of a Δlys2Δlys5 deletion strain. Our hypothesis is that expression of ScLYS2-ScLYS5 in a Fusarium Δppt1Δlys2 mutant would restore lysine prototrophy but not secondary metabolite biosynthesis. Yet, for still unknown reasons transformation of a Fusarium Δlys2 mutant with the yeast ScLYS2 coding region (between Fusarium promoter and terminator) did not yield LYS+ transformants. Interestingly, mutation of PPT1 strongly reduced specific production (amount metabolite per biomass) of the terpenoids deoxynivalenol and culmorin, and of butenolide in a lysine supplemented liquid medium. We also noticed that volatile production was markedly different, e.g. 2-methyl-1-butanol and 3-hydroxy-2-butanone accumulated at the end point of cultivation (4 weeks) only in Δppt1 and Δppt1Δlys2 double mutants but were not found in wild-type or Δlys2 mutant headspace, while a terpenoid (putatively annotated as a-longifolene) was detected only in wild-type and Δlys2 mutants. The impact of disruption of FgPPT1 is therefore more complex and pleiotropic than anticipated, so that reduced virulence cannot be attributed specifically to lacking PKS and NPS products.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W38

Wednesday 6th April
14:00 - 16:00

ALCALDE Eugenio *(1), FRASER Paul D (1)*
(1) School of Biological Science, Royal Holloway University of London, Egham, UK

Metabolite profiling in fungi, a platform to correlate the effect of single mutation across cellular metabolism

Metabolomics is a recent addition to the functional genomics toolkit, enabling the analysis of all low-molecular weight metabolites produce by a biological system ((1)). The predominant target for the microbial metabolomics applications has been the elucidation of metabolic pathways and their fluxes (2-5). The metabolome of an organism is the response of the genome to its environment. Therefore, in order to characterise an organism and its potential at a systems level, the development and implementation of robust metabolomic/metabolite profiling methodologies and their correlation with the genome/transcriptome are essential. Phycomyces blakesleeanus, a mucoral filamentous fungus, and Xanthophyllomyces dendrorhous, a basidiomycete yeast, are very distant organisms but they are both of commercial interest. Providing renewable biosources of industrial high value compounds such as carotenes, other isoprenoids (ubiquinone and sterols), organic acids and fatty acids that can be exploited by the industry. Several mutants in the Y-carotene biosynthetic pathway in Phycomyces and Xanthophyllomyces have been isolated. GC-MS and UPLC-PDA analysis has been performed in both organisms in order to compare different carotene mutant and wild-type strains. The results reveal that perturbations in the carotene pathway have widespread effects on the steady state metabolite levels across cellular metabolism in these fungi. The platform proposed is universal and can be applied to other organisms.

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(3) N. Saito, M. Robert, S. Kitamura, R. Baran, T. Soga, H. Mori, et al., J. Proteome Res. 5 (2006) 1979-87.
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POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W39

Wednesday 6th April
14:00 - 16:00

Gilbert Matthew (1)
(1) USDA, Agricultural Research Service, Food and Feed Safety Unit, New Orleans, LA, USA

Whole genome comparison of *Aspergillus flavus* L-morphotype strain 3357 (type) and S-morphotype strain 70

Aspergillus flavus is a saprophytic fungus that can invade and contaminate agronomically important crops. When colonizing a host crop, the fungus produces a number of toxic and/or pigmented secondary metabolites such as aflatoxin and cyclopiazonic acid. Understanding the growth, development, virulence and toxin production by members of this species is vital to developing strategies for remediation. Members of this species may be divided into two morphologically distinct categories; the L-morphotype (Large sclerotia) and the S-morphotype (Small sclerotia). These morphotypes differ significantly in the amount of aflatoxin produced, the development of reproductive structures, and in pigment production. The whole genome sequencing and annotation of two A. flavus strains, strain 70 (S-morphotype) and strain 3357 (L-morphotype), has recently been completed. Genome annotation of strain 70 was completed using the software program Maker which integrated gene predictions from augustus and genemark with RNA-seq and protein homology evidence. We identified 472 genes unique to strain 70 and 367 genes unique to strain 3357. A whole genome comparison has revealed a genetic basis for differences observed in the two morphotypes. Variant analysis revealed several potentially high impact mutations and analysis of secondary metabolic clusters revealed significant differences in the potential for metabolite biosynthesis. Finally, we functionally characterized a metabolic cluster that is present in strain 70 but absent in strain 3357.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W40

Wednesday 6th April
14:00 - 16:00

MORIN-SARDIN Stéphanie *(1), JANY Jean-Luc (1), ARTIGAUD Sébastien (2), BERNAY Benoît (3), PICHEREAU Vianney (2), COTON Emmanuel (1), MADEC Stéphanie (1)*
(1) Laboratoire Universitaire de Biodiversité et Ecologie Microbienne (LUBEM), Université de Bretagne Occidentale, Brest, France
(2) Laboratoire des Sciences de l'Environnement Marin (LEMAR), CNRS, IRD, Ifremer, Institut Universitaire Européen de la Mer, Université de Bretagne Occidentale, Brest, France
(3) Plateforme Proteogen, SFR ICORE, Université de Caen Basse-Normandie, Caen, France

Use of a proteomic approach for the determination of *Mucor spp.* adaptation markers to dairy environment

The Mucor genus includes a large number of ubiquitous fungal species found in the environment, on raw materials or foodstuffs. These species are regularly encountered as contaminants of meat, fruits, vegetables or processed products, such as dairy products. In these contexts, their uncontrolled growth may induce undesirable effects including off-flavours, anomalous textures or discolorations. On the other hand, several Mucor species also present a technological interest, highly contributing to the texture and the sensory characteristics of many fermented products such as cheeses. This dichotomy raises the question of the adaptation of some species and potentially of domestication processes. A recent work focusing on the effect of abiotic factors on *Mucor spp.* growth (1) reported that Mucor strains belonging to species regularly used for their technological potential showed higher optimal growth rates than contaminant strains on cheese analog and cheese compared to synthetic medium. In this context, we studied the molecular mechanisms involved in the adaptation of technological species to dairy environment. Four technological, contaminant or non-cheese related Mucor species were grown on synthetic Potato Dextrose Agar (PDA) or cheese analog (Cheese Agar - CA) media during 7 days at 14°C and proteomes were compared using bidimensional gel electrophoresis. Differentially expressed proteins on PDA versus CA medium were analyzed by nanoLC-MALDI TOF/TOF followed by database searching using the Mascot program. Out of the 438 targeted proteins, 80% (351) were identified. Functional characterization steps are under progress in order to determine the main metabolic pathways involved in the adaptation to cheese matrices.

(1) Morin-Sardin et al., 2016. Food Micro. In press.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W41

Wednesday 6th April
14:00 - 16:00

DIRNBERGER Benedict *(1), VALERIUS Oliver (1), FEKETE-SZÜCS Enikő (1), MEISTER Cindy (1), TEICHERT Ines (2), GERKE Jennifer (1), TROPPENS Danielle (1), BRAUS Gerhard H. (1)*
(1) Institute of Microbiology & Genetics, Departement of Molecular Microbiology & Genetics Georg-August-University Göttingen, Germany
(2) Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, Germany

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

A. nidulans sexual and asexual differentiation are linked to significant differences in formation of secondary metabolites and the formation of various distinct cell types in the different developmental programmes (1). A comparative approach to investigate differences in the proteomes of fungal cells of different developmental programmes was initiated. 1981 proteins could be identified in the vegetative mycelium, 1874 proteins were identified in hyphae where sexual development was induced (dark conditions) and 1889 proteins in hyphae where asexual development was induced (illumination). Hülle cells are unique cell types of Aspergilli and are linked to the sexual programme. These globose thick-walled cells presumably support the formation of cleistothecia which are closed sexual fruiting bodies. A protocol for the enrichment of Hülle cells was established where 963 proteins could be identified. An enzyme of the monodictyphenone cluster as well as the dimethylallyltransferases XptB/XptC are specifically expressed in sexual mycelium and in Hülle cells. The current status of this proteomic comparison will be presented.

(1) Bayram et al., 2016; Fung. Genet. Biol. 87, 30-53

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W42

Wednesday 6th April
14:00 - 16:00

LALUCQUE Herve *(1), MALAGNAC Fabienne (1), GAUTIER Valérie (1), GROGNET Pierre (1), CHAN HO TONG Laetitia (1), SILAR Philippe (1)*
(1) Université Denis Diderot, Laboratoire Interdisciplinaire des Energies de Demain (LIED) UMR 8236, Case courrier 7040 Lamarck, 75205 Cedex13, Paris, France

Identification and characterization of two genes required in the control of a cell degeneration in the filamentous fungi *Podospora anserina*

For several years, we use the coprophilous fungus *Podospora anserina* to study a cell degeneration called Crippled Growth (CG) triggered by an epigenetic and cytoplasmic element. In the wild-type strain, this element is produced during stationary phase and eliminated at growth renewal. However, in some particular growth conditions, the element is not removed from growing hyphae triggering CG. Previous results showed that CG is controlled by two MAPK modules, the PaNox1 NADPH oxidase, and IDC1, a protein with unknown activity, but that was recently proposed to be the scaffold of the PaMpk1 MAPK module. Recently, we published that PaSo, a protein required for anastomosis, is also involved for CG development. Here, we describe the identification and characterization of two new partners involved in the control of CG, IDC2 and IDC3. Data show that IDC2 and IDC3 likely act downstream of PaNox1 to regulate the PaMpk1 MAPK. We will present a thorough analysis of the phenotypes of IDC2 and IDC3 mutants and phylogenetic analyses of the IDC2 and IDC3 proteins. We will also present the determination of the localization of IDC3 and of the Epichloe festucae homologues of the IDC2 protein in *P. anserina* mycelia.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W43

Wednesday 6th April
14:00 - 16:00

GRANAHAN Ashling (1)
(1) Centre for Environmental Research Innovation and Sustainability (CERIS), School of Science, Institute of Technology Sligo, Ash Lane, Ireland

Investigating serine proteases in basidiomycete *Coprinopsis Cinerea* fruiting body development&nutrient acquisition from humic rich environments.

The basidiomycete *Agaricus bisporus* is the most cultivated mushroom worldwide and the mushroom industry represents the largest horticultural sector in Ireland. Basidiomycete fungi also have a clear ecological role, where the depolymerisation of biopolymers is the main process in the cycling of carbon, with litter decomposition in temperate forests mainly driven by fungal activity. They constitute a major fraction of the living biomass responsible for efficient degradation of numerous recalcitrant organic compounds in soil litter and the humic layer. *Coprinopsis cinerea*, (formally *Coprinus cinereus*), the ink cap mushroom, is a model mushroom that is used to study basidiomycetes due to its ease of cultivation and fast growth cycle.
This research is focused on the role of serine proteases in basidiomycete fruiting body development and nutrient acquisition from humic-rich environments. Sequence analysis of the *C. cinerea* genome has revealed seven potential serine proteases. The function of these enzymes and their role in the life cycle of the mushroom, from nutrient acquisition to fruiting body development will be investigated through promoter profiling. Results to date report on the construction of expression plasmids and the isolation of the serine protease promoters by PCR.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W44

Wednesday 6th April
14:00 - 16:00

METIN Banu *(1), DOGEN Aylin (2), ILKIT Mehmet Macit (3), HEITMAN Joseph (4)*
(1) Istanbul Sabahattin Zaim University, Department of Food Engineering, Istanbul, Turkey
(2) Mersin University, Department of Pharmaceutical Microbiology, Mersin, Turkey
(3) Cukurova University, Department of Microbiology, Adana, Turkey
(4) Duke University, Department of Molecular Genetics and Microbiology, Durham, NC, USA

Characterization of the mating type locus and investigation of sexual reproduction of the opportunist black yeast *Exophiala (Wangiella) dermatitidis*

This study was aimed at characterizing the mating type locus (MAT) and investigating the sexual reproduction of the pathogenic black fungus *Exophiala (Wangiella) dermatitidis*. In ascomycetous fungi, mating is controlled by a single mating type (MAT) locus. The MAT locus involves two unique, completely different sequences in the two mating types, and because of this, they are termed idiomorphs rather than alleles. In fungi belonging to the Pezizomycotina subphyllum, each MAT idiomorph encodes an alpha domain or an HMG domain transcription factor. Although the alpha domain and the HMG domain transcription factor genes are indispensable components, structural features of the MAT locus, including, the length, the gene content, and the transcriptional directions of the genes differ from fungus to fungus. To characterize the MAT locus of *E. dermatitidis*, its genome was first searched for the HMG domain gene and the SLA2 and the APN2 genes surrounding it. Next, PCR primers were designed within the HMG gene and one in SLA2 and one in APN2 to amplify a longer region. *E. dermatitidis* strains isolated in previous studies were grown on Malt extract agar (MEA) and DNA extraction was performed. The extracted DNAs and the designed primers will be used in PCR to amplify the MAT regions of the E. dermatitidis strains. The PCR products obtained will be sequenced and the sequences will be analyzed to determine whether they have HMG or alpha domain gene sequences. The ratio of the two mating types will provide clues as to how E. dermatitidis reproduces in its natural habitat; that is, whether it reproduces clonally, that is asexually, or sexually. In addition, if sequences from the other mating type are observed, the length, the limits, and the gene content of the MAT locus will be determined. This way, the first black fungal MAT locus to be characterized will be *E. dermatitidis.* Furthermore, the MAT locus of *E. dermatitidis* will be compared to the MAT loci of other ascomycetous fungi to gain clues on the evolution of this locus. The strains determined to be suitable for mating as a result of the sequence analysis will be inoculated on suitable media to observe sexual reproduction. This work is important both for advancing molecular genetic studies, and for understanding the nature of this opportunistic pathogen.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and physiology
CS7W45

Wednesday 6th April
14:00 - 16:00

BANANI Houda *(1), SPADARO Davide (1), MARCET-HOUBEN Marina (2), BALLESTER Ana-Rosa (3), ABBRUSCATO Pamela (4), GONZÁLEZ-CANDELAS Luis (3), GABALDÓN Toni (2)*
(1) DiSAFA - Dept. Agricultural, Forestry and Food Sciences and AGROINNOVA – Centre of Competence for the Innovation in the Agroenvironmental Sector, University of Torino, Grugliasco, Turin, Italy
(2) Bioinformatics and Genomics Programme. Centre for Genomic Regulation (CRG), Barcelona, Spain
(3) Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Valencia, Spain
(4) Bioeconomy Unit, Parco Tecnologico Padano, Lodi, Italy

Genome mining and characterization of secondary metabolites pathways of the postharvest pathogen *Penicillium griseofulvum* PG3

Penicillium griseofulvum causes blue mould decay in stored apples, which is the most important postharvest disease of pome fruit. This pathogen is known to produce potent mycotoxins, such as patulin. The genome of *P. griseofulvum* strain PG3 isolated from rotten apples in Italy was sequenced, and important secondary metabolites (SM) clusters were analysed in order to gain insight into mycotoxins synthesis in PG3 in vitro and during disease development on fruit. The analysis of the sequenced PG3 genome (29.3 Mb) shows that *P. griseofulvum* is branched off after the divergence of *P. oxalicum* but before the divergence of *P. chrysogenum*. Genome-wide analysis of P. griseofulvum PG3 genes revealed putative gene clusters for patulin, griseofulvin and roquefortine C biosynthesis. Besides the bioinformatic analysis of these SM gene clusters, we quantified their production by *P. griseofulvum* PG3 in vitro and on apples during the course of infection. Furthermore, the SM detected during apple infection were closely examined by studying the expression kinetics of their key genes under controlled conditions. These findings provide relevant information to understand the molecular basis of mycotoxins biosynthesis in *P. griseofulvum*, to permit further research directed towards blocking their synthesis in order to avoid consumer health threats and increase food safety.

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and Physiology
CS7W46

Wednesday 6th April
14:00 - 16:00

LA STARZA Sonia Roberta *(1), MICCOLI Cecilia (1), REVERBERI Massimo (1), FANELLI Corrado (1), O'BRIAN Greg (2), PAYNE Gary (2)*
(1) Department of Environmental Biology, University of Rome "Sapienza", Rome, Italy
(2) Department of Plant Pathology, North Carolina State University, Raleigh, North Carolina, USA

Role of a secondary metabolism gene cluster in the pathogenic interaction between *Aspergillus flavus* and *Zea mays*

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

POSTER SESSION ABSTRACTS
Session CS7 Metabolism and Physiology
CS7W47

Wednesday 6th April
14:00 - 16:00

ROMSDAHL Jillian *(1), BLACHOWICZ Adriana (2), CHIANG Yi-Ming (1), YAEGASHI Junko (1), VENKATESWARAN Kasthuri (2), WANG Clay (1)*
(1) Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, School of Pharmacy, Los Angeles, California, USA
(2) Biotechnology and Planetary Protection Group, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California, USA

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

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

Supported by 2012 Space Biology NNH12ZTT001N grant #19-12829-27.

POSTER SESSION ABSTRACTS
Session CS8 Adaptation to xenobiotics
CS8W1

Wednesday 6th April
14:00 - 16:00

PERROT Thomas *(1), DEROY Aurélie (1), SAIAG Fanny (1), KEBBI-BENKEDER Zineb (2), MOREL-ROUHIER Mélanie (1), SORMANI Rodnay (1), COLIN Francis (3), DUMARCAY Stéphane (2), GÉRARDIN Philippe (2), GELHAYE Eric (1)*
(1) INRA, Lorraine University, Interactions Arbres Microorganismes, Vandoeuvre-les-Nancy, France
(2) Université de Lorraine, INRA, Laboratoire d'Etudes et de Recherche sur le Matériau Bois , Vandoeuvre-les-Nancy, FRANCE
(3) AgroParisTech, LERFOB, Nancy, France

Functional variability of the detoxification system in wood-decaying fungi

Fungi play a key role in the organic matter recycling and some of them; especially basidiomycetes are the most efficient microorganisms to degrade lignocellulosic materials. To perform such degradation, these organisms have developed different strategies based on oxidative processes either using specific degradation enzymes or producing hydroxyl radicals by non-enzymatic Fenton reactions. Wood decaying fungi are thus in contact with many compounds resulting from wood decay and also compounds already present in wood. Among these latters, wood extractives (flavonoids, terpenoids, stilbenes&hellip) are potentially toxic. To cope with this potential toxic environment, fungi have developed detoxification system involving multigenic families such as cytochrome P450 monooxygenases (involved in the first oxidation step of detoxication) and glutathione transferases (acting in the second conjugaison step). It has been showed that different isoforms of fungal glutathione transferases are able to interact differently with extracts of various wood species. However, their roles in the fungal endogenous metabolism remain mysterious. We have particularly worked on glutathione transferases from the white-rot *Trametes versicolor* focusing on isoforms belonging to the omega class (GSTO). Among the sixteen TvGSTOs identified in this fungus, we report here the biochemical characterization of nine isoforms.

POSTER SESSION ABSTRACTS
Session CS8 Adaptation to xenobiotics
CS8W2

Wednesday 6th April
14:00 - 16:00

VALETTE Nicolas *(1), GELHAYE Eric (1), MOREL-ROUHIER Mélanie (1)*
(1) Université de Lorraine, INRA, Interactions arbre/microorganismes, Labex ARBRE, Nancy, France

Characterization of small proteins secreted by lignolytic fungi

Saprophytic fungi play an important biological and ecological role in forest ecosystems. Indeed, thanks to many extracellular enzymes, they are able to degrade all wood components, a key step for carbon recycling. During this process, wood releases various molecules, called extractives, which can be toxic for fungal cells. This is the reason why these microorganisms have developed extensive detoxification systems. Interestingly, a transcriptomic analysis revealed that numerous up-regulated genes involved in extractive response in *Phanerochaete chrysosporium* are still of unknown function. Among them, one group called small secreted proteins (SSP) has been highlighted. A SSP from *P. chrysosporium* has been produced as a recombinant protein *in Escherichia coli* and purified. First results show that this SSP has several interesting structural features. Indeed, it can forms a kind of hydrogel depending on pH. Moreover, the protein is highly stable being insensitive to temperature, denaturating agents or reductants. Since homologues have been identified in ectomycorrhizal or litter degrading fungi, its role could be related to detoxification or stress response in fungi. We are currently assessing this point by developing genetic tools in *P. chrysosporium* to decipher the physiological role of these proteins in stress conditions.

POSTER SESSION ABSTRACTS
Session CS8 Adaptation to xenobiotics
CS8W3

Wednesday 6th April
14:00 - 16:00

CARTER Helen *(1), CLAKE Helen (1), DELLER Siân (1), SCOTT Eileen (1), CSUKAI Michael (1)*
(1) Syngenta, Jealott's Hill International Research Centre, Bracknell, UK

Advances in chemical genetic tools and impact on the research and development of novel crop protection products

There is strong demand within agriculture and horticulture for ever-improving control of crop pests and diseases. In order to support the discovery and development of new active ingredients a wide range of chemical genetic tools are utilised and are under constant development. Historically many of these tools used the model organism *Saccharomyces cerevisiae*, but improvements in genomic information and tools make it possible to perform research directly in field-relevant plant pathogens. Some of the wide range of tools used in support of new fungicide discovery will be outlined, including: Forward genetic tools, which have long been the mainstay of mode of action diagnosis, provide an unbiased approach for the identification of the molecular target of a chemical inhibitor. If resistant mutants can be generated, the technique can now be applied to any pathogen, providing a reference genome is available and single nucleus cells/spores can be isolated. Libraries of engineered model organism strains. Chemistry with lower resistance risk is often associated with the inability to isolate resistant mutants in the plant pathogen of interest. In this case unbiased genetic tools are still of value and model organisms are employed. Haploinsufficiency or overexpression-induced resistance within a yeast library can provide information on proteins or protein families targeted by novel chemistry. Hypotheses can then be based on the mutations seen, and compared to information from additional phenotypes such as biochemical pathway inhibition and microscopic observations.

POSTER SESSION ABSTRACTS
Session CS8 Adaptation to xenobiotics
CS8W4

Wednesday 6th April
14:00 - 16:00

KELLY Emily *(1), CENTENO Carla (1), SOTO Charles (1), CAFARO Matias (1)*
(1) University of Puerto Rico, Mayaguez Campus, Mayaguez, USA

Induced expression of laccase enzyme by xenobiotics: Amplified laccase sequences from genomic DNA extracted from manglicolous fungi of Puerto Rico

Lignolytic fungi in mangrove ecosystems are the main organisms involved in carbon recycling from lignin. Fungi, have specific enzymes involved in lignin degradation, such as lignin and Mn peroxidases, and laccases. Previous studies have shown that lignolytic fungi can also degrade polycyclic hydrocarbons (PAHs). In order to study laccase enzyme expression in manglicolous fungi, we isolated fungi from wood and decaying leaves in Bahia Sucia, Cabo Rojo and La Parguera, Lajas. To induce lignolytic enzyme expression the azo dye Congo Red and naphthalene were used because of their similar chemical structure to lignin, thus serving as proxy for degradation studies of the latter. Fungi were grown in a liquid basal medium with Congo Red or naphthalene as their only carbon and energy source at 28° C, 150 rpm. As a positive control we used *Asperguillus flavus*. Genomic DNA was extracted from the six samples used in the degradation assay. For further identification of the samples primers ITS 4 and ITS 5 were used. Also laccase sequences were amplified from genomic DNA extracted using primers LAC 2 FOR and LAC 3 REV for the six samples used in the degradation assay. Some of the species isolated were *Penicillium citrinum*, *Fusarium solani*, *Aspergillus flavipes, Purpureocillium lilacinum*, *Aspergillus caelatus*, and *Phoma sp*. Sequences were compared to other ascomycete laccase sequences on the databases.

POSTER SESSION ABSTRACTS
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CS8W5

Wednesday 6th April
14:00 - 16:00

ROSSI Antonio *(1), MENDES Niege (1), PERSINOTI Gabriela (1), SILVA-ROCHA Rafael (2), SANCHES Pablo (1), MARTINEZ-ROSSI Nilce (1)*
(1) São Paulo University, Ribeirão Preto Medical School , Department of Genetics, Ribeirão Preto, SP, Brazil
(2) São Paulo University, Ribeirão Preto Medical School, Department of Cellular and Molecular Biology, Ribeirão Preto, SP, Brazil

Comprehensive analysis of the transcriptional profile and alternative splicing in the dermatophyte *Trichophyton rubrum* exposed to undecanoic acid

Dermatophytes are fungi that colonize keratinized tissues, whose infective capacity is associated with the keratinolytic activity. These pathogens cause cutaneous mycoses restricted to the skin, nails, and hair, do not cause a pandemic, but can be invasive in immunocompromised patients. Fatty acids have antifungal properties, and undecanoic acid (AUN) (C11, saturated) is the most toxic of the series C7 to C18. Thus, our aim was to characterize by RNA-seq analysis the transcriptome of *T. rubrum* responsive to UDA. Mycelia were exposed to UDA for 3 or 12 hours in biological triplicate, and the resulting RNA was sequenced generating approx 58 million of reads per library. The sequences were filtered, aligned with the reference genome, and differential analysis identified 385 and 210 genes, respectively modulated in response to 3 and 12 hours exposure. These genes are related to several physiological processes including the degradation and drug efflux, and pathogenesis. Following, we identified about 100 genes exhibiting exon skipping, and a large number of genes showing intron retention after exposure or not to UDA. These genes showing alternative splicing are associated with various metabolic functions such as translation, vesicular transport, ribosomal biogenesis, and pre-mRNA processing. These results expand the knowledge of the molecular mechanisms involved in the survival response upon exposure to the AUN.
Financial Support: FAPESP, CNPq, CAPES, FAEPA.

POSTER SESSION ABSTRACTS
Session CS8 Adaptation to xenobiotics
CS8W6

Wednesday 6th April
14:00 - 16:00

LENGYEL Szabina (1), RASCLE Christine (1), MARCATO Riccardo (2), GAGEY Marie-Joseph (1), SELLA Luca (2), FAVARON Francesco (2), ***CHOQUER Mathias*** *(1)*
(1) University of Lyon 1, CNRS, BAYER Cropscience, Lyon, France
(2) Department TeSAF, University of Padova, Legnaro, Italy

Botrytis cinerea **transcriptomic response to the active substance eugenol**

Plant essential oils are well known for their antimicrobial and antifungal activities. Eugenol (4-allyl-2-methoxyphenol) is the main component of clove oil which is extracted from clove buds, but it is also found in cinnamon or basil oils. The precise mechanism of its antifungal effect is still unclear and, to address its mode of action, a microarray analysis was performed on treated and untreated cultures of *Botrytis cinerea*. This fungus is a plant-pathogen causing grey mould on more than 200 dicotyledonous plants and resulting in huge crop losses. The transcriptional response of *B. cinerea* to eugenol was analysed and category enrichment was only observed for down-regulated genes. Significant enrichment of down-regulated genes was found in ergosterol biosynthesis, DNA replication factors, glutathione pathway and Botrydial toxin biosynthesis.

POSTER SESSION ABSTRACTS
Session CS8 Adaptation to xenobiotics
CS8W7

Wednesday 6th April
14:00 - 16:00

CONINX Laura *(1), RUYTINX Joske (1), OP DE BEECK Michiel (2), MORIN Emmanuelle (3), COLPAERT Jan (1)*
(1) Hasselt University, Hasselt, Belgium
(2) Lund University, Lund, Sweden
(3) INRA, Nancy, France

Characterization of a ZIP transporter, SlZRT1, in the ectomycorrhizal Basidiomycete *Suillus luteus*

Zn is an essential nutrient, but toxic when present in excess. To assure growth and reproduction in changing environments, all cells make use of homeostatic pathways to tightly control availability of this element in the cytoplasm. Membrane transporters and intracellular and extracellular chelating metabolites and proteins play a crucial part in the maintenance of optimal cytoplasmic Zn concentrations. In this study we report the characterization of a member of the ZIP (ZRT/IRT-like protein) family of transporter proteins, ZRT1 of the ectomycorrhizal fungus Suillus luteus. Within this fungal species, different ecotypes showing distinct tolerance levels towards external Zn concentrations exist. ZIP transporters are known to increase cytoplasmic Zn levels by transporting Zn across the plasma membrane or by mobilizing stored Zn from intracellular compartments. SlZRT1 was first investigated in silico and a phylogenetic tree was constructed to predict the resulting protein"s function. Bio-informatics data illustrated that SlZRT1 has a ZIP signature sequence and that it most likely functions as a plasmamembrane localized Zn importer. Heterologous expression in yeast was performed to verify the predicted function and localization of the protein. A gene expression analysis via RT-qPCR was performed to establish whether SlZRT1 expression was affected by external Zn concentrations. It was shown that in *S. luteus SlZRT1* was higher expressed when grown in the presence of limited external Zn. When grown in the presence of excess external Zn, the expression of the transporter is turned down. All together these data indicate a key role for SlZRT1 in cellular Zn homeostasis of the ectomycorrhizal fungus S. luteus. Future experiments will reveal if this transporter functions differentially in *S. luteus* genotypes showing a different tolerance level towards Zn and how it contributes to Zn homeostasis in different developmental stages.

POSTER SESSION ABSTRACTS
Session CS8 Adaptation to xenobiotics
CS8W8

Wednesday 6th April
14:00 - 16:00

BILLARD Alexis (1), AZEDDINE Saad (1), BACH Jocelyne (1), AUDEON Colette (1), LANEN Catherine (1), FILLINGER Sabine (1), ***DEBIEU Danièle*** *(1)*
(1) BIOGER, INRA, AgroParisTech, Thiverval-Grignon, France

Genetic and biochemistry analyses of the natural resistance of the fungicide fenhexamid in the phytopathogenic fungus *Botrytis pseudocinerea*

The *Botrytis* species complex responsible for grey mould disease on grapevine is composed of two species: *Botrytis cinerea* the major one (about 90%) and *Botrytis pseudocinerea*. Despite their genetic polymorphism, these species cannot be morphologically distinguished. However, they do differ in their response to several fungicides, especially to the sterol biosynthesis inhibitor fenhexamid. While *B. cinerea* is sensitive to this hydroxyanilide, *B. pseudocinerea* is naturally resistant. Enzyme assays showed that in *B. pseudocinerea* the fenhexamid target enzyme, the sterol 3-ketoreductase was less sensitive to fenhexamid. In addition, a synergic effect between fenhexamid and sterol 14A-demethylation inhibitors (DMIs) known to inhibit Cyp51, a cytochrome P450 monooxygenase was observed in *B. pseudocinerea*. These results could suggest detoxification of fenhexamid by cytochromes P450. The cyp684 gene showing the strongest similarity to cyp51 among all *B. cinerea* cytochrome P450 genes was found strongly overexpressed in the presence of fenhexamid in *B. pseudocinerea*. In this work, we studied separately the effect of *B. pseudocinerea* erg27 polymorphism, erg27 encoding 3-ketoreductase, and of the recently identified cytochrome P450 gene, cyp684, on resistance to fenhexamid. The objective is to determine their respective implication in resistance. Experiments were conducted by exchange between erg27*B.cinerea* and erg27*B.pseudocinerea* in *B. cinerea* and by cyp684 deletion in *B. pseudocinerea*. In parallel, metabolization studies are conducted to identify metabolites and test their activity on *Botrytis spp*.

POSTER SESSION ABSTRACTS
Session CS8 Adaptation to xenobiotics
CS8W9

Wednesday 6th April
14:00 - 16:00

MARTINOVA Veronika *(1), RUYTINX Joske (1), COLPAERT Jan (1)*
(1) Hasselt University, Hasselt, Belgium

Cu and Cd tolerance in *Suillus luteus* populations from Norway and Belgium

Contamination with heavy metals poses risks both to human health and the ecosystems and with greater awareness of its detrimental effects, remediation of heavy-metal contaminated soils has been an important concern in ecological restoration. Both Cu and Cd are toxic to plants and soil organisms. Even though Cu is a micronutrient, it is harmful at elevated concentrations, causing extensive cell damage. On the other hand, Cd is nonessential and it is toxic even at small concentrations. Biodiversity is reduced in sites polluted with these metals and there is great selection pressure on the organisms to withstand elevated concentrations of these elements. One of the organisms that has adapted to the contamination in these areas is *Suillus luteus*, a basidiomycete fungus that forms ectomycorrhizal associations with Scots pine (*Pinus sylvestris*). Ectomycorrhizal fungi are known to help the associated plants to cope with various types of environmental stress, *S. luteus* being intensively studied for its role in protection of the plants from heavy metals. In the presented work we investigate the presence of Cu or Cd tolerance in *S. luteus* populations coming from a Cu mine spoil in Norway (17 fungal isolates) and several sites polluted by metal ore smelters in Flanders, Belgium (12 isolates). Dikaryotic mycelia were grown on solid Fries medium containing 6 different concentrations of the studied metals. After 10 days of growth they were harvested and their dry weight was determined. The freeze dried mycelia were subjected to heavy metal determinations by means of inductively coupled plasma optical emission spectrometry (ICP-OES). We calculated the EC50 (half maximal effective concentration) for each isolate and found a continuum of tolerance for both metals in every population. However, isolates coming from heavier polluted sites presented higher EC50 values for the respective metal. Furthermore, the heavy metal determination in the mycelium will shed light into whether the isolates exclude or accumulate the metal, aiding further investigations into potential tolerance mechanisms of this fungus.

POSTER SESSION ABSTRACTS
Session CS8 Adaptation to xenobiotics
CS8W10

Wednesday 6th April
14:00 - 16:00

COX Belinda *(1), DODHIA Kejal (1), HARPER Lincoln (1), OLIVER Richard (1),* ***FRAN Lopez*** *(1)*
(1) Centre for Crop Disease Management, Department of Environment and Agriculture, Curtin University, Perth, Australia

Development and applications of digital PCR and Loop-mediated isothermal amplification for fungicide resistance detection

With the development of new molecular technologies, we are moving away from traditional fungicide resistance monitoring methods to rapid identification and early detection of development of resistance in the field.Digital PCR (dPCR) is a quantitative high-throughput, sensitive, fast and cost effective method to screen for genetic mutations in mixed populations. Samples are partitioned on a silicon chip into thousands of individual reactions. Fluorescently labelled probes that target single nucleotide polymorphisms (SNP) are incorporated in the reaction, to facilitate end point digital detection and quantification of mutant alleles. A dPCR assay has been developed targeting mutations Y136F and S509T in the Cyp51 gene of *Blumeria graminis f. sp. hordei* (Bgh), the causal agent of barley powdery mildew. These mutations have previously been found in Australia, and have shown to be associated with resistance to triazole fungicides. Barley infected leaf samples were collected from fields across Australia, DNA pooled together and screened on a single chip, to determine the mutation rates in the field. Results showed all Australian samples carried the Y136F mutation. For the S509T mutation, there were high levels in Western Australia, Victoria and Tasmania, and medium levels in New South Wales, but was not detected in any of the samples analysed from Queensland. Loop mediated isothermal amplification (LAMP) is a simple, highly specific and rapid method for amplification and detection of DNA. The simplicity of this technique, coupled with a portable platform, make it highly suitable for in situ diagnostic applications. In addition to simple detection of any pathogens, LAMP can be used to detect SNPs. This is particularly useful in cases where resistance to fungicide is caused due to a SNP in the sequence of the gene encoding the fungicide target. Mutations I365S and the combination 369P + N373S in Os-1, the target gene of fungicide iprodione in the grape pathogen *Botrytis cinerea*, have been previously correlated with widespread resistance to iprodione in the field. SNP-typing LAMP has been used to successfully detect these mutations in strains of B. cinerea allowing for the quick discrimination of *B. cinerea* wild type and resistant strains. The implications of dPCR and LAMP methodologies for the management of fungicide resistance are discussed.

POSTER SESSION ABSTRACTS
Session CS9 New tools for fungal biology
CS9W1

Wednesday 6th April
14:00 - 16:00

MARSCHALL Robert *(1), SCHUMACHER Julia (1), TUDZYNSKI Paul (1)*
(1) Institute of Plant Biology and Biotechnology, WWUM, Münster, Germany

Chasing stress signals: new applications of the redox-sensitive GFP2 and the total ROS/superoxide detection kit in *Botrytis cinerea*

Reactive oxygen species (ROS) are produced in all cells that depend on molecular oxygen. They are produced in highly conserved processes either as byproducts or as results of evolved enzymatic reactions. Besides their high damaging potential for DNA and lipids, ROS can serve as signaling molecules in various developmental processes. Due to the important role of ROS, many studies aim to uncover and understand ROS production, detoxification and influence on the redox state. In *Botrytis cinerea* we characterized the specific H2O2 and superoxide production ability of the wild type and mutants of different signaling pathways under (un)stimulated conditions. We were able to show that the oscillation-like pattern of superoxide is directly influenced by the addition of oxidative, osmotic as well as calcium stress agents. Moreover, we confirmed the varying levels of H2O2 production of the wild type and already analyzed mutants (e.g. BcLtf1, BcNoxA/B) by an exact fluorometric measurement. We monitored redox state changes by using modified versions of the redox sensitive GFP2-system [1], integrated in the cytosol, the endoplasmic reticulum and mitochondria. We chased stress signals (e.g. H2O2, NaCl and SDS) in the wild type and the mentioned mutants and analyzed the influence of the stress signals on the redox state in the different compartments.

[1] Heller, J., Meyer, A. J., Tudzynski, P. (2012). *Molecular plant pathology*, *13*(8), 935-947.

POSTER SESSION ABSTRACTS
Session CS9 New tools for fungal biology
CS9W2

Wednesday 6th April
14:00 - 16:00

BOECKER Simon *(1), WANKA Franziska (1), SÜSSMUTH Roderich D. (2), ARENTSHORST Mark (3), RAM Arthur F. J. (3), MEYER Vera (1)*
(1) Berlin University of Technology / Applied and Molecular Microbiology, Berlin, Germany
(2) Berlin University of Technology / Biological Chemistry, Berlin, Germany
(3) Leiden University / Molecular Microbiology and Biotechnology, Leiden, Netherlands

Conditional gene expression in *Aspergillus niger* - perspectives and limitations of inducible expression systems

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

[1] V. Meyer, F. Wanka, J. van Gent, M. Arentshorst, C. A. M. J. J. van den Hondel, A. F. J. Ram, Appl. Environ. Microbiol. 2011, 77, 2975–2983
[2] F. Wanka, T. Cairns, S. Boecker, C. Berens, A. Happel, X. Zheng, J. Sun, S. Krappmann, V. Meyer, Fungal Genet. Biol. 2015, Epub ahead of print

POSTER SESSION ABSTRACTS
Session CS9 New tools for fungal biology
CS9W3

Wednesday 6th April
14:00 - 16:00

PILLAI Aneesh *(1), KAUTTO Liisa (1), LIN Chi-Hung (1), SUN Angela (1), TE'O Junior (2), PACKER Nicki (1), NEVALAINEN Helena (1)*
(1) Macquarie University, Sydney, Australia
(2) Queensland University of Technology, Queensland, Australia

Expression of functional human ST6GAL1 in *Trichoderma reesei*

Sialyltransferases can be used to add sialic acid to glycoproteins to study and modify their functional properties including alteration in metastatic potential of cancer cells, change in drug resistance and differentiation of stem cells. Here we have expressed a functional human sialyltransferase, ST6Gal1, in *Trichoderma reesei* with a view of improved availability of the enzyme. Two expression cassettes were designed, one with the mCherry gene which encodes a fluorescent protein that enables initial screening of transformants and tracking of secretion, and one without mCherry. A truncated and codon optimised ST6Gal1 cDNA was expressed under the *T. reesei* cbh1 promoter. ST6Gal1 was found to be secreted into the culture medium, established by Western blotting with ST6Gal1 specific antibodies. Two main forms, 40 kDa and 60 kDa of the secreted ST6Ga1 were observed indicating processing/degradation of the heterologous product. Southern blot analysis revealed the integration of multiple copies of ST6Gal1 gene into the fungal genomic DNA, with at least one of the transformants having the expression cassette integrated in the cbh1 locus. A lectin based high-throughput enzyme activity assay was developed and the results were validated using mass spectrometry.

POSTER SESSION ABSTRACTS
Session CS9 New tools for fungal biology
CS9W4

Wednesday 6th April
14:00 - 16:00

GROTTOLLI Alessandro (1), SANSEVERINO Walter (2), AIESE CIGLIANO Riccardo (2), BECCACCIOLI Marzia (1), FANELLI Corrado (1), REVERBERI Massimo (1), ***SCALA Valeria*** *(1)*
(1) Department of Environmental Biology, University of Rome "Sapienza", Rome, Italy, Roma, Italy
(2) Sequentia Biotech SL, Parc Cientific Barcelona, Barcelona, Spain, Barcelona, Spain

Gene deletion and genome perturbation

Fusarium verticillioides (Fv) causes ear rot disease in maize and produces fumonisins, mycotoxins toxic to humans and livestock. Gene deletion is a molecular approach effective in identifying genes or gene clusters function. In this study, the linoleate diol synthase-coding gene, lds1, was deleted in a strain of Fv. Since significant difference in the genome emerged between our isolate and the reference strain (Fv7600), deposited in the BROAD institute, we re-sequenced our wild type as well as the lds1-deleted mutants originated from our naïve strain. Significant differences in genome sequences emerged between our wild type and the two lds1-mutants further than the trivial deletion of the lds1 locus. Tests performed through a bioinformatic approach, highlighted significant differences in the our wild type as well as the two lds1-deleted mutants, such as single nucleotide polymorphisms (SNPs), small deletion/insertion polymorphisms (DIPs) and structural variations (SVs). These differences have been validated through Sanger sequencing. This variation affect also the expression of other gene located downstream the variation. The results led us to consider the possibility that the effect of a punctual transformation event might cause an overall genomic instability, and that recombination practices may potentially be responsible of unexpected, stochastic and henceforth off-target rearrangements throughout the genome.

POSTER SESSION ABSTRACTS
Session CS9 New tools for fungal biology
CS9W5

Wednesday 6th April
14:00 - 16:00

O'DONNELL Ronan *(1), HERNON Alan (2), WILLIAMS Katherine (3), TUOHY Maria (4), HENEGHAN Mary (1)*
(1) School of Life Sciences, Department of Science, Institute of Technology Sligo, Sligo, Ireland
(2) National Institute of Bioprocessing, Research and Training, Dublin, Ireland
(3) Institute for Organic Chemistry, University of Hanover, Hanover, GEermany
(4) Molecular Glycobiotechnology Group, Biochemistry, School of Natural Sciences, National University of Ireland, Galway, Ireland

Development of a Talaromyces emersonii 'Molecular toolkit', enabling the efficient expression of designer enzymes/enzyme cocktails for industrial applications.

Talaromyces emersonii is an aerobic, thermophilic ascomycete. In this species a broad range of enzymatic activities relating to cellulose, hemicellulose and pectin hydrolysis have been identified. The thermostable nature of these enzymes means that they are very valuable for industry; they can be used in the production of glucose through complete hydrolysis of crystalline cellulose, for processing materials such as leather, or as a food additive to improve the quality of the finished product. In industrial processes such as these, elevated temperatures are often useful to increase reaction rates and improve the efficiency of reactions. *T. emersonii* has been shown to outperform species such as *Trichoderma* and *Aspergillus* that are typically used for industrial enzyme production. Molecular biological techniques must be developed to transform *T. emersonii*, in order to increase product output and generate an industrially viable enzyme producer. In this research, methods of transformant selection have been identified, and regulatory elements from *T. emersonii* have been isolated. Plasmids that will be used in homologous gene expression have been designed. Transformation systems typically used in fungi will be tested and compared, in order to optimise the transformation of *T. emersonii*. This will generate a highly efficient procedure for delivery of genetic material.

POSTER SESSION ABSTRACTS
Session CS9 New tools for fungal biology
CS9W6

Wednesday 6th April
14:00 - 16:00

COSTA Patrícia *(1), COSTA Aline (2), PRADELLA José (1)*
(1) Laboratório Nacional de Ciência e Tecnologia do Bioetanol (CTBE), Centro Nacional de Pesquisa em Energia e Materiais (CNPEM) , Campinas, Brazil
(2) Faculty of Chemical Engineering, State University of Campinas, Campinas, Brazil

Cumulative mutation and new selection strategies for *Trichoderma harzianum* hypercelulolytic mutants

The use of classical mutagenesis techniques have been an attractive strategy to increase production of glicohidrolases from filamentous fungi, but the definition of an efficient selection method for filamentous fungi mutants remains a challenge. This study is aimed to the realization of the development of the new mutant *Trichoderma harzianum* PCE6 originated from the wild strain *T. harzianum* P49P11, as the production by mutagenic treatment using ethyl-methyl sulfonate (EMS) chemical agent, followed by selection using optimal selection strategies. The presented strategy comprehends of two rounds of cumulative mutation with EMS. The potential mutants obtained were selected by submerged cultivation in catabolic repression conditions, carried out in mini-fermenation using cellulose as inductor and increasing concentrations of glucose. The enzymatic extracts obtained by the mini-fermentation were then tested by mini-hydrolisis of standard pre-treated sugarcane bagasse. The selected strains using this methodology possessed advantages over the *T. harzianum* P49P11 wild strain and its PCE6 mutant, achieving superior cellulase and lower foam-generation production. This is a new strategy for direct selection of mutants for the bioethanol production process. In the near future these mutants will be sequenced in relevant regions of the DNA and compared with the parental and wild strains, indicating potential genes of interest aming cellulases overproduction.

POSTER SESSION ABSTRACTS
Session CS9 New tools for fungal biology
CS9W7

Wednesday 6th April
14:00 - 16:00

RUIJTEN Philip *(1), VAN LAARHOVEN Karel (1), PEETERS Loes (1), HUININK Henk (1), ADAN Olaf (1)*
(1) Eindhoven University of Technology, Eindhoven, Netherlands

Hyphal growth and water: the role of porous materials

Water in the indoor environment is quantified by the relative humidity, RH. In equilibrium, this RH determines the water activity and water content of porous materials, the substrate on which indoor fungi grow. The micro-geometric properties of the substrate influence the relation between the RH, the water activity and the water content. The influence of water activity on fungal growth is well established. The influence of water content, however, has been overlooked so far. Therefore, a real-time, non-destructive video microscopy method was developed and used to investigate the growth rate of *Penicillium rubens* on various substrates with controlled water content. Growth on gypsum was studied for samples both equilibrated with RH, and soaked with solutions of a specific water activity. It was found that moisture content and water activity have distinct effects: a higher moisture content leads to earlier colonization and higher hyphal extension rate. Subsequently, the behavior of *P. rubens* on glass is currently under investigation. This second substrate has the advantage of excluding nutrients as a parameter in the system, as well as an optical advantage. Only physiological events which do not require nutrients could be monitored. Therefore, time of germination and initial extension rate as a function of ambient relative humidity are analyzed. This is done using regular, as well as porous glass, thereby introducing again as a variable in the system.

POSTER SESSION ABSTRACTS
Session CS9 New tools for fungal biology
CS9W8

Wednesday 6th April
14:00 - 16:00

ARIF Rabia (1), LEE Siu Fai (2), ***SALEEM Muhammad (****1)*
(1) University of the Punjab, Botany Department , Lahore, Pakistan
(2) University of the Punjab, Botany Department , Lahore, Pakistan
(3) University of Melbourne, Genetics Department, Melbourne, Australia

Evaluating potential short sequence repeats (SSRs) markers and predictions of post- translational modifications of mating type A and translational Elo

Sordaria fimicola is found worldwide but little is known about its genetic variations and population structure in any region. To bridge this knowledge gap, we attempted to develop molecular markers to study the genetic diversity and population structure in the natural strains of *S. fimicola*. Based on the whole genome sequence of *Sordaria macrospora* we targeted 23 genomic regions that contain short sequence repeats (SSR). Chemical characterizations of post-translational modifications (PTMs) in proteins are critical to understand the regulatory mechanisms involving modified proteins and their role in different cellular processes. In this work the role of different modifications and their interplay, involving the regulating mating activity of MAT-A gene and TEF-1 gene, is investigated by various bioinformatics tools. Among these is the YinOYang prediction method, which predicts Yin Yang sites in proteins (sites, where O-glycosylation and phosphorylation may compete with each other). PTMs for kinase mediated phosphorylation and propeptide cleavage sites on Arginine/Lysine were also analysed. In addition to PTMs, an 18S small subunit rRNA gene and a 28S large subunit rRNA gene were also targeted and sequenced. Genetic homogeneity was observed between *S. fimicola* strains originating from the North- and South-facing slopes of the Evolution Canyon in case of SSRs and ITS region. The findings favour the alternative hypothesis that non-genetic mechanisms underpin successful adaptation to highly contrasting climatic conditions in this species.

POSTER SESSION ABSTRACTS
Session CS9 New tools for fungal biology
CS9W9

Wednesday 6th April
14:00 - 16:00

HAN Zhiping *(1), KAUTTO Liisa (1), SUN Angela (1), NEVALAINEN Helena (1)*
(1) Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, Australia

Secretion of proteases by *Scedosporium aurantiacum*

Scedosporium aurantiacum is an opportunistic filamentous fungus increasingly isolated from the sputum of cystic fibrosis (CF) patients, and is especially prevalent in Australia. Secreted proteases are classified as virulent factors in many fungal diseases. We have compared the profiles between a high-virulence clinical isolate and a low-virulence environmental strain of *S. aurantiacum* in different carbon sources (glucose, casein, mucin), in order to map out proteases secreted by this organism and identify proteases that may have a functional role in lung infection. While serine (trypsin and elastase) and aspartic proteases were produced by both strains on a medium containing mucin to mimic composition of the CF sputum, the enzyme activities were significantly higher (around 5-50 x) in the clinical isolate compared to the environmental strain. About 80% of the activity corresponded to serine proteases, which makes this group of enzymes a good candidate for closer investigation. Proteases secreted by the clinical isolate were further analyzed by mass spectrometry which confirmed the presence of a trypsin and an aspartic protease; however, elastase could not be identified. The work is progressing into cloning of the genes encoding the main serine proteases, pending on the availability of the annotated genome, and exploring the effects of the enzymes on lung cell cultures in vitro.

POSTER SESSION ABSTRACTS
Session CS9 New tools for fungal biology
CS9W10

Wednesday 6th April
14:00 - 16:00

BORMANN Joerg *(1), MENTGES Michael (1)*
(1) University of Hamburg, Biocenter Klein Flottbek, Department of Molecular Phytopathology, Hamburg, GERMANY

Real-time imaging of hydrogen peroxide dynamics in vegetative and pathogenic hyphae of *Fusarium graminearum*

Balanced dynamics of reactive oxygen species in the phytopathogenic fungus *Fusarium graminearum* play key roles for development and infection. To monitor those dynamics, ratiometric analysis using the novel hydrogen peroxide (H2O2) sensitive fluorescent indicator protein HyPer-2 was established for the first time in phytopathogenic fungi. H2O2 changes the excitation spectrum of HyPer-2 with an excitation maximum at 405 nm for the reduced and 488 nm for the oxidized state, facilitating ratiometric readouts with a maximum emission at 516 nm. HyPer-2 analyses were performed using a microtiter fluorometer and confocal laser scanning microscopy (CLSM). Addition of external H2O2 to mycelia caused a steep and transient increase in fluorescence excited at 488 nm. This can be reversed by the addition of the reducing agent dithiothreitol. HyPer-2 in *F. graminearum* is highly sensitive and specific to H2O2 even in tiny amounts. Hyperosmotic treatment elicited a transient internal H2O2 burst. Hence, HyPer-2 is suitable to monitor the intracellular redox balance. Using CLSM, developmental processes like nuclear division, tip growth, septation, and infection structure development were analyzed. The latter two processes imply marked accumulations of intracellular H2O2. Taken together, HyPer-2 is a valuable and reliable tool for the analysis of environmental conditions, cellular development, and pathogenicity.

POSTER SESSION ABSTRACTS
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CS9W11

Wednesday 6th April
14:00 - 16:00

MARK Caddick (2), ***HARB Omar*** *(1), HERTZ-FOWLER Christiane (2), ROOS David (1), JASON Stajich (3)*
(1) University of Pennsylvania, Philadelphia, USA
(2) University of Liverpool, Liverpool, UK
(3) University of California, Riverside, Riverside, USA

FungiDB: A functional genomic resource for fungi and oomycetes

FungiDB (http://FungiDB.org) is a free online database that enables data mining and analysis of the pan-fungal and oomycetes genomic sequences and functional data. As part of the Eukaryotic Pathogen Bioinformatics Resource Center (http://EuPathDB.org), FungiDB enables users to conduct sophisticated and integrated searches via an intuitive web-based graphical system. FungiDB contains genome sequence and annotation for over 70 species spanning the Oomycota water molds, fungi from Ascomycota, Basidiomycota, zygomycete and chytrid lineages; including pathogenic species from the *Cryptococcus*, *Histoplasma*, and *Coccidioides* genera. In addition to genomic sequence data and annotation, FungiDB includes whole genome polymorphism data, selected transcriptomic data based on RNA sequence, microarray experiments and all expressed sequence tag data from GenBank. All genomes in FungiDB are run through a standard analysis pipeline that generates additional data such as signal peptide and transmembrane domain predictions, GO term and EC number associations and orthology profiles. Input from the community (images, files, PubMed records, etc) can be added to FungiDB records (ie. gene pages) via user comments; these comments become immediately visible and searchable. The graphical user interface in FungiDB allows users to conduct in silico experiments that leverage the available data and analyses. For example, a search in FungiDB can identify all genes in Candida albicans that do not have orthologs in mammals, have a predicted signal peptide, are annotated as a kinase and are expressed under conditions of high oxygen stress. Results from any search can be further analyzed visually using a companion genome browser or through analysis tools such as genome ontology and metabolic pathway enrichment. All results and searches can be saved in a user"s profile or downloaded in multiple formats.

POSTER SESSION ABSTRACTS
Session CS9 New tools for fungal biology
CS9W12

Wednesday 6th April
14:00 - 16:00

KYEONGCHAE Cheong *(1), KIM Ki-Tae (1), JEON Jongbum (1), LEE Yong-Hwan (2)*
(1) Fungal Bioinformatics Laboratory, Seoul National University, Seoul, South Korea
(2) Department of Agricultural Biotechnology,Seoul National University, Seoul, South Korea

EMGD: Eukaryotic Mitochondrial Genome Database

Mitochondria in the eukaryotic cells convert carbon source into energy and contain its own genome, of which length is relatively short in comparison to the nuclear genome. Although large number of genomes were publicly released, there is still no comprehensive sequence repository and gene annotation platform for mitochondrial genomes. We established Eukaryotic Mitochondrial Genome Database (EMGD; http://emgd.riceblast.snu.ac.kr/) to provide better platform for comparative and evolutionary analyses. A total of 375 eukaryotic mitochondrial genomes including 267 pathogenic and saprotrophic fungi were collected and dissected for taxon-specific or class-specific features such as genome length, GC ratio, numbers of ORFs and tRNAs, and codon usages. To identify the conserved genes throughout eukaryotic mitochondrial genomes, gene clustering with tribeMCL was also conducted. Circular SNU Genome Browser was implemented in the web site to graphically display mitochondrial genomes. Taken together, EMGD would be a user-friendly and comprehensive platform for comparative and evolutionary analyses of mitochondrial genomes.

POSTER SESSION ABSTRACTS
Session CS9 New tools for fungal biology
CS9W13

Wednesday 6th April
14:00 - 16:00

IFTIKHAR Sehrish *(1), ALI SHAHID Ahmad (2), AHSAN HALIM Sobia (2)*
(1) Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan
(2) Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Lahore, Pakistan

In silico screening for identification of novel anti-fungal inhibitors of SDH by molecular docking, pharmacophore modeling and virtual screening.

Early blight is distributed worldwide, and is one of the most important foliage diseases caused by *Alternaria solani* (Ellis and Mart) Jones and Grout. The protective pesticides are mainly used to control the potato diseases however these pesticides do not always control the disease. Moreover, exponential increase in the use of pesticides has caused the development of pesticide-tolerant pathogen strains. Most of the fungicide inhibits respiratory system, protein and RNA synthesis of fungi. Inhibition of respiratory enzymes has severe consequences. Succinate dehydrogenase or respiratory Complex II is an enzyme complex is the only enzyme that participates in both the citric acid cycle and the electron transport chain. SDH inhibitors (SDHI) act as fungicides, and specifically inhibit fungal respiration; hence play an important role in the integrated management programs of many plant diseases. In the present work, in silico modeling approach was used to screen novel anti-fungal agents that targets SDH. Hence the three dimensional (3D) structure of *Alternaria solani* SDH was modeled. Virtual screening was conducted based on ligand based pharmacophore modeling and structure based docking approaches. Pharmacophore model was built by the existing 18 fungicides and used to screen 17,900,742 compounds retrieved from «Drug Like» category of ZINC database. The screening resulted in 50,000 compounds that were docked into SDH model by Autodock4. The top 1% of the docked hits (500 compounds) was re-scored by MOE. The docked poses of 500 compounds were inspected visually. Based on docking energies and interactions, 12 compounds were selected finally. The selected compounds mediate multiple hydrophobic and hydrophilic interactions within the ligand binding site of SDH. The selected compounds are now under experimental validation process. We believe these compounds can function as a starting point for the discovery of promising new SDHI fungicide candidates.

POSTER SESSION ABSTRACTS
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CS9W14

Wednesday 6th April
14:00 - 16:00

HERZOG Robert *(1), BÖLKER Michael (2), HENNICKE Florian (1)*
(1) Junior Research Group Genetics and Genomics of Fungi, Senckenberg Gesellschaft für Naturforschung, Frankfurt am Main, Germany
(2) Philipps-University Marburg, Department of Biology, Marburg, Germany

Carboxin resistance transformation of the poplar mushroom *Agrocybe aegerita*

The black poplar mushroom *Agrocybe aegerita* is a cosmopolitan commercially grown choice edible mushroom, which is also used as a model basidiomycete to study fruiting body formation. Here, we report transformation of the monokaryotic standard strain *A. aegerita* AAE-3-13 by using protoplasts derived from monokaryotic oidia. With the wild type strain AAE-3-13 being sensitive to the systemic fungicide carboxin (Cbx), sequence data from the whole-genome sequence of the parental dikaryotic strain *A. aegerita* AAE-3 were used to introduce a single point mutation (His237 to Leu) into the *A. aegerita* gene encoding the iron-sulfur protein subunit of succinate dehydrogenase. The mutated version of this gene conferred carboxin resistance (CbxR) when introduced into A. aegerita AAE-3-13. We used a linearised CbxR plasmid and suppose that it is integrated into the native *A. aegerita* succinate dehydrogenase iron-sulfur protein subunit locus. This dominant selection marker and the protoplast transformation protocol at hand, the edible mushroom *A. aegerita* has become available to molecular genetics approaches for functional characterisation of genes involved in mushroom development.

POSTER SESSION ABSTRACTS
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CS9W15

Wednesday 6th April
14:00 - 16:00

NØDVIG Christina Spuur *(1), HOOF Jakob Blæsbjerg (1), MORTENSEN Uffe Hasbro (1)*
(1) Technical University of Denmark, Kgs. Lyngby, Denmark

Oligonucleotide directed mutagenesis of *Aspergilli* genomes using CRISPR-Cas9 technology

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

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CS9W16

Wednesday 6th April
14:00 - 16:00

DÖRNTE Bastian (1), ***KÜES Ursula*** *(1)*
(1) Georg-August-Universität Göttingen, Göttingen, Germany

New trp1+ based marker system for sequential transformation of *Coprinopsis cinerea*

A mayor bottleneck in DNA-mediated transformation of fungi is the limited number of available selective marker genes, especially when sequential transformations of the same strain are necessary. In the model basidiomycete *Coprinopsis cinerea*, the trp1+ gene is commonly used as selection marker for transformation to complement trp1 auxotrophies. The trp1+ encoded tryptophan synthase is a bifunctional enzyme which catalysis the final two reactions in the biosynthetic pathway of tryptophan. The N-terminal A-domain is responsible for the conversion of indole-3-glycerol-phosphate into indole, while the C-terminal B-domain catalyzes the subsequent production of tryptophan from serine and indole. The trp1.1,1.6 mutant allele used in *C. cinerea* hosts for transformation carries a mutation in each domain, which prevents the strain from completing tryptophan biosynthesis. Taking advantage of this situation, we developed from a new set of vectors containing either just the A- or the B-domain encoding sequences (trpA and trpB). The new marker set enables the independent complementation of the distinct mutations in separate transformations. First, trpB needs to be transformed and indole must be added to the regeneration medium. The second transformation with trpA allows production of indole. Both markers have successfully been used in successive co-transformations with other genes.

POSTER SESSION ABSTRACTS
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CS9W17

Wednesday 6th April
14:00 - 16:00

SCHÄPE Paul *(1), KWON Min Jin (1), LENZ Swantje (1), NITSCHE Benjamin (1), MEYER Vera (1)*
(1) Department Applied and Molecular Microbiology, Institute of Biotechnology, TU Berlin, Berlin, Germany

A gene co-expression network as a tool to predict functional modules in *Aspergillus niger*

Aspergillus niger is a well-established cell factory in biotechnology used for the production of organic acids and enzymes since almost hundred years and with a published genome sequence since 2008. However, the function of only 2% of its predicted 14,000 genes has been studied so far and about 50% of the annotated ORFs encode hypothetical proteins. Gene co-expression network analysis is a powerful approach for the functional annotation of uncharacterized genes. It aims to find genes with a consistent, correlated expression pattern across phenotypically diverse samples or experimental conditions. Genes within shared expression profiles are tightly connected and are predicted to function in the same regulatory and/or functional pathway («guilt-by-association» approach). In order to identify tightly connected genes in *A. niger*, we have established a transcriptomics database using Affymetrix microarray data published for *A. niger*. The database includes 158 different cultivation conditions reflecting different carbon and nitrogen sources, starvation and stress conditions, conditions related to temporal and spatial stages during its life cycle, different cultivation concepts and many more. Using Bioconductor, pairwise correlation coefficients were calculated and pairs with a Spearman score higher than 0.7 were considered to have significant co-expression relationship and were connected by an edge in the network. The resulting gene co-expression network was furthermore enriched with a network created for shared putative transcription factor binding sites, a network predicting protein-protein interactions based on orthology to Saccharomyces cerevisiae and a network considering physical chromosomal co-localization. The functional modules predicted by the final network were investigated for gene content and validated based on published data for the function of known secretory pathway genes. These analyses supported the biological relevance of these modules, suggesting that the co-expression network obtained presents a valuable predictive tool for functional annotation of *A. niger* genes.

POSTER SESSION ABSTRACTS
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CS9W18

Wednesday 6th April
14:00 - 16:00

LEE Jungkwan *(1), KANG Yunhee (1), KIM Mi Ran (2), LEE Seung-Ho (2)*
(1) Department of Applied Biology, Dong-A University, Busan, South Korea
(2) Ginseng Research Division, National Institute of Horticultural & Herbal Science, Eumseong , South Korea

Enrichment and density quantification of the ginseng root rot pathogen *Cylindrocarpon destructans*

The soil-borne fungus *Cylindrocarpon destructans* is one of the most important pathogens causing root rot disease in ginseng cultivation and produces antimicrobial radicicol. The slow growth of this fungus compared with other plant pathogenic and saprophytic fungi in soil disturbs isolation of this fungus from soil and infected ginseng. We developed a selective medium for *C. destructans* using radicicol produced by this fungus. Supplementing of radicicol to medium inhibited the mycelia growth of other fungi but did not affect the growth of *C. destructans*. In addition, conidia germination of other fungal species except for *C. destructans* was inhibited in submerged culture supplemented with radicicol. After incubating soil that contains the fungal conidia in the medium for 48 h, we extracted genomic DNA and performed real-time PCR using specific primers for *C. destructans*. Amplification was successfully performed even when the soil sample contains only one conidium. This study provides a very efficient tool for isolating *C. destructans* and enrichment using this medium can be used for density quantification of this fungus in soil.

POSTER SESSION ABSTRACTS
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CS9W19

Wednesday 6th April
14:00 - 16:00

DELABONA Priscila *(1), RODRIGUES Gisele Nunes (1), ZUBIETA Mariana (1), LIMA Deise Juliana (1), CRUCELLO Aline (2), HORTA Maria Augusta Crivelente (2), FARINAS Cristiane Sanchez (3), PRADELLA José Geraldo Da Cruz (1), SEIBOTH Bernhard (4)*
(1) Brazilian Bioethanol Science and Technology Laboratory (CTBE), Brazilian Centre of Research in Energy and Materials (CNPEM), Campinas, Brazil
(2) Center for Molecular Biology and Genetic Engineering (CBMEG), University of Campinas (UNICAMP), Campinas, Brazil
(3) Embrapa Instrumentation, Agroenergy Laboratory, São Carlos, Brazil
(4) Institute of Chemical Engineering, University of Technology of Vienna, Vienna, Austria

Expression of biomass-degrading enzymes by xyr1 overexpression in *T. harzianum*

Recent demands for the production of biofuels from renewable carbon sources have led to increased interest in the hydrolysis of cellulose. A new strain of *T. harzianum* isolated from the Amazon rainforest was found to be a potential candidate for the formulation of optimized enzyme mixtures for the saccharification of plant biomass. The present results for GH production by *T. harzianum* cultivated under sugar cane bagasse submerged fermentation are superior to the values reported in the literature by *T. reesei* Rut 30 [1]. A proteomic study of *T. harzianum* grown in sugarcane bagasse identified 24 different glycoside hydrolases and four CBM proteins, within 12 different CAZy families. Most of the cellulase genes are regulated in a consistent manner, suggesting a fine-tuned cooperation of the respective transcription factors (TFs). Xylanase regulator 1 (XYR1) is considered the main activator of cellulase and hemicellulase gene expression, because its deletion eliminates cellulase induction by all inducers and also impairs the induction of different hemicellulase genes involved in xylan and arabinan degradation. To construct a strain constitutively expressing xyr1 the pRLMex30 plasmid [2] and the pki1 promoter was used. The results showed that the genes expression (CBHI, GH10, GH11, GH3, EGII, EGIII and AA9) and the enzymatic productivity using sugarcane bagasse bioreactor fermentation was increased by xyr1 overexpression in *T. harzianum*.

[1] Delabona, P.S., Cota, J., Hoffmam, Z.B., Paixao, D.A., Farinas, C.S., Cairo, J.P., Lima, D.J., Squina, F.M., Ruller,R.,Pradella, J.G. (2013) Bioresour Technol 131: 500-507
[2] Mach-Aigner, A.R., Pucher, M.E., Steiger, M.G., Bauer, G.E., Preis, S.J., and Mach, R.L. (2008) Appl Environ Microbiol 74: 6554-6562.

POSTER SESSION ABSTRACTS
Session CS9 New tools for fungal biology
CS9W20

Wednesday 6th April
14:00 - 16:00

APPELS Freek *(1), KRIJGSHELD Pauline (1), JANSEN Kaspar (2), WÖSTEN Han (1)*
(1) Utrecht University, Utrecht, Netherlands
(2) Delft University of Technology, Netherlands

Mycelium Design

Our economy that is based on finite resources should convert to a sustainable system. The use of fungal mycelium can contribute to this change. Fungi degrade plant-based materials such as low quality agricultural waste and turn these in an interconnected network of hyphae, called mycelium. This mycelium can be used as a novel biomaterial. In our research, *Schizophyllum* commune mycelium is grown in liquid media and subsequently subjected to chemical and physical treatments. The effects of these treatments on the properties of the mycelium were evaluated by tensile measurements. Both chemical and physical treatments impact the properties of the mycelium. Mycelium material with plastic, rubber, leather and paper-like properties have been obtained. Mycelium properties are also influenced by environmental conditions such as CO2 concentration. These findings suggest that fungal mycelium can be used to replace oil-based products and thus will contribute to the transformation to a sustainable economy.

POSTER SESSION ABSTRACTS
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CS9W21

Wednesday 6th April
14:00 - 16:00

MIYAUCHI Shingo *(1), NAVARRO David (1), GRIGORIEV Igor (2), LIPZEN Anna (2), BERRIN Jean-Guy (1), ROSSO Marie-Noëlle (1)*
(1) Biodiversité et Biotechnologie Fongiques, INRA, Université Aix Marseille, Marseille, France
(2) US Department of Energy Joint Genome Institute (JGI), California, USA

Applied Biomass Conversion Design for Fungal Green Technology (ABCDEFGT) for comparative fungal transcriptomics

The genomes of plant-associated fungi have recently been found to contain a striking diversity of lignocellulolytic enzymes. We have been studying transcriptomic and secretomic data to identify which particular groups of enzymes are responsible for the degradation of lignocellulose in complex plant biomass. The comparative analysis of fungi using the multi-omics approach gives insight into the strategies of the deconstruction of complex plant materials and potentially allows the design of combinations of enzymes for biotechnological applications. We have developed a workflow to simplify the analysis and interpretation of this combined transcriptomic and secretomic data. The workflow, Applied Biomass Conversion Design for Fungal Green Technology (ABCDEFGT) is made of customised R scripts incorporating self organising maps (SOMs), weighted gene correlation network analysis (WGCNA), and differential gene expression analysis (DESeq2). The workflow; 1) produces simple graphic outputs of genome-wide transcription; 2) enables the selection of genes based on the similar expression patterns with significant log2 fold changes; and 3) facilitates the integration of secretomic data. We investigated the early response of the saprotrophic white-rot fungus *Pycnoporus coccineus* to various carbon sources using ABCDEFGT. We identified 162, 41, and 39 up-regulated genes and the corresponding enzymes potentially involved in the degradation of pine (softwood), aspen (hardwood), or wheat straw (cereal) respectively. These included genes coding for plant cell wall degrading enzymes (ie. xylanases, CBM1-carrying proteins, class II peroxidases) and H2O2-generating enzymes (ie. peptidases, hydrophobins). The large number of the genes for the growth with pine was indicative of intensive metabolic adaptations to toxic compounds, exemplified by the finding of five genes coding for cytochrome P450. We also performed inter-species comparisons with *P. coccineus*, *P. cinnabarinus*, and *P. sanguineus* grown under the identical conditions. The results highlighted the importance of oxido-reductases, glycoside hydrolases, peptidases, expansins, and hydrophobins in the early response to complex plant-derived substrates. Our analysis demonstrates that ABCDEFGT is an RNA-seq data-mining pipeline suitable for comparative transcriptomics of non-model organisms.

POSTER SESSION ABSTRACTS
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CS9W22

Wednesday 6th April
14:00 - 16:00

PEPE Alessandra *(1), SBRANA Cristiana (2), FERROL Nuria (3), GIOVANNETTI Manuela (1)*
(1) Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy
(2) CNR, Institute of Agricultural Biology and Biotechnology, UOS Pisa, Pisa, Italy
(3) Department of Soil Microbiology and Symbiotic System, Estación Experimental del Zaidín, CSIC, Granada, Spain

An in vivo experimental system for gene expression analyses of extra-radical mycorrhizal networks

Arbuscular mycorrhizal fungi (AMF) establish beneficial mutualistic symbioses with land plants, receiving carbon in exchange for mineral nutrients absorbed by the extraradical mycelium (ERM) spreading from mycorrhizal roots into the soil. In a bi-dimensional experimental system (sandwich system), a *Funneliformis coronatus* isolate showed low hyphal self-recognition ability and self-incompatibility, resulting in lower-interconnected ERM compared with *Funneliformis mosseae* and *Rhizophagus intraradices*. With the aim of obtaining in vivo-produced ERM for gene expression analyses, the sandwich system was modified by wrapping *Cichorium intybus var. foliosum* colonised roots in a nylon net to facilitate ERM collection. ERM produced by *F. mosseae*, *R. intraradices* and *F. coronatus* was used for RNA extraction and the experimental system was validated by analysing ERM cDNA for AMTs gene expression of the three AMF by RT-PCR, using specific primers designed on available (*F. mosseae, R. irregularis*) and new (*F. coronatus*) AMT sequences. The analyses showed that AMT transporter genes were differentially expressed in the three AMF species and that relative transcription levels of *F. coronatus* ERM were significantly lower compared to the other two AMF.

POSTER SESSION ABSTRACTS
CS9W23

Wednesday 6th April
14:00 - 16:00

TWARUSCHEK Krisztian (1), SPÖRHASE Pia (1), WIESENBERGER Gerlinde (1), ***ADAM Gerhard*** *(1)*
(1) Univ. of Natural Resources and Life Sciences, Vienna (BOKU), Dept. of Applied Genetics and Cell Biology, Tulln, Austria

New plasmids allowing positive-negative selection for fungal transformation and efficient Cre-loxP mediated marker recycling

In filamentous fungi disruption of multiple genes of interest in the same strain (e.g. to test for redundant gene function) is a difficult task due to the limited availability of different reliable and efficient selection markers. In *S. cerevisiae*, the Cre-lox system can be efficiently used to remove loxP-flanked resistance cassettes. Activation of Cre gene expression from an inducible promoter (PXYN1) [1] was inefficient to evict the selection marker in a self-excising cassette in *Fusarium graminearum*. To reduce the screening effort, we have created a series of hybrid fusion genes which allow positive selection of transformants in the first step and subsequent negative selection for marker removal. The constructs consist of *Herpes simplex* thymidine kinase (HSV-TK) to which the commonly used drug resistance markers hph, nptII and nat1 (conferring resistance to hygromycin B, geneticin and nourseothricin) were fused c-terminally. For removal of the loxP flanked resistance cassettes protoplasts of transformants were directly treated with purified Cre recombinase protein [2]. Loss of HSV-TK containing cassette can be selected by restoration of resistance to 5-fluoro-2-deoxyuridine (5-F2DU). The fusion genes expressed under the *Trichoderma* PKI promoter are also conferring antibiotic resistance in *E. coli*, allowing straightforward construction of disruption plasmids (e.g. by Gibson assembly).

[1] MG Steiger et al. (2011) Appl. Environ. Microbiol., 77, 114-121.
[2] O Mizutani et al. (2012) Appl. Environ. Microbiol., 78, 4126-4133.

POSTER SESSION ABSTRACTS
Session CS9 New tools for fungal biology
CS9W24

Wednesday 6th April
14:00 - 16:00

BRANKOVICS Balázs *(1), VAN DIEPENINGEN Anne D. (1), VAN DER LEE Theo A.j. (3), WAALWIJK Cees (3), DE HOOG G. Sybren (1)*
(1) CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands
(2) Wageningen University and Research Centre, Wageningen, Netherlands

GRAbB: Genomic Region Assembly by Baiting, a new niche for genomic research

Although, the costs of sequencing the whole genome of fungal species are approaching the costs of multi locus sequencing, many research groups hesitate to enter the field of genomics, because they lack high performance computers that are generally used for genome assemblies. Our group has developed a program, GRAbB (Genomic Region Assembly by Baiting), that can selectively assemble regions of the genome from next generation sequencing reads. By only assembling the region of interest, the process of assembly is computationally less demanding and can be handled by a regular computer. By applying this approach it is feasible to assemble mitochondrial genomes, plasmid sequences and/or viral genomes. In addition, the program can extract homologous sequences based on a reference or explore the vicinity of known sequences, like bar-coding genes. The homology approach is a valuable tool to extract regions for comparative analysis or screen for the presence or absence of genes. Basically, research objectives that could be tackled by PCR-based approaches can be performed in silico with GRAbB using the reads generated by next generation sequencing with the added advantage that further experiments can be run on the same data set. This also means that existing read files can be used for further analysis. We demonstrate the applicability of our program to a diverse range of applications: assembly of mitochondrial genomes, rDNA regions (18S rRNA - ITS1 - 5.8S rRNA - ITS2 - 28S rRNA - IGS), barcoding markers, RNA genomes of viruses and mating type locus sequences. For these analyses we used WGS data from different *Fusarium oxysporum*, *F. graminearum* and *F. poae* strains. GRAbB is capable to extract the specific region even if the reference sequence is from a related species, exemplified by the fact that it was capable to extract the sequence of the mating type locus from strains having either idiomorph using the same reference.

POSTER SESSION ABSTRACTS
Session CS9 New tools for fungal biology
CS9W25

Wednesday 6th April
14:00 - 16:00

HOOF Jakob *(1), NØDVIG Christina Spuur (1), MORTENSEN Uffe Hasbro (1)*
(1) Department of Systems Biology, Technical University of Denmark (DTU), Kgs. Lyngby, Denmark

Genetic engineering in the *Aspergillus genus*

The genus *Aspergillus* comprises more than 300 species, and enormous diversity exist within the group. All the known species are currently in the pipeline for full genome sequencing. This will eventually open up for a need to perform genetic engineering endeavors, which requires that the species is amenable to it. We have recently shown that the CRISPR-Cas9 gene editing system is a versatile and useful technology to apply to *Aspergillus* species where no genetic markers is available. We therefore set out to examine how the CRISPR-Cas9 system performs in a selection of newly sequenced *Aspergillus*, where there are no reports of genetic engineering. Due to size of this project, we have decided to use the project for educational purposes where DTU students at various levels systematically attempt to test whether it is possible to transform the *Aspergillus* species. If possible, they investigate how CRISPR-Cas9 works in the different strains, and establish pyrG mutant strains.

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Benjamin A. Horwitz
Faculty of Biology, Technion
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Novozymes fact sheet

Company profile
Novozymes is the world leader in bioinnovation. Our business is industrial enzymes and microorganisms.

Enzymes are proteins, and in nature they initiate biochemical reactions in all living organisms. It is enzymes that convert the food in our stomachs to energy and turn the falling leaves in the forest to compost.

Novozymes finds enzymes in nature and optimize them for use in industry. In industry, enzymes replace chemicals and accelerate production processes. They help our customers make more from less, while saving energy and generating less waste.

Enzymes are widely used in laundry and dishwashing detergents where they remove stains and enable low-temperature washing and concentrated detergents.

Other enzymes improve the quality of bread, beer and wine, or increase the nutritional value of animal feed. Enzymes are also used in the production of biofuels where they turn starch or cellulose from biomass into sugars which can be fermented to ethanol.

These are just a few examples as we sell enzymes to more than 40 different industries.

Like enzymes, microorganisms have natural properties that can be put to use in a variety of processes. Novozymes supplies a range of microorganisms for use in agriculture, animal health and nutrition, industrial cleaning and wastewater treatment.

Novozymes is quoted on NASDAQ OMX Copenhagen (NZYM B).

Headquarters and affiliates
- Novozymes' headquarters are located in Bagsvaerd just outside of Copenhagen, Denmark
- Production in Argentina, Brazil, Canada, China, Denmark, England, India and the United States.
- Affiliates and sales offices in more than 30 countries

History
Novozymes was founded in 2000 in a demerger from pharmaceutical company Novo Nordisk. Novozymes' roots date back to the 1920s when *Novo Terapeutisk Laboratorium* and *Nordisk Insulinlaboratorium* were established in Copenhagen, Denmark. Enzyme production began in 1941.

Executive leadership team
- President & CEO: Peder Holk Nielsen
- Corporate Functions: EVP & CFO Benny D. Loft
- Research, Innovation & Supply: EVP & COO Thomas Videbæk
- Agriculture & Bioenergy division: EVP Tina Sejersgård Fanø
- Food & Beverages division: EVP Andrew Fordyce
- Household Care & Technical division: EVP Anders Lund

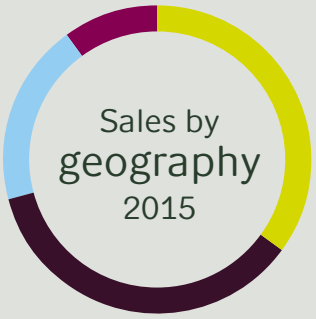
Sustainability
Together, we find biological answers for better lives in a growing world. Let's Rethink Tomorrow. This is Novozymes' purpose statement. Novozymes strives to have great impact by balancing good business for our customers and our company, while spearheading environmental and social change.

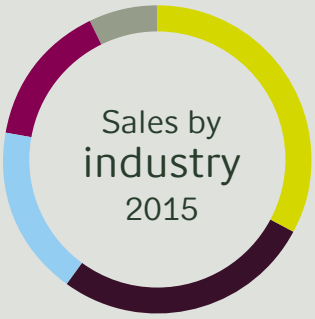
In 2015, the worldwide application of our products helped our customers reduce their CO_2 emissions by an estimated 60 million tons. It is our ambition to enable a 100 million ton reduction in CO_2 emissions in 2020.

The Dow Jones Sustainability Index, a global sustainability benchmark, ranked Novozymes among the top 3 percent of companies in the chemical industry sector in 2015. Novozymes received a gold medal in the RobecoSAM Sustainability Yearbook in 2010-2013 and a bronze medal in 2014-2015.

Key figures

- Sales: DKK 14.002 billion (2015)
- EBIT margin: 27.7% (2015)
- Market share in industrial enzymes: 48% (2015)
- R&D: We invest ~14% of our revenue in research and development. Novozymes holds over 7,000 granted or pending patents
- Workforce: 6,485 employees. Denmark (2,715), North America (1,322), Asia Pacific (1,815), Europe (256), Latin America (377) (January 2016)





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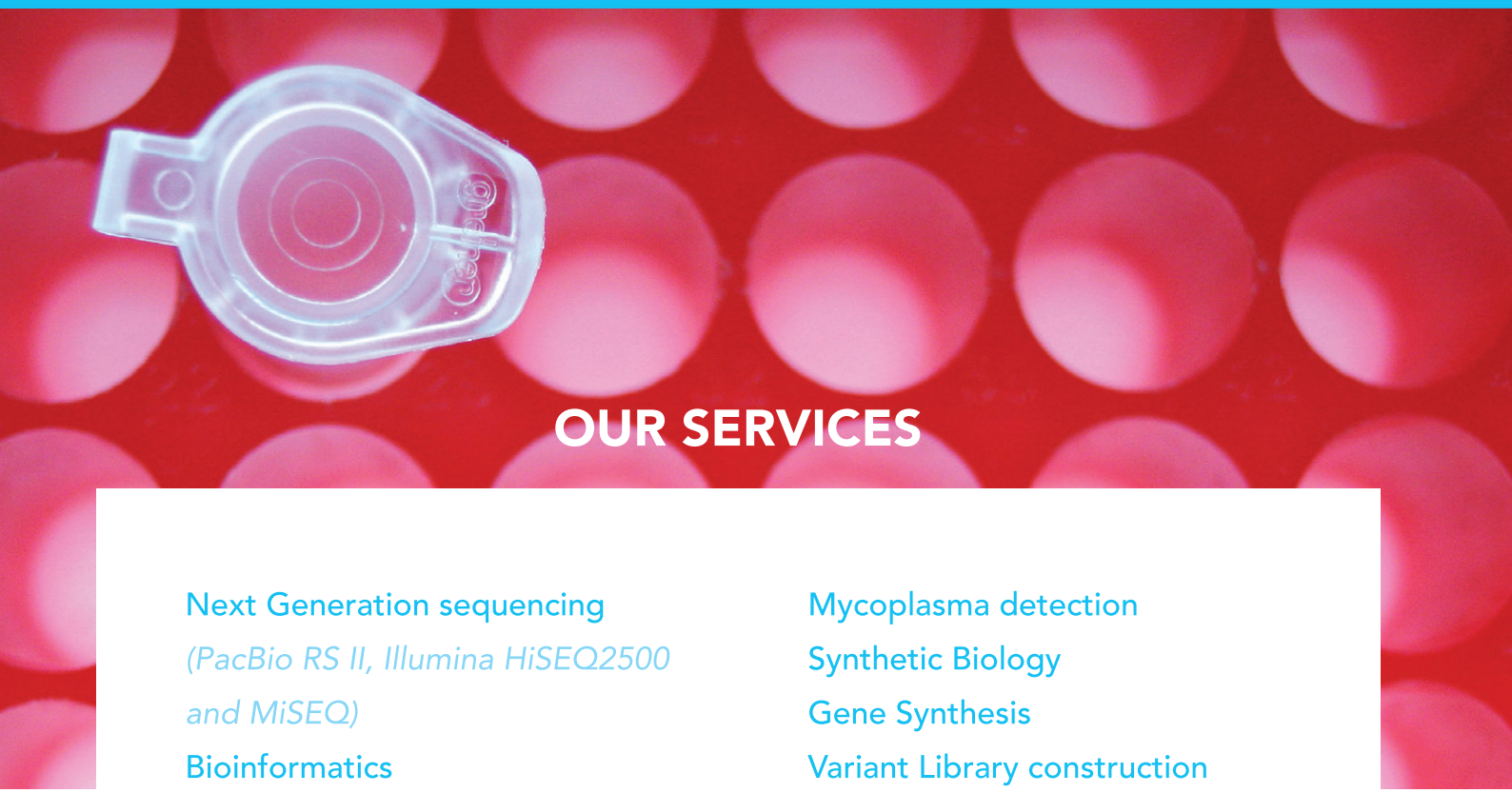

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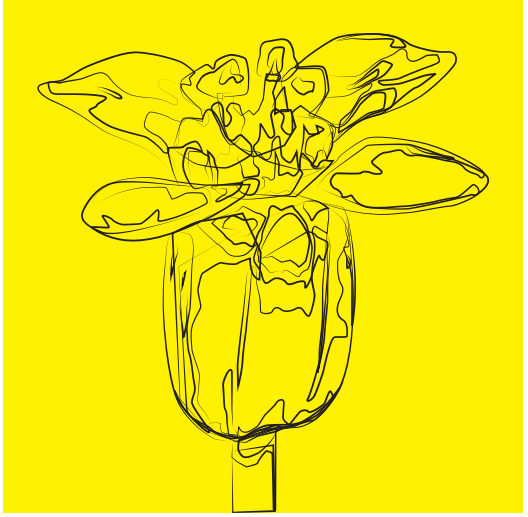

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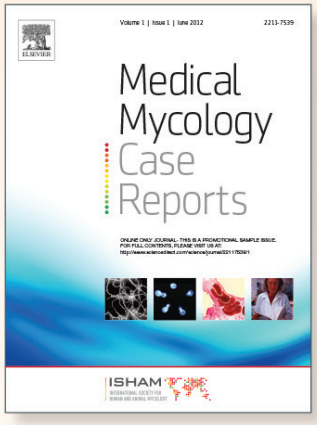

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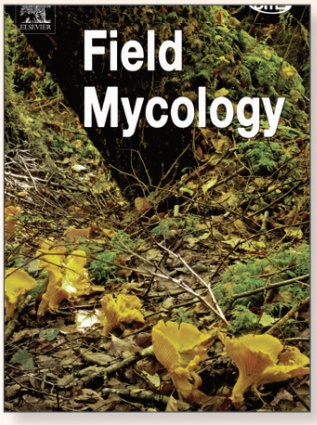

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