ECFG14
HAIFA 2018

14th European Conference on Fungal Genetics

February 25-28, 2018, Haifa, Israel

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WELCOME

Welcome to ECFG14! The unifying theme of the conference is fungal genetics, linking cell signaling and dynamics, gene expression, genome sequence and structure, interactions with hosts and symbiotic partners, molecular evolution, ecology, and environmental and applied mycology. The Technion is especially pleased to host this conference, with its interdisciplinary combination of fundamental and technological topics. Satellite workshops will add fields of interest including a focus on specific organisms, and on topics of immediate agricultural or medical issues. The Technion and the city of Haifa are honored to host this international meeting, and we hope you also had a chance to experience the cultural viewpoints of our small but vibrant Mediterranean city, as well as other points of historical, cultural and natural interests which comprise the diverse country of Israel.

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SCIENTIFIC PROGRAM

Sunday, February 25, 2018

09:00-18:00 **SATELLITE WORKSHOPS**

16:00-19:30 ***ECFG14 Conference Registration***

18:00-19:30 ***WELCOME RECEPTION***

19:30-20:00 **OPENING SESSION**

OPENING REMARKS:

- **Benjamin A. Horwitz,** Co-Chair, ECFG14
- **Peretz Lavie,** President, Technion-Israel Institute of Technology
- **Yehuda Assaraf,** Dean, Faculty of Biology, Technion-Israel Institute of Technology
- **Marc-Henri Lebrun,** President of ECFG
- **Yona Yahav,** Mayor of Haifa

20:00-20:45 **KEYNOTE LECTURE 1**

Chair: **Oded Yarden,** The Hebrew University of Jerusalem, Israel

20:00 **Arturo Casadevall**
Johns Hopkins University, USA
Thoughts on the Origin of Fungal Virulence

Monday, February 26, 2018

Location: **TECHNION - FACULTY OF MEDICINE**

From 08:30 ***Registration***

09:00-09:45 **KEYNOTE LECTURE 2**

Chair: **Daniel Kornitzer,** Technion-Israel Institute of Technology, Israel

09:00 **Judith Berman,** Tel Aviv University, Israel
Genome and population dynamics in pathogenic yeasts

Monday, February 26, 2018

09:45-12:45 PLENARY SESSION 1: DEVELOPMENT AND CELL BIOLOGY

Sponsored by: BRITISH MYCOLOGICAL SOCIETY

Chairs: **Stefanie Pöggeler,** Georg-August University Göttingen, Germany
Han Wösten, Utrecht University, Netherlands

09:45 **Philippe Silar,** *Université Paris Diderot, France*
The crippled growth epigenetic cell degeneration of podospora anserina, an overview

10:15 **André Fleissner**, *Technische Universitaet Braunschweig, Germany*
Fungal Dialogs: Intra- and interspecies communication in filamentous fungi

10:45-11:15 Coffee Break

11:15 **Meritxell Riquelme**, *Centro de Investigación Científica y de Educación Superior de Ensenada, CICESE, Mexico*
The secretory pathway of cell wall building enzymes in *Neurospora crassa* hyphae

11:45 **JinRong Xu**, *Purdue University, USA*
A-to-I RNA editing during sexual reproduction in filamentous ascomycetes

12:15 **Alex Brand,** *University of Aberdeen, UK*
Elucidating three pathways that contribute to directional growth regulation in *Candida albicans* hyphae

12:45-13:45 Lunch Break

13:45-15:30 POSTER SESSION 1

15:30-18:30 *Concurrent Sessions*: CS1; CS2; CS3; CS4

15:30-18:30 CS1: DEVELOPMENT AND CELL BIOLOGY

Chairs: **Miguel A. Peñalva**, CSIC, Spain
Oded Yarden, The Hebrew University of Jerusalem, Israel

15:30 **Takeshita Norio,** *University of Tsukuba, Japan and Karlsruhe Institute of Technology (KIT), Germany*
Pulses of Ca2+ coordinate actin assembly and exocytosis for stepwise cell extension

15:55 **George Diallinas,** *National and Kapodistrian University of Athens, Greece*
Roles of the AP-2 and AP-1 complexes in apical cargo sorting, endocytosis and polar growth in fungi

16:20 **Miguel A. Peñalva**, *CSIC Centro de Investigaciones Biológicas, Spain*
Endocytic recycling underlies the polarized hyphal mode of life

16:45-17:15 Coffee Break

Monday, February 26, 2018

CS1: DEVELOPMENT AND CELL BIOLOGY (cont'd)

17:15 **Stefanie Pöggeler**, *Georg-August University, Germany*
Pexophagy and fruiting-body formation in *Sordaria macrospora*

17:40 **Elke-Martina Jung**, *Friedrich Schiller University, Germany*
Fungal growth and development visualization in *Schizophyllum commune*

18:05 **Ursula Kües,** *University of Göttingen, Germany*
Fruiting body development in the Basidiomycete *Coprinopsis cinerea*

15:30-18:30 CS2: APPLIED AND INDUSTRIAL MYCOLOGY

Sponsored by: NOVOZYMES

Chairs: **Taina Lundell,** University of Helsinki, Finland
Yitzhak Hadar, The Hebrew Univesity of Jerusalem, Israel

15:30 **Peter Punt**, *Dutch DNA Biotech, The Netherlands*
A unique protease regulatory gene from *Trichoderma reesei*

15:55 **Markku Saloheimo**, *VTT Technical Research Centre of Finland Ltd., Finland*
Controlling the expression of the *Trichoderma reesei* subtilisin protease gene *slp2* leads to improved biotherapeutic protein production

16:20 **Stefany Solano**, *Institute of Integrative Biology, UK and Universidad Nacional, Costa Rica*
Genomic annotation of a mannosylerithritol lipid yeast producer as an approach to unravel its metabolic pathway

16:45-17:15 *Coffee Break*

17:15 **Taishi Inoue**, *Graduate School of Agricultural Science, Tohoku University, Japan*
Comparative analysis for transcription start sites of enolase genes in *Aspergillus oryzae* and *Aspergillus nidulans*

17:40 **Aurore Labourel**, *INRA, Aix Marseille University, France*
Basidiomycetes secretomes: an under-exploited tank of new plant-polysaccharide-depolymerizing enzymes

18:05 **Mari Mäkinen**, *University of Helsinki, Finland*
Transcriptomics of white rot fungal decay of spruce wood and bioconversion of waste lignocellulose substrates by the Polyporales species *Phlebia radiate*

15:30-18:30 CS3: PHYSIOLOGY AND METABOLISM Green Hall

Chairs: **Richard A. Wilson,** University of Nebraska-Lincoln, USA
Dov Prusky, Agricultural Research Organization, Volcani Center, Israel

15:30 **Nicholas Talbot,** *University of Exeter, UK*
Investigating the biology of plant tissue invasion and cell-to-cell movement by the rice blast fungus *Magnaporthe oryzae*

CS3: PHYSIOLOGY AND METABOLISM (cont'd)

15:55 **Michael Thon,** *University of Salamanca, Spain*
Evolution of host range is associated with carbohydrate and protein metabolism in *Colletotrichum* spp.

16:20 **Martin Tegelaar,** *Utrecht University, Netherlands*
Functional distinction of hyphal compartments

16:45-17:15 *Coffee Break*

17:15 **Lars Voll,** *Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany and Philipps-University Marburg, Germany*
Foliar sugar accumulation enhances priming of the salicylic acid-mediated defense response

17:40 **Richard B. Todd,** *Kansas State University, USA*
Branched chain amino acid biosynthesis genes and regulators in *Aspergillus nidulans*

18:05 **Johan Philipp Benz**, *Technical University of Munich, Germany*
A taste for 'sour' sugars: characterization of a highly efficient D-galacturonic acid metabolism in the basidiomycete yeast genus *Rhodosporidium*

15:30-16:45 CS4: SYMBIOSIS AND ENDOPHYTES

Chairs: **Carolyn Young,** Noble Research Institute, USA
Amir Sharon, Tel Aviv University, Israel

15:30 **Gregor Langen,** *University of Cologne, Germany*
Characterization of the molecular mechanisms underpinning local and systemic responses in root-microbe multispecies interactions

15:55 **Annegret Kohler**, *Institut National de la Recherche Agronomique, France*
The impact of fungal genotypes and species on the transcriptomic landscape of ectomycorrhizal symbioses

16:20 **Carolyn Young,** *Noble Research Institute, USA*
Seed transmitted endophytes of crop wild relatives

16:45-17:15 *Coffee Break*

17:15 **Stanley Freeman,** *Agricultural Research Organization, Volcani Center, Israel*
Interactions between three symbiotic fungi associated with an invasive ambrosia beetle and their host trees in Israel

17:40 **Gunther Doehlemann**, *University of Cologne, Germany*
Ustilaginomycete yeasts as hub organisms in leaf microbial communities

18:05 **Daohong Jiang,** *State Key Laboratory of Agricultural Microbiology, China*
Exploring hypovirulence-associated DNA mycovirus to be a natural fungicide

Tuesday, February 27, 2018

09:15-09:45 KEYNOTE LECTURE 3

Chair: **Dov Prusky,** Agricultural Research Organization, Volcani Center, Israel

09:15 Sophien Kamoun, *The John Innes Centre, UK*
The EMBO Lecture:
BLASTOFF-Keeping up with the plant destroyers

09:45-10:45 PLENARY SESSION 2: FUNGAL-HOST INTERACTIONS

Chairs: **Regine Kahmann,** Max Planck Institute for Terrestrial Microbiology, Germany
Anita Sil, University of California San Francisco, USA

09:45 Neil Gow, *University of Aberdeen, UK*
Use of antibodies from single human B cells and C-type-lectin probes to map the dynamic cell surface of fungal pathogens

10:15 Alga Zuccaro, *University of Cologne, Germany and Max-Planck-Institute for Terrestrial Microbiology, Germany*
Effector-mediated suppression of extracellular ATP-triggered immunity by the root endophyte *Serendipita indica*

10:45-11:15 Coffee Break

11:15 Antonio Di Pietro, *Universidad de Córdoba, Spain*
Host adaptation in the cross-kingdom pathogen *Fusarium oxysporum*

11:45 Chengshu Wang, *Chinese Academy of Sciences, China*
Genetics and molecular biology of fungus-insect interactions

12:15 Amir Sharon, *Tel Aviv University, Israel*
Is fungal A-PCD the Achilles heel of plant and human killer pathogens?

12:45-13:45 Lunch Break

13:45-15:30 POSTER SESSION 2

15:30-16:45 *Concurrent Sessions*: CS5; CS6; CS7; CS8

15:30-18:30 CS5: GENOMES, CHROMOSOMES AND EPIGENETICS

Sponsored by: AB ENZYMES

Chairs: **Michael Freitag,** Oregon State University, USA
Shay Covo, The Hebrew University of Jerusalem, Israel

15:30 Like Fokkens, *University of Amsterdam, Netherlands*
Pathogenicity chromosomes as Trojan horses: the costs of mobile DNA in pathogen evolution

15:55 Michael Habig, *Environmental Genomics, Germany*
Function or Drive – Why are the Accessory Chromosomes of *Zymoseptoria tritici* maintained*?*

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CS5: GENOMES, CHROMOSOMES AND EPIGENETICS (cont'd)

16:20 **Ting-Fang Wang,** *Academia Sinica, Taiwan*
Genome-wide mapping of meiotic recombination products generated by hybrid crossing of two *Trichoderma reesei* wild isolates QM6a and CBS999.97

16:45-17:15 Coffee Break

17:15 **Astrid Mach-Aigner,** *TU Wien, Austria*
Long non-coding RNA meets transactivator for regulation of cellulase expression

17:40 **Yi Liu,** *University of Texas Southwestern Medical Center, USA*
DNA replication is required for circadian clock function by regulating rhythmic nucleosome composition

18:05 **Shay Covo,** *The Hebrew University of Jerusalem, Israel*
Genomics of fungal DNA repair

18:15 **Michael Freitag,** *Oregon State University, USA*
Facultative heterochromatin in *Fusarium*: Control of gene regulation by Polycomb Repressive Complex 2

15:30-18:30 CS6: FUNGAL-HUMAN INTERACTIONS

Chairs: **Christophe d'Enfert,** Institut Pasteur, France
Ronen Ben-Ami, Tel Aviv Medical Center, Israel

15:30 **Neta Shlezinger**, *Memorial Sloan Kettering Cancer Center, USA*
Sterilizing immunity in the lung relies on targeting fungal apoptosis-like programmed cell death

15:55 **Bettina Böttcher**, *Friedrich Schiller University, Germany and Leibniz Institute for Natural Product Research and Infection Biology - Hans Knoell Institute, Germany*
Extracellular alkalinization drives the development of pathogenic *Candida albicans* biofilms

16:20 **Bhawna Yadav**, *University of Aberdeen, UK*
Role of *MNN1* gene family in *Candida albicans* in mannan structure and host immune recognition

16:45-17:15 Coffee Break

17:15 **Falk Hillmann,** *Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute (HKI), Germany*
Copper and redox homeostastis of human pathogenic fungi are targeted by the mycophagous amoeba *Planoprotostelium fungivorum*

17:40 **Udita Roy,** *Technion-Israel Institute of Technology, Israel*
Structural and functional analysis of CFEM proteins for hemoglobin iron acquisition

18:05 **Ulrich Terpitz,** *Julius Maximilian University, Germany*
Microscopic analysis of filamentous fungi and their interaction with immune cells

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15:30-18:30 CS7: FUNGI IN THE ENVIRONMENT

Chairs: **Petr Baldrian,** Institute of Microbiology of the CAS, Czech Republic
Nina Gunde-Cimerman, University of Ljublijana, Slovenia

15:30 **Fred Asiegbu,** *University of Helsinki, Finland*
Mycobiome and metatranscriptomic analysis of asymptomatic and symptomatic Norway spruce trees naturally infected by the conifer pathogen *Heterobasidion* spp

15:55 **Petr Baldrian,** *Institute of Microbiology of the CAS, Czech Republic*
Fungi in the forest ecosystem: habitats, diversity, and contribution to ecosystem processes

16:20 **Daria Feldman**, *The Hebrew University of Jerusalem, Israel*
Are small secreted proteins (SSPs) regulators of secondary metabolism in the white rot fungus *Pleurotus ostreatus?*

16:45-17:15 Coffee Break

17:15 **Sebastian Franz Josef Micheller,** *Swiss Federal Institute of Technology, Zürich, Switzerland*
Bacteria-induced defense responses in the filamentous fungus *Coprinopsis cinerea*

17:40 **Cene Gostinčar**, *University of Ljubljana, Slovenia*
The curious case of the black yeast *Hortaea werneckii* and its genome duplication

18:05 **Zheng Wang,** *Naval Research Laboratory, USA*
Omics and epigenetic investigation of radiation resistance mechanism in melanized fungi

15:30-18:30 CS8: PLANT-FUNGAL INTERACTIONS

Chair: **Maggie Levy,** The Hebrew University of Jerusalem, Israel

15:30 **Nicole Ludwig,** *Max Planck Institute for Terrestrial Microbiology, Germany*
A protein complex formed by four *Ustilago maydis* effectors is essential for virulence

15:55 **Maggie Levy,** *Robert H. Smith faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel*
A novel *Botrytis cinerea* MFS transporter provides tolerance towards glucosinolate breakdown products and act as virulence factor

16:20 **Ayako Tsushima,** *The University of Tokyo, Japan and RIKEN, Japan*
Transposable elements contribute to the evolution of genomic diversity between strains of the plant pathogenic fungus *Colletotrichum higginsianum*

16:45-17:15 Coffee Break

17:15 **Thorsten Langner**, *Norwich Research Park, UK*
Structure-guided engineering of synthetic immune receptors against the blast fungus

17:40 **Anna Avrova,** *James Hutton Institute, UK*
Rhynchosporium commune effectors and their potential role during barley colonisation

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CS8: PLANT-FUNGAL INTERACTIONS (con'd)

18:05 **Sayo Kodama**, *Kyoto Prefectural University, Japan*
MTF4 is a key transcription factor downstream of MOR required for plant-derived signal dependent appressorium development in *Colletotrichum orbiculare*

Wednesday, February 28, 2018

09:00-09:45 KEYNOTE LECTURE 4

Chair: **Benjamin A. Horwitz,** Technion-Israel Institute of Technology, Israel

09:00 **Hanna Johannesson,** *Uppsala University, Sweden*
The fungal individual: an evolutionary perspective

09:45-12:45 PLENARY SESSION 3: EVOLUTION AND MOLECULAR ECOLOGY

Sponsored by: ZYMERGEN

Chairs: **Luis Corrochano,** University of Seville, Spain and
Francis Martin, Institut National de la Recherche Agronomique (INRA), France

09:45 **Eva Stukenbrock,** *Max Planck Institute for Evolutionary Biology, Germany and Christian-Albrechts University of Kiel, Germany*
The role of histone-methyltransferases KMT1 and KMT6 in genome stability and chromatin organization in the wheat pathogen *Zymoseptoria tritici*

10:15 **Laszlo Nagy,** *BRC-HAS, Hungary*
Complex multicellularity in fungi: the genetic underpinnings of convergent origins

10:45-11:15 *Coffee Break*

11:15 **Toni Gabaldón,** *CRG, Spain*
Recent evolution of the opportunistic human pathogen *Candida glabrata*

11:45 **Li-Jun Ma,** *University of Massachusetts Amherst, USA*
Genome evolution and functional adaptation of the species complex *Fusarium oxysporum*

12:15 **Stephen J. Mondo**, *Joint Genome Institute, USA*
Widespread adenine N6-methylation of active genes in fungi

12:45-13:45 ***Lunch Break*** *Dining Room*

13:45-15:00 POSTER SESSION 3 Foyer

Wednesday, February 28, 2018

15:00-18:00 *Concurrent Sessions: CS9; CS10; CS11; CS12*

15:00-18:00 CS9: SYNTHETIC BIOLOGY AND SECONDARY METABOLITES Ruth Auditorium

Sponsored by: MDPI TOXINS

Chairs: **Luis Larrondo,** Pontificia Universidad Católica de Chile, Chile
Axel Brakhage, Hans-Knöll-Institut (HKI), Germany

15:00 **Dominik Mojzita,** *VTT Technical Research Centre of Finland Ltd., Finland*
SES: Universal expression system for fungi

15:25 **Christian Derntl**, *TU Wien, and University of Natural Resources and Life Sciences, Austria*
Inducible expression of secondary metabolites using a synthetic transcription factor in an essentially background-free system

15:50 **Tina Netzker**, *Leibniz Institute for Natural Product Research and Infection Biology (HKI), Germany*
Genome-wide chromatin mapping of *Aspergillus nidulans* reveals BasR, a novel regulator of bacteria-triggered fungal natural product biosynthesis

16:15-16:45 Coffee Break

16:45 **Luis Larrondo**, *Pontificia Universidad Católica de Chile, Chile*
Fungal optogenetics: imagening biotechnological applications and imaging gene expression

17:10 **Rebecca Shapiro**, *Broad Institute, and MIT, USA*
A CRISPR Cas9-based gene drive platform for studying complex genetic interactions in *Candida albicans*

17:35 **Rasmus J.N. Frandsen**, *The Technical University of Denmark, Denmark*
Design of an *Aspergillus nidulans* cell factory for the production of the natural food pigment carmine based on an artificial type II/III PKS system

15:00-18:00 CS10: ANTIFUNGALS AND FUNGICIDES

Chairs: **Sabine Fillinger,** INRA, France
Nir Osherov, Tel Aviv University, Israel

15:00 **Gustavo H. Goldman**, *Universidade de São Paulo, Brazil*
The *Aspergillus fumigatus* CrzA transcription factor activates chitin synthase gene expression during the caspofungin paradoxical effect

15:25 **Andreas Mosbach**, *Syngenta Crop Protection AG, Switzerland*
Studies on anilinopyrimidine resistance and mode of action in *Botrytis cinerea* suggest a mitochondrial target

15:50 **Michael Bromley**, *University of Manchester, UK*
Regulation of Azole Resistance in Aspergillus fumigatus

16:15-16:45 Coffee Break

Wednesday, February 28, 2018

CS10: ANTIFUNGALS AND FUNGICIDES (cont'd)

16:45 **Liang Ma**, *Tel Aviv University, Israel*
UDP-KDG, a transient antifungal metabolite, weakens fungal cell wall partly by inhibition of UDP-galactopyranose mutase

17:10 **Jane Usher,** *University of Exeter, UK*
Attenuating the emergence of drug resistance by harnessing synthetic lethal interactions in a model organism

17:35 **Eduardo H. Goulin**, *Instituto Agronômico, Brazil*
RNA interference in *Colletotrichum abscissum,* causal agent of citrus postbloom fruit drop

15:00-18:00 CS11: EVOLUTION AND TAXONOMY

Sponsored by: MDPI GENES

Chairs: **Bruce McDonald,** ETH Zürich, Switzerland
Ursula Kües, University of Goettingen, Germany

15:00 **Remco Stam**, *Technical University of Munich, Germany*
The evolutionary history of global Ramularia collo-cygni epidemics

15:25 **Ivan Ayuso-Fernandez,** *CSIC, Spain*
Evolutionary convergence in fungal ligninolytic enzymes

15:50 **Nani Maryani Martawi**, *Wageningen University and Research, The Netherlands and Universitas Sultan Ageng Tirtayasa (UNTIRTA), Indonesia*
Genetic variation of Indonesian *Fusarium oxysporum* f.sp. *cubense* isolates and their pathogenicity on wild and cultivated banana species

16:15-16:45 *Coffee Break*

16:45 **Milica Zlatkovic**, *University of Novi Sad, Serbia*
Neofusicoccum *parvum*: Genetic uniformity and invasive spread in the Western Balkans

17:10 **Torda Varga**, *Biological Research Centre, Hungarian Academy of Sciences, Hungary*
Understanding the evolution of mushroom-forming fungi: macro-evolutionary analyses of a 5300-species phylogeny of Agaricomycetes

17:35 **Christophe d'Enfert**, *Institut Pasteur, France*
A *Candida albicans* population genomics study

Wednesday, February 28, 2018

15:00-18:00 CS12: CELL REGULATION AND SIGNALLING

Chairs: **Alfredo Herrera-Estrella,** Center of Research and Advanced Studies, Mexico
Benjamin A. Horwitz, Technion-Israel Institute of Technology, Israel

15:00 **Monika Schmoll**, *AIT Austrian Institute of Technology GmbH, Austria*
A phosphodiesterase is responsible for the block of cellulase gene expression in light in *Trichoderma reesei*

15:25 **Marcel René Schumann**, *TU Braunschweig, Germany*
The Ca^{2+}-binding penta-EF-hand protein PEF-1 is part of a fungal resistance mechanism against cell fusion-induced lysis and membrane-destabilizing antifungals

15:50 **Ying Huang,** *Nanjing Normal University, China*
The *Schizosaccharomyces pombe* PPR protein Ppr10 associates with a novel protein Mpa1 and acts as a mitochondrial translational activator

16:15-16:45 *Coffee Break*

16:45 **Alfredo Herrera-Estrella**, *Center of Research and Advanced Studies, Mexico*
Molecular signaling in the response to injury of *Trichoderma atroviride*

17:10 **Leandro J. Assis**, *Universidade de São Paulo - USP, Brazil*
Regulation of *Aspergillus nidulans* CreA-mediated catabolite repression by the F-box proteins Fbx23 and Fbx47

17:35 **Kobi Simpson-Lavy,** *Tel Aviv University, Israel*
A regulated protein aggregation controls glucose response in *S. cerevisiae*

18:00-18:30 CLOSING SESSION

Chair: **Benjamin A. Horwitz,** Co-Chair, ECFG14

- ***Closing remarks***
- **Poster prize awards**
- ***Announcement of the next meeting:***
  Massimo Reverberi, *University of Rome Sapienza, Italy*

19:30 CONFERENCE DINNER (optional)

The dinner will be held at the Knights' Halls (Ulamot Ha'Abirim) in Old Acre (Akko).

Keynote Lecture Abstracts

KEYNOTE LECTURE 2
(Monday, February 26, 2018 09:00)

Genome and population dynamics in pathogenic yeasts

Judith Berman
Molecular Microbiology & Biotechnology, Tel Aviv University, Ramat Aviv, Israel

Rapid responses to acute stresses are essential for stress survival and adaptation. An excellent microbial model for this process is the rapid emergence of drug responses in fungal pathogens. Fungi can adapt and survive drug concentrations that inhibit the growth of progenitor cells. They often do so via large effect size mutations involving whole chromosomes, chromosome arms as well as some point mutations, in a manner similar to cancer cells responding to chemotherapy drugs. Specifically, they undergo ploidy switches, alter chromosome copy number (become aneuploid) and undergo frequent loss of heterozygosity, via recombination and chromosome mis-segregation. In addition, fungi can respond with increased cell to cell variability in their response to drugs with small or large populations of cells acquiring the ability to grow in drug concentrations that inhibit others. This property has been ignored clinically but is likely to be an important factor in how well infections respond to antifungal therapies. Overall, fungi exhibit a broad repertoire of different mechanisms that enable rapid and transient generation of genomic diversity, upon which the strong selective pressure of antifungal drugs can act to facilitate improved survival and growth of the fungal pathogen during antifungal therapy.

KEYNOTE LECTURE 3
(Tuesday, February 27, 2018 09:15)

The EMBO Lecture:
BLASTOFF-Keeping up with the plant destroyers

Sophien Kamoun
The Sainsbury Laboratory, The John Innes Centre, Norwich, UK

Infectious plant diseases cause havoc to world agriculture and threaten to slow laudable efforts to launch a second green revolution to meet the food security needs of a booming world population. Filamentous pathogens such as the rice blast fungus, wheat stripe and stem rust, the Irish potato famine pathogen, and many others continue to trigger recurrent epidemics with far reaching consequences. In this talk, I will discuss how it is possible to perform cutting-edge research and significantly advance knowledge on economically important pathosystems, particularly in the post-genomics era. I will focus on the blast fungus *Magnaporthe oryzae*, a devastating cereal killer that infects the crops wheat, barley and rice, which together are staple food for a majority of the world population. Together with collaborators Ryohei Terauchi (Kyoto University, Japan) and Mark Banfield (John Innes Centre, UK), we gained an unprecedented level of detail of the molecular interactions that define host-pathogen recognitions by solving the crystal structures of fungal effectors in complex with plant proteins and reconstructing the evolutionary history of these molecular interactions. Our aim is to build on these discoveries to drive both basic and applied plant pathology. We have started to develop a thorough understanding of the biophysical properties of pathogen effector binding to host proteins and their consequences on pathogenesis and immunity. Such knowledge, along with related mechanistic and evolutionary studies, will guide the retooling of the plant immune system towards resistance to diseases. Ultimately, we will deliver traits and non-transgenic cultivars for breeding disease resistance in crops.

KEYNOTE LECTURE 4
(Wednesday, February 28, 2018 09:00)

The fungal individual: an evolutionary perspective

Hanna Johannesson
Department of Organismal Biology, Uppsala University, Uppsala, Sweden

The history of life has been driven by evolutionary transitions in individuality, *i.e.*, the aggregation of autonomous individuals to form a new, higher-level individual. Theoretical expectations for such transitions are well defined. First, grouping has to yield benefits, arising for example from cooperation amongst members of the lower level of the group. On the other hand, conflicts between members of the group may arise and should be minimized, which can be achieved for example by ensuring genetic homogeneity, since intraorganismal diversity would mean that selfish variants, deleterious to the group-level, could evolve. In fungi, the concept of individuality is challenging. This is due to the fact that a fungal mycelium can contain millions of genetically diverse nuclei, each capable of giving rise to new mycelia. Furthermore, the fungal kingdom provides examples of transitions in individuality from homo- to heterokaryosis. In my research-group, we have studied the interaction between nuclei and mycelia in several fungal systems, but have focused much of our recent effort to understand individuality in the filamentous ascomycete *Neurospora tetrasperma*. This species has recently (approximately 1 MYA) evolved a mating system (pseudohomothallism) in which mating-type heterokaryosis is dominant throughout the life cycle. In *N. tetrasperma*, selection can act at different levels: while nuclei can compete in their replication and transmission into short-lived asexual spores, at the level of the heterokaryotic individual cooperation between nuclear types is required to produce the long-lived sexual spores. I will outline recent results on conflicts and cooperation among nuclei in heterokaryons of *N. tetrasperma*, and generalize our findings to shed light on evolutionary transitions in individuality.

Plenary Lecture Abstracts

PLENARY SESSION 1: DEVELOPMENT AND CELL BIOLOGY - Sponsored by: British Mycological Society (Monday, February 26, 2018 09:45)

The Crippled Growth epigenetic cell degeneration of Podospora anserina, an overview

Philippe Silar

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Fungi frequently undergo phenotypic changes. In the case of filamentous fungi, the change often manifests itself as sectors of different morphology and color on the thallus. These changes may have a genetic or an epigenetic origin. For 20 years, my lab has been studying a complex and intriguing phenotypic change: the Crippled Growth (CG) cell degeneration of *Podospora anserina*. Early analysis revealed CG to be epigenetic and linked to the spreading in hyphae of a cytoplasmic and infectious factor. This factor can be at will induced and cured by simple stimuli, stationary phase and stress, respectively. Through a classical genetic approach, *i.e.*, the search of mutants altering CG, we have been able to show that the degeneration likely stems from the abnormal activation of the “Cell Wall Integrity” MAP kinase cascade. I will summarized what we know about CG, especially I will present our analyses of the IDC mutant affected in the MASTRINO complex that are unable to undergo CG, but also that of what is likely the master locus enabling this cell degeneration by promoting the growth- and stress-regulated misactivation of the MAP kinase cascade.

PLENARY SESSION 1: DEVELOPMENT AND CELL BIOLOGY - Sponsored by: British Mycological Society (Monday, February 26, 2018 10:15)

Fungal dialogs: intra- and interspecies communication in filamentous fungi

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In many filamentous fungi, germinating spores undergo mutual attraction and fusion, thereby forming a supracellular network, which further develops into the mycelial colony. Within mature colonies, hyphal branches merge thereby increasing interconnectedness. Germling and hyphal fusion in *Neurospora crassa* employ an unusual signaling mechanism, in which the two fusion partners take turns in signal sending and receiving. The highly coordinated cellular behavior is mediated by an intricate signaling network, which includes two MAP kinase cascades and fungal specific factors, such as the SO protein. Function and activity of these proteins are highly dependent on their subcellular localization, indicated by the alternating membrane recruitment of the MAK-2 MAP kinase and SO during the tropic cellular interaction.

Recent studies identified SIP-1, a protein of unknown function, as an interaction partner of SO. While both proteins co-localize during the cell-cell interaction, their dynamics differ in isolated non-interacting germlings. While SO is fully cytoplasmic under these conditions, SIP-1 localizes to the plasma membrane of the growing germ tube in an alternating manner comparable to the dynamics during cell fusion. These observations indicate that germlings rapidly alternate between two physiological stages, which are probably associated with the initiation of cell-cell interactions.

In addition, we recently found that the cell-cell communication mechanism first described in *N. crassa* is highly conserved in the phytopathogenic grey mold *Botrytis cinerea*. During germling fusion, the MAK-2 and SO homologs of this fungus show dynamics identical to the ones observed in the red bread mold. Germinating spores of *N. crassa* and *B. cinerea* readily undergo interspecies interactions, suggesting that also the so far unknown signals and receptors are conserved. Interestingly, germling fusion in *B. cinerea* is fully absent during growth on plant surfaces, which induce pathogenic development, suggesting that cell fusion and host infection are two alternative, mutually exclusive developmental routes.

PLENARY SESSION 1: DEVELOPMENT AND CELL BIOLOGY - Sponsored by: British Mycological Society (Monday, February 26, 2018 11:15)

The secretory pathway of cell wall building enzymes in *Neurospora crassa* hyphae

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Neurospora crassa has been at the vanguard of biochemical and genetics research for over a century. Remarkably, this filamentous fungus has become as well a magnificent model system to study polarized cell growth. We have used *N. crassa* to identify and analyze by high-resolution live fluorescence imaging key players of the secretory processes leading to a polarized delivery of vesicles at sites of cell wall growth, i. e. tips and septa. While chitin synthases (CHSs) are contained in microvesicles (chitosomes) that concentrate at the core of the Spitzenkörper (Spk) at hyphal apices, and β-1,3-glucan synthases are contained in macrovesicles that occupy the outer layer of the Spk, the mechanisms of traffic and sorting of these enzymes from synthesis to delivery sites remain largely unexplored. We have shown that distinct Rab GTPases are the likely candidates that mediate the vesicular journey along the hyphae towards the apex, where the octameric exocyst complex mediates vesicle tethering at the apical plasma membrane. Our current efforts are oriented towards understanding further the secretory pathway/s followed by the cell wall biosynthetic nanomachineries. We have focused on CHS-4; its localization at the apex and septa depends on CSE-7, a putative ER-based chaperone, but is independent of the exocyst. We are characterizing an extensive endomembranous network of tubules and sheets, presumably the secretory compartments associated with the biogenesis of CHS-4.

PLENARY SESSION 1: DEVELOPMENT AND CELL BIOLOGY - Sponsored by: British Mycological Society (Monday, February 26, 2018 11:45)

A-to-I RNA editing during sexual reproduction in filamentous ascomycetes

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Fungi, like plants, do not have ADAR orthologs and are believed to lack A-to-I RNA editing, which is the prevalent mRNA editing in animals. However, genome-wide A-to-I editing occurs specifically during sexual reproduction in the wheat scab fungus *Fusarium graminearum*. Unlike those in animals, majority 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 pseudogenes with the UAG stop codon in their ORFs. Furthermore, *F. graminearum* differs from animals in the sequence-preference and structure-selectivity of editing sites. Genome-wide A-to-I editing also specifically occurs during sexual reproduction in *Neurospora crassa, N. tetrasperma,* and *F. verticillioides*. Some of the editing sites are conserved in these fungi and they may be functionally related to their stage-specific functions during ascus or ascospore development. Unlike in humans, RNA editing in fungi preferentially targets As in hairpin loops, which is similar to the anticodon loop of tRNA targeted by ADATs, and nonsynonymous editing events in fungi are generally beneficial and favored by positive selection. RNA editing occurred before ascus development but became prevalent during ascosporogenesis. Overall, our results indicate that A-to-I editing in fungi occurs specifically during sexual reproduction with ADAR-independent mechanisms, is generally adaptive, and may be functionally related to other stage specific genetic phenomena.

PLENARY SESSION 1: DEVELOPMENT AND CELL BIOLOGY - Sponsored by: British Mycological Society

(Monday, February 26, 2018 12:15)

Elucidating three pathways that contribute to directional growth regulation in *Candida albicans* hyphae

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The production of hyphae is strongly linked to pathogenesis during superficial mucosal infections and the life-threatening disseminated disease, invasive candidiasis. Hyphae constitute the 'Special Weapons And Tactics' capability deployed by *C. albicans* during mucosal and endothelial cell layer invasion. Hyphae are equipped with adhesins and secreted effectors but these are only effective if hyphal guidance mechanisms are operational to direct penetrative growth into host tissue. We have identified three distinct hyphal phenotypes in which the ability to respond normally to external cues is attenuated or lost. Each of the three phenotypes is produced by specific grouping of mutant or gene deletion strains. The functional links within some groupings are emerging. For example, a sinusoidal growth morphology is observed when Pxl1 or its putative kinase, Ptk2, are deleted. However, in other cases the relationship between the proteins in a grouping is less clear, demonstrating that there are significant gaps in our understanding of polarised growth in *C. albicans*. Our aim is to elucidate these signalling pathways and generate an integrated model of how hyphal guidance signals influence the molecular machinery that drives polarised growth.

PLENARY SESSION 2: FUNGAL-HOST INTERACTIONS
(Tuesday, February 27, 2018 09:45)

Use of antibodies from single human B cells and C-type-lectin probes to map the dynamic cell surface of fungal pathogens

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The cell surface of fungal pathogens encodes a suite of molecules that are recognised by pattern recognition receptors of the innate immune system resulting in the activation of immune defences. Differences in the cell wall composition of different fungi and or the same fungus organisms growing in different morphologies and in differing environments generates a moving target for immune recognition. Progress in our understanding the nature of the immune response is described using two types of novel tools. We used cloned human monoclonal antibodies from individual B cells from patients who recovered from a recent fungal infection and the lectin binding domains of a range of mannan-detecting C-type lectin receptors as probes to map dynamic changes in the cell wall. The human mAbs also showed significant potential as diagnostics and as immune compatible therapeutic reagents. We show that immune relevant epitopes revealed by Fc-lectin mapping can be diffuse or clustered, superficial or buried in the cell wall. The immune reactivity of different fungal cell surfaces was not necessarily related to the phylogenetic relationship between organisms, and mannan-detecting lectins discriminated between different types of mannans on the cell surface and between yeast, hypha and other morphologies of the same fungus. Finally the growth cycle of a fungus was shown to be accompanied by temporal masking or unmasking of particular mannan epitopes during batch culture. These experiments demonstrate that the fungal cell surface is ordered, complex and dynamically changing, making immune recognition a challenging process requiring the concerted action of multiple immune receptors operating singly and in combination.

PLENARY SESSION 2: FUNGAL-HOST INTERACTIONS
(Tuesday, February 27, 2018 10:15)

Effector-mediated suppression of extracellular ATP-triggered immunity by the root endophyte *Serendipita indica*

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Extracellular adenosine 5'-triphosphate (eATP) is an essential signaling molecule that mediates different cellular processes through its interaction with membrane-associated receptor proteins in animals and plants. eATP regulates plant growth, development and responses to biotic and abiotic stresses. Its accumulation in the apoplast induces ROS production and cytoplasmic calcium increase mediating a defense response to invading microbes. We showed that the root endophyte *Serendipita indica* can overcome this response by secreting the ecto-5`-nucleotidase *Si*E5'NT in the apoplast of its plant hosts. *Si*E5'NT hydrolyzes eATP to adenosine, functioning as a compatibility factor. Arabidopsis lines expressing extracellular *Si*E5'NT are significantly better colonized and have reduced eATP levels and defense responses compared to the control lines upon fungal challenge, suggesting that *Si*E5'NT serves as a tool for manipulation of host eATP signaling.

PLENARY SESSION 2: FUNGAL-HOST INTERACTIONS
(Tuesday, February 27, 2018 11:15)

Host adaptation in the cross-kingdom pathogen *Fusarium oxysporum*

Cristina López Díaz[1], David Turrà[1], Tania Ribeiro Fernandes[1], Dilay Hazal Ayhan[2], Stefania Vitale[1], **Antonio Di Pietro**[1], Li-Jun Ma[2]
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Fungal pathogens pose a severe threat to human health and food security. These organisms often show exquisite host adaptation, but also undergo rapid evolution leading to shifts or expansions in the host range. The genetic mechanisms of pathogen-host adaptation remain poorly understood. In the soil-inhabiting vascular wilt fungus *Fusarium oxysporum,* individual isolates tend to exhibit high specificity towards a given plant host, while the species complex collectively attacks more than a hundred different crops. In addition, *F. oxysporum* is also an emerging human pathogen that provokes lethal systemic infections in immunocompromised individuals. Remarkably, a single field isolate of this fungus can kill tomato plants, immunodepressed mice and insects. By following a combination of reverse genetics and experimental evolution approaches, we found that *F. oxysporum* uses multiple strategies to adapt to different host environments. These include the recruitment of conserved cell signaling pathways or hijacking of host regulatory mechanisms. Fungal populations obtained after serial passages through different environments displayed significant alterations in growth, development and virulence phenotypes compared to the original clone. Re-sequencing of evolved populations revealed changes both at the nucleotide and chromosome level, many of which were fixed in the population. Our results suggest that genome plasticity acts as a major evolutionary driver in *F. oxysporum*, and that host adaptation involves trade-offs between developmental programs favouring infection versus proliferation.

Genetics and molecular biology of fungus-insect interactions

Chengshu Wang
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Fungal pathogens of insects play important roles in regulating insect populations in nature, most of which are ascomycetes. The species like *Metarhizium* spp. and *Beauveria bassiana* has been developed as environmentally friendly biocontrol agents against different insect pests. Comparative and phylogenomic analyses revealed that fungal entomopathogenicity is polyphyletic and more closely related to phytopathogens instead of mammalian pathogens. Relative non-insect pathogens, similar expansions of insect cuticle degrading enzymes reflect a convergent evolution unique for fungus-insect interactions. Genomics analysis of different *Metarhizium* species with varied host range indicated that the specialist species with a narrow host range diverged first and then the transitional species with intermediate host ranges evolved and followed by the generalists in association with protein family expansions. Similar to plant pathogens, an array of effector-like proteins are also encoded by animal pathogens despite that the gene-for-gene relationship is still suspected in fungus-animal interactions. Our molecular biology studies indicated that the insect fungi like *M. robertsii* evolved with a lineage-specific collagen-like protein to camouflage cell wall components for evading insect-host immunities. Divergent LysM proteins encoded in insect pathogens such as *B. bassiana* can bind chitin to suppress chitin-induced immune responses in insects and or protect fungal cell wall against chitinases. Besides the polypeptide effectors, we also found that secondary metabolites biosynthesized by insect pathogens could also be deployed to inhibit host innate immune responses and thereby facilitate fungal propagation within insect body cavity. Our studies advanced the understanding of the evolution and effector mechanisms of fungus-host interactions.

PLENARY SESSION 2: FUNGAL-HOST INTERACTIONS
(Tuesday, February 27, 2018 12:15)

Is fungal A-PCD the Achilles heel of plant and human killer pathogens?

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Apoptotic-like programmed cell death (A-PCD) has emerged as an important mechanism, involved in and affecting fungal development, survival, and pathogenicity. This talk will examine fungal A-PCD in a retrospective to prospective point of view, with emphasis on the role of A-PCD in pathogenicity.

Studies in the late 90s first revealed that yeasts undergo cell death with similar phenotypes to mammalian apoptosis. While the necessity of a suicidal machinery in a single-cell organism remained controversial, studies in filamentous species supported the existence of an operative A-PCD machinery, which has been associated with stress adaptation, development, and pathogenicity. In the necrotrophic plant pathogen *Botrytis cinerea,* A-PCD was observed during the establishment phase, and the protective role of the anti-A-PCD protein BcBir1 was found be necessary for virulence of this fungus. Similar findings were recently found in the human pathogen *Aspergillus fumigatus.* In both cases, the host immune response, while very different, initiated A-PCD in the pathogen as a defense strategy. The components of the A-PCD machinery are therefore potential targets for antifungal drugs that will trigger A-PCD. Indeed, genome search for potential candidates reveals a repertoire of putative fungal homologues of known apoptosis-related proteins; however, analyses of these homologues showed a surprisingly low level of functional conservation. Furthermore, domain search and in-depth genome analyses showed that fungi lack the entire upstream parts of animal apoptotic machinery, and miss most of the important apoptotic domains. Hence, while similar in phenotype, fungal A-PCD seems substantially different in regulation and mechanism from animal apoptosis. Recent studies support the involvement of mitochondria homeostasis and metabolism in regulation of fungal A-PCD, but further investigation is necessary in order to gain better insight of the process and identify key A-PCD regulators that might be used as targets for A-PCD-inducing antifungal drugs

PLENARY SESSION 3: EVOLUTION AND MOLECULAR ECOLOGY - Sponsored by: Zymergen
(Wednesday, February 28, 2018 09:45)

The role of histone-methyltransferases KMT1 and KMT6 in genome stability and chromatin organization in the wheat pathogen *Zymoseptoria tritici*

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Zymoseptoria tritici is a plant pathogenic fungus specialized to infect wheat (*Triticum aestivum*). The genome of the sequenced reference isolate comprises 21 chromosomes of which eight are accessory chromosomes. These chromosomes are highly instable during meiosis and mitosis, transcriptionally repressed and show enrichment of repetitive elements and of heterochromatic histone marks. To elucidate the role of heterochromatin associated histone modifications on genome stability and transcriptional regulation in *Z. tritici,* we created deletion mutants of the methyltransferases KMT6 and KMT1 that are responsible for H3K27me3 and H3K9me3, respectively. We combined experimental evolution, genetics and high-resolution microscopic analyses to follow the impact of these deletions on chromosome and genome stability. Furthermore, we used ChIPseq, whole genome sequencing and RNAseq to compare changes in chromatin and genome structure and differences in gene expression between mutant and wild type strains. Analyses of genome and ChIPseq data from the Δ*kmt1* mutants reveal dramatic chromatin reorganization, genome rearrangements and formation of neochromosomes likely mediated by transposable element activation. The Δ*kmt6* mutant however displays an increased stability of the accessory chromosomes compared to the wild type under normal growth conditions *in vitro*. Based on these results we conclude a strong impact of H3K9me3 in chromatin and genome organization and an important role of H3K27me3 for the stability of accessory chromosomes.

Complex multicellularity in fungi: the genetic underpinnings of convergent origins

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Fungi represent one of the few lineages that evolved complex multicellularity. In contrast to animals, plants, red and brown algae, however, fungi evolved complex multicellular structures convergently, providing a phylogenetically tractable system for understanding the genetic bases of a major evolutionary transition. Fungal fruiting bodies are some of the most typical manifestations of complex multicellularity, with the highest levels of multicellular organization evolved in fungi, comparable to simple animals and plants in terms of complexity levels. To understand the genetic bases of fruiting body development and its evolutionary origins, we apply various computational and high throughput approaches and phylogenetic comparative strategies in the Basidiomycota. Through comparisons of the developmental transcriptomes of 5 Agaricomycete species with diverse morphologies, we could identify conserved and lineage specific genes that show developmentally relevant expression patterns and thus represent candidates for implementing key events of fruiting body development. Complex multicellularity in fungi comprise not only fruiting bodies, but also rhizomorphs, sclerotia and an array of other, mostly reproductive structures. Comparisons of the developmental program of such structures to that of fruiting bodies can highlight some of the general principles of the development of complex multicellular structures in fungi. Such data, combined with whole genome comparisons shed light on the evolution of developmentally relevant genes across fungi and reveal some of the general principles of the evolution of increasingly complex multicellular organisms.

PLENARY SESSION 3: EVOLUTION AND MOLECULAR ECOLOGY - Sponsored by: Zymergen
(Wednesday, February 28, 2018 11:15)

Recent evolution of the opportunistic human pathogen *Candida glabrata*

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Candida glabrata is an opportunistic fungal pathogen that ranks as the second most common cause of systemic candidiasis. Despite its genus name, this yeast is more closely related to the model yeast *Saccharomyces cerevisiae* than to other *Candida* pathogens, and hence its ability to infect humans is thought to have emerged independently. Moreover, *C. glabrata* has all the necessary genes to undergo a sexual cycle, but it is considered an asexual organism due to the lack of direct evidence of sexual reproduction. To reconstruct the recent evolution of this pathogen, and find footprints of sexual reproduction, we assessed genomic and phenotypic variation across 33 globally-distributed *C. glabrata* isolates. We cataloged extensive copy number variation, which particularly affects genes encoding cell-wall associated proteins, including adhesins. The observed level of genetic variation in *C. glabrata* is significantly larger than that found in *Candida albicans*. This variation is structured into seven deeply divergent clades, which show recent geographical dispersion and large within-clade genomic and phenotypic differences. We show compelling evidence of recent admixture between differentiated lineages, and of purifying selection on mating genes, which provide first evidence for the existence of an active sexual cycle in this yeast. Altogether, our data point to a recent global spread of previously genetically isolated populations and suggest that humans are only a secondary niche for this yeast.

PLENARY SESSION 3: EVOLUTION AND MOLECULAR ECOLOGY - Sponsored by: Zymergen
(Wednesday, February 28, 2018 11:45)

Genome evolution and functional adaptation of the species complex *Fusarium oxysporum*

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Fusarium oxysporum is a highly adaptive species complex causing destructive and intractable wilt diseases across a broad spectrum of plant hosts. The *Fusarium* comparative genomics demonstrates that horizontal transfer of entire chromosomes conveys pathogenicity and contributes directly to the niche adaptation. This discovery establishes *F. oxysporum* as an effective model to investigate horizontal transfer in eukaryotes, and the pathogenicity chromosomes provide a focal point to investigate the genetic mechanisms that underlie pathogenesis against different plant hosts. Using two *F. oxysporum* strains, Fo5176 and Fo47, which give distinctive phenotypes when inoculating the root of Arabidopsis ecotype Col-0, we have established the *F. oxysporum*-Arabidopsis systems capturing either the pathogenic or the non-pathogenic interaction. A comprehensive RNAseq experiment at 12, 24, 48 and 96 hours post inoculation revealed distinct signaling pathways involved in the pathogenic versus non-pathogenic interactions. These results highlight the dynamic nature of fungal-plant interactions.

PLENARY SESSION 3: EVOLUTION AND MOLECULAR ECOLOGY - Sponsored by: Zymergen
(Wednesday, February 28, 2018 12:15)

Widespread adenine N6-methylation of active genes in fungi

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N6-methyldeoxyadenine (6mA) is a noncanonical DNA base modification present at low levels in plant and animal genomes, but its prevalence and association with genome function in other eukaryotic lineages remains poorly understood. Here we report that abundant 6mA is associated with transcriptionally active genes in early-diverging fungal lineages. Using single-molecule long-read sequencing of 16 diverse fungal genomes, we observed that up to 2.8% of all adenines were methylated in early-diverging fungi, far exceeding levels observed in other eukaryotes and more derived fungi. 6mA occurred symmetrically at ApT dinucleotides and was concentrated in dense methylated adenine clusters surrounding the transcriptional start sites of expressed genes; its distribution was inversely correlated with that of 5-methylcytosine. Our results show a striking contrast in the genomic distributions of 6mA and 5-methylcytosine and reinforce a distinct role for 6mA as a gene-expression-associated epigenomic mark in eukaryotes

Concurrent Session Abstracts

CS1: DEVELOPMENT AND CELL BIOLOGY
(Monday, February 26, 2018 15:30)

Pulses of Ca2+ coordinate actin assembly and exocytosis for stepwise cell extension

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Many eukaryotic cells grow by extending their cell periphery in pulses. The molecular mechanisms underlying this process are not yet fully understood. Here we present a comprehensive model of stepwise cell extension by using the unique tip growth system of filamentous fungi. Live-cell imaging analysis, including superresolution microscopy, revealed that the fungus *Aspergillus nidulans* extends the hyphal tip in an oscillatory manner. The amount of F-actin and secretory vesicles (SV) accumulating at the hyphal tip oscillated with a positive temporal correlation, whereas vesicle amounts were negatively correlated to the growth rate. The intracellular Ca2+ level also pulsed with a positive temporal correlation to the amount of F-actin and SV at the hyphal tip. Two Ca2+ channels, MidA and CchA, were needed for proper tip growth and the oscillations of actin polymerization, exocytosis, and the growth rate. The data indicate a model in which transient Ca2+ pluses cause depolymerization of F-actin at the cortex and promote SV fusion with the plasma membrane, thereby extending the cell tip. Over time, Ca2+ diffuses away and F-actin and SV accumulate again at the hyphal tip. Our data provide evidence that temporally controlled actin polymerization and exocytosis are coordinated by pulsed Ca2+ influx, resulting in stepwise cell extension.

Takeshita et al, PNAS, 114(22):5710-06, 2017.

Roles of the AP-2 and AP-1 complexes in apical cargo sorting, endocytosis and polar growth in fungi

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Filamentous fungi provide excellent systems for investigating the role of the AP complexes in polar growth. Using *Aspergillus nidulans,* we recently showed that AP-2 and AP-1 are essential for polarity maintenance and growth. Surprisingly, the role of AP-2 is clathrin-independent and this is in line with a sequence analysis showing that the AP-2 β subunit (β2) lacks a clathrin-binding domain. AP-2 interacts with endocytic markers SlaB and SagA and the lipid flippases DnfA and DnfB in the sub-apical collar region of hyphae. AP-2 was also shown to be critical for proper apical membrane lipid and cell wall composition. Our findings supported that the AP-2 complex of dikarya has acquired, in the course of evolution, a specialized clathrin-independent function necessary for fungal polar growth. In ECFG14 we will present most recent findings concerning the specific roles of AP-1 in apical cargo sorting and fungal growth.

CS1: DEVELOPMENT AND CELL BIOLOGY
(Monday, February 26, 2018 16:20)

Endocytic recycling underlies the polarized hyphal mode of life

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An idiosyncratic feature of filamentous fungi is that their vegetative phase consists of tube-shaped cells (hyphae) that grow exclusively by apical extension. Therefore polarized growth is a distinctive feature of hyphal organisms, and the one that underlies their capacity to colonize substrates or, in the case of pathogenic species, to invade tissues. To sustain the strikingly fast rates of growth, the secretory pathway must efficiently deliver to the apex both the lipids accounting for the extension in plasma membrane surface and the enzymes that synthesize the cell wall in the hyphal tip dome. Our studies with one of these physiologically crucial cell-wall modifying enzymes demonstrated that its polarized localization to the apical dome is mediated by endocytic recycling, which streamlines the delivery of this type of cargoes to their site of action to facilitate rapid apical extension.

CS1: DEVELOPMENT AND CELL BIOLOGY
(Monday, February 26, 2018 17:15)

Pexophagy and fruiting-body formation in *Sordaria macrospora*

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The homothallic filamentous ascomycete *Sordaria macrospora* is an ideal model organism to study multicellular fruiting-body development. The fungus lacks asexual reproduction and is strictly dependent on the sexual cycle for the production of ascospores.

Supply and homeostasis of nutrients are important issues for sexual development. We therefore analyzed the role of non-selective autophagy during fruiting-body formation. Autophagy is a degradation process in which eukaryotic cells digest their own cell constituents. We have characterized conserved components of the autophagic machinery and could show that autophagy is an essential and constitutively active process to sustain high energy levels for multicellular development. In contrast to non-selective bulk autophagy, selective autophagy is characterized by cargo receptors, which bind specific cargos such as superfluous or damaged organelles, and target them for autophagic degradation. Using the core autophagy protein ATG8 as bait, GFP-Trap analysis followed by liquid chromatography mass spectrometry (LC/MS) identified a putative homolog of the human autophagy cargo receptor neighbour of BRCA1 (NBR1) in *S. macrospora*. Fluorescence microscopy revealed that SmNBR1 co-localizes with SmATG8 at autophagosome-like structures and in the lumen of vacuoles. Delivery of SmNBR1 to the vacuoles requires SmATG8. Both proteins interact in an LC3 *nteracting region (LIR)-dependent manner. Deletion of Smnbr1* leads to impaired vegetative growth under starvation conditions, and reduced sexual spore production under non-starvation conditions. The human *nbr1* homolog partially rescues the phenotypic defects of the fungal *Smnbr1* deletion mutant. The *Smnbr1* mutant can neither use fatty acids as a sole carbon source, nor form fruiting bodies under oxidative stress conditions. Fluorescence microscopy revealed that degradation of a peroxisomal reporter protein is impaired in the *Smnbr1* deletion mutant. Thus, SmNBR1 is a cargo receptor for peroxisomes in filamentous ascomycetes.

CS1: DEVELOPMENT AND CELL BIOLOGY
(Monday, February 26, 2018 17:40)

Fungal growth and development visualization in *Schizophyllum commune*

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The basidiomycete *Schizophyllum commune* belongs to the white rot fungi and is relevant for wood degradation worldwide. As early colonizer of tree wounds and after forest fire, the fungus has also phytopathogenic importance. Its high competitive ability is based on the recognition of other fungi and bacteria, the production of specific extracellular metabolites and a strategy of fast growth. The fungal cytoskeleton, composed of a complex network of microtubules and actin structures, has a major impact on transport of vesicles as well as endo- and exocytosis processes. Visualization of the actin cytoskeleton in actively growing hyphae was performed with Lifeact-GFP. Thereby cortical actin patches were visualized at cell tips and clamps and as well as in subapical cells, preceded septation. The actin cytoskeleton in living hyphae during septum development shows close association with nuclear division. Clamp cell formation, typical of many model basidiomycetes including *S. commune*, indicated an aggregation of actin filaments to ring structures at the future site of nuclear division. Additionally, GFP-labeling of histone H2B enables visualization of nuclear movement and mitosis events in monokaryotic and dikaryotic cells. Further fast nuclear exchange in anastomoses after mating could be shown.

CS1: DEVELOPMENT AND CELL BIOLOGY
(Monday, February 26, 2018 18:05)

Fruiting body development in the Basidiomycete Coprinopsis cinerea

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Fruiting body development in *Coprinopsis cinerea* follows a conserved scheme defined by day and night phases, with well predictable distinct stages over the time. Fruiting starts with primary hyphal knot (PHK) formation in the dark, followed by light-induced compact aggregates, secondary hyphal knots (SHKs) in which stipe and cap tissues differentiate. Primordium development (P1 to P5) takes five days to culminate on day 6 of development in karyogamy, meiosis and subsequent basidiospore production which parallel fruiting body maturation. Development is regulated at distinct steps by light, temperature, humidity, nutrients, CO_2 and metals like copper. Light signals induce formation of SHKs, differentiation of tissues within the growing primordia, and karyogamy. Without light, 'dark stipes' are formed from SHKs with elongated stipes and underdeveloped caps. Copper addition has the same effect in all conditions. Lack of aeration before light-induced formation of SHKs fully blocks fruiting. When SHKs have been formed prior to blockage of air, development arrests. Block in aeration at P1 to P3 leads in light to outgrowth of 'dark stipe'-like structures. When plates are air-sealed at the P4 or P5 fruiting body maturation happens with reduced sporulation. Mutants in light reception can form 'dark stipes' in light while the phenotype in others may arise from defects in regulation by aeration. Most mutants in fruiting are however simply blocked at specific steps. In a collection of about 1500 strains, mutations did not evenly distribute over the complete pathway. High numbers of mutants are available in hyphal know formation, comparably few in the subsequent steps up to P3. Larger sets of mutants exist for P4 and P5 when karyogamy and meiosis have to occur in the basidia and fruiting body maturation has to be initiated. Mutant numbers may reflect the genetic complexity of specific steps in fruiting.

A unique protease regulatory gene from Trichoderma reesei

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The reduction of unwanted endogenous proteases has already been an important target for strain improvement of fungal host strains used in protein production for many years. Targeted deletion of specific proteases has been used extensively for this purpose. Surprisingly, only very little is known about regulatory circuits that specifically control fungal protease production. The only protease-specific regulator gene discovered to date is the *prtT* gene from *Aspergillus niger*, which encodes a canonical Zn2-Cys6 activator protein involved in the expression of a wide range of protease genes (Punt *et al*., 2008). Interestingly, homologues of *prtT* are only found in *Aspergillus* species, whereas no *prtT* homologue is present in *Trichoderma reesei*. We aimed to discover a similar protease master switch in our research. *T. reesei* mutants with strongly reduced overall protease levels and strongly reduced expression of a number of protease genes were obtained using a biological screen for the selection of protease-deficient mutants (Braaksma *et al*., 2008). Genome sequencing of a number of these strains followed by SNP analysis revealed that several of these mutants carried mutant alleles from a single gene, which we termed *pea1* for *protease-expression-affected.* Disruption of this gene in both *T. reesei* and *Fusarium sp.* confirmed the role of *pea1* in protease gene expression. Intriguingly, the encoded protein does not show any similarity to known regulatory proteins, indicating that a completely new regulatory circuit may be governing protease gene expression in *T. reesei*, which opens the way to further research in this area.

Controlling the expression of the *Trichoderma reesei* subtilisin protease gene *slp2* leads to improved biotherapeutic protein production

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T. reesei is capable of high levels of protein production, but is also an active secretor of proteases. This hinders the production of many sensitive therapeutic hormones and cytokines that are by nature easy to degrade. Even antibodies which are thought to be relatively stable molecules are susceptible to protease degradation. The subtilisin like protease SLP2 is one of the most abundant proteases produced by *T. reesei* and causes degradation of a range of therapeutic proteins such as antibodies and interferon alpha 2b. To control the expression of *slp2* gene we tried to delete it, but that strategy affected the growth and sporulation of the strain. Alternatively we developed RNA hairpin expressing vectors to silence its messenger RNA or we simply exchanged the promoter of *slp2* to attempt to reduce its expression. In a mAb production strain these approaches reduced the secreted protease activity by half and improved the antibody yield by 2-fold, with the highest production level reaching 7.6 g/L. The heavy chain quality was strikingly improved after controlling the SLP2 protease activity. Likewise, silencing *slp2* also dramatically improved the production of interferon alpha 2b, allowing levels of 7.9 g/L to be reached.

Genomic annotation of a mannosylerithritol lipid yeast producer as an approach to unravel its metabolic pathway

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Mannosylerithritol lipids (MELs) are amphiphilic molecules of interest to industry due to their broad range of potential applications, including use as biosurfactants. MEL production has been reported in *Ustilaginomycetes* as a secondary metabolite, involving a biosynthetic cluster of five genes. There are four types of MELs, categorized as A to D, each of which varies according to the number of acetylations and acylations. MELs are produce as mixtures of the four types at different ratios depending on the species. We have focused our study into the mechanisms behind MEL production on *Pseudozyma graminicola*, which has been reported to primarily produce MEL-C (85%). We sequenced and assembly its genome, using the PacBio platform, into 34 contigs with a total of 19.57 Mb. Subsequently, RNAseq data, was used to facilitate gene calling and annotation leading to the identification of 6602 genes. We have identified the MEL cluster, as well the presence of pathogenic clusters which has been reported in species such as *U. maydis* and *S. reilianum*. From our phylogenic analysis we have observed that *P. graminicola* shares more synteny to the aforementioned species than to other *Pseudozyma*. However, unlike *U. maydis* and *S. reilianum, P. graminicola* is reported to be an endophyte and not a biotrophic pathogen. In addition to genome characterization we have developed a transformation protocol and used this to investigate the role of genes putatively involved in MEL production, including three regulatory genes. Intriguingly, three of the mutants have very strong morphological phenotypes. We will report our findings, including the impact of these mutants on MEL production.

Comparative analysis for transcription start sites of enolase genes in *Aspergillus oryzae* and *Aspergillus nidulans*

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Aspergillus oryzae is a domesticated filamentous fungus used for Japanese fermentation industries and has been well known for its high ability of starch assimilation. In *A. oryzae*, transcription of glycolytic genes is induced at high level in the presence of fermentable carbon sources such as glucose. This feature would be important for the growth in industrial culture conditions of *A. oryzae*. However, it has not been evaluated whether or not the transcriptional induction of glycolytic genes is a specific feature to *A. oryzae* compared with the related species existing in natural environments such as *Aspergillus nidulans*.

Our previous studies in *A. oryzae* uncovered that the enolase gene (*AoenoA*), one of the most strongly expressing glycolytic genes, has two alternative transcription start sites (TSSs) dependent on the difference of two types of carbon source; the TSS located at -510 nt upstream of the start codon (+1) is strictly used under condition with nonfermentable carbon sources and another TSS located at -36 nt is used under condition with fermentable carbon sources.

To elucidate the conservation of alternative TSSs in *enoA* among the genus *Aspergillus*, we investigated the TSS usage of *A. nidulans enoA* (*AnenoA*) under glucose or acetate culture condition. Although 5' RACE analysis revealed that *AnenoA* had also two TSSs located at around -440 nt and -19 ~ -67 nt, transcription from the downstream TSS was not preferentially observed under glucose condition unlike in *AoenoA*. In addition, Northern blot and qRT-PCR analyses showed that the induction levels of the transcript from the downstream TSS in the presence of glucose were approx. 18-fold in *AnenoA* and approx. 100-fold in *AoenoA* compared those in the presence of acetate. These results indicated that transcription from the downstream TSS under glucose condition is markedly upregulated in *AoenoA* compared to that in *AnenoA*.

Basidiomycetes secretomes: an under-exploited tank of new plant-polysaccharide-depolymerizing enzymes

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Fungi are essential for the global carbon cycle as they use plant biomass by producing enzymes that degrade plant cell wall polysaccharides into metabolizable sugars. Plant-polysaccharide-depolymerizing enzymes are of great interest to biotechnology, as the products of their catalysis can be used as precursors in the processes that generate bio-based products, e.g., fuels and chemicals (1). The enzymes degrading or modifying plant polysaccharides are classified as carbohydrate-active enzymes (CAZymes) and are divided into families according to their amino acid sequence and structural similarity (2). The CAZy database is organized into families of glycoside hydrolases, carbohydrate esterases, polysaccharide lyases, glycosyltransferases, and auxiliary activities. Our knowledge of basidiomycetes regarding their ability to decompose plant polysaccharides is limited compared to the wealth of information on ascomycetes, due largely to the traditional and well-established industrial relevance of several ascomycetes. In nature, basidiomycetes are the most efficient degraders of woody biomass and therefore contain a huge potential for applications in various industries, which has so far remained largely unexplored (3,4). The identification of lignocellulose-degrading enzymes is based on their sequence similarity to members of known sequence-based families of CAZymes on the CAZy database. But key enzymes of industrial interest might not be homologous to known CAZymes, preventing their identification and characterization. One of the most promising approaches to discover new catalytic functions is the analysis of the secreted enzymes (secretomes) produced when fungi are cultured on lignocellulosic substrates. This approach undertaken in our group thanks to –omic approaches has led to the identification of several putative new CAZymes among the secretomes of a white-rot basidiomycete fungus, *Pycnoporus coccineus* (5).

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Transcriptomics of white rot fungal decay of spruce wood and bioconversion of waste lignocellulose substrates by the Polyporales species *Phlebia radiata*

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White rot Basidiomycota species secrete an array of carbohydrate-active enzymes and lignin-modifying oxidoreductases, in order to decompose wood lignocellulose polysaccharides and lignin. *Phlebia radiata*, the type species of the genus *Phlebia*, in phlebioid clade of the order Polyporales, is an ecologically important species able to decompose efficiently the main components of plant cell wall (1) and presenting high applicability in biotechnological processes, especially in production of ethanol from non-pretreated lignocellulose waste materials by a single-step process (2).

Genome sequencing of *P. radiata* resulted in construction of the mitochondrial genome (3) and in high-quality assembly and annotation of the nuclear genome. Transcriptomics by RNA-seq and proteomics analysis upon a six-week cultivation on Norway spruce wood confirmed the up-regulation of plant-cell wall degradation associated genes needed for white rot type of decay and production of the corresponding proteins upon fungal colonization of wood (4). Especially, the lignin-modifying class-II peroxidases together with glyoxal and alcohol oxidases were abundantly produced on wood indicating an initial oxidative attack against lignin units, while dynamic changes in quantities of LiP and MnP enzymes were detected, and several AA9 lytic polysaccharide monooxygenase encoding genes were highly up-regulated.

Expression of lignocellulose-decomposition associated genes and enzyme activities were studied while *P. radiata* was grown on lignocellulose waste materials and simultaneously producing ethanol under semi-aerobic conditions. RT-qPCR results supported the importance of specific genes coding for activities against cellulose, hemicellulose and lignin, also in conversion of the waste substrates. Indications on regulation of specific genes involved in glucose and xylose metabolism were obtained under fermentation conditions resulting in ethanol production from waste lignocelluloses, which will be discussed.

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CS3: PHYSIOLOGY AND METABOLISM
(Monday, February 26, 2018 15:30)

Investigating the biology of plant tissue invasion and cell-to-cell movement by the rice blast fungus *Magnaporthe oryzae*

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Magnaporthe oryzae is the causal agent of rice blast, one of the most serious diseases affecting rice production. The fungus is also the causal agent of wheat blast, a disease that now threatens wheat production in South America and South Asia. During plant infection, *M. oryzae* forms a specialised infection structure called an appressorium. The infection cell generates enormous turgor, focused as mechanical force to breach the rice cuticle. Re-polarisation of the appressorium requires a hetero-oligomeric septin complex that organises a toroidal F-actin network at the base of the appressorium. This allows the fungus to invade epidermal cells and develop biotrophic invasive hyphae. Septin-mediated plant infection is controlled by NADPH oxidase activity and a regulated burst of reactive oxygen species occurs within the appressorium. The process is regulated by a turgor sensing protein kinase, which can sense when optimal appressorium turgor is achieved and the switch to polarised growth is triggered. A pressure-mediated cell cycle checkpoint is also necessary for initiation of septin activation and the re-orientation of the cortical F-actin cytoskeleton. Once tissue is invaded the fungus undergoes differential expression and secretion of a large repertoire of effector proteins that are delivered to the apoplastic space which surrounds invasive hyphae, or directed into plant cells. The fungus also undergoes distinct physiological changes, including activation of enzymes associated with utilisation of a broad spectrum of carbon sources, as we as distinct secondary metabolic pathwys. *M. oryzae* suppresses plasmodesmatal immunity in order to facilitate its spread from cell-to-cell in plant tissue. This is controlled by a specific MAP kinase signalling pathway and requires septin-dependent hyphal constriction to enable the fungus to spread rapidly in rice tissue.

CS3: PHYSIOLOGY AND METABOLISM
(Monday, February 26, 2018 15:55)

Evolution of host range is associated with carbohydrate and protein metabolism in *Colletotrichum* spp

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Colletotrichum spp. cause anthracnose disease on a wide range of agronomically important plant species and exhibit a broad diversity of host range, host specificity and reproductive behaviors. We leveraged the growing number of genome sequences available for *Colletotrichum* spp. and performed a comparative analysis of gene content to find associations with host range and host specificity. The predicted protein sequences from each species were classified into protein families using a variety of tools. Hierarchical clustering of gene family and functional domain assignments, and phylogenetic analyses revealed lineage specific losses of secreted carbohydrate-active enzymes (CAZymes) and protease encoding genes in species that have narrow host range as well as expansions of these families in the acutatum and gloeosporioides species complexes. Members of these species complexes are broad host range pathogens, suggesting that the higher number in CAZy and protease diversity may be associated with the ability to infect multiple host species. This result highlights the similarity in both secretomes and whole proteomes of these species complexes and suggests that their gene family content, especially their repertoires of CAZymes and peptidases are the product of recent, lineage specific expansions of these families independently in each species complex. Interestingly, phylogenetic analyses of the CAZyme and peptidase families revealed that, in contrast to our expectations, gene loss in other *Colletotrichum* species is as important, if not more important force driving the evolution of gene family size. These results are consistent with the idea that different lifestyles, hosts and host tissues present different types of carbohydrate substrates to the pathogen this is reflected by each species' CAZyme and peptidase repertoire.

CS3: PHYSIOLOGY AND METABOLISM
(Monday, February 26, 2018 16:20)

Functional distinction of hyphal compartments

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Hyphae grow of higher fungi grow at their apex and are compartmentalized by septa that have a central pore enabling cytoplasmic streaming. Peroxisome-derived Woronin bodies however can plug these pores. Incidence of plugging increases in time switching the unicellular organization of young hyphal compartments into a multicellular one in older compartments. It was assessed whether the multicellular organization contributes to apical growth of hyphae and how growth is affected when Woronin bodies are absent. Hyphae of the wildtype strain and a Δ*hexA* strain that lacks Woronin bodies had a similar morphology and growth rate. A total of 58% and 17% of the hyphae continued growing, respectively, after dissecting the 2nd compartment. Growth rate of these hyphae was not affected, even when the carbon or nitrogen source was limiting. Dissection at a fixed position of 400 µm from the apex revealed that all wild-type and Δ*hexA* hyphae stopped growing when the first septum was positioned 400 µm from the apex, while 81 % and 57% of the hyphae, respectively, continued growing when the first septum located 400 µm from the tip. When apical compartments were dissected, normal growth from subapical compartments was recovered in wild type hyphae but not in Δ*hexA* hyphae. Together, we showed for the first time that apical compartments are self-sustaining in growth

CS3: PHYSIOLOGY AND METABOLISM
(Monday, February 26, 2018 17:15)

Foliar sugar accumulation enhances priming of the salicylic acid-mediated defense response

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We have investigated the role of carbohydrate partitioning and allocation in Arabidopsis source leaves in the compatible interaction with the fungal hemibiotroph *Colletotrichum higginsianum*, which exhibits an initial biotrophic and an ensuing necrotrophic colonization phase.

Arabidopsis mutants with impaired starch turnover are more susceptible towards *C. higginsianum* infection and a strong negative correlation between diurnal carbohydrate accumulation and fungal proliferation is evident in the investigated mutants. Our results demonstrate that mutants suffering from nocturnal carbon shortage show a dampened salicylic acid (SA) response that impairs defense especially during the necrotrophic colonization phase.

On the other hand, Arabidopsis double mutants lacking the sucrose transporters SWEET11 and SWEET12 show constantly elevated carbohydrate levels and are more resistant towards *C. higginsianum*. Analysis of YFP reporter plants as well as single and double mutants suggests that a lack of these transporters does not affect pathogen nutrition during the initial biotrophic phase. Instead, our data identify enhanced priming of the SA pathway in *sweet11/sweet12* double mutant as the cause of increased resistance.

CS3: PHYSIOLOGY AND METABOLISM
(Monday, February 26, 2018 17:40)

Branched chain amino acid biosynthesis genes and regulators in *Aspergillus nidulans*

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The branched chain amino acids (BCAA) leucine, isoleucine, and valine are important precursors for biosynthesis of proteins and secondary metabolites. The BCAA biosynthesis pathway is well characterized in *Saccharomyces cerevisiae*. However, recent work on BCAA pathway enzymes in *Aspergillus* revealed differences in the number of genes for several steps. The genes for the final two steps of leucine biosynthesis, catalyzed by β-isopropylmalate dehydrogenase (β-IDH) and BCAA aminotransferase (BAT), have not yet been characterized in the Aspergilli. The BATs also catalyze the final step of isoleucine and valine production. In *S. cerevisiae*, there is one β-IDH gene and two BAT genes. Using protein sequence similarity we identified two β-IDH genes in *A. nidulans*, *leuD* and *leuE.* We show that deletion of *leuD*, but not *leuE*, causes leaky leucine auxotrophy. The *leuD*Δ *leuE*Δ double mutant is a strict leucine auxotroph indicating that both genes encode functional enzymes. Quantitative RT-PCR reveals that *leuE* up-regulation compensates for loss of *leuD*. We identified, using protein sequence similarity, six *A. nidulans* BAT genes, *batA-F*. Deletion of these six genes separately does not confer BCAA auxotrophy. However, the double deletion mutant lacking the two most highly expressed BAT genes is a BCAA auxotroph, suggesting that these two genes encode the predominant biosynthetic enzymes and the other *BAT genes* may have evolved new roles. Two of the other BAT genes lie in the aspercryptins secondary metabolism gene cluster and likely catalyze biosynthesis of unusual BCAA components of aspercryptins. We have characterized the regulation of leucine biosynthesis pathway genes by the transcription factor LeuB. The *leuB*Δ mutant is a leaky leucine auxotroph. We have identified a LeuB paralog, LeuR, by sequence similarity. Deletion of *leuR* does not confer leucine auxotrophy. However, the *leuB*Δ *leuR*Δ double mutant is a strict leucine auxotroph, indicating that LeuR also regulates leucine biosynthesis.

CS3: PHYSIOLOGY AND METABOLISM
(Monday, February 26, 2018 18:05)

A taste for 'sour' sugars: characterization of a highly efficient D-galacturonic acid metabolism in the basidiomycete yeast genus *Rhodosporidium*

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Saccharides released during the degradation of lignocellulosic material (e.g. for biorefinery purposes) are traditionally fermented using strains of *Saccharomyces cerevisiae*, which however has only a very limited ability to metabolize all available sugars. Thus, major efforts have to be undertaken to engineer catabolic pathways for sugars such as D-xylose, L-arabinose and D-galacturonic acid (GalA) in this ascomycete yeast. Still, the metabolism of some of these monosaccharides remains slow. Better catabolic pathways have to be transferred from fungal strains with a high ability to utilize these monosaccharides.

The oleaginous basidiomycete yeasts *Rhodosporidium toruloides* and *Rhodotorula mucilaginosa* were discovered as saprophytes colonizing for example pectin-rich olives and wine grapes and are known to be able to produce large quantities of carotenoids in short time. However, they also thrive on GalA, the main component of the pectin backbone. This is remarkable, since GalA is an oxidized sugar and therefore energetically difficult to catabolize by fungi. In addition, the molecular mechanisms behind the pectinolytic capabilities of basidiomycete fungi are almost entirely unknown.

In this work, we characterized the GalA metabolism pathway of these special yeasts on multiple levels. Following physiological growth assays with multiple strains, RNAseq analyses were performed in *R. toruloides* to identify genes that are differentially expressed on GalA and pectin. Moreover, RB-TDNAseq, a recently reported novel method for fitness scoring in barcoded TDNA-transformed populations, was used to identify targets affecting fitness during growth on GalA. This way, we identified the enzymes being involved in the GalA catabolism, which were heterologously expressed and their kinetics determined. Moreover, sugar transporters and a novel transcription factor putatively regulating the GalA metabolism were identified. Taken together, our results demonstrate that the genes from *R. toruloides* and *R. mucilaginosa* are promising candidates for rational engineering of an efficient GalA metabolism for example in *S. cerevisiae*.

CS4: SYMBIOSIS AND ENDOPHYTES
(Monday, February 26, 2018 15:30)

Characterization of the molecular mechanisms underpinning local and systemic responses in root-microbe multispecies interactions

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This project aimed to understand how host-microbiota interactions in natural soil shape and are shaped by local and systemic responses to beneficial and pathogenic root associated fungi. In order to address this, we established a reductionist approach which takes advantage of a gnotobiotic natural soil-based split root system to identify plant and microbe-derived transcripts that locally and systemically affect these interactions. Focus was on the role of local and root to root systemic signalling events on barley root associated fungal microbes. We studied single and joint root infection of barley with the cereal root rot fungal pathogen *Bipolaris sorokiniana* and with the beneficial root endophyte *Serendipita vermifera*. The consequences for local and systemic plant immunity were studied by analysis of reciprocal transcriptional responses to fungal colonization. Fungal cell wall degrading enzymes like chitinases and β-1,3-exoglucanases of *S. vermifera* were highly induced indicating that antagonism might play a role in protecting plant roots. However, transcriptomic analysis of tripartite interaction in the split-root system suggested plausible role of symbiont-modified plant responses in reducing colonization by the pathogen.

CS4: SYMBIOSIS AND ENDOPHYTES
(Monday, February 26, 2018 15:55)

The impact of fungal genotypes and species on the transcriptomic landscape of ectomycorrhizal symbioses

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Ectomycorrhizal (ECM) fungi are essential for forest ecosystems. In exchange for carbohydrates, ECM fungi offer an improved mineral supply to trees. Proper functioning of ECM implies differentiation of specific tissues and communication between partners. Fungal hyphae surround root tips of host trees and penetrate between cells to form a symbiotic interface dedicated to nutrient exchange. Hyphae extending from mycorrhizal roots, the extraradicular mycelium, explore the soil for essential elements such as N and P and transfer them to the host.

The aim of this study is to understand how ECM fungi regulate the expression of their gene repertoire to communicate with their host tree, to develop specialized morphological structures and to exchange nutrients. Is the "symbiosis toolbox" expressed by colonising ECM fungi the same within a species? How different is the set of symbiosis-related transcripts used by two phylogenetically- and ecologically-distant species, i.e. the basidomycete *Laccaria bicolor* and the ascomycete *Cenococcum geophilum*? And are the transcriptome alterations taking place in their host tree, *Populus*, similar?

To tackle these questions, we performed a series of RNA-seq analyses on different developmental stages of *Laccaria bicolor S238N – Populus tremula x alba* ECM and associated extraradicular mycelium, fruiting body and free-living mycelium grown on increasing levels of NH_4^+, Pi or glucose. Further, changes in gene expression triggered by *C. geophilum* and seven *L.bicolor* geographical accessions during the interaction with *Populus* roots were compared.

We identify symbiosis-upregulated genes with a putative role in plant-fungal interaction, but we also show that ectomycorrhiza development recruits gene networks involved in hyphal aggregation and in N-P-C homeostasis. We highlight the effect of genetic variability on the regulation of symbiosis-related gene expression. These genome-wide transcript profiling allowed us to identify candidate genes for further functional analyses aiming to better understand how ECM fungi fulfill their important role in forest ecosystems.

CS4: SYMBIOSIS AND ENDOPHYTES
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Seed transmitted endophytes of crop wild relatives

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Crop wild relatives have potential to provide improved genetic traits (biotic and abiotic stress resistance) through breeding with closely related domesticated crop species. In addition to the introgression of genetic traits, crop wild relatives could represent a source of beneficial symbionts that may improve crop productivity. Grasslands provide sources of many crop wild relatives and are among the largest and most widely distributed ecosystem representing 26% of the world land area. Grasses from the subfamily Pooideae are often infected with endophytic *Epichloë* species that represent dominant members of the plant's microbiome and provide protection to their host from biotic and abiotic stresses. Although *Epichloë* species associate with many crop wild relatives, these endophytes are not found within domesticated cereal crops such as wheat or rye. We examined wildryes from North America and *Hordeum* species from Asia to identify seed transmitted endophytes that could be utilized for improving cereal crops. Using PCR to detect presence or absence of genes encoding bioactive alkaloid biosynthesis steps, mating type genes and other informative markers, we identified endophyte infected host lines and determined the endophyte taxa and diversity associated within each host species. Scanning electron microscopy and confocal laser scanning microscopy were used to examine the endophyte colonization patterns. We observed some host species had epiphytically growing mycelia, conidiogenous cells and conidia on the leaf surface in addition to the endophytic intercellular growth. Synthetic associations of wheat and rye with the endophytes from *Hordeum* species could be established but endophyte compatibility was poor resulting in severely stunted and developmentally delayed host plants. Yet, epiphyllous hyphae could be observed on the infected rye and wheat blades similarly to what is seen in the native host. Understanding mechanisms of endophyte host compatibility may provide insight to overcome the barriers of moving *Epichloë* species to domesticated cereal crops.

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Interactions between three symbiotic fungi associated with an invasive ambrosia beetle and their host trees in Israel

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The ambrosia beetle, *Euwallacea* nr. *fornicatus* (Coleoptera: Scolytinae), is a new invasive species to Israel, reported in 2009. Consequently, it was found that *E. fornicatus* is comprised of a complex of at least three cryptic species that harbor different symbiotic fungal species, all highly specific to their beetle hosts. Recently, a monophyletic group of Fusaria within the *F. solani* species complex was defined, each with exclusivity to their *Euwallacea* ambrosia species. Isolations from *E.* nr. *fornicatus* female beetle mandibular mycangia and brood galleries in the attacked host trees revealed the presence of three novel fungal symbiotic species*: Fusarium euwallaceae*, *Graphium euwallaceae* and *Paracremonium pembeum*. The spores act as feed once the mature adult female beetles colonize the host plant, where they tunnel the brood galleries and release the spores that germinate, generating mycelial layers that eventually line the galleries and serve as fungal gardens for the developing larvae. Upon beetle attack, the mandibular mycangia comprise 99% of the total spore count of *F. euwallaceae*. *G. euwallaceae*, the main food source for developing larvae, appears on mass approximately a week after establishment of the new gallery. The role of *P. pembeum* is not yet clear. *F. euwallaceae* acts as a pathogen and is responsible for host plant damage. Xylem inoculations with each of the three fungi alone indicated that only *F. euwallaceae* survives for long periods but spreads very slowly, while the other symbionts disappear rapidly from live issue and survive in dead xylem after beetle emergence. The beetle attacks more than 50 tree species in Israel but only ten (representing different botanical families) are suitable for reproduction, among them in descending order, *Acer negundo, A. buergerianum, Quercus robur*, *Q. pedunculiflora, Platanus* spp., *Ricinus communis* and *Persea americana.* The symbiotic fungal interactions and their roles in host plants will be discussed.

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Ustilaginomycete yeasts as hub organisms in leaf microbial communities

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To facilitate co-habitation of resource-limited niches such as plant leaves, microbes have evolved mechanisms to collaborate or compete with others. Resulting microbial communities build integral networks, which are constantly disturbed by biotic and abiotic factors. This requires continuous responsiveness and re-calibration of microbe-microbe interactions. Key to a dynamic network stability are microbial hubs, i.e. microbes that are disproportionally important in shaping microbial communities. Recent findings suggest a particular importance of the negative interactions of antagonistic microbes for the stabilization of microbial communities. In wild *Arabidopsis thaliana* populations, we identified an Ustilaginomycte yeast of the genus Moesziomyces as a hub microbe [(1,2)].

This yeast, which is closely related to plant pathogenic smuts, antagonizes several bacterial members of the *A. thalania* leaf microbiome and also represses infection by an oomycete pathogen. We therefore hypothesize that Moesziomyces in particular and fungal yeasts of the class Ustilaginomycetes in general are crucial factors for microbial community structure. To test this hypothesis on the functional level, we have established a high quality annotated genome sequence. RNA sequencing of Moesziomyces sp. was performed to identify genes being functionally relevant for microbe-microbe interactions. An efficient transformation system allows reverse genetic approaches to verify and identify the function of identified candidate genes in microbe-microbe-plant interactions. In a parallel approach, we address the question, how the yeast-like lifestyle of Ustilaginomycete yeasts like Moesziomyces sp. contributes to their role in multitrophic interactions. To this end, we generated a synthetic self-compatible Moesziomyces sp. strain, which is analysed regarding growth mode, as well as for an eventual ability to invade and colonize plant tissue.

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Exploring hypovirulence-associated DNA mycovirus to be a natural fungicide

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Mycoviruses are widespread in various groups of fungi. Some mycoviruses may confer hypovirulence to their hosts, and could have potential to be used as biological control agents. Cryphonectria hypovirus 1 which was successfully used to control chestnut blight in Europe countries was a classic example. However, the potential of biological control with mycoviruses is extremely restricted by some limiting factors. Mycoviruses are usually vertically transmitted *via* conidia or *via* sexual spores occasionally, and horizontally transmitted *via* hyphal anastomosis which is controlled by non-self-recognition system; Most mycoviruses are absent of intercellular phase and without any transmission vector. Furthermore, hypovirulence-associated strains are very weak in nature since they could not infect plants. Recently, more and more mycoviruses were isolated in various crop pathogenic fungi, and researches suggested that the transmission properties among mycoviruses may not be the same, and some mycoviruses could transmit among vegetative incompatible individuals. *Sclerotinia sclerotiorum* is a notorious fungal crop pathogen with wide host range, it attacks rapeseed (*Brassicae napus*) and causes huge losses (more than 2.0 billion Yuan RMB) at the middle and lower reaches of the Yangtze River where more 85 % of rapeseed of China is planted. We isolated a DNA virus (named *Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1*, SsHADV-1) from a hypovirulent strain DT-8. Our research showed that SsHADV-1 has strong infectivity that its purified particles could infect intact hyphae of S. sclerotiorum directly, virus could enter into vegetative incompatible strain through strain DT-8 when the two strains were dual cultured. We further found that SsHADV-1 could mutualistically interact with mycophagous insect (Lycoriella ingénue), and use it as transmission vector. Spraying hyphal fragments of Strain DT-8 on aerial parts of rapeseed plants could control stem rot of rapeseed efficiently. Our findings suggested that SsHADV-1 could be used to control Sclerotinia diseases with glorious potential.

CS5: GENOMES, CHROMOSOMES AND EPIGENETICS - Sponsored by AB ENZYMES
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Pathogenicity chromosomes as Trojan horses: the costs of mobile DNA in pathogen evolution

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Pathogenic *Fusarium oxysporum* (*Fo*) strains are host-specific and cluster genes involved in infection together with transposons on separate, dispensable pathogenicity chromosomes. Horizontal transfer of a pathogenicity chromosome can transform a non-pathogenic strain into a pathogenic one, yet for this soil fungus pathogenicity is the exception rather than the rule. This suggests that the costs of obtaining a pathogenicity chromosome counterbalance the positive effects of being able to infect a new host. One example of such a cost is the fact that transposons that reside on a pathogenicity chromosome may colonize and disrupt the rest of the genome.

We investigate the level and timescale of genome colonization by transposons from a transferred chromosome by reconstructing transferred chromosomes and subsequent transposon insertion events within several distinct *Fo* clonal lines. Moreover, we apply experimental evolution using strains that obtained pathogenicity chromosomes in the lab, to study genome dynamics directly after horizontal transfer, *in vitro* and *in planta*. Together these two approaches will give insight into the dynamics of genome organization and the likelihood of emergence of new diseases through horizontal chromosome transfer.

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Function or Drive – Why are the Accessory Chromosomes of *Zymoseptoria tritici* maintained*?*

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The genome of the fungal wheat pathogen *Zymoseptoria tritici* comprises a set of accessory chromosomes that has persisted at least since the speciation of *Z. tritici* approx. 11,000 years ago. The long-term maintenance of these chromosomes is at odds with the frequently observed loss of one or more of these chromosomes in isolates collected in a wide variety of environments. Here we performed two types of experiments to address the functional role and the underlying mechanisms of the maintenance of accessory chromosomes in *Z. tritici*. We tested the functional relevance of these chromosomes using a forward genetics approach based on isogenic whole chromosome deletion strains in the reference isolate IPO323. Surprisingly, the presence of accessory chromosomes is associated with a fitness cost *in planta*, but dependent on the host genotype. An associated fitness cost should favor complete loss, not maintenance, of the chromosomes in the fungal population. However, additional mating experiments *in planta* between isolates differing in their set of accessory chromosomes subsequently revealed a possible explanation for the chromosomes' persistence. Unpaired accessory chromosomes, i.e. present only in one parental strain, are found to segregate in a non-mendelian manner during meiosis, leading to an overrepresentation of these unpaired accessory chromosomes in the resulting ascospores. Moreover, this segregation distortion was shown to be the result of a female meiotic drive, which was in turn influenced by environmental factors. We conclude that the maintenance of the accessory chromosomes in the fungal population is caused by meiotic drive and suggest that these chromosomes represent a novel group of selfish genetic elements.

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Genome-wide mapping of meiotic recombination products generated by hybrid crossing of two *Trichoderma reesei* wild isolates QM6a and CBS999.97

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Meiosis is a specialized cell cycle that generates genetically divergent gametes or the sexual spores in fungi. Genome-wide DNA double-stranded breaks (DSBs) are spontaneously generated during meiotic prophase and then repaired by homologous recombination using two non-sister homologous chromosomes as templates instead of the sister chromatids. Interhomolog recombination ultimately leads to chiasmata which hold homologous chromosomes together until the transition from metaphase I to anaphase I to ensure proper chromosome segregation. The evolutionarily conserved endonuclease Spo11 is responsible for the initiation of the majority of meiotic DSBs in most studied experimental model organisms. The absence of Spo11 usually results in infertility due to abnormal chromosomal alternations, disrupted meiosis, apoptosis or the production of aneuploid gametes and sexual spores. We show that three *Trichoderma* species (*T. reesei, T. atroviride* and *T. virens*) each contains a conserved *spo11* gene. Removal of the *T. reesei spo11* gene resulted in no apparent defect in the entire sexually developmental process, including the formation of stromata, perithecia, asci and ascospores. To further reveal the molecular mechanism of meiotic recombination in the presence and absence of *spo11*, we have applied both next- and third-generation sequencing platform to generate high-resolution genetic maps before and after sexual crossing of two *T. reesei* wild isolates QM6a and CBS999.97. Our results will be useful for applying sexual crossing to industrial strain improvements.

Long non-coding RNA meets transactivator for regulation of cellulase expression

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Trichoderma reesei is a saprotrophic fungus that is used in industry for production of carbohydrate-active enzymes such as cellulases. We discovered an intergenic region as a regulatory factor that influences the expression of these enzymes. It turned out that this region actually codes for a long non-coding RNA (*HAX1*). Interestingly, the length and consequently the length of *HAX1* seem to have evolved during the strain selection process done for industrial purposes. We observed a *HAX1*-dependent phenotype in differently good enzyme producing strains. The essential transactivator of the cellulases (i.e. the Xylanase regulator 1) and *HAX1* do not only share a palindromic sequence, *HAX1* also bears an unusual number of binding sites for the transactivator. We wished to learn if and how those two interact and how exactly the regulation of gene expression works on the molecular level. Based on our recent findings we are able to present a model on this regulatory mechanism.

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DNA replication is required for circadian clock function by regulating rhythmic nucleosome composition

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Although the coupling between circadian and cell cycles allows circadian clocks to gate cell division and DNA replication in many organisms, circadian clocks were thought to function independently of cell cycle. Here we show that DNA replication is required for circadian clock function in *Neurospora*. Genetic and pharmacological inhibition of DNA replication abolished both overt and molecular rhythmicities by repressing *frequency* (*frq*) gene transcription. DNA replication is essential for the rhythmic changes of nucleosome composition at the *frq* promoter. The FACT complex, known to be involved in histone disassembly/reassembly, is required for clock function and is recruited to the *frq* promoter in a replication-dependent manner to promote replacement of histone H2A.Z by H2A. Finally, deletion of H2A.Z uncoupled the dependence of circadian clock on DNA replication. Together, these results establish circadian clock and cell cycle as interdependent coupled oscillators and identify DNA replication as a critical process in circadian mechanism.

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Genomics of fungal DNA repair

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All organisms operate many proteins that protect their chromosomes from the continuous damage they suffer every day. Most of what we know about DNA damage response in fungi comes from model organisms like *Saccharomyces cerevisiae*; it is unknown to what extent the data obtained in model organisms is relevant to the entire fungal kingdom. We compared 100 DNA damage response genes across the genomes of ascomycete fungi and found significant divergent from the current dogma. First, while the MRE11 gene is highly conserved its partner at the MRX complex RAD50 is far less conserved at least in the classes of Saccharomycotina, Sordariomycetes and Dothideomycetes. Second, the major recombinase of the eukaryotic world Rad51 is far less conserved than its paralog Rad55 in the orders of Sordariomycetes and Dothideomycetes, suggesting that Rad55 can act as a standalone recombinase. Third, on one hand Dothideomycetes lack the conserved holiday junction resolvase MUS81, on the other hand they show high degree of conservation of SRS2. This combination suggests that the balance between crossover and non-crossover is tilted towards the latter. We also examined the transcription response of *Fusarium oxysporum* to DNA damage. Surprisingly, the nucleotide excision repair machinery, UVDE or PHR1 are not stimulated by UV. Moderate degree of stimulation of other DNA repair genes by UV is observed in fungi exposed to 50J but not 200J. Interestingly UV repair genes are induced during the transition of conidia to germilings. In contrast to UV, there is a massive transcriptional response to MMS in *Fusarium oxysporum*. Most interesting is the over expression of nucleotide repair genes. In summary, our DNA damage response analysis is the most comprehensive one ever done in filamentous fungi. We show significant divergence of the response from the one described in model organisms such as *Saccharomyces cerevisiae*.

Facultative heterochromatin in *Fusarium*: Control of gene regulation by Polycomb Repressive Complex 2

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Polycomb Group (PcG) proteins generate facultative heterochromatin by trimethylating histone H3 lysine 27 (H3K27me3). Members of the conserved Polycomb Repressive Complex 2 (PRC2) include the H3K27 methyltransferase, KMT6, and its binding partners SUZ12, EED, and –at least sometimes– MSL1. In humans, mutation of PRC2 components result in developmental defects, inherited diseases and sporadic cancers. Deletion of *kmt6*, *eed*, or *suz12* in *Fusarium graminearum* leads to complete loss of H3K27me3, which is accompanied by pleiotropic developmental defects, but deletion of *msl1* has no such drastic effects. Close to a quarter of all genes are increased in expression by more than fourfold in *kmt6*, *eed*, or *suz12* mutants, and many are involved in development, secondary metabolism and pathogenicity. We will report on the *in vivo* and *in vitro* effects of PRC2 subunit mutations. Minor changes in the primary sequence of KMT6 resulted in complete or intermediate loss of function. Cytology and ChIP-seq showed partial mislocalization of KMT6-GFP in some of these strains. Several mutations affected the allosteric regulation of KMT6 by the EED or SUZ12 subunits. To uncover suppressors of H3K27me3 silencing, and to identify functional equivalents of PRC1 (an animal complex that binds H3K27me3 yet does not exist in fungi), we developed a forward genetics approach, relying on de-repression of a *neo* reporter gene in a reliably silenced region. Dozens of primary mutants, called *defective in silencing* (*dis*) or *drug response attenuated* (*dat*), were classified by distinct growth phenotypes and global gene expression patterns evidenced by transcriptome sequencing. Mutations in *dis* and *dat* genes were identified by bulk segregant analyses followed by sequencing. The first mutant, *dis1*, carries a truncated *eed* gene, and completely phenocopies the deletion mutant we constructed. We will report on additional components of the PcG silencing system.

CS6: FUNGAL-HUMAN INTERACTIONS
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Sterilizing immunity in the lung relies on targeting fungal apoptosis-like programmed cell death

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Humans inhale mold conidia daily and typically experience lifelong asymptomatic clearance. Conidial germination into tissue-invasive hyphae can occur in individuals with defects in myeloid function, though the mechanism of myeloid cell-mediated immune surveillance remains unclear. By monitoring fungal physiology in vivo, we demonstrate that lung neutrophils trigger programmed cell death with apoptosis-like features in *Aspergillus fumigatus* conidia, the most prevalent human mold pathogen. An anti-apoptotic protein, AfBIR1, opposes this process by inhibiting fungal caspase activation and DNA fragmentation in the murine lung. Genetic and pharmacologic studies indicate that AfBIR1 expression and activity underlies conidial susceptibility to NADPH oxidase-dependent killing and, in turn, host susceptibility to invasive aspergillosis. Immune surveillance exploits a fungal apoptosis-like programmed cell death pathway to maintain sterilizing immunity in the lung.

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Extracellular alkalinization drives the development of pathogenic *Candida albicans* biofilms

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Candida albicans is the fourth most common cause of nosocomial bloodstream infections that are often initiated by adherence of host cells as well as medical indwelling devices. The formation of fungal biofilms goes along with development of multilayered and polymorphic cell communities surrounded by an extracellular matrix, which promotes high resistance against antifungal treatments.

Biofilm formation is essentially linked to robust filamentation, since filamentation-defective *C. albicans* strains display thinner biofilms. When grown on host-relevant alternative carbon sources, such as amino acids, the fungus can raise of environmental pH. This process leads to hyphal morphogenesis and can be observed *in vitro* and within immune cells. Here, the transcription factor Stp2 was shown to be the key regulator, because its loss led to an impaired alkalinization. In contrast to planktonic cells, the growing biofilm develops nutrient and gas gradients, with increasing abundance of alternative carbon sources. Thus, we propose that *C. albicans* actively alkalinizes the environment during biofilm maturation. Therefore, our goal is to develop methods to measure extracellular pH in biofilms.

In vitro tracing of pH development was facilitated by using pH micro-electrodes and preliminary results identified a diminished pH rise in biofilms of *C. albicans stp2*Δ deletion mutants in comparison to the wild type strain SC5314. This pH defect correlated with thinner biofilms and a reduction in biomass under static and shear flow conditions. Thus, we predict that the transcription factor Stp2 interconnects amino acid metabolism and biofilm formation. As heterogeneous structures, biofilms comprise environmental microniches and we applied pH-sensitive fluorescent dyes to follow in a non-invasive manner the distribution of pH microscopically. Three-dimensional profiling revealed large neutralized areas in a mature biofilm that was grown in an unbuffered, initially acid medium.

The study of pH-dependent biofilm formation will reveal potential novel targets to combat this cause of severe infections.

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Role of *MNN1* gene family in *Candida albicans* in mannan structure and host immune recognition

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Cell surface molecules are significant in host-pathogen interactions as the host defence system is capable of recognizing a range of cell surface pathogen-associated molecular patterns (PAMPs) with the help of specific pathogen recognition receptors (PRRs).

The outer *N*-mannan-rich microfibrillar layer of the *Candida albicans* cell wall is complex, heterogeneous and differs in chemistry in different cell morphotypes. The *N*-mannan is composed of an α-1,6 mannose backbone with side chains predominantly of α-1,2 mannose capped with α-1,3 mannose. Because the α-1,3 mannose capping sugars are likely to be important for mannan recognition by C-type lectins and other mannan-recognising PRRs we aimed to create and characterise a complex mutant lacking all six members of the Mnn1 α-1,3 mannosyltransferases. .

A previous study reported the characterization of individual null mutants of the *MNN1* family members, however, with the exception of the *mnn14* null, none of the other mutants displayed any defects – most likely due to functional redundancies between the family members (1).

We created a *mnn1*Δ[6] mutant, disrupting all twelve alleles of *MNN1* family, using CRISPR-Cas9 mediated gene disruption. Analysis of mannan structure by NMR in ten independent *mnn1Δ*[6] mutants showed an expected loss of peak corresponding to α-1,3 mannan residues and longer mannan side chains suggesting that α-1,3 mannose capping contributed to side-chain termination. However, no change in cell wall porosity was observed in the hextuple mutants. Immune recognition by human monocytes and mouse RAW macrophage cell line measured by cytokine production were altered in the *mnn1Δ*[6] mutants.

Our results thus suggest for the first time that the terminal capping mannan residues regulate mannan chain length in *C. albicans*, and PAMP recognition by host PRRs leading to altered recognition and immune response.

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Copper and redox homeostastis of human pathogenic fungi are targeted by the mycophagous amoeba *Planoprotostelium fungivorum*

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Candida, Aspergillus and *Cryptococcus sp.* are the leading causative agents of systemic mycoses. Many of their representatives have also been isolated from non-human sources, suggesting that their virulence potential might have been partially shaped in their natural niche as a strategy to counteract environmental predators. Previously we demonstrated that basic phagocytic interactions of filamentous fungi are conserved between soil amoeba and macrophages. We have now established a new natural amoeba model system to study evolutionary forces that could have supported the generation and maintenance of virulence traits in human pathogenic fungi. *Planoprotostelium fungivorum* is an exclusively mycophagous amoeba, widely spread in nature, which we have successfully isolated and completely sequenced its genome. Large-scale feeding experiments revealed a broad prey spectrum including several yeasts and filamentous fungi. With an extreme efficiency, the yeasts were recognized, phagocytosed and killed in a range of few minutes. Filamentous fungi, like *A. fumigatus*, were too large to be ingested, but could be killed by a “lysocytosis”-like mechanism, which included the punctuate opening of the hyphae, followed by efflux of the cytoplasmic content. As *Candida parapsilosis* was found to be a preferential prey, we used this pathogen in a dual-transcriptome approach to identify targets of this predatory-prey interaction. Amoeba-responsive genes included those involved in the elevated metal efflux, oxidative stress response, filamentuous growth and secreted lipase, suggesting predatory selection pressure on these important virulence determinants. As a killing mechanism, we propose mobilization of internal copper resources leading to an impaired oxidative stress response and intoxication inside of the acidic phagolysosome. Constructed deletion mutants for the most promising targets will further reveal if traits that have originated to counteract with natural predators could also have supported the resistance against innate immune cells.

CS6: FUNGAL-HUMAN INTERACTIONS
(Tuesday, February 27, 2018 17:40)

Structural and functional analysis of CFEM proteins for hemoglobin iron acquisition

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Candida albicans is a human commensal microorganism that can cause life-threatening systemic infections in immunocompromised individuals. The human host invests substantial efforts into withdrawing iron from potential pathogens. To overcome this, *C. albicans* has evolved a pathway for heme-iron scavenging from hemoglobin. This pathway includes a network of secreted and GPI-anchored extracellular proteins containing the cysteine-rich CFEM domain, which can extract heme from hemoglobin and transfer the heme across the cell envelope from one CFEM protein to the next, until delivery to the endocytic pathway. The crystal structure of the secreted CFEM protein Csa2 reveals that the CFEM domain adopts a novel helical-basket fold consisting of six α-helices, stabilized by four disulfide bond between eight conserved cysteine residues. Site-directed mutants of these cysteines suggest they play no role in heme binding or transfer. The planar heme molecule is bound between a flat hydrophobic platform located on top of the helical basket and a flexible N-terminal loop. Uniquely, an aspartic acid residue serves as the axial heme-iron ligand. Mutational analysis of the Csa2 protein surface identified mutants that are able to bind heme and extract it from hemoglobin, but that are defective in heme transfer. These mutants may identify a site of interaction between CFEM proteins that mediates heme transfer. Our results, which reveal the molecular details of the mechanism used by a fungal pathogen to overcome host nutritional immunity, may enable the development of novel antifungal therapies.

CS6: FUNGAL-HUMAN INTERACTIONS
(Tuesday, February 27, 2018 18:05)

Microscopic analysis of filamentous fungi and their interaction with immune cells

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Due to their tiny habitus, their complex organisation, and their strong auto-fluorescence, fungal hyphae are not easily analysed by microscopy. Super-resolution techniques that are circumventing the resolution limit caused by the diffraction of light are of special interest for the mycological research. We evaluated the potential of diverse novel techniques in the investigation of fungal cells and their interaction with immune cells. We used direct stochastic optical reconstruction microscopy (*d*STORM) to investigate the interplay of natural killer (NK) cells with *Aspergillus fumigatus* and the distribution of the pattern recognition receptor dectin-1 in macrophages. Furthermore, we exploited the inherent blinking behaviour of eYFP for single-molecule localisation and reconstruction to reveal the spatial distribution of the rhodopsin CarO in hyphae of *Fusarium fujikuroi*. *In vivo* confocal laser scanning microscopic (CLSM) analysis of membrane-stained murine macrophages (MH-S) revealed the time course of engulfment of fungal conidia, yielding data that are suitable for subsequent bioinformatical analysis. In order to test the influence of mutations or different treatments in the dynamics of fungal spore germination we recently developed the ImageJ toolbox HyphaTracker. This toolbox is used to process microscopic acquisitions (movies) of conidial germination. Regions of interest (ROIs) are extracted, which are analysed for their area, circularity, and timing. ROIs originating from germlings crossing other hyphae or the image boundaries are omitted during analysis. Each conidium/hypha is identified and related to its origin, thus allowing subsequent categorization. The efficiency of HyphaTracker was proofed and the accuracy was tested on simulated germling and bright field microscopic images of conidial germination of rhodopsin-deficient *F. fujikuroi* mutants, revealing earlier and faster germination of the CarO deficient mutant. This toolbox will also be useful in the research on pathogenic fungi to investigate the influence of drugs or mutations on the early hyphal development.

CS7: FUNGI IN THE ENVIRONMENT
(Tuesday, February 27, 2018 15:30)

Mycobiome and metatranscriptomic analysis of asymptomatic and symptomatic Norway spruce trees naturally infected by the conifer pathogen *Heterobasidion* spp

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Microorganisms are ubiquitous residents of forest tree tissues living in close symbiosis with their hosts. An important role of plant microbiome in maintaining the host fitness is supported by numerous studies. Despite significant progress achieved in our understanding of the factors affecting the composition of microbial communities associated with trees, very little is known about the effect of plant pathogens on their structure. Similarly not much is known on the effect of beneficial fungal biota on the disease dynamics and progression. We investigated the effect of *Heterobasidion* root and butt rot disease on fungal communities associated with Norway spruce. The sequenced ITS2 data were analyzed using the mothur standard operation pipeline. Canonical analysis of principle coordinate (CAP) and principal coordinates analysis (PCoA) were used to visualize the fungal community structure. To identify differentially secreted host transcripts and *in-planta* expressed pathogen genes, the processed RNA-seq data were mapped against genome assembly of Norway spruce and *Heterobasidion annosum* respectively. Our results demonstrate that diseased and asymptomatic trees significantly differed in the structure of the fungal communities residing in their sapwood, but not in other anatomic regions. Each of the investigated tissues (sapwood, bark, needles and suberized roots) harbored a unique fungal community. *Heterobasidion* infected trees were also more susceptible to co-infection by other saprotrophic wood degrading fungi. Furthermore, inspite of considerable overlap in the number of shared mycobiomes, metatranscriptomic analysis revealed pronounced differences in gene expression pattern among the individual symptomatic and asymptomatic trees. The distance-based linear model analysis showed that expression levels of several genes with a predicted role in host defense (e.g. NBS-LRR disease resistance protein) were significantly correlated with the abundance of *in planta* expressed gene transcripts of *Heterobasidion* spp. Our results indicate that plant pathogens may cause significant changes in the structure of microbial communities associated with trees.

CS7: FUNGI IN THE ENVIRONMENT
(Tuesday, February 27, 2018 15:55)

Fungi in the forest ecosystem: habitats, diversity, and contribution to ecosystem processes

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Globally, forests represent highly productive ecosystems that act as carbon sinks where soil organic matter is formed from residuals after biomass decomposition as well as from rhizodeposited carbon. Fungi inhabit various forest habitats: foliage, the wood of living trees, the bark surface, ground vegetation, roots and the rhizosphere, litter, soil, deadwood, rock surfaces, invertebrates, wetlands or the atmosphere, each of which has its own specific features, such as nutrient availability or temporal dynamicy and specific drivers that affect fungal abundance and the composition of their communities as well as the nature of ekosystém processes where fungi participate. Fungi are especially important in those forest habitats where decomposition of plant organic matter takes place and in soil, where they represent the necessary symbionts of trees and other plants, responsible for providing their hosts with nutrition. Many ffungi inhabit or even connect multiple habitats, and are thus incorporated in ekosystem processes in a very complex way. Forests are dynamic on a broad temporal scale with processes ranging from short-term events over seasonal ecosystem dynamics, to long-term stand development after disturbances such as fires or insect outbreaks and microbes respond to and mediate the changes that occur. We are now starting to appreciate the relative role of fungi in the forest microbiome that appears to show that they are largely responsible for plant biomass decomposition and their activity, especially of those taxa that are associated with root of host plants, show the peak of activity during the summer vegetation season in the temperate and boreal forests. Among temperate and boreal biomes, forests seem to be those most dependent on fungal activity.

Baldrian P. 2017: Forest microbiome: Diversity, complexity and dynamics. FEMS Microbiology Reviews 41:109-130.

Žifčáková L et al. 2017: Feed in summer, rest in winter: microbial carbon utilization in forest topsoil. Microbiome 5:122.

CS7: FUNGI IN THE ENVIRONMENT
(Tuesday, February 27, 2018 16:20)

Are small secreted proteins (SSPs) regulators of secondary metabolism in the white rot fungus *Pleurotus ostreatus* ?

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Understanding the functions of small-secreted proteins (SSPs) in fungi is at its early stages, and considered the least characterized component of the fungal secretome. The production of SSPs is associated with pathogens and symbionts, who usually have a high proportion of species-specific SSPs, many of which have been shown to function as effectors. SSPs have also been identified in saprophytic fungi, suggesting alternative, yet unknown, roles. We have identified 3 genes (*ssp1*, *2* and *3*) in *Pleurotus ostreatus* encoding proteins that have been annotated as SSPs. These genes exhibited a ~4,500- fold increase in expression, 24 hr following exposure to 5-hydroxymethylfurfural (HMF), a compound inhibitory to yeasts, which is formed during pre-treatment of plant biomass. HMF is efficiently degraded by the *P. ostreatus* wild type (PC9) strain. SSPs, aryl-alcohol oxidases (AAOs) and the intracellular aryl-alcohol dehydrogenases (AADs) were also induced after exposure to other aryl-alcohols, known substrates and inducers of AAOs, and during idiophase (after the onset of secondary metabolism). A knockdown strain of *ssp1* (KD*ssp1*) exhibited reduced expression of AAO-and AAD-encoding genes after exposure to HMF. Conversely, a strain overexpressing *ssp1* (OE*ssp1*) exhibited elevated expression of genes encoding AAOs and ADD, and a 3-fold increase in enzymatic activity of AAOs. Quantitative secretome analysis of the KD*ssp1,* OE*ssp1* and the parental PC9 strain, in 8, 10 and 13-day-old cultures were used to monitor the effect of SSP levels on protein accumulation over time. The genetic manipulations introduced conferred a time shift in the secretion pattern: OE*ssp1* entered the idiophase earlier than PC9, while the converse was observed for KD*ssp1*. We propose that SSPs have roles in saprophytes, and suggest that in *P. ostreatus* they function as part of the regulation of fungal transition from primary to secondary metabolism during the idiophase, such as occurs with the ligninolytic system.

CS7: FUNGI IN THE ENVIRONMENT
(Tuesday, February 27, 2018 17:15)

Bacteria-induced defense responses in the filamentous fungus *Coprinopsis cinerea*

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In the past few years, our laboratory has taken a reductionistic confrontation approach to study molecular defense strategies of the filamentous coprophilic fungus *Coprinopsis cinerea (Cc)* against Gram-positive and Gram-negative (G+ and G-) bacterial competitors. Thereby, antibacterial mechanisms of *Cc* were identified, comprising the novel peptide-based antibiotic copsin [1] as well as putative fungal enzymes hydrolyzing bacterial quorum sensing molecules of the homoserine lactone class [2]. Besides these constitutive defense lines of *Cc,* RNAseq data revealed the upregulation of (antibacterial) genes within its vegetative mycelium onto challenge with G+ and G- bacteria respectively. Since the two sets of upregulated fungal genes overlap to a high degree (Kombrink; manuscript in preparation), the presence of a common elicitor of *Cc* defense gene induction can be hypothesized. Furthermore, confrontation assays with axenic bacterial **C**ell **F**ree **S**upernatants (CFSs) showed similar fungal induction potentials as bacterial cell culture challenges, pointing towards a secreted compound. (Stöckli; unpublished). To confirm this hypothesis of bacteria-induced defense responses in *Cc* via secreted bacterial elicitor(s), fungal strains were constructed in which promoters of upregulated antibacterial genes are fused to the dTomato reporter gene. Hereby, the inductive potential of bacterial CFS fractions and peptidoglycan preparations can be tracked via immunoblotting or microfluidic and fluorescence-microscopic devices. Once the bacterial elicitor/s is identified and structurally characterized, the nature of the fungal receptor/s sensing the elicitor/s could be revealed by using *Cc* defense gene knockout strains. Overall, the successful identification of bacterial elicitor/s and fungal receptor/s could enable comparative studies on how conserved this inter-kingdom communication is between bacteria and other fungi of the same or different ecotypes as *Cc*.

[1] Essig *et al.,* 2014; The Journal of Biological Chemistry, 289(50).

[2] Stöckli *et al.,* 2016; Fungal Genetics and Biology, 102.

CS7: FUNGI IN THE ENVIRONMENT
(Tuesday, February 27, 2018 17:40)

The curious case of the black yeast *Hortaea werneckii* and its genome duplication

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The black yeast *Hortaea werneckii* (Pezizomycotina, Dothideomycetes, Capnodiales) is notable for its extreme halotolerance, being able to thrive at near-saturating concentrations of sodium chloride, but also growing in normal media without added salt. Cellular mechanisms behind its physiology are of considerable scientific interest, also due to their possible applications. However, nearly two decades of research on *H. werneckii* extremotolerance and taxonomy have been hampered by its diploid genome since the very beginning. The genome of the species has been published in 2013, but it was of poor quality due to suboptimal assembly of conserved duplicated regions. Four years later this led to a publication of a second genomic sequence, improved by the use of long-read, single-molecule sequencing. Analysis of duplicated genes showed that their coding sequences differed in almost 10% of nucleotides, while in 400 bp long upstream regions of these genes the level of mismatches was around 15%. The level of divergence and the structure of the predicted mating locus led to the conclusion that the diploid *H. werneckii* genome is most likely a consequence of a relatively recent endoreduplication rather than intra- or interspecific hybridisation. However, the conclusions that can be drawn from a single genome sequence are necessarily limited. In order to further investigate the phylogeny of *H. werneckii* and address the origin of its diploidisation, we re-sequenced 11 strains of the species isolated from different habitats around the world, as well as a strain of the related *Hortaea thailandica*. Results of the comparative genomic analyses of these data will be presented with a special focus on the whole genome duplication of *H. werneckii* and the challenges such a genome represents to wet laboratory and bioinformatic work.

CS7: FUNGI IN THE ENVIRONMENT
(Tuesday, February 27, 2018 18:05)

Omics and epigenetic investigation of radiation resistance mechanism in melanized fungi

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The proliferation of melanized fungi under cosmic radiation on spacecraft, as well as the presence of numerous melanized fungal species in the damaged nuclear reactor at Chernobyl suggest that these fungi are resistant to ionizing radiation (IR). However, the molecular mechanisms that contribute to radiation resistance in melanized fungi are not well understood. The DNA repair machinery for these organisms, for example, is not extraordinary. To identify novel responses to IR in fungi, then, we have investigated two radiation-resistant, melanized yeasts: the ascomycete Wangiella dermatitidis and the basidiomycete Cryptococcus neoformans. In both organisms, unmelanized cells were only moderately susceptible to acute IR exposure. Initial investigations of the epigenetic response of W. dermatitidis to IR stress suggest that survival mechanisms do not strictly involve DNA methylation, but may include alteration of histone modifications. Additionally, we completed an -omics investigation of the C. neoformans radiation response. RNA-seq analysis of melanized and unmelanized C. neoformans treated with a sublethal, acute dose of gamma radiation (200Gy) revealed IR-responding genes including DNA repair enzymes, cell cycle proteins, fatty acid catabolic enzymes, and antioxidants. The most highly regulated genes, however, encoded novel hypothetical proteins containing various motifs associated with mitochondrial RNA stability, DNA binding, and protein secretion. Deleting these genes resulted in susceptibility to IR. In contrast to previous reports, moreover, approximately 800 genes were significantly regulated by L-DOPA, a substrate for melanization in C. neoformans. Analysis of transcriptome data using a machine learning approach verified that expression levels of a distinct cluster of 102 genes were lower in the melanized cells than in the non-melanized cells. This cluster of genes was negatively regulated in melanized cells but positively regulated in non-melanized cells upon exposure to IR. This radiation modulation was additionally correlated to a shotgun metabolomics analysis.

CS8: PLANT-FUNGAL INTERACTIONS
(Tuesday, February 27, 2018 15:30)

A protein complex formed by four *Ustilago maydis* effectors is essential for virulence

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Ustilago maydis is a biotrophic fungal pathogen, which causes smut disease in its host plant maize. During colonization *U. maydis* secretes about 250 effector proteins to suppress plant defense responses and manipulate the host physiology for its own benefit. A majority of these proteins lack functional annotations and their role in virulence remains to be determined.

Effectors were categorized based on their expression pattern during the lifecycle of *U. maydis*. By focusing on the most highly upregulated genes during the initial interaction, we were able to identify three effectors whose deletion mutants were no longer able to cause disease. Deletion mutants of these three effectors, named *stp2*, *stp3* and *stp4* (stop after penetration) were still able to form appressoria and penetrate the plant, but arrested in the epidermal cell layer. The arrest was accompanied by plant defense responses including a disruption of the plant plasma membrane surrounding the fungal hyphae. A similar phenotype was observed for the previously described effectors *stp1* and *pep1* (Döhlemann *et al.)*. Co-IP/MS experiments using each of these essential effectors revealed that Stp1, Stp3, Stp4 and Pep1 form an effector complex. Interestingly, complex members did not show specific interactions with Stp2 or plant proteins. Our newest findings suggest that not only the presence of the individual complex members, but the formation of the complex itself is necessary for a successful colonization. We will report on our current efforts to identify the cellular compartment where the complex resides, the plant proteins it interacts with and will speculate on the function of the complex and its members.

CS8: PLANT-FUNGAL INTERACTIONS
(Tuesday, February 27, 2018 15:55)

A novel *Botrytis cinerea* MFS transporter provides tolerance towards glucosinolate breakdown products and act as virulence factor

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Glucosinolates are secondary metabolites that accumulate mainly in cruciferous plants. Glucosinolates hydrolytic products have antifungal properties and play a role in plants` resistance against pathogens. *Botrytis cinerea*, a necrotrophic pathogen, has variable sensitivity to glucosinolates. Here we studied the mechanisms of tolerance to glucosinolates in the fungus *B. cinerea*. Exposure of *B. cinerea* to glucosinolate breakdown products induced expression of *Botrytis cinerea* major facilitator superfamily transporter that involved in Isothiocyanates detoxification (*BcmfsI*). *B. cinerea* inoculated on wild-type *Arabidopsis thaliana* or on plants transgenic for high glucosinolate level activated *BcmfsI* expression to higher levels than *B. cinerea* on glucosinolate-deficient *A. thaliana* mutants. *B. cinerea* strains lacking functional MFSI (Δ*BcmfsI*) were more sensitive to glucosinolate breakdown products *in vitro* and *in planta*. Δ*BcmfsI* strains were less virulent on wild-type *Arabidopsis* plants, but not on glucosinolate-deficient mutants. We demonstrate here that the MFSI transporter is a virulence factor that increases tolerance of the pathogen to glucosinolates. We also demonstrate that *BcmfsI* can confer tolerance to hydrolytic products of glucosinolate in the yeast *Saccharomyces cerevisiae*.

CS8: PLANT-FUNGAL INTERACTIONS
(Tuesday, February 27, 2018 17:15)

Transposable elements contribute to the evolution of genomic diversity between strains of the plant pathogenic fungus *Colletotrichum higginsianum*

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The members of the genus *Colletotrichum* cause anthracnose disease on a broad range of crops and have a devastating economic impact. Further, the interaction between *Colletotrichum higginsianum* and its host, the model plant *Arabidopsis thaliana*, has been a useful pathosystem to study fungal hemibiotrophic infection of plants. Genomic analyses of other fungal plant pathogens have revealed that fast-evolving genomic compartments are often enriched in genes encoding effectors, which are diverse, small, secreted proteins involved in pathogenicity. In order to investigate if genomic compartmentalization exists in *C. higginsianum*, we sequenced the genome of strain MAFF305635-RFP from Japan with PacBio RSII, yielding an assembly of 49.8 Mb consisting of 28 contigs. This assembly was compared to the genome of strain IMI349063 from Trinidad and Tobago (Zampounis *et al.*, 2016). The two strains are closely related, sharing 88.2 % of sequence (≥ 99 % identity, ≥ 15 kb). However, this analysis revealed the presence of extensive genomic rearrangements between the two strains. Among these, 6 inter-chromosomal translocations and 4 intra-chromosomal inversions were identified. Whole-genome comparisons also revealed the presence of strain-specific regions in the genome of this species including regions encoding strain-specific effector candidates. Interestingly, large-scale genomic rearrangements and strain-specific regions tend to associate with transposable elements. This result suggests that mobile elements may increase the genomic plasticity of this pathogen by contributing to homology-based recombination through their repetitive sequences and direct disruption through transpositions. The novel insights from this study will help pathogenicity-related gene mining of *C. higginsianum* by considering its dynamic genomic changes.

CS8: PLANT-FUNGAL INTERACTIONS
(Tuesday, February 27, 2018 17:15)

Structure-guided engineering of synthetic immune receptors against the blast fungus

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Plant pathogens secrete a plethora of effector proteins to enable colonization of their hosts. These effectors interact with intracellular plant proteins to alter their function and promote infection. Plants are generally effective at fighting off pathogens and have evolved an effective immune system, including immune receptors of the nucleotide-binding, leucine-rich repeat proteins (NLR) class. However, NLRs tend to have a narrow recognition spectrum limiting their value in modern agriculture. Here, we present a strategy to improve NLR-mediated plant immunity using structural information of effector-target complexes. The fungus *Magnaporthe oryzae* (syn. *Pyricularia oryzae*) is one of the most devastating plant pathogens causing blast disease on a wide range of monocot hosts, including rice, wheat, and millet. The *M. oryzae* effector AVR-Pik is recognized by the rice NLR pair Pik1/2 through binding to a heavy metal associated (HMA) domain that has integrated into Pik-1. We identified a sequence related effector, APikL2 (AVR-Pik like 2), which is conserved in nearly all *M. oryzae* isolates. Similar to AVR-Pik, APikL2 binds to HMA containing proteins and structural analyses revealed a common HMA-binding interface between these two effectors. However, APikL2 is not recognized by the NLR pair Pik1/2 and because it is widespread in *M. oryzae* is a high value target for blast disease resistance development. We combined sequence alignments and structure-based information derived from effector-target complexes to identify polymorphic residues around the effector-HMA binding interface that could define binding specificity of the NLR. We then introduced these residues into the HMA-domain of the Pik1 NLR to generate synthetic immune receptors that bind APikL2 and thus carry expanded effector binding spectra. Our work highlights how basic understanding of the biochemical and biophysical basis of pathogen-host interactions can be used to retool plant immunity and generate novel immune receptor functionalities.

CS8: PLANT-FUNGAL INTERACTIONS
(Tuesday, February 27, 2018 17:40)

02. Plant-fungal interactions

Rhynchosporium commune effectors and their potential role during barley colonisation

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For over a century *Rhynchosporium commune* has remained one of the most destructive and economically important pathogens of barley. *R. commune* is a hemibiotroph with a prolonged asymptomatic phase. Following conidia germination on the leaf surface and cuticle penetration, fungal hyphae spread between the host epidermal cells without directly penetrating them.

Sequencing of the *R. commune* genome, and transcriptomes from germinated conidia and an early time point during barley infection, led to identification of putative effectors with roles in the interaction with the host plant. Some of the identified effectors, including a secreted chorismate mutase and a family of LysM domain proteins, may be responsible for the delay in symptoms development. The *R. commune* genome contains an extended family of genes encoding proteins with one or more LysM domains. LysM1, LysM5 and LysM7 contain just one LysM domain, while LysM2 - two domains, LysM3 - three domains, LysM4 and LysM6 - four domains. In addition, two genes code for enzymes containing LysM domain pairs, a subgroup C chitinase, which also contains a different chitin-binding motif, and a putative peptidoglycan lytic transglycosidase. Transcriptional upregulation of *R. commune* secreted chorismate mutase and several genes encoding LysM domain proteins at the start of barley colonisation suggests their importance during the early stages of interaction with the host. The ability of LysM2 and LysM3 produced using *Pichia pastoris* to bind chitin and chitosan suggest their role in prevention of the host immune response to chitin. Chorismate mutase on the other hand might be involved in manipulation of SA mediated defences in barley resulting in a compatible interaction. Targeted gene silencing of candidate effectors will help to determine their importance for pathogenicity.

CS8: PLANT-FUNGAL INTERACTIONS
(Tuesday, February 27, 2018 18:05)

MTF4 is a key transcription factor downstream of MOR required for plant-derived signal dependent appressorium development in *Colletotrichum orbiculare*

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Many plant pathogenic fungi including *Colletotrichum* species form a specialized infection structure, called an appressorium. Appressorium formation relies on perception of physical and biochemical signals at the plant surface. In cucumber anthracnose fungus *C. orbiculare,* the morphogenesis-related NDR (nuclear Dbf2-related) kinase pathway (MOR) is crucial for translating plant-derived signals for appressorium development (Kodama *et al*., 2017). To determine the downstream target of MOR, we performed whole genome transcript profiling of the NDR kinase Cbk1-AS strain, the MOR component *pag1* mutant strain and the wild type strain during appressorium formation. In the wild type strain, several transcription factor-encoding genes were upregulated on the host plant compared with artificial substrates. Among them, we identified 10 genes as *MTF* (MOR-related transcription factor) *1-10* that were downregulated in Cbk1-AS and/or *pag1* mutants compared with the wild type. Targeted gene deletion analysis of *MTF1-10* showed that *mtf4* mutant phenotypes were closely similar to *pag1* mutant. Introduction of the constitutively active variant of Cbk1 had no effect on *mtf4* mutant phenotypes. However, introduction of overexpression construct of *MTF4* to *pag1* mutant complemented *pag1* defects. These results indicate that *MTF4* acts downstream of MOR for appressorium morphogenesis induced by plant-derived signals. To test the response of *MTF4* to plant signals, we analyzed its cellular localization by fluorescent protein tagging. The Mtf4-GFP was recognized at nuclei during appressorium formation *in planta*, whereas no distinct localization of Mtf4-GFP was observed *in vitro*. The conditional inactivation of Cbk1 and *pag1* deletion decreased nuclear localization of Mtf4-GFP *in planta*. These results suggest that MOR-dependent localization of Mtf4 responds to plant-derived signals during appressorium development. Therefore, *MTF4* is a key downstream transcription factor of MOR in *C. orbiculare* for appressorium development in response to plant-derived signals.

SES: Universal expression system for fungi

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We have developed a novel orthogonal expression system (SES) that functions in a wide spectrum of eukaryotic organisms. The expression system is based on a synthetic transcription factor (sTF) that regulates expression of the target gene via a sTF-dependent promoter. The sTF expression is driven by an engineered, universal core promoter that provides a low, but sufficient expression level of the sTF. The sTF expression is constitutive in all tested growth conditions and developmental and growth stages. The sTF-dependent promoter regulating the expression of the target gene also contains a similar type of universal core promoter, making the whole expression system independent of the host's native regulation and therefore functional in diverse species. The combination of multiple sTF-binding sites and the core promoters enables specific adjustment over a wide range of target gene expression levels, from very low to very high. This expression system provides robust, stable, and tunable expression levels of target genes in a broad spectrum of host organisms with numerous applications in metabolic engineering and protein/enzyme production. Furthermore, it greatly simplifies the genetic tools needed for the construction of novel production hosts, including those with undeveloped know-how for the heterologous gene expression.

The method for selecting the universal core promoters, constructing the expression systems, and demonstrating their performance in diverse fungal species will be presented. In addition, the utility of the expression system will be demonstrated on heterologous gene expression in novel fungal hosts, which were previously not genetically modified.

Inducible expression of secondary metabolites using a synthetic transcription factor in an essentially background-free system

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Fungal secondary metabolites (SM) are a highly diverse group of compounds with many different biological activities; many can be used for medicinal purposes. For their industrial production, mainly the native hosts are used, which can be problematic regarding cultivation conditions (expensive additives, induction of SM production). An approach to circumvent these problems is heterologous expression in fungi. We have constructed a novel synthetic transcription factor for the industrial workhorse *Trichoderma reesei*, which facilitates strong and tightly controllable gene expression of its target genes using the cheap inducer estradiol. *T. reesei* is industrially used to produce cellulases and hemicellulases with outstanding production rates. Recently, we have identified a pleiotropic regulator of the secondary metabolism (1). The over-expression of this transcription factors results in a reduction of the native SM production in this fungus which possess already only few SM-encoding genes. The major SM of *T. reesei* are sorbicillinoids, whose synthesis can be completely shut off by deleting their main regulator (2). Additionally, we have identified and established a set of loci for targeted gene insertions, which allow insertion of SM encoding genes, *e.g.* the genes for biosynthesis of the pharmaceutical Lovastatin. All these findings and innovations enable strong and inducible expression of SM without the production of any mentionable side-products.

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- Derntl C, Rassinger A, Srebotnik E, Mach RL, Mach-Aigner AR. 2016. Identification of the Main Regulator Responsible for Synthesis of the Typical Yellow Pigment Produced by Trichoderma reesei. Appl Environ Microbiol 82:6247-6257.

Genome-wide chromatin mapping of *Aspergillus nidulans* reveals BasR, a novel regulator of bacteria-triggered fungal natural product biosynthesis

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Microorganisms can produce a plethora of secondary metabolites (SMs), which often have pharmacological potential (1). In nature, microorganisms live in multispecies communities, in which the produced SMs are often used as signal molecules. Mimicking the natural habitat in the laboratory by mixed fermentation experiments has been developed into a useful strategy to identify new SMs (2). We have been intensively studying the interaction between the model organism *Aspergillus nidulans* and the soil bacterium *Streptomyces rapamycinicus*, which leads to the activation of the silent fungal orsellinic acid (*ors*) gene cluster (3). Essential for the *ors* gene cluster activation is the activity of the lysine-acetyltransferase GcnE, which specifically acetylates lysine 9 and 14 of histone H3 during the co-cultivation (4). Furthermore we could show that the exchange of several amino acids of histone H3 in *A. nidulans* resulted in major changes in the penicillin, sterigmatocystin and orsellinic acid biosynthesis (5). This specific microbial interaction provides an excellent model system to study molecular and regulatory mechanisms underlying interspecies crosstalk. A genome-wide chromatin immunoprecipitation (ChIP) analysis was performed to analyse the distribution of the acetylation events during the interaction. Our data reveal major changes in the fungal chromatin landscape induced by the bacterium and led to the identification of the transcription factor BasR, required for the bacteria-induced activation of secondary metabolism.

(1) Macheleidt et al. (2016) Annu Rev Genet, (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

CS9: SYNTHETIC BIOLOGY AND SECONDARY METABOLITES - Sponsored by: MDPI TOXINS
(Wednesday, February 28, 2018 16:45)

Fungal Optogenetics: imagening biotechnological applications and imaging gene expression

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Light is a strong environmental cue. Learning how to harness it as a means to control gene expression opens the doors to new strategies to reprogram cell function. Thus, we have adopted different optogenetics strategies to utilize light as an orthogonal signal to control gene expression in yeast. *Saccharomyces cerevisiae* is naturally incapable of seeing light and therefore, we have design optogenetic switches to tune gene expression in this organism. Thus, now we can efficiently induce gene expression up to 1300-fold in *S. cerevisiae* and control biotechnological relevant phenotypes such as flocculation by switching on/off the lights.

We have developed these optogenetic switches utilizing LOV domains obtained from *Neurospora crassa*. The latter 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 dimerization that results in strong transcriptional activation, in a light-intensity dependent manner.

We have also adopted optogenetic approaches to further delve into Neurospora's circadian and light-responses. In doing so, we were able to genetically program 2D-images in this organism. Thus, we can project a photograph on top of a Neurospora carrying a luciferase reporter under the control of a light responsive promoter and obtain back a bioluminescent pattern mimicking the original image. Thus, we have established a live canvas in which images are genetically processed and reconstituted with real-time dynamics. Such technology not only allows studying light-responses with great resolution, but is also provides a powerful substrate for artistic projects. MN-FISB, FONDECYT 1171151, HHMI International Research Scholar Research Program

A CRISPR Cas9-based gene drive platform for studying complex genetic interactions in *Candida albicans*

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The opportunistic pathogen *Candida albicans* a the leading cause of fungal infection worldwide. Yet, genetic analysis in this clinically important pathogen remains cumbersome because it is a diploid organism that requires sequential allele knockouts to generate homozygous null mutants. This issue is further compounded when double mutant lines need to be generated, such as when studying critical genetic networks modulating virulence pathways. Here, we have developed a CRISPR-Cas9-based 'gene drive' platform for rapid, precise, and efficient genome editing in *C. albicans,* enabling applications for global genetic analysis of fungal pathogenesis. In our gene drive system, a modified DNA donor molecule is used that acts as a selfish genetic element, replaces the targeted site, and propagates to replace any additional wild-type locus it encounters. Coupling this approach with newly identified mating-competent haploid *C. albicans* lineages, we can rapidly and efficiently create diploid *C. albicans* strains that are double homozygous deletion mutants, enabling us to create large scale double-deletion libraries and analyze complex genetic interactions networks in *C. albicans* for the first time. We demonstrate the power of this technology by generating two double-gene deletion libraries, targeting factors involved in either drug efflux or cellular adhesion. By screening these libraries for sensitivity to antifungal perturbations or biofilm growth, we identify central regulators of these pathways, and determine how genetic interaction networks shift under diverse environmental conditions. This platform transforms our ability to perform complex genetic interaction analysis of virulence traits in *C. albicans* and could be readily extended to other clinically important fungal pathogens.

Design of an *Aspergillus nidulans* cell factory for the production of the natural food pigment carmine based on an artificial type II/III PKS system

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The natural red food pigments carmine (E120/cochineal) and carminic acid have been utilized by humans since 3000 BC. Industrial production of the pigments are based on extraction from the scale insect *Dactylopius coccus*. The market value of carmine is ~200 million USD/year, however, the market price experiences large year-to-year fluctuations due to changing production levels. Carminic acid is a glucosylated non-reduced aromatic polyketide that includes an octaketide with a C2-C15/C5-C14/C7-C12 backbone fold. The natural biosynthetic pathway remains enigmatic, and only a single enzyme, a C-glucosyltransferase (DcUGT2) responsible for the last step in the pathway, has been identified. We can here present the first fermentable microbial cell factory for the production of carminic acid, the dye component of carmine. The major challenge for construction of the cell factory was the lack of known natural PKS systems for forming the polyketide core of carminic acid. However, *de novo* biosynthesis was achieved by constructing an artificial PKS system, by combining a plant type III PKS (OKS) with an aromatase (ZhuI) and a cyclase (ZhuJ) from a bacterial type II PKS system. Co-expression of the three genes in *Aspergillus nidulans* resulted in production of the desired aromatic structure (flavokermesic acid anthrone). Further analysis of the strain revealed the production of flavokermesic acid and kermesic acid, both known downstream intermediates in the natural *D. coccus* pathway, showing that endogenous *A. nidulans* monooxygenases can catalyze several essential steps of the pathway. Final conversion to carminic acid was achieved by introducing *DcUGT2*. The artificial PKS system represents the first successful hyphenation of type II and III PKS systems. This synthetic biological solution can be generalized to form the first programmable PKS platform which allows for the production of a wide variety of different aromatic polyketides, by offering control over polyketide chain length and folding pattern.

CS10: ANTIFUNGALS AND FUNGICIDES
(Wednesday, February 28, 2018 15:00)

The *Aspergillus fumigatus* CrzA transcription factor activates chitin synthase gene expression during the caspofungin paradoxical effect

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Aspergillus fumigatus is an opportunistic fungal pathogen that causes invasive aspergillosis (IA), a life-threatening disease in immunocompromised humans. The echinocandin caspofungin, adopted as a second-line therapy in combating IA, is a β-1,3-glucan synthase inhibitor, which, when used in high concentrations, reverts the anticipated *A. fumigatus* growth inhibition, a phenomenon called the "caspofungin paradoxical effect" (CPE). The CPE has been widely associated with increased chitin content in the cell wall due to a compensatory upregulation of chitin synthase-encoding genes. Here, we demonstrate that the CPE is dependent on the cell wall integrity (CWI) mitogen-activated protein kinase $MpkA^{MPK1}$ and its associated transcription factor (TF) $RlmA^{RLM1}$, which regulate chitin synthase gene expression in response to different concentrations of caspofungin. Furthermore, the calcium- and calcineurin-dependent TF CrzA binds to and regulates the expression of specific chitin synthase genes during the CPE. These results suggest that the regulation of cell wall biosynthetic genes occurs by several cellular signaling pathways. In addition, CrzA is also involved in cell wall organization in the absence of caspofungin. Differences in the CPE were also observed between two *A. fumigatus* clinical isolates, which led to the identification of a novel basic leucine zipper TF, termed ZipD. This TF functions in the calcium-calcineurin pathway and is involved in the regulation of cell wall biosynthesis genes. This study therefore unraveled additional mechanisms and novel factors governing the CPE response, which ultimately could aid in developing more effective antifungal therapies.

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Studies on anilinopyrimidine resistance and mode of action in *Botrytis cinerea* suggest a mitochondrial target

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Anilinopyrimidine (AP) fungicides are used worldwide for the control of different ascomycetes, including the grey mould pathogen *Botrytis cinerea*. Currently, the mode of action of APs is proposed to be inhibition of methionine biosynthesis (http://www.frac.info/). However, although they were introduced more than 20 years ago, neither the mechanism(s) of resistance nor their molecular target have been determined. We combined different approaches to discover and validate mutations resulting in loss of sensitivity towards APs in *B. cinerea*.

UV mutagenesis followed by fungicide selection was used to generate resistant mutants in the lab. Resistance mechanisms were determined by next generation sequencing and validated by reverse genetics. This led to the identification of eight different nuclear genes in which specific non-synonymous nucleotide polymorphisms were independently conferring resistance. One of these genes, a homologue of the mitochondrial ABC transporter Mdl1 in yeast, was also found to be responsible for loss of sensitivity in a proportion of field samples displaying the AniR1 phenotype. However, a majority of AniR1 samples did not carry one of the mutations identified by UV screening.

A mapping population from a cross between an AniR1 isolate and a sensitive reference was generated, and the resistance locus determined by bulked segregant analysis. Resistance was linked to a region on chromosome 10, which was further delimited to the causal mutation by an iterative reverse genetic procedure. The resistance gene is homologous to the mitochondrial NADH kinase Pos5 of yeast. Phenotyping of deletion and overexpression mutants, as well as enzymatic tests suggested that APs act by perturbation of Bc-Pos5 activity. Mitochondrial and cell biology explorations showed a very elusive phenotype and complex interplay between the molecular target and the regulation of cell cycle progression. Further studies will be required to better understand the components involved and ascertain Bc-Pos5 as the primary target in *Botrytis*.

Regulation of Azole Resistance in Aspergillus fumigatus

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Aspergillus fumigatus is the most important airborne mould pathogen and allergen worldwide. Estimates suggest that over 3 million people have invasive or chronic infections that lead to in excess of 600,000 deaths every year. Very few drugs are available to treat the various forms of aspergillosis and we rely predominantly on the azole class of agents (Itraconazole, Voriconazole, Posoconazole and the recently licensed Isavuconazole). Resistance to the azoles is emerging. For individuals that are infected with a resistant isolate the mortality rate exceeds 88%. Therapy failure is in part attributed to delays in administering alternative therapies so methods to rapidly detect resistance is critical. While resistance in around 50% of clinical isolates has been linked to modification of the gene encoding the target of the azoles, *cyp51A*, our understanding of what leads to resistance in the remaining strains is lacking.

We have undertaken a programme of work to identify transcriptional and post transcriptional regulators of azole resistance. We have discovered a cohort of transcription factors and kinases including the CCAAT-binding complex, Negative Cofactor 2 and ssn3 that modulate azole tolerability. With a view to understanding which genes are directly regulated by these transcription factors, we have performed genome-wide protein-DNA interaction analysis using ChIP-seq. We have identified that these transcription factors bind the promoters of genes known to be associated with azole tolerance including *cyp51A* the drug transporter *cdr1B*. Our Chip-seq data provides evidence to suggest that these regulators bind the promoters of many genes however do not always modulate their expression. We are currently exploring the role of ssn3 and a number of other kinases in the regulation of azole resistance.

UDP-KDG, a transient antifungal metabolite, weakens fungal cell wall partly by inhibition of UDP-galactopyranose mutase

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Can accumulation of a normally transient metabolite affect fungal biology? UDP-4-keto-6-deoxy-glucose (UDP-KDG) represents an intermediate stage in conversion of UDP-glucose to UDP-rhamnose. Under natural conditions, UDP-KDG is undetectable in living cells because it is quickly converted to UDP-rhamnose by the enzyme UDP-4-keto-6-deoxyglucose-3,5-epimerase/-4-reductase (ER). We previously found that deletion of the *er* gene in *Botrytis cinerea* resulted in accumulation of UDP-KDG to levels that were toxic to the fungus due to destabilization of the cell wall. Here we show that these negative effects are at least partly due to inhibition by UDP-KDG of the enzyme UDP-galactopyranose mutase (UGM), which reversibly converts UDP-galactopyranose (UDP-Gal*p*) to UDP-galactofuranose (UDP-Gal*f*). An enzymatic activity assay showed that UDP-KDG inhibits the *B. cinerea* UGM enzyme with a Ki of 221.9 µM. Deletion of the *ugm* gene resulted in strains with weakened cell wall and phenotypes that were similar to those of the *er* deletion strain, which accumulates UDP-KDG. Gal*f* residue levels were completely abolished in the Δ*ugm* strain and reduced in the Δ*er* strain, while over-expression of the *ugm* gene in the background of a Δ*er* strain restored Gal*f* levels and alleviated the phenotypes. Collectively, our results show that the antifungal activity of UDP-KDG is due to inhibition of UGM and possibly other nucleotide sugar-modifying enzymes, and that the rhamnose metabolic pathway serves as a shunt that prevents accumulation of UDP-KDG to toxic levels. These findings, together with the fact that there is no Gal*f* in mammals, support the possibility of developing UDP-KDG or its derivatives as antifungal drugs.

Attenuating the emergence of drug resistance by harnessing synthetic lethal interactions in a model organism

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Drug resistance has emerged as a huge probem in many areas of medicine from cancer to infectious diseases. This is driving the development of novel therapeutic strategies. One that is gaining ground, is multi-target therapy, where combinations of drugs targeting different components of a disease network are deployed. A major impediment to this approach is the characterisation of sutiable targets for combination therapies. To date, most combinatorial therapy targets have been selected based on previous biological knowledge of drug mode of action and/or mechanisms of resistance, severely constraining the number of proteins that can be targeted.

Unbiased genome-wide screens will reveal many more components of the interaction networks of known drug targets, which could then be targeted in combination therapies. To test this principle in the context of antimicrobial resistance we have implemented an unbiased genome-wide screening technology, SGA analysis, facilitating characterisation of pair-wise synthetic genetic interactions. We performed an SGA screen with a *Candida glabrata* PDR1^{+} gain of function allele. *PDR1* encodes a transcriptional regualtor and gain of function mutations in this gene, are the principal mediator of fluconazole resistance in *C. glabrata*. We identified a *gcn5* null mutation as one negative synthetic interatin with *PDR1^{+}.* We showed that deletion of *GCN5* and/or chemical inhibition of the protein Gcn5, are snythetically lethal with *PDR1^{+}.* These data demonstrate that deletion or chemcial inhibition of a *PDR1^{+}* synthetically lethal gene results in cellular death if wild-type *PDR1* mutates to a *PDR1^{+}* FLZ resistance conferring allele.

RNA interference in *Colletotrichum abscissum,* causal agent of citrus postbloom fruit drop

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Citrus is an economically important culture for many countries worldwide, being Brazil the world highest producer of sweet oranges and orange juice. The great concern for this culture is the severe incidence of several pathogens causing relevant economically losses. Among the fungal diseases post-bloom fruit drop (PFD) causes dramatically losses in production, it is characterized by damages in the blossoms that causes fruit infeasibility and early drop, the *Colletotrichum abscissum* is one of the causal agent. The control of this pathogen is based in chemical spraying that contribute to the resistance development over the time. Fungicides compounds have action in different targets, as the succinate dehydrogenase, which plays a hole in the tricarboxylic acid cycle and also in mitochondrial electron transport chain, making this enzyme a target to several fungicides. New technologies are being applied every year to better understand the pathogens biology, and it can contribute to plant diseases control as an alternative to chemical control. The RNA interference emerge as a potential technology for gene function studies as well as an approach for pathogens control. Here we investigate the presence and functionality of the RNAi machinery of *C. abscissum* and test genetically whether the chemically pre-defined fungal SDHi target may represent a promising target gene in HIGS plants. In addition, the mutants generated for this studies made possible the fungus infection process investigation. Furthermore, knockdown mutants of succinate dehydrogenase subunit gene resulted in morphological and pathogenicity changes. Therefore, we concluded that the RNA interference is an important tool that can be exploited to disease control.

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The evolutionary history of global Ramularia collo-cygni epidemics

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Ramularia Leaf Spot (RLS) has emerged as a global threat for barley production. Late appearance of unspecific RLS symptoms made that only with molecular diagnostics the fungus *Ramularia collo-cygni* (Rcc) could be detected as the biotic factor of disease. Recent research has shed more light on the biology and genomics of the pathogen, the cause of the recent global spread remains unclear.

To address urging questions, about life-cycle, transmission, and quick adaptation to control measures, we de-novo sequenced the genome of Rcc. Additionally, we sequenced fungal RNA from 6 different conditions to improve annotation. This resulted in a high quality draft assembly of about 32 Mb, with only 78 scaffolds (N50: 2.1 Mb). The overall annotation enabled the prediction of 12.346 high confidence genes. Genomic comparison revealed that Rcc has significantly diverged from related *Dothidiomycetes*, however without obtaining species-specific genome features.

To evaluate the species-wide genetic diversity, we resequenced the genomes of 19 Rcc isolates from multiple geographic locations and diverse hosts and mapped sequences to our reference genome. Admixture analyses show that Rcc is world-wide genetically uniform and that samples do not show a strong clustering on either geographical location or host species. Most samples cluster closely together, with the exception of three outliers.

Analysis of linkage disequilibrium shows that in the world-wide sample set there are signals of recombination and thus sexual reproduction, however these signals largely disappear when excluding the three outliers samples, suggesting that the main global expansion of Rcc comes from clonally propagating populations. We further analysed the historic population size (Ne) of Rcc using Bayesian simulations, which provides additional indicators for the timing of population expansions.

Ultimately, we discuss how recombination, clonal spreading and lack of host-specificity could further support global epidemics and place Rcc the category of high-risk plant pathogen.

Evolutionary convergence in fungal ligninolytic enzymes

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The development of similar solutions for the same selective pressure is a common event in biological systems. During the last decades evolutionary convergence has been extensively investigated using bioinformatic tools. Here we track the ability to degrade lignin as a phenotypic trait in fungi, and analyze its evolution in Polyporales, where many wood-rotting species are included. Recent studies on oxidative biodegradation of lignin established the origin of wood-rotting fungi in the Carboniferous period, associated with the production of the first ligninolytic peroxidases. The subsequent evolution of these enzymes in Polyporales is analyzed by ancestral sequence reconstruction and heterologous expression of synthetic genes (ancestral enzyme resurrection). Lignin degradation during the evolution of these organisms started with the production of peroxidases generating Mn^{3+}, a diffusible oxidizer acting on the minor phenolic moiety of lignin. Later, these enzymes acquired the ability to oxidize nonphenolic lignin directly at a surface oxidation site, while improving their stability at acidic pH where ligninolysis occurs in nature. However, the appearance of the surface tryptophan responsible for lignin oxidation was not an isolated event in the evolution of ligninolytic peroxidase. Ancestral enzyme resurrection showed the molecular changes that led to the appearance of the same surface oxidation site in two distant peroxidase lineages. By characterization of the resurrected enzymes, we demonstrate convergent evolution at the amino-acid level during the evolution of fungal peroxidases, and track the different changes leading to phylogenetically-distant ligninolytic peroxidases from ancestors lacking the ability to degrade lignin.

Genetic variation of Indonesian *Fusarium oxysporum* f.sp. *cubense* isolates and their pathogenicity on wild and cultivated banana species

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Fusarium wilt of banana – also known as Panama disease – is caused by the soil-borne fungus *Fusarium oxysporum* f.sp. *cubense* (Foc). Foc strains have been divided into races (1, 2 and 4) based on their pathogenicity towards certain banana varieties. The recent outbreak of Foc Tropical Race 4 (FocTR4) is threatening the global banana production and thus endangers food security, livelihoods of smallholders and the banana export trade. A better understanding of Foc pathogenic diversity is necessary to ensure a long-term solution for resilient banana cropping systems. We isolated a set of 75 Foc strains from Indonesia, the center of origin of both banana and Foc, which was phenotyped on an explorative set of wild and cultivated banana varieties comprising the well-known edible triploids Grand Naine and Gros Michel, and the wild diploids *Musa acuminata* var. *malaccensis* "Pahang" and "Pisang Rejang". Moreover, each strain was genetically characterized by genotyping-by-sequencing using DArTSeq technology. Analyses of variance showed a highly significant banana host x Foc isolate interaction component, indicating specific gene action. The majority of Foc strains was highly pathogenic on both Grand Naine and Gros Michel and were therefore classified as FocTR4, which was also confirmed by FocTR4-specific molecular diagnostics. However, FocTR4 isolates clearly differed in their aggressiveness towards Grand Naine, suggesting underlying genetic variation despite their generally accepted clonal nature. Foc strains that were solely pathogenic on Gros Michel were classified as Foc Race1 and negative for the FocTR4 diagnostic. Interestingly, "Pahang" and "Pisang Rejang" were highly resistant to the majority of Foc strains, including the most aggressive FocTR4 isolates. These data will contribute to a better understanding of the genetic basis of host-pathogen interactions in Foc-banana pathosystem.

Neofusicoccum *parvum*: Genetic uniformity and invasive spread in the Western Balkans

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During the past decade, various forest and ornamental trees and shrubs in the Western Balkans region have been damaged by outbreaks of canker and die-back disease. These have been caused by species of the Botryosphaeriaceae, including the globally distributed *Neofusicoccum parvum*. The aims of this study were to determine genetic diversity and structure between populations of *N. parvum* from Serbia and Montenegro. This was achieved using DNA sequence data of the internal transcribed spacer (ITS) rDNA, translation elongation factor 1-alpha (TEF 1-α), β-tubulin-2 (BT2) and microsatellite markers. The study included populations of *N. parvum* from Continental (CR) and Mediterranean (MR) climatic regions. *Neofusicoccum parvum* was shown to have a low gene and genotypic diversity across the region. The CR and MR populations of *N. parvum* were found to be partially differentiated from each other and no genotypes and only a single haplotype were shared between the two regions. The low genetic diversity of *N. parvum* on non-native trees and comparison of populations from other regions of the world suggests that this species has most likely been introduced into Western Balkans, possibly through the movement of diseased plants. *Neofusicoccum parvum* populations appear to have been influenced by recent founder events from an unknown source population.

Understanding the evolution of mushroom-forming fungi: macro-evolutionary analyses of a 5300-species phylogeny of Agaricomycetes

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The Agaricomycetes is one of the most morphologically and taxonomically diverse fungal lineages (comprised of 20000 species). It contains most of the iconic mushroom-forming fungi, yet, the macro-evolutionary mechanisms that have shaped its extant taxonomic and morphological diversity are barely known. To address this question, a large multigene phylogeny of Agaricomycetes was assembled, consisting of 5284 species and three loci (4835 nrLSU; 1253 RPB2 and 721 ef1-a sequences), including 1386 newly sequenced taxa. Maximum Likelihood trees were inferred along a phylogenomic backbone of 103 species and chronograms were estimated by applying temporal information of eight fungal fossils. Using BAMM (Bayesian Analysis of Macroevolutionary Mixtures) 40-60 core shifts (i.e. posterior-to-prior odds ratio ≥ 5) in diversification rate per tree were detected. Most core shifts were inferred in the Agaricales, suggesting that it has the largest speciation and extinction dynamics across Agaricomycete orders. Further, it was found that at least one mass extinction event has likely occurred during the evolution of the class (CoMET model and TESS analyses). Next, we investigated if morphological innovations found in fruiting bodies could underlie differences in diversification rate across Agaricomycete species. Some of the most typical evolutionary innovations of mushroom forming fungi are related to spore protection and propagation. Therefore it was tested whether three hallmark traits, the presence or absence of cap, the enlarged hymenial surface (by gills, pores, etc.) and the presence or absence of protective veil tissues have influenced diversification of the class. First the transition rates between character states (BayesTraits V.2.0) were estimated to reconstruct ancestral states and directionality in the evolution of these traits. This was followed by analyses of character state dependent diversification (BiSSE or MuSSE models), to test the effect of traits on species diversification. This study contributes to understanding the macro-evolutionary history of Agaricomycetes and to a better understanding of the evolution of mushroom-forming fungi.

A *Candida albicans* population genomics study

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Candida albicans is a diploid yeast species responsible for life-threatening infections in hospitalized patients and also the most frequent fungal commensal of humans. 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. While a parasexual cycle, involving mating between two diploid individuals carrying opposite mating types followed by random chromosome loss without meiosis has been described, no direct evidence for its occurrence in humans has been provided.

We have now sequenced the diploid genomes of 182 *C. albicans* isolates from healthy carriers, and superficial and invasive infections. Population structures defined using different molecular markers (SNPs, indels, CNVs) recapitulate that inferred from MLST, indicating predominantly clonal reproduction. Yet, events of introgression are observed, consistent with (rare) parasexuality events in the human host. As expected in a clonal diploid population, strain-specific recessive lethal and deleterious alleles were shown to accumulate. Interestingly, we have observed that a specific clade grouping *C. albicans* isolates with a number of distinctive phenotypic features and niche restriction shows reduced genetic diversity despite worldwide dissemination. Our analysis indicates that reduced virulence in these isolates is likely to result from the accumulation of homozygous nonsense mutations in several genes with known contribution to *C. albicans* pathogenicity.

A phosphodiesterase is responsible for the block of cellulase gene expression in light in *Trichoderma reesei*

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Plant cell wall degradation is of major importance for sustainable production of materials and fuel in the future. The biotechnological workhorse *Trichoderma reesei* produces the required enzymes as well as heterologous proteins for industrial applications.

We showed previously, that cellulase gene expression is regulated by light in *T. reesei* and that the photoreceptor ENV1 is involved in this process. Thereby, light tolerance with respect to cellulase production is increased in early high producer mutants such as QM9414. However, in the wild-type QM6a, cellulase levels drop dramatically in light. ENV1 is essential for elevated cAMP levels during growth in light and exerts its function at least in part via the cAMP pathway. It was assumed that ENV1 acts by dampening the function of phosphodiesterases. We tested this hypothesis by investigating mutants in the phosphodiesterase genes *pde1* and *pde2* in *Neurospora crassa*, which showed only minor regulatory effects with respect to cellulase formation or gene regulation upon growth on cellulose.

In contrast, in *Trichoderma reesei* we found that indeed PDE1 dampens transcript levels of the major cellulase gene *cbh1* in light on cellulose and this effect is mediated by ENV1. Accordingly, the light dependent growth defect of mutants lacking ENV1 prevails in double mutants with phosphodiesterases. Additionally our first data also suggest an effect on secondary metabolism.

In summary, we found an important contribution of a phosphodiesterase to cellulase gene expression as well as rewiring of the associated light dependent signaling pathway between *T. reesei* and *N. crassa*.

The Ca^{2+}-binding penta-EF-hand protein PEF-1 is part of a fungal resistance mechanism against cell fusion-induced lysis and membrane-destabilizing antifungals

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To establish a mycelial colony, germinating vegetative spores of *Neurospora crassa* fuse with each other and form a supracellular network. Fusion pore formation involves highly controlled cell wall breakdown and plasma membrane merger. These steps bear the risk of cell lysis and death by membrane rupture.

We identified the Ca^{2+}-binding penta-EF-hand protein PEF-1 as part of a proposed membrane repair mechanism. Subcellular localization and live-cell imaging revealed that PEF-1 is recruited to the fusion point of lysing germling pairs. Additionally, PEF-1 accumulates at the plasma membrane after treatment with antifungal and membrane-destabilizing drugs, such as nystatin or the plant defense compound tomatine. The treatment with tomatine also results in PEF-1 recruitment to septa and occlusion of the septal pore. Consistent with this finding, the growth of a *pef-1* knock-out mutant on medium containing tomatine is highly impaired, compared to the wild type strain.

In addition, PEF-1 functions appear to be conserved in the fungal kingdom. For example, the PEF-1 homologue of the grey mold *Botrytis cinerea* also shows membrane recruitment after tomatine treatment. Moreover, our data indicate that Pef-1p, as part of a repair mechanism, promotes survival of the human fungal pathogen *Candida albicans* inside of macrophages.

We hypothesize that, membrane damage results in the influx of calcium, which activates PEF-1, which in turn mediates plasma membrane repair. Further studies aim to characterize the molecular bases of this repair mechanism in different fungi. Since PEF-1 mediates resistance to the phytoanticipin tomatine, we are currently also testing its contribution to fungal virulence using *B. cinerea* as a model. Further studies will also investigate the role of Pef-1p -as a potential pathogenicity factor- during infections by *C. albicans*.

The Schizosaccharomyces pombe PPR protein Ppr10 associates with a novel protein Mpa1 and acts as a mitochondrial translational activator

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The pentatricopeptide repeat (PPR) proteins characterized by tandem repeats of a degenerate 35-amino-acid motif function in all aspects of organellar RNA metabolism, many of which are essential for organellar gene expression. In this study, we report the characterization of a fission yeast Schizosaccharomyces pombe PPR protein, Ppr10 and a novel Ppr10-associated protein, designated Mpa1. The ppr10 deletion mutant exhibits growth defects in respiratory media, and is dramatically impaired for viability during the late-stationary phase. Deletion of ppr10 affects the accumulation of specific mitochondrial mRNAs. Furthermore, deletion of ppr10 severely impairs mitochondrial protein synthesis, suggesting that Ppr10 plays a general role in mitochondrial protein synthesis. Ppr10 interacts with Mpa1 in vivo and in vitro and the two proteins colocalize in the mitochondrial matrix. The ppr10 and mpa1 deletion mutants exhibit very similar phenotypes. One of Mpa1`s functions is to maintain the normal protein level of Ppr10 protein by protecting it from degradation by the mitochondrial matrix protease Lon1. Our findings suggest that Ppr10 functions as a general mitochondrial translational activator, likely through interaction with mitochondrial mRNAs and mitochondrial translation initiation factor Mti2, and that Ppr10 requires Mpa1 association for stability and function.

Molecular signaling in the response to injury of *Trichoderma atroviride*

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Wound response in multicellular eukaryotes is essential for survival and is highly conserved in plants and animals. In our laboratory, we discovered that the filamentous fungus *Trichoderma atroviride* responds to injury by triggering hyphal regeneration and the formation of asexual reproductive structures. Transcriptomic analyses revealed that the mechanism of response to this stimulus is similar to that of animals and plants. Furthermore, biochemical data implicate ROS, eATP, and Ca^{2+} as signaling molecules, suggesting that the injury response mechanism is highly conserved in the three eukaryotic kingdoms.

We have recently found that mutants affected in the MAPKs Tmk1 and Tmk3 do not conidiate in response to injury. It is particularly interesting that, in addition to this phenotype, a D*tmk*1 mutant does not regenerate. Using this information, we defined a set of regeneration genes based on the transcriptomics analyses of mutants defective in this process, as well as, upon treatment of the wild type strain with drugs that block signaling pathways intervening in regeneration. Furthermore, we evaluated the injury response in mutants of the RNAi synthesis machinery of *T. atroviride*. The *Δdcr2* and *Δrdr3* strains presented a dramatic defect in regeneration ability and asexual reproduction in response to injury. To understand the molecular processes affected by the absence of the RNAi pathway, we performed transcriptomic analysis of the WT and *Δdcr2* strains subjected to injury, showing that signaling processes, DNA repair and cell cycle progression are essential to overcome this stress and are affected in the *Δdcr2* mutant.

Regulation of *Aspergillus nidulans* CreA-mediated catabolite repression by the F-box proteins Fbx23 and Fbx47

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The attachment of one or more ubiquitin molecules by SCF (Skp-Cullin-Fbox) complexes to protein substrates targets them for subsequent degradation by the 26S proteasome, allowing the control of numerous cellular processes. Glucose-mediated signalling and subsequent carbon catabolite repression (CCR) are processes relying on the functional regulation of target proteins, ultimately controlling the utilization of this carbon source. In the filamentous fungus *Aspergillus nidulans*, CCR is mediated by the transcription factor CreA which modulates the expression of genes encoding biotechnologically-relevant enzymes. Although CreA-mediated repression of target genes has been extensively studied, less is known about the regulatory pathways governing CCR and this work aimed at further unravelling these events. The Fbox protein Fbx23 was identified as being involved in CCR and the Δ*fbx23* mutant presented impaired xylanase production in repressing (glucose) and de-repressing (xylan) conditions. Mass spectrometry showed that Fbx23 is part of a SCF ubiquitin ligase complex that is bridged via the protein kinase GskA to the CreA-SsnF-RcoA repressor complex, resulting in the degradation of the latter in de-repressing conditions. Upon the addition of glucose, CreA dissociates from the ubiquitin ligase complex and is transported into the nucleus. Furthermore, casein kinase subunits A and B are important for CreA function during glucose signalling although the exact role of phosphorylation in CCR remains to be determined. The addiction of glucose makes the protein kinase GskA to leave the nucleus and lose the interaction with Crea-SsnF-RcoA repressor complex, the deletion of GskA shows a sick phenotype and impaired hydrolytic enzymes secretion. In summary, this study unravelled novel mechanistic details underlying CreA-mediated CCR and provides a solid basis for studying additional factors involved in carbon source utilization which could prove useful for biotechnological applications.

Keywords: CCR, F-box, SCF complex, CreA, GskA, CkiA, CkiB, xylanase.

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CS12: CELL REGULATION AND SIGNALLING
(Wednesday, February 28, 2018 17:35)

A regulated protein aggregation controls glucose response in *S. cerevisiae*

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The ability to respond to available nutrients is critical for all living cells. The AMP-activated protein kinase (SNF1 in *S. cerevisiae*) is a central regulator of metabolism that is activated when energy is depleted. We found that SNF1 activity in the nucleus is regulated by controlled relocalisation of the SNF1 activator Std1 into puncta at the NVJ. This process is regulated by glucose through the activity of the previously uncharacterized protein kinase Vhs1 and its substrate Sip5, a protein of hitherto unknown function. Unphosphorylated Sip5 associates with Std1 and prevents its accretion. Reversible Std1 puncta formation occurs under non-stressful, ambient conditions, creating inclusion bodies in the form of a liquid drop, and utilizes the Hsp40, Hsp70 and Hsp104 chaperones, similarly to the aggregation of toxic or misfolded proteins such as those associated with Parkinson's, Alzheimer's and CJD diseases. Our results reveal a controlled, non-pathological, physiological role of protein aggregation in the regulation of a major metabolic cellular pathway.

Poster Session Abstracts

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

A fungal specific cohesin subunit regulates developmental biology in *Fusarium oxysporum*

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Cohesin is an essential protein complex that assures sister chromatid cohesion and participates in DNA repair, transcription and maintenance of chromosome structure. The cohesin subunit rad21 has several paralogs; each paralog dictates the role of cohesin in different developmental stages such as mitosis and meiosis. We identified three rad21 paralogs in the fungal pathogen *Fusarium oxysporum*. One paralog is probably the canonical essential rad21 protein. Another is the meiosis specific gene rec8. The third one is non-conserved rad21 paralog (rad21nc) that was identified only in a small subset of fungi and could be horizontally transferred to *F. oxysporum* from *Fusarium solani*. All rad21 paralogs are expressed in *F. oxysporum* although the canonical paralog is expressed much more than the others. *F. oxysporum* strains deleted for the rad21nc paralog exhibit irregular conidiation, delayed maturation of hyphae, and defects in spore germination under mitosis stress. These phenotypes are leading us to hypothesize that rad21nc paralog functions in a checkpoint-like response during fungal development. The possible role of such checkpoint protein in fungal pathogenesis and will be further discussed.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Gul-1 mediates cell wall remodeling via the cot-1 pathway in *Neurospora crassa*

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In *Neurospora crassa,* impaired function of the NDR kinase COT-1 results in markedly thickened cell walls. This effect is partially suppressed by inactivation of *gul-1* (an RNA-binding protein involved in translational regulation of cell wall remodeling proteins). Using electron microscopy, we determined that inactivation of *gul-1* also results in improved characteristics of the *cot-1* cell wall and septa. These observations coincide with our findings that 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. The *gul-1* mutant was also found to be almost 2-fold more sensitive to a chitin synthase inhibitor (Nikkomycin Z), when compared to the wild type. We suggest that GUL1 contributes to changes in cell wall morphology and composition in a manner that confers the suppressive effect on *cot-1*. We found that *gul-1* is involved in regulation of the expression of several cell wall remodeling genes such as glucan/chitin synthases and chitinase in a manner which is at least partially independent of the classic cell wall integrity pathway. Our RNASeq analysis revealed that GUL-1 affects transcript abundance of at least 25 genes involved in cell-wall remodeling via the COT-1 pathway. Moreover, GUL-1 was also found to regulate additional pathways such as transmembrane transport as well as amino acid metabolism. Based on catRAPID algorithm analysis mRNAs of some of the differentially-expressed cell wall-related genes have been predicted to physically interact with the GUL-1 protein. RNA antisense purification (RAP) analysis coupled with mass spectrometry is currently being employed to determine GUL-1-mRNA interactions, including the possibility that GUL-1 binds its own transcript.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

iPool-Seq: A novel functional genomics approach to identify fungal insertion mutants enabling large-scale virulence factor mapping in plant fungal interactions

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Fungal plant-pathogens require virulence factors for the successful infection of their hosts. The elucidation of virulence factors is essential to shed light on fungal infection mechanisms and can pave the way to engineer resistant plants. *Ustilago maydis* has a large arsenal of putative virulence factors, but methods for comprehensive *in vivo* analyses are lacking. Here, we developed insertion Pool-Sequencing (iPool-Seq), which enables fast and reliable identification of virulence factors from insertional mutant pools. iPool-seq has a highly efficient Next-Generation Sequencing library generation with unbiased genome-wide incorporation of adapters. Inserted adapters contain unique molecular identifiers (UMIs) which facilitate the accurate assessment of insertional mutant fitness. We identified 16 significantly depleted mutants in a pool of 195 *U. maydis* insertional mutants. Strikingly, among the top hits we identified recently characterized essential *U. maydis* effectors ApB73, Pep1 and Pit2, demonstrating that iPool-Seq is functional under *in vivo* conditions. Moreover, we confirmed the impaired virulence of three candidate mutants via individual infection assays and confocal microscopy of infected plants with WGA-AF488 staining. In summary, iPool-Seq promises to be a versatile tool to identify fitness and colonization factors of plant-infecting or colonizing microbes. iPool-seq may be applicable in various plant-pathogen systems to identify virulence factors due to its highly sensitive and quantitative nature and has the potential to elucidate virulence factors on a genome-wide scale.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Modification of the genetic backround of *Mucor circinelloides* using a plasmid free CRISPR/Cas9 system

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The CRISPR/Cas9 genome editing system has been developed and optimized for several different organisms. In fungi, the transformation strategy includes a plasmid containing the crRNA, the trans-activating crRNA (tracrRNA) and the CRISPR-associated (Cas) nuclease, which are expressed together. In this study, we developed a plasmid free CRISPR/Cas9 system to modify the genetic background of *Mucor circinelloides*.

M. circinelloides is a carotene producing Mucoromycotina fungus used as a model organism in various molecular microbiological studies. Genetic manipulation of Mucoromycotina species based on homologous recombination is difficult to achieve and the mitotic stability of the resulting transformants is often low.

To optimize the plasmid free CRISPR/Cas9 system, we disrupted the *M. circinelloides carB* gene encoding the carotenogenic enzyme, phytoene dehydrogenase. PEG mediated protoplast transformation was used to introduce the Cas9 enzyme and a synthesized, *carB* specific gRNA with or without a DNA fragment containing the deletion cassette into the fungus. Successful disruption of the *carB* gene resulted in white colonies. If only the gRNA and Cas9 were transferred into the protoplasts, molecular analysis of the transformants indicated a 2300 nt gap upstream from the PAM sequence. If we co-transformed Cas9 and gRNA with the deletion cassette, the double-strand breaks of the DNA were repaired by homologous recombination.

After the optimization of the system, we have started to disrupt the three HMG-CoA reductase genes (*hmgR1*, *hmgR2* and *hmgR3*) of *M. circinelloides* separately and together. HMG-CoA reductase catalyses the rate-limiting step of the isoprene biosynthesis. Our results suggested that *hmgR1* plays an important role in the adaptation to the low temperature while disruption of *hmgR2* and *hmgR3* resulted in increased sensitivity to statins. The latter genes proved to be determining for the general isoprenoid biosynthesis as their combined disruption was lethal.

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POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Paxillin protein PaxB and actinin-like protein AcnA are required for cytokinesis via regulating actin ring assembly in *Aspergillus nidulans*

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Cytokinesis, as the final step of cell division, plays an important role in fungal growth and proliferation. In filamentous fungus *Aspergillus nidulans*, abnormal multinuclear or non–nucleated cells induced by defected cytokinesis will cause defected hyphal growth and sporulation. Previous studies have demonstrated that proper cytokinesis is accompanied with actin ring formation and contraction, which are regulated by (SIN) septation–initiation network that is consist of several conserved components. In our previous study, we found that actinin-like protein AcnA is essential for cytokinesis but underlined relationship between AcnA and SIN or between AcnA and actin cytoskeleton are not known yet.

In the present study, we have identified a cytoskeletal protein paxillin PaxB has a similar phenotype to that of AcnA, suggesting it is also essential for cytokinesis. In the absence of AcnA or PaxB, a key component of SIN pathway -MobA, was unable to contract at the predict septation site. Comparably, loss of function of SIN pathway could affect localization of AcnA and PaxB at the septation site. These results suggest that two cytoskeletal proteins AcnA and PaxB and the SIN pathway are reciprocal required to drive the proper cytokinesis. Moreover, deletion of *acnA* or *paxB* caused actin rings disappeared， which implies that AcnA and PaxB are crucial for actin ring formation. In addition, deletion of *acnA* leads to undetectable PaxB at the septation site, in comparison, deletion of *paxB* did not affect the location of AcnA but block its contraction during cytokinesis, which demonstrate that AcnA and PaxB are required for the function of each other. In *paxB* deletion mutant strains, septation and sporulation defects can be rescued by overexpressed *acnA*. These data suggest that AcnA and PaxB probably have an overlapping function for the proper function of SIN pathway and the formation of actin ring in *A. nidulans*.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Sem1, 26S proteasome degradation and cellular redox state

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Sem1 is *bona fide* lid subunit of the proteasome. The role of this intrinsically disordered protein was investigated in the multicellular model organism *Aspergillus nidulans* as the human corresponding gene is essential. We found that Sem1 from *A*. *nidulans* is required for oxidative stress response, mitochondria integrity and proteasome assembly. Sem1 is not required for vegetative fungal growth but it is essential for cellular differentiation and coordination of secondary metabolites. Oxidative stress response in the wild type included increased transcriptional levels of detoxificating enzymes and proteasomal subunits *semA* and *rpn11*, whereas the mutant strain exhibited damaged and dysfunctional mitochondria. EM revealed increased number of 20S proteasomes in Δ*semA* mutant strain with double the catalytic activity compared to the complementation strain. This enhanced degradation rate of 20S proteasome presumably serve the purpose of dealing with accumulation of damaged proteins due to oxidative stress.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Altering *Neurospora Crassa* MOB2A exposes specific functions and affects its interaction with the NDR kinase COT1

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MOB (MPS-1 binding) proteins act as activating subunits which are required for NDR kinase 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. Phosphorylation has been suggested to play a role in the regulation of MOB function. Two Tyr residues (Tyr117 and Tyr119) of MOB2A can potentially undergo phosphorylation. These residues 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). *mob-2a(Y117E,Y119E)* in a Δ*mob-2b* background is a temperature-sensitive mutant that exhibited extremely slow growth at 34^{o}C (optimal for the wild type). When cultured at a semi-restrictive temperature (32^{o}C) a significant reduction in growth rate (75%) and in distance between branches (50%) was observed. We have also determined that MOB2 proteins negatively regulate conidial germination and that even though MOB2A and MOB2B have some overlapping functions, MOB2B cannot compensate for MOB2A's role in conidiation and germination. In addition to their role in a-sexual development, MOB2A/B are also involved in the sexual reproductive cycle as mutating MOB2A/B affected perithecial development. Even though Tyr117 and Tyr119 do not reside within the predicted MOB2A-COT1 physical interface (based on the yeast model), altering these residues also affected the genetic and physical interactions between MOB2A and COT1, as determined by yeast two hybrid analyses.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Dynamics of a Kinetochore Protein Dam1 during pathogenic development in the Rice-Blast fungus

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Filamentous pathogenic fungi exhibit multiple morphological forms, associated with changes in cell polarity. Development in the rice-blast fungus *Magnaporthe oryzae* during vegetative hyphal growth proceeds by apical extension and lateral branching, while pathogenic development requires polarised elongation of the germ tube followed by an isotropic expansion of its tip into the infection structure called appressorium. These developmental transitions are tightly regulated by correct mitotic progression, nuclear segregation and migration. The kinetochore is a multi-protein complex that brings about high-fidelity nuclear segregation during anaphase. The fungus-specific outer kinetochore protein Dam1 plays a crucial role in maintaining the spindle structure, and thus in proper nuclear segregation and viability in yeasts. In addition to kinetochore localisation, Dam1 is also seen associated with microtubules in fission yeast. *In vitro* studies with *S. cerevisiae* proteins show Dam1 complex as oligomeric rings and patches around microtubules. However, the requirement of Dam1 function for viability and its localisation during cell cycle progression vary among different yeast species. Here, we found that the GFP-tagged Dam1 puncta(e) localise to the nucleus at the onset of mitosis, and intensify during nuclear segregation and migration in the filamentous fungus *M. oryzae*. Further, loss of Dam1 function (*dam1*Δ) led to prolonged mitosis with sluggish nuclear movement and difficulty in segregation, resulting in delayed and aberrant appressorium formation. Interestingly, GFP-Dam1 also localised to the tip of the growing hyphae or germ tubes during interphase, suggesting its role in cell polarity. The *dam1*Δ mutant showed reduced hyphal growth and aberrant hyphal morphology. Thus, our findings indicate that *Magnaporthe* Dam1, in addition to its role in nuclear division, likely has a broader function in the dynamics of the microtubular network and/or cell polarity even during interphase.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Different genes of the ubiquitin-proteasome system regulate development and virulence in the cereal pathogen *Fusarium graminearum*

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The ubiquitin-proteasome system (UPS) uses ubiquitin to mark proteins for rapid proteolysis and is a universal process in eukaryotes, including humans. Malfunctions in the UPS may result in severe diseases.

The fungal plant pathogen *Fusarium graminearum* is a major pathogen of cereals world-wide. During the initial infection on wheat flower leaves *Fusarium graminearum* forms two morphologically distinct structures, non-invasive runner hyphae and invasive compound appressoria. Transcriptome analyses of runner hyphae and infection cushions identified 158 genes most likely involved in the ubiquitin-proteasome system.

Disruption of two different F-box proteins and one ubiquitin-conjugating enzyme resulted in fungal mutants deficient in different aspects of development such as hyphal branching, sexual and vegetative propagation, and virulence.

A detailed proteomic analysis of the mutants identified changes in pathways relevant to the observed mutant phenotypes. Furthermore, motif analysis was done using all ubiquitin remnant peptides in an ubiquitin remnant motif antibody scan, followed by LC-MS/MS analysis. Proteins with altered ubiquitination will be presented and discussed in the context of the mutants phenotypes.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Regulators of fungal asexual development FlbE and FlbD orchestrate in vegetative hyphae the long-distance tip-to-nucleus dynamics of the transcription factor FlbB

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Asexual spores are the main vehicle for dissemination of filamentous fungi. In the model filamentous fungus *Aspergillus nidulans* asexual sporulation (conidiation) is induced by a transduction mechanism that connects signals received at the tip of hyphae with the transcriptional activation of *brlA*, the first conidiation-specific gene. Here we describe that the tip-to-nucleus dynamics of the transcription factor FlbB is controlled in space and time by the interactions with developmental regulators FlbE and FlbD. The former enables the apical localization of FlbB, and the role of its functional domains is characterized. Expression of an FlbE::FlbB::GFP chimera enables apical localization but inhibits nuclear accumulation, blocking conidiation. The insertion of a T2A viral peptide between FlbE and FlbB, which causes the split of a single mRNA (*flbE::mrfp::t2A::flbB::gfp*) into two proteins (FlbE::mRFP::T2A and FlbB::GFP), partially restores the nuclear accumulation of FlbB and conidia production. This suggests that, once at the tip, FlbB/FlbE interaction is apparently inhibited to initiate a basipetal transport to nuclei. We have also analyzed the dependence of this movement on microtubules, dynein and importin-α KapA. The nuclear levels of FlbB decrease with those of FlbD, but increase in the absence of the repressor of asexual development, NsdD. Overall, results show a dynamic interaction pattern of FlbB with other regulators of development in order to control the induction of conidiation

POSTER SESSION 1
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Development and cell biology

Interplay between GPI-anchored α-1,6-mannanase Dfg5 and MAPK signaling in cell wall remodeling of the mycoparasite *Trichoderma atroviride*

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The cell wall plays a critical role in fungal cells. It not only protects the cell from hazardous environmental factors but also allows the fungus to assess its environment and activate signaling pathways in response to it. The wall consists of a cross-linked matrix of glucans, chitins, and cell wall proteins. Many of the integral cell wall proteins are produced as glycosylphosphatidylinositol (GPI)-anchored proteins, which are membrane-bound proteins with enzymatic, antigenic and adhesive function.

Using a membrane-based yeast two-hybrid screening in *Trichoderma atroviride* – a prominent mycoparasite that kills and feeds on other fungi - we identified a GPI-anchored glycosidase protein of the GH76 family as a putative interactor of the Gpr1 membrane receptor. GH76 proteins are α-1,6-mannanases suggested to be involved in cross-linking of glycoproteins into the cell wall, where they are proposed to act as transglycosylases.

Our structural and functional analyses show that *T. atroviride* α-1,6-mannanase is a GPI-anchored Dfg5 protein that is required for hyphal morphogenesis and cell wall remodeling. Δ*dfg5* mutants exhibited reduced radial growth, likely due to a hyphal elongation defect, however were not impaired in biomass production. Further, Δ*dfg5* mutants better assimilated glucose and some of its related substances compared to the WT, but were impaired in utilization of sucrose and some polyols. Increased release of mannose was detected after addition of an *Aspergillus niger* mannosidase to the mutants' fermentation broth, what supports the hypothesis that mannose-containing oligosaccharides (e.g. galactomannans) are incorporated to the cell wall by the Dfg5. In addition, elevated levels of non-covalently entrapped cell wall proteins but a decrease in total proteins secreted into the growth medium were detected in Δ*dfg5* strains. We show that expression of *dfg5* is Tmk1 dependent, whereas the chitin and glucan synthases are under its suppression. Further, the expression of glucan synthase genes *gel1* and *smi1* is governed by Dfg5.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Localization and stability of the velvet protein VE-1 and its orthologous VeA in *Neurospora crassa*

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The Velvet regulators are a family of proteins with a conserved domain that help to coordinate fungal growth, differenciation and secondary metabolism in fungi. In *Aspergillus nidulans* VeA is a light-dependent developmental regulator that activates sexual development and inhibits conidiation. Mutations in *veA* results in constitutive conidiation that is independent of light, and VeA forms a complex with photoreceptors. The *Neurospora* crassa genome contains a homolog of *veA*, *ve-1*, that encodes a protein, VE-1, with a nuclear localization sequence. The *ve-1* mutant has defects in aerial hyphal growth and increased conidiation.

We have characterized the localization and stability of VE-1 using a strain with a tagged version of VE-1. We detected VE-1 in vegetative mycelia and in aerial hyphae during conidial development when the fungus was grown in the light. In the dark, however, VE-1 was detected in vegetative hyphae and was absent in aerial hyphae despite the presence of *ve-1* mRNA. We characterized the stability of VE-1 in a series of mutants in the protein degradation pathway and with a chemical inhibitor of the proteasome. We propose that the absence of VE-1 in aerial hyphae in the dark is due to VE-1 degradation through the proteasome with FWD-1 acting as an adaptor protein during this process.

To investigate if the regulation by light of VE-1 stability was present in other fungi we have cloned a tagged version of the *A. nidulans veA* gene in *N. crassa*. The new strain will allow us to characterize the stability and localization of the *A. nidulans* protein in *N. crassa* and to identify possible conserved regulatory mechanisms between the two fungi.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Pleomorphism in *Zymoseptoria tritici*: adaptation and response to environmental stimuli

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Adaptation and invasion of host tissue are enabled by switching between different cellular morphologies in some pathogenic fungi. This switch may be triggered by different environmental stimuli. Although the mechanisms involved in morphological changes are well known for dimorphic human pathogens, only few studies have investigated these phenomena in plant pathogens. Generally, fungal plant pathogens grow as filamentous over and inside the plant tissue to cause disease. However, the ability of some fungal species to switch between one or more morphologies, the biological function of the different cellular morphologies, and the genetic basis controlling it, are understudied. Here we investigated the responsiveness of the devastating wheat pathogen *Zymoseptoria tritici* (Zt) to environmental signals controlling growth form transition. Furthermore, we used a RNA-Seq approach to identify candidate genes involved in the dimorphism. All the seven tested stimuli affected the cellular morphology in the four tested Swiss Zt isolates. We focus here on the effects of carbon deprivation and high temperature stress (at 27°C). Carbon starvation induces a fast response with blastospores rapidly switching to hyphal growth within a few hours after the application of the stress. After 96 hours, ~60% of the cells presented hyphal growth. High temperature also promoted a rapid morphological response with hyphal induction after four hours of incubation. Strikingly, we also observed the formation of structures similar to chlamydospores and pseudohyphae under this condition. These structures were never before described in Zt, but are reported in other pathogenic fungi. Specially the chlamydospores are reported as long-term survival structures helping to escape from harsh environments. The viability, resistance, and pathogenicity of chlamydospore-like cells will be investigated further. We provide evidences of the sensible regulatory mechanisms by which Zt isolates detect changes in the environment and respond to these changes by promoting a diversity of cellular morphologies, according to the sensed stimuli.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Pharmacological and transcriptomic analyses of a *B. cinerea* flavohemoglobin deficient mutant demonstrate that nitric oxide affects germination, DNA replication and cell cycle

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Nitric oxide (NO) is a highly reactive molecule with fundamental roles in the biology of all living systems. The participation of NO in developmental processes has been described in several fungal species. However, the nature of the mechanisms and factors being affected by NO in fungi is poorly characterized. *Botrytis cinerea* is a plant pathogenic fungus which has attracted much attention given its wide host range and its necrotrophic life style. The production of NO by the fungus has been demonstrated as well as its detoxification by means of a flavohemoglobin enzyme encoded by gene *Bcfhg*1. It has been suggested that the physiological functions of the flavohemoglobin could be related to its involvement in the modulation of endogenous NO levels produced by the fungus during specific developmental stages.

Pharmacological studies in which germinating spores and mature mycelium were exposed either to NO donors or to NO scavengers indicate that NO affects germination. From the data obtained, in combination with the results derived from the analysis of the response of *ΔBcfhg*1 germinating spores to NO, it can be concluded that NO exerts and inmediate and transitoy effect on germination efficiency, on germ tube elongation and on nuclear division rate. Global expression analysis of *ΔBcfhg*1 in these conditions detected major changes in the expression pattern with about one third of the genes predicted in the *B. cinerea* genome responding to exposition to NO. Functional enrichment analysis allowed to identify links between exposition to NO, growth arrest and down-regulation of “DNA replication”, “nucleolus” and “cell cycle” genes.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

The small GTPase ArfA controls secretion, morphology, and growth in *Aspergillus niger* via actin ring positioning

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In filamentous fungi, growth and protein secretion occurs predominantly at the hyphal tip. This requires coordinated regulation of multiple processes, including vesicle trafficking, exocytosis, and endocytosis, which are facilitated by a complex cytoskeletal apparatus. In this study, functional analyses of the small GTPase ArfA from *Aspergillus niger* demonstrate that this protein functionally complements the *Saccharomyces cerevisiae* ARF1/2, and that this protein is essential for *A. niger*, where it regulates hyphal growth rates, protein secretion, and hyphal morphology. ArfA co-localizes to Golgi equivalents and post-Golgi carriers, but not the endoplasmic reticulum in hyphae. Moreover, localization of the endocytic machinery, visualized via fluorescent tagging of the actin ring, was found to be abnormal in ArfA under- and overexpressed conditions, indicating that ArfA mechanistically regulates secretion by affecting actin ring positioning. Finally, we provide evidence that secretion in *A. niger* occurs subapically, which may be a mechanism to compensate for defective and/or excess secretion at the hyphal apex due to ArfA misregulation. Taken together, our results demonstrate that ArfA fulfils multiple functions in the secretory pathway of *A. niger*. We propose that ArfA is a critical regulator that controls the endocytotic machinery at the hyphal apex.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Perithecial development in *Neurospora crassa*, as observed by thick sectioned correlative microscopy

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The development of sexual fruiting bodies is a critical stage in completing the life cycle of many filamentous fungi. During the sexual phase of *Neurospora crassa* development, the immature fruiting body (protoperithecium) significantly increases in size to eventually form the pear-shaped melanised structure comprised of layered cell wall material engulfing a centrum, in which ascogenous tissue develops. Mature ascospores are shot from the ostiloe (an opening at the top of the perithecial neck). While light and electron microscopy are commonly used to analyse perithecial structure, each have their limitations in providing detailed analysis of these structures. Using a thick sectioning (20-30 µM) procedure, we have established a protocol that provides the possibility of combining light and scanning electron microscopy to improve the visualization of developing perithecia.

We used cryostat sections with mild sample preparation. Tissue samples were either pre- or post-stained with fluorescent markers. Following light microscopy imaging tissue samples were further prepared for high resolution scanning electron microscopy (SEM) imaging. The combination of these methods allows the assessment of the integrity of the layered cell wall, ascospore development and orientation and provides the option for detailed structural analysis of normal and impaired fruiting body development. This simple correlative microscopy approach can be widely applied to the study of samples of other tissues and can also be combined with gene/protein localisation for high resolution tissue morphology analyses.

POSTER SESSION 1
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Development and cell biology

Self-organization of intracellular structures in the apical segment of growing *Neurospora crassa* hyphae

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N.crassa hyphae grow by apical extention at rates of about 20-30 µm/min forming lateral branches. Hypha consists of segments 10-20 µm in diameter and 50-100 µm length, each containing about 20-30 nuclei 2-3 µm in diameter, asynchronously dividing every 80-90 min. Plasma membrane and the cell wall are permanently formed *de novo* at the hyphal apex with the participation of endoplasmic reticulum, Golgi apparatus, actin cytoskeleton, microtubuls, and mitochondria. All these structures are located in the apical segment in a well-defined order which arises *de novo* during conidia outgrowth or lateral branch development. This order is supported constantly during apical growth. A detailed description is needed to evaluate the role of morphogens, forces or fields which create and support the mentioned order and to unravel communicative mechanisms of its players. An integral part of apical development is lack of H+-ATPases, the main membrane potential generators, in the hyphal apices (~100 µm from the tip) The result of such intrahyphal segregation is creation of a profound local electric field-(~100 V/m), and an electric current generation comparable in magnitude with a proton pump current. We have shown that growth rate of isolated hyphal apices decreased, the diameter narrowed, and their lateral branching was inhibited, but the length of the apical segment (150 — 300 µm) did not change as well as the intercalary one (50 — 100 µm). Molecular detailes of hyphal septation are well documented. Unfortunately they can't provide understanding of what kind of sources and fields determine the length of a hyphal segment and keep the first septum at a distance of 150-300 µm from the apex. Mechanisms of apical growth control by the nuclei are also not yet clear.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Recruitment of tRNA PolIII promoters in CRISPR/Cas9 system to edit genome of *Aspergillus niger* and the application in strain development

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As a powerful tool for fast and precise genome editing, the CRISPR/Cas9 system has been applied in few organisms of filamentous fungi that greatly improved the efficiency of gene alteration. However, the delivery of the guide RNA (gRNA) has been so far the bottleneck of performing CRISPR mutagenesis on *Aspergillus* species. Here we report a modification of gRNA expression driven by a series of endogenous tRNA promoters, which include the tRNA gene plus preceding 100-basepair of upstream sequence. Cotransformation of Cas9-expressing plasmid with gRNA linear DNA demonstrated that 97% of 37 tRNA promoters are able to produce mature gRNA for Cas9 targeting in *Aspergillus niger*. When gRNA(s) and Cas9 were expressed in a single extrachromosomal plasmid, the efficiency of single gene disruption was achieved as high as 98%, and the duplex targeting efficiency was around 68%. Along with a homologous recombination donor, our CRISPR/Cas9 system resulted in 94% efficiency of gene knock-in in the *kusA*$^{-}$ strain, which was 100% of targeting integration. Relied on robust and reliable regulatory strength of tRNA promoter, our results provide a wide avenue of genome editing in *A. niger* and in other filamentous fungi. In addition, using tRNAs as polymerase III promoter to lead gRNA expression in the CRISPR/Cas9 system can be easily extended to other organisms than fungi.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Implication of a MADS box protein (MCM1) for spontaneous perithecium development in *Podospora anserina*

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The ascomycete *Podospora anserina* is a heterothallic filamentous fungus found in herbivore dungs, commonly used in laboratories as a model system, and for which the complete life cycle is reproducible *in vitro*. The main objective of our team is to better understand the global process of the development of fruiting body, named perithecia, induced by fertilization. In this context, three mutants, named *pfd3*, *pfd9* and *pfd23* (for « promote fruiting body developement ») obtained by UV mutagenesis, were selected in view of their abilities to promote barren perithecium development without fertilization. By complete genome sequencing of *pfd3* and *pfd9* mutants, we identified point mutations in the *MCM1* gene and validated this gene by complementation. We also showed that the *pfd23* mutant was mutated in *MCM1*. MCM1 proteins are MADS box transcription factors that control diverse developmental processes in plants, metazoans, and fungi. Here, we present the complete functional characterization of the three *pfd* mutants, as well as that of the deleted strain. In particular, we showed that *ΔMCM1* and *pfd3* were sterile and that the development of spontaneous perithecia needed the presence of wild-type nuclei in the mycelium, whereas *pfd9* et *pfd23* were able by themselves to spontaneously produce perithecia.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Cytogenomic and transcriptomic approaches to understand nuclear cycle of rusts

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Fungal life cycles are characterised by predominant haploid stages, while in most cases diploid nuclei occur only in a single cell (the basidium or the ascus, in Basidiomycota and Ascomycota respectively), following karyogamy between haploid nuclei and immediately followed by meiosis. Rust fungi are also reported as obeying to this general rule, with the basidium as the single diploid cell. The haploid cycle in rust fungi is divided in two stages, the first comprising monokaryotic cells (from basidiospores to pycniospore formation), and the second, following plasmogamy, comprising dikaryotic cells (from pycniospore conjugation through aeciosporic and urediniosporic stages to karyogamy occurring in teliospore leading to the diploid basidium). Recently, flow cytometric studies to quantify rusts genome size showed the presence of 1C, 2C and a low proportion of 4C nuclei in different stages of the urediniosporic cycle of several rust fungi, namely *Hemileia. vastatrix*. These results suggest the presence of diploid nuclei that supposedly only occur in teliospores, and are compatible with the occurrence of karyogamy and meiosis prior to urediniospore formation, although endopolyplody or other parasexuality phenomena cannot be ruled out. In this work we combined cytogenomic techniques with a transcriptomic approach along the infection cycle of *H. vastatrix* in *Coffea arabica* leaves to confirm the occurrence of diploid nucleus and the expression of genes related with karyogamy and/or meiosis aiming to enlighten the nuclear cycle of rusts.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Growth advantage in stationary phase (GASP) in Eukaryotes

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Introduction: The Growth Advantage in Stationary Phase (GASP) phenomenon reflects the remarkable versatility of cells to tolerate stressful conditions. This commonly used strategy for microbial survival has been widely observed and established with different strains of bacteria.

In this study, we tested whether the GASP phenomenon exists in eukaryotes. To this end, a strain of *Saccharomyces cerevisiae,* termed *Sc404* had been isolated from a prolonged period in stressful environment, in the form of a two-year old bottled beer, containing 5% ethanol. This isolated strain was studied and compared to its parental strain *Safale* in various parameters related to GASP.

Methods: The growth of *Sc404* strain was compared to its parental strain, *Safale*, both in optimal and sub-optimal conditions, and competition experiments of the two strains were performed. In addition, the tolerance of these two strains to stressful environments, including heat, high osmotic pressure, acidity and ethanol was compared. Accordingly, a full genome sequence of both strains was determined and compared.

Results: Indeed*, Sc404* presented a significant advantage in survival and growth under the tested stressful conditions over the parental strain, including large amounts of ethanol (20%), basic environment (pH=10) and at high osmotic pressure. Interestingly, in optimal conditions both strains showed similar growth rates. Serial dilutions and growth assays demonstrate that the difference between the strains is irreversible, and therefore genetic in origin. Genomic sequence analysis of the two strains shows dramatic changes in *Sc404* which apparently lost about 10% of it genome. Importantly, main stress sensing molecular machineries in the cell are heavily mutated.

Discussion: Our data thus reveal that the remarkable GASP phenomenon is indeed present in *S. cerevisiae*, showing improvement in survival under various stresses. Moreover, these results suggest that GASP is a general phenomenon which might play a role in the evolution of various kingdoms.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Towards a sexual cycle in *Aspergillus niger*

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In recent years an increasing number of filamentous fungi previously considered asexual, such as *Aspergillus fumigatus*, have been shown to possess a cryptic sexual cycle, triggered only under highly specific conditions. However, for around 20% of the known fungal species, including *Aspergillus niger*, a sexual cycle has not been found yet. *A. niger* is a relevant industrial microorganism, currently used as a versatile cell factory for the production of organic acids and enzymes. The discovery of a sexual cycle in *A. niger* would not only broaden the current biological knowledge of this organism but also allow to unravel new strategies for strain improvement.
In this study, we report some strong indications of the sexual potential of *A. niger*.
First, sclerotia-like structures were observed in the sequenced *A. niger* strain ATCC 1015, containing the MAT1-1 gene, when plated in combination with an opposite mating type *A. niger* strain. Sclerotia are considered pre-mature sexual structures which can mature to cleistothecia, the latter being an important requirement for the occurrence of a sexual cycle in *Aspergilli*. The observed sclerotia-like structures are often formed at the contact zones between the colonies derived from the two strains, suggesting an interaction between them. Also, the typical presence of liquid droplets on top of the formed structures can be often observed.
Second, a reduction of the normal asexual conidiation can be observed when the two opposite mating type strains are plated together on the same plate. This reduction is even more drastic when sclerotia-like structures are formed. Overall, our findings strongly indicate that steps towards a complete sexual cycle of *A. niger* can be achieved.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Genetic analysis of Hsp70 phosphorylation sites reveals a role in *Candida albicans* cell and colony morphogenesis

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Heat shock proteins are best known for their role as chaperonins involved in protein folding, but they can also participate in cellular regulatory pathways, e.g. via their phosphorylation. Hsp70/Ssa1 is a central cytoplasmic chaperonin in eukaryotes, which was shown to participate *i.a.* in cell cycle regulation in the yeast *Saccharomyces cerevisiae*. Here we analyze the role of Ssa1 phosphorylation in the morphogenesis of the fungus *Candida albicans,* a common human opportunistic pathogen. *C. albicans* can assume alternative yeast and hyphal (mold) morphologies, an ability that contributes to its virulence. We identified 11 phosphorylation sites on *C. albicans* Ssa1, of which 8 were only detected in the hyphal cells. Genetic analysis of 10 of these sites revealed allele-specific effects on growth at high temperature, cell and colony morphology, and resistance to cell wall-active drugs. The pleiotropic effects of many Ssa1 mutations are consistent with the large number of Ssa1 client proteins, whereas the lack of concordance between the phenotypes of the different alleles are consistent with the possibility that different sites on Ssa1 can affect interaction with specific classes of client protein, and that modification of these sites can play cellular regulatory roles.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Cell polarity and cell morphogenesis in *Ustilago maydis*

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Localization of positional cues to distinct cell domains is critical for the generation of cell polarity, cell morphogenesis, septum positioning, and organelle distribution. We are interested in understanding how positional cues govern cell morphogenesis and nuclear position in *Ustilago maydis*, a member of the Basidiomycota. *U. maydis* exhibits a yeast-like and a filamentous form. Additional morphologies are generated by interaction with its hosts maize (*Zea mays*) and teozintle (*Zea mays subsp. parviglumis* and *subsp. mexicana*), suggesting that plant signals modulate fungal morphogenesis. Thus, *U*. *maydis* provides excellent opportunities to understand how positional cues control polarized growth in the different morphologies.

We identified genes for cell polarity and cell morphogenesis using a genetic screen to isolate mutants with altered colony and cell morphology. One of the genes identified codes for a protein with similarity to fission yeast Tea4, a SH3 protein that determines the axis of polarized growth (Martin et al., 2005). *U. maydis tea4* codes for a multidomain protein of 1684 amino acid residues. The presence of some domains only in Tea4 homologues in the Basidiomycota, suggests that Tea4 performs additional functions in this group of fungi. Another gene identified codes for a protein of 1698 amino acid residues with similarity to *S. pombe* Tea1, a Kelch domain protein, that is a key determinant of directionality of polarized growth (Mata and Nurse, 1997). UmTea1 is a multidomain protein. Tea1 homologues in the Ascomycota and Basidiomycota contain Kelch repeats located near the amino terminus of the protein. Analysis of the Um*tea4* and Um*tea1* null phenotype indicates that UmTea4 and UmTea1 are important positional markers for polarized growth. We also uncovered novel roles for Tea4 and Tea1 in other processes that contribute to the generation of normal cell shape in *U. maydis*. Here we describe the results of this analysis.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Global analysis of the molecular roles, localizations and interactomes of F-box proteins during fungal development

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Multiprotein complex, Skp-Cul-Fbox (SCF) E3 ubiquitin ligases, are the largest family of E3 ligases that are responsible for marking of target proteins with ubiquitin and subsequent proteasome-dependent degradation. SCF E3 ligases are involved in many cellular processes, including transcription, cell-cycle control by determining protein levels of target proteins. The F-box component of the SCF complex is essential for the substrate specificity of the SCF complex by recruiting target proteins for ubiuitination. In this study, we have systematically investigated the molecular functions of 73 F-box or F-box-like protein encoding genes of the eukaryotic model system *Aspergillus nidulans*. Deletion of 73 *fbx* genes revealed that only 8-10 % of the *fbx* genes are required for proper fungal development and light response. Only *fbx25,* which encodes SconB necessary for sulphur metabolism*,* is essential for fungal growth and survival. 50 % of the F-box proteins (30-35) are associated with the SCF complexes through the adaptor SkpA protein during fungal development. Several F-box proteins show development and stress specific interactions with the SkpA protein. 30 % of the F-box proteins are exclusively localized to the nuclear fraction whereas the rest show other localization patterns including, cytoplasmic, hyphal tip and plasma membrane. High scoring F-box proteins (Fbx1 to Fbx48) interact with more than 1500 proteins including SkpA and CullinA. These data suggest that F-box proteins interact with at least 15% of the total proteome and control developmental responses to environmental stimuli and stresses.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

Genetic and Biochemical Characterization of GH72 glucanosyltransferases shows they function in attaching glycoproteins into the fungal cell wall

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The Neurospora crassa genome encodes five GH72 family glucanosyltransferases, and four of these enzymes (GEL-1, GEL-2, GEL-3, and GEL-5) have been found to be present in the cell wall proteome. We carried out an extensive genetic analysis on the roles of these four glucanosyltransferases in cell wall biogenesis and demonstrated that the enzymes are required for the formation of a normal cell wall. As suggested by the proteomic analysis, we found that multiple glucanosyltransferases were being expressed in N. crassa cells and that different combinations of the enzymes are required in different cell types. The combination of GEL-1, GEL-2, and GEL-5 is required for the growth of the vegetative hyphae, while the GEL-1. GEL-2, GEL-3 combination is needed for the production of aerial hyphae and conidia. Our data demonstrates that the enzymes are redundant with partially overlapping activities, which provides the fungus with a robust cell wall biosynthetic system. Characterization of glucanosyltransferase-deficient mutants demonstrated that the incorporation of cell wall glycoproteins was severely compromised. Interestingly, we found that the mutant cell walls contained more β-1,3-glucan than the wild type cell wall. Our results demonstrate that the GH72 glucanosyltransferases are not needed for the incorporation of β-1,3-glucan into the cell wall, but they are required for the incorporation of cell wall glycoprotein into the cell wall. We further demonstrated tht the enzymes are capable of cross-linking cell wall glycoproteins and cell wall glucans.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Development and cell biology

P loop-NTPases in developmental processes of *Coprinopsis cinerea*

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The self-compatible homokaryotic strain AmutBmut with defects in both mating-type loci is a mutant of *Coprinopsis cinerea* for studying fruiting body development. Proto159 is a derived mutant of AmutBmut with a defect in the formation of primary hyphal knots (PK) as a first step of light-regulated fruiting body development and in sclerotia formation in the dark. Proto159 shows a somewhat slower growth rate and pigments the mycelium and the agar beneath dark-brown. The mutation in the defective strain Proto159 was found to be suppressed by transformation of a specific gene that belongs to the *NWD2* family. *NWD2* genes encode proteins with an N-terminal NACHT domain which is an evolutionary conserved domain that serves in signal transduction and is named after four different types of P-loop NTPases (NAIP, CIITA, HET-E and TP1). The NACHT domain contains a typical NTP binding site (P-loop, Walker A motif), a Walker B motif, a charged amino acid at a specific conserved position, a GRRxE motif, and a GxP motif of STAND NTPases. *NWD2* genes exist only in some Agaricales (e.g. *Amanita muscaria, Agaricus bisporus*, *Moniliophthora roreri,* and *Laccaria bicolor)* including *C. cinerea* where they have been multiplied to represent in total 36 genes, most of which are seen to locate in transposon-rich and telomere regions of chromosomes. All the encoded proteins have the N-terminal NACHT domains while these have variably been fused to different C-terminal protein halves (e.g. tandem WD40 repeats). More *NWD2* genes were subcloned and used in transformations of Proto159. Introduction of some genes altered mycelial properties, blocked the brown staining of mycelium and the agar, and induced primary hyphal knot and sclerotia formation and sometimes fruiting.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

Regulation of interrelated aromatic amino acid and vitamin biosyntheses in *Coprinopsis cinerea*

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Chorismate from the shikimate pathway acts as common precursor in the biosyntheses of aromatic amino acids and PABA. Their production is regulated by feedback and cross-pathway controls. Tryptophan biosynthesis in *Coprinopsis cinerea* is mediated by four enzymes anthranilate synthase Trp3, the tri-functional Trp2 (with TrpG, TrpF and TrpC domains), Trp4, and the bi-functional tryptophan synthase Trp1 (with TrpA and TrpB domains). Ectopic transformation of *trp3* and *trp1* copies into the fungal genome causes a paradoxical phenomenon: Transformation with either trp^+ gene halves the numbers of viable prototrophic transformants as compared to cotransformations with a non-trp^+ vector. Cotransformation of both trp^+ genes reduces number of transformants further. Loss of transformants reflects feedback inhibition above a critical level that irreversibly shuts off the Trp production with growth inhibition as consequence. Lethal effects can be further enhanced by addition of Trp precursors and also through cross-pathway control by addition of other aromatic amino acids. We split Trp1 into its units TrpA and TrpB. TrpA and TrpB can complement each one of two mutations the defective allele *trp1-1,1-6*. Their cotransformation into *trp1-1,1-6* mutants gives prototrophic clones. Thus, indole produced by TrpA is transferred to TrpB for Trp production. Depending on specific mutations and resulting steric effects on the lockable 2.5 Å tunnel between TrpA and TrpB as pathway for indole transfer, such transfer is not always possible from TrpA unit to a TrpA-mutated full-length Trp1 protein or from a TrpB-defective Trp1 protein to TrpB. Now, we plan to uncouple the domains of Trp2 and of the PABA-synthase whose TrpG-like domains might be interchangeable. Trp2 with a defective TrpF domain is expected to accumulate the intermediate PRA which in bacteria can serve in synthesis of the HMP precursor of thiamin. *C. cinerea* lacks a respective *thi5* gene which we introduce from a foreign host.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

Cellulolytic enzyme genes are significantly upregulated by most of the mutations that cause defects in the ligninolytic activity in the white-rot fungus *Pleurotus ostreatus*

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White-rot fungi play an important role in the global carbon cycle because wood lignin is almost exclusively biodegraded by them in nature. The oyster mushroom *Pleurotus ostreatus* is frequently used for biochemical and genomic studies of lignin biodegradation. Recently, we identified three genes, *wtr1*, *chd1* and *pex1*, in which mutations cause defects in the ligninolytic activity, RBBR decolorization and wood lignin degradation, in *P. ostreatus*. Here, we show results of comparative RNA-seq analysis to identify genes of which expression are significantly affected by aforementioned mutations when they are grown on sawdust media. It was shown that many genes probably involved in lignin degradation were significantly downregulated in the single-gene disruptant of the three genes. We also found that many genes encoding cellulolytic enzymes, putative four endo-β-1, 4 glucanases belonging to GH6 and GH7, cellobiose dehydrogenase, many copper-dependent lytic polysaccharide monooxigenases belonging to AA9, and an exo- or endo- glucanase belonging to GH131, are significantly upregulated (70-22 folds in RPKM values) by *chd1* or *pex1* disruption, but not by *wtr1* disruption. It was shown that extracellular enzymes obtained from mutant UVJ3-3, the *chd1-1* mutant, grown on sawdust medium saccharified Avicel much more efficiently than the wild-type strain PC9, which is consistent with the result of RNA-seq. We recently isolated new mutants (total five) defective in the ligninolytic activity, and genes responsible for the phenotype of some mutants were identified. qRT-PCR analysis revealed that aforementioned cellulolytic enzyme genes are also significantly upregulated in four out of five mutants, while genes probably involved in ligninolysis are downregulated in all of the five mutants. These suggest gene regulation mechanisms switching between ligninolytic mode and cellulolytic one in *P. ostreatus* although it remains unclear whether the change in gene expression is the cause or result of the defects in ligninolysis.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

Disruption of the NAD cycle as a potential approach to manage fungal plant pathogens

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Fungal plant pathogens are major threat to food security and impose severe economic burden. Therefore, there is a continuous need to develop new strategies to manage fungal plant pathogens. We suggest the NAD pathway as a target for pesticide development. NAD oxidation status determines the metabolic fate of the cell. NAD is also consumed by sirtuins histone deacetylases. These proteins remove acetyl groups from histone and thus regulate gene expression and chromatin accessibility. Sirtuins convert NAD to nicotinamide; the latter is a vitamin that was shown to inhibit sirtuins. Wurtele et al. found that uptake of nicotinamide can reduce the severity of *Candida albicans* infection in mice. The mode of fungal toxicity was inhibition of the fungal specific sirtuin, Hst4. We were able to show that nicotinamide is fungistatic to plant pathogens albeit in high concentrations in vitro, on tomato slices and on cherry tomato berries. Nicotinamide inhibits hyphal growth much more than conidial germination. Fungal cells response to nicotinamide by over-expression of transcription factors in agreement with its role as a histone deacetylase inhibitor. In order to increase the efficiency of nicotinamide we thought to inhibit an enzyme that further metabolize it in the cell. Nicotinaldehyde inhibits in vitro pnc1 a nicotinamidase. Pnc1 is part of the NAD salvage pathway. Nicotinaldehyde is fungistatic but its mode of action is probably not through increase of the cellular concentration of nicotinamide. First, unlike nicotinamide, nicotinaldehyde inhibits germination much more than hyphal growth. Second, the cellular response to nicotinaldehyde is through over-expression of genes related to redox potential in the cell and not chromatin or transcription. Pnc1 is overexpressed by nicotinaldehyde suggesting it does inhibit the NAD salvage pathway. Currently, we further study the mode of action of nicotinamide and nicotinaldehyde in order to develop a novel strategy to manage fungal diseases.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

The role of respiration in canavanine tolerance – reviving the Goldilocks legend

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The yeast *Saccharomyces cerevisiae* is a petite-positive species, i.e., it can survive without respiration. This unique feature makes *S. cerevisiae* an excellent model for testing the role of respiration in stress tolerance. We exposed the respiration-proficient wild type (WT) and respiration-deficient (petite) strains to heat shock, the toxic amino acid thialysine, and the antifungal fluconazole, yet we did not observe any difference in colony-forming ability between WT and petites. In contrast, colony formation was severely inhibited in petites when grown on plates containing canavanine, the toxic analog of arginine. Interestingly, the significant differences observed on plates between respiration-proficient and respiration-deficient strains were not observed in liquid media containing canavanine. Under these conditions, there was no differential effect on glucose consumption. We then hypothesized that, due to a low diffusion rate on agar media, ethanol accumulation in the colony synergizes with canavanine to cause toxicity, yet there was no synthetic delay when combining canavanine, glucose, and ethanol in plates. Alternatively, the slow diffusion rate into the colony may limit the availability of nutritional factors. While increasing glucose concentration did not improve colony formation of petite mutants in the presence of canavanine, increasing arginine did. Next, we found that petite mutants produce less arginine than WT cells, indicating that when exposed to sublethal doses of canavanine, the low diffusion rate of arginine building blocks combined with petites` inherent deficiency to produce arginine results in the inhibition of colony formation. We then examined colony formation of WT cells when grown on a non-fermentable carbon source. Surprisingly, no colonies were formed, and canavanine was fungistatic in liquid media. We conclude that both lack of respiration and complete dependence on respiration are synthetic with canavanine in colony-formation inhibition, but through different mechanisms.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

Investigation of the role of *hxn* genes in the nicotinic acid catabolic process of *Aspergillus nidulans*

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Although many microorganisms can utilize nicotinic acid as a sole nitrogen source in nature, the nicotinate catabolic process was studied only in a limited number of prokaryotes. The eukaryotic utilization routes are completely unknown. In our laboratory we started to reveal the nicotinate utilization process in *A. nidulans*. Since the 1970s we know that conversion of nicotinate to 6-hydroxynicotinate (common first step of catabolism from prokaryotes to eukaryotes) by Purine hydroxylase II depends on a regulator *hxnR* and the induction by nicotinate or 6-hydroxynicotinate. We identified 11 co-regulated genes involved in nicotinate utilization and organized in three clusters (*hxnP/S/T/Y/R/Z*, *hxnX/W/V* and *hxnM/N*). In order to study their function we systematically deleted each of the *hxn* genes and the nicotinate utilization properties of the deletion mutants were screened by growth tests.

Here we present the expression profile of *hxn* genes and nicotinate utilization properties of the deletion strains. According to the transcript profile, the *hxn* genes are inducible by nicotinate, are non-inducible in strains carrying *hxnRΔ* mutation and show strong constitutive expression in $hxnR^c$ (*hxnR* constitutive allele) background. The growth tests indicated that HxnS, HxnT and HxnY are involved in the initial steps of the catabolism and the pathway splits up to alternative routes after the first step. HxnV, HxnX, HxnW, HxnM and HxnN act downstream to the step in which the compounds of the alternative routes are converted into a common intermediate. Identification of the intermediate metabolites was aided by GC-MS analysis. Here we outline the preliminary scheme of the nicotinate catabolism in *A. nidulans*.

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POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

Spatial heterogeneity of glycogen and its metabolizing enzymes in hyphal tip cells of *Aspergillus nidulans*

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Glycogen is a homopolymer of glucose and a ubiquitous cellular-storage carbon. This study investigated which *Aspergillus nidulans* genes are involved in glycogen metabolism. Gene disruptants of predicted glycogen synthase (*gsyA*) and glycogenin (*glgA*) genes accumulated less cellular glycogen than the wild-type strain, indicating that GsyA and GlgA synthesize glycogen like other eukaryotes. The gene disruption of *gphA* encoding glycogen phosphorylase increased the amount of glycogen more during the stationary phase that accompanies carbon-source limitation. Fluorescence-tagged GsyA and GphA were distributed in the cytosol and formed punctate and filamentous structures, respectively. Carbon starvation elongated the GphA filaments and increased their numbers. These structures were more frequently located in the basal regions of tip cells and in their adjacent cells than in the apical regions of tip cells. Cellular glycogen visualized using a fluorescent glucose analog accumulated in cytoplasmic puncta that were more prevalent in the basal regions like GsyA. The colocalization of glycogen and GsyA at punctate structures in the tip and sub-apical cells probably represents the cellular machinery for synthesizing glycogen. More frequent colocalization in the basal, than in the apical regions of the tip cells indicated that the tip cells differentiate the subcellular regions to synthesizing glycogen. Our findings of glycogen, GsyA and GphA distribution evoke the spatial heterogeneity of glycogen metabolism in fungal hyphae.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

Itaconic acid production from D-xylose by *Aspergillus terreus*

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Itaconic acid (2-methylenesuccinic acid; IA) is a five-carbon dicarboxylic acid, frequently used as a building block chemical for the synthesis of plastics, coatings and resins. IA is commercially produced by large-scale submerged fermentations employing the filamentous Ascomycete fungus *Aspergillus terreus* and using molasses or hydrolized corn starch as primary carbon sources. The objective of this study was to test whether IA can be produced on D-xylose in concentrations and specific yields ($Y_{p/s}$) similar to D-glucose by *A. terreus*.

Production of IA is the result of the metabolic overflow of primary metabolism. High ($Y_{p/s}$>0.8) molar yields on D-glucose require high (>10%, w/v) concentrations of carbon, strong aeration and carefully set cultivation parameters, of which Mn(II) ion limitation is the most prominent. When D-glucose was replaced with D-xylose under identical fermentation conditions, the plot depicting specific IA yield vs. initial carbon concentration was notably different. Maximum IA yield was significantly reduced ($Y_{p/s}$=0.55), but it was achieved at a relatively low (5%, w/v) initial D-xylose concentration. Any further increase above this level did not affect yield, which was, however, subject to severe Mn(II)-related regulation. Mn(II) ion concentrations as low as 5 ppb decreased IA yield on D-xylose by 15%. In contrast to the situation on D-glucose, IA yield did not drop below 0.3 on D-xylose even in the presence of 1000 ppb of Mn(II) ions. In conclusion, while it is possible to produce IA from D-xylose by *A. terreus*, the technology has to be improved considerably to be competitive with traditional glucose-based fermentations.

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POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

A lariat branch point motif-interrupted spliceosomal twin intron in *Aspergillus nidulans*

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In the primary transcript of nuclear genes, coding sequences – exons –alternate with non-coding sequences – introns. The latter are removed and former are joined to create the mRNA ORF that translates into the functional peptide product. Ubiquitous intron splicing provides a means of post-transcriptional regulation of expression by coupling alternative splicing with nonsense-mediated mRNA decay, hardily addressed in fungi. We use spliceosomal twin introns ("stwintrons") as model systems to study spliceosomal introns and their excision. Stwintrons are unconventional intervening sequences where a standard "internal" intron interrupts one of the three canonical splicing motifs of an "external" intron, and that consequently, can only be removed by consecutive splicing reactions. Previously, we have characterised stwintrons where the internal intron interrupts either the donor- or the acceptor sequence of the external intron (**). We have demonstrated that stwintrons can emerge by the appearance of a new intron within a pre-extant intron, consistent with mechanisms of intron gain from an endogenous origin. Here we present a new type of stwintron in which the internal intron is nested in the conserved sequence element around the lariat branch point adenosine of the external intron. This particular lariat branch point motif-interrupted stwintron is a recently evolved feature in *Aspergillus nidulans* and we show that it emerged by an alternative mechanism which involves intronisation of exonic sequences on either side of a pre-extant standard intron.

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(**) Flipphi et al. (2013) Fungal Genet. Biol. 57:48; Ág et al. (2015) Fungal Genet. Biol. 85:7; Fekete et al. (2017) Nucleic Acids Res. 45:9085; Flipphi et al. (2017) Fungal Biol. Biotechnol. 4:7

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

Carbon starvation and carbon limitation stress responses in *Aspergillus nidulans*

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Genome-wild transcriptional changes caused by carbon stress in *Aspergillus nidulans* cultures were analyzed. Carbon starvation and carbon limitation stresses were induced by transferring exponentially phase hyphae, pre-grown on glucose, into carbon source free and lactose containing media, respectively. Up-regulation of genes involved in autolytic cell wall degradation was characteristic for carbon starved cultures and genes encoding amino acid catabolic enzymes were more active in starving than in lactose-fed cultures. In contrast, up-regulations of *galX* and *galR* encoding regulators of D-galactose degradation as well as *lacpA* and *lacpB* lactose permeases were characteristic for cultures growing on lactose. Genes of lactose and hemicellulose utilizing enzymes (including members of the D-galactose oxidoreductive pathway) as well as genes involved in ribosome biogenesis and amino acid biosynthesis were more active on lactose than under carbon starvation conditions. Despite of these differences, the two stresses caused surprisingly similar changes in the transcriptome because more than 80 % of carbon stress genes responded to the two treatments in a similar manner. As an example, 80 out of the 90 carbon stress induced extracellular enzyme genes showed up-regulation in both stresses. Not surprisingly, the secretomes of 2 d cultures were also very similar.

Our results support the view that lactose as a ß-galactoside is utilized by *A. nidulans via* pathways involved in hemicellulose degradation. Although *A. nidulans* can grow well on this disaccharide, transferring mycelia from glucose to lactose means a real stress which induces a very similar stress response to that detected under carbon starvation.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

AreB, the nitrogen and carbon regulator in *Aspergillus nidulans*

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Nitrogen and carbon repression general regulatory systems modulate the expression of target genes participating in utilization of alternative nitrogen/carbon sources, resulting in transcription only when preferred sources are limiting. In *Aspergillus nidulans,* the GATA transcription factor AreB was identified as nitrogen regulator (Conlon et al., 2001) but its activity depends on both nitrogen and carbon source (Dzikowska et al., 2012).

Transcriptomic analysis of *areB* deletion strain grown under different carbon/nitrogen conditions has shown that areB participates both in nitrogen and carbon regulation. Observed effects are, at least partially, indirect as the expression of several transcription factor coding genes is changed in *areB* deletion strain.

areB gene encodes three different proteins which differ at their N-terminal part. This results from a differential splicing and selection of two different promoter regions and start codons. To explore the mechanism of transcription regulation by areB we analyzed the changes in expression levels of three *areB* transcripts (*areBα, areBβ, areBγ*) grown under carbon/nitrogen repressing/de-repressing conditions. The qPCR analysis shows that the three *areB* transcripst are expressed in different proportions and this depends on carbon/nitrogen conditions.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

Regulation of arabinose-induced gene expression in *Aspergillus niger*

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The AraR transcription factor of *Aspergillus niger* encodes a Zn(II)2Cys6 transcription factor required for the induction of several arabinolytic genes. One of the target genes of AraR is AbfA encoding an arabinofuranosidase that is specifically induced on arabinan and arabinose in an AraR-dependent way. Expression of AbfA as well as other arabinolytic genes in *A. niger* requires the presence of L-arabinose as an inducer to activate AraR.

With the goal to isolate mutants that constitutively express arabinolytic genes independent on the presence of L-arabinose as an inducer, we designed a positive selection method using the arabinose-responsive promoter (*PabfA*) fused to the acetamidase (*amdS*) reporter gene. Expression of the *amdS* gene enables the fungus to grow on acetamide as the sole nitrogen source. Hence, mutants constitutively expressing the *amdS* gene can be selected on agar-plates with acetamide as a N-source. Growth analysis of the *PabfA-amdS* reporter strain indicated that *abfA* is specifically induced by arabinose, arabitol and arabinan. The *in vivo* reporter strain was also used to monitor carbon catabolite repression control. The *PabfA-amdS* reporter was repressed by glucose, fructose and sorbitol in a concentration dependant manner. CreA is important in mediating carbon catabolite repression and deletion of the *creA* gene in the *PabfA-amds* reporter strain abolished repression by glucose, fructose and sorbitol. Interestingly, the *PabfA-amdS* reporter construct in the Δ*creA* background was induced not only by arabinose but also by xylose indicating a regulatory overlap between AraR and XlnR transcription factors.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

Exp5, a *Magnaporthe oryzae* nucleo-cytoplasmic receptor involved in plant pathogenesis

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Karyopherins are involved in the translocation of proteins and/or RNAs between the nucleus and the cytoplasm. The *Magnaporthe oryzae* karyopherin Exp5 protein is the orthologue of the human karyopherin exportin-5 and the *Saccharomyces cerevisiae* Msn5. The *M. oryzae* Δ*exp5* mutant is strongly impaired in plant disease symptoms production. Therefore, to understand the role of this karyopherin during plant infection we studied Exp5 RNA and protein cargoes. Several t-RNA synthetases, seven subunits of the 26S proteasome and key components of pathogenesis-related signal transduction pathways (MAPKs Pmk1 and Mps1) and fungal metabolism (Tps1) were found to immunoprecipitate with Exp5. An important set of mitochondrial proteins including TIM44 and TOM70 also interacted with Exp5, which suggest that mitochondrial dysfunction can be contributing to the strong root infection defects exhibited by Δ*exp5*. Additional deficiencies such as accumulation of small RNAs derived from transposon elements and $tRNA^{Met}$ in the mutant supports the involvement of Exp5 in ncRNA metabolism and tRNA transport. Overall, our results suggest that Exp5 is required for the transport of specific classes of proteins and ncRNAs, several of which contribute to *M. oryzae* pathogenicicity.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

Additional function of KaeA, the subunit of KEOPS/EKC complex?

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Kae1p, the subunit of KEOPS/EKC complex, is a highly conserved (Galperin and Koonin 2010), metal-binding protein belonging to the ASKHA (Acetate and Sugar Kinase, Hsp70 chaperone proteins and Actin) protein superfamily (Mao et al., 2008). It has been shown that Kae1p participates in the universal tRNA modification (t6A) (Perrochia L. et al, 2013). However, several published results suggest that Kae1p may also participate in other cellular processes. Both in *S. cerevisiae* (Kisseleva-Romanova et al, 2006) and in human cells (Costessi et al., 2012), participation of the KEOPS/EKC complex in transcription was suggested. Our published (Dzikowska et al., 2015) and unpublished results (i.a. NGS transcriptomic analysis of the *kaeA* mutants versus *kaeA*$^{+}$ strain, KAEA cellular localization studies and t6A modification studies in *kaeA* mutants) suggest that also in the model filamentous fungus, *Aspergillus nidulans*, Kae1p may participate not only in t6A modification but also in regulation of transcription.

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POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

HapX iron sensing in *Aspergillus fumigatus* involves the interaction with the monothiol glutaredoxin GrxD

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Aspergillus fumigatus is a ubiquitous saprophytic mold, which causes 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 and iron excess are mediated by the bZIP transcription factor HapX, which functions *via* physical interaction with the heterotrimeric CCAAT-binding complex. During iron starvation, iron consuming pathways are repressed and iron uptake is activated. During iron overload, the cell is detoxified from iron by activation of vacuolar iron storage [1].

Currently, the molecular mechanisms of iron sensing by HapX are unknown and remain to be elucidated. As shown for iron regulators in other ascomycetes, *A. fumigatus* HapX senses the cellular iron status most likely by interaction with other regulators, like monothiol glutaredoxin (GrxD). We applied a co-immunoprecipitation approach for identification of possible GrxD as well as HapX interaction partners. VENUS-tagged GrxD and MYC-tagged HapX proteins were enriched from crude cell extracts by GFP-Trap and MYC-Trap, respectively. Immunoprecipitated proteins were identified by nano LC-MS/MS measurement. HapX co-precipitated during $GrxD^{VENUS}$ enrichment under iron starvation, sufficiency and excess. *Vice versa,* GrxD was co-enriched during ^{MYC}HapX immunoprecipitation. The interaction of GrxD and HapX was subsequently confirmed *in vivo* by bimolecular fluorescence complementation analysis. In line with the *in vivo* results, recombinant *A. fumigatus* GrxD and HapX proteins were also co-purified with an unknown Fe-S ligand *in vitro* from *Escherichia coli*. In summary, these data provide first evidence that HapX iron sensing in *A. fumigatus* involves the interaction with GrxD.

Reference

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POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

Host and environmental regulation of cercosporin biosynthesis: a genetic perspective

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Cercospora zeae-maydis, a hemibiotrophic foliar pathogen of maize, produces cercosporin, a phytotoxic perylenequinone, during the necrotrophic stage of pathogenesis. Although the cercosporin biosynthetic (*CTB*) gene cluster has been identified, little is known about the molecular regulation of cercosporin production during pathogenesis. Through a combination of forward and reverse genetics, we identified a suite of non-cluster genes that positively or negatively regulate cercosporin biosynthesis. These include genes implicated in environmental sensing, signal transduction, nutrient acquisition, primary metabolism, chromatin remodeling, and the circadian clock. From this information, a new working model describing the regulation of cercosporin biosynthesis has emerged.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

New elements in the regulation of carotenoid biosynthesis in *Fusarium*

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Fusarium species produce a large variety of secondary metabolites ranging from toxins, antibiotics to pigments. Among the latter is the carotenoid neurosporaxanthin (NX), synthesized after illumination and presumably involved in protection against oxidative stress. In *Fusarium fujikuroi* and *Fusarium oxysporum*, light induces transcription of structural genes *carRA*, *carB*, *carO, and carT, involved in NX biosynthesis.*

Until now, no specific transcription factors of the *car* genes have been demonstrated to bind to their promoters. However, deep-pigmented strains accumulating large NX amounts have been characterized as well as the gene responsible of this phenotype: *carS*. An increase of *carS* mRNA*,* modulated by the Tet-on system, has shown to reduce the accumulation of NX, supporting the role of CarS as a repressor. The protein CarS does not bind to the *carRA* promoter, a key gene in the NX pathway, but it has two RING finger domains characteristic of ubiquitin ligases, suggesting a control by interaction with other regulatory proteins. In order to find putative specific transcription factors, pull-down experiments with biotinylated *car* promoters have been carried out and putative candidates genes have been identified.

RNA-seq data of the the upstream 5' region of *carS* in *F. fujikuroi* and *F. oxysporum* led to identify a non-annotated transcript in their genomes, that we named *carP*. In *F. fujikuroi*, expression of *carP* is enormously affected by the *carS* mutation. Present data indicate that *carP* is a lncRNA transcribed in the same direction that *carS*. Deletion of *carP* provokes a down regulation of the structural *car* genes and an increase of *carS* mRNA, which correlate with a lack of NX accumulation. Altogether indicate a complex regulation of carotenogenesis in *Fusarium* in which participate not only regulatory proteins but also a lncRNA, currently under detailed study.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

Proteomic analysis of temperature dependent total proteins from *Trichoderma guizhouence* NJAU4742 by using rice straw as sole carbon sources under solid-state culture condition

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Trichoderma guizhouence NJAU4742 is a mesophilic filamentous fungus isolated from compost, which plays a vital role in prompting plant growth through acting as plant growth promoting and owns a capacity to degrade various agricultural wastes. The metabolic rate is inhibited by the parameter of temperature, especially the ability of decomposing lignocellulosic materials, however, the biological mechanism is not clear. In this study, various extracellular and intracellular proteins synthesized by *T. guizhouence* NJAU4742 by using rice straw as sole carbon sources were explored by using the quantitative proteomic approach Sequential Windowed Acquisition of all Theoretical fragment ions (SWATH) with Data Dependent Acquire (DDA) mode for relative quantification. The results indicated that 1464 proteins were all identified in all the treatments (cultured at 20 °C, 28 °C and 37 °C) including many intracellular regulatory proteins and extracellular hydrolytic enzymes (cellulases, hemicellulases, lignin-degrading enzymes, proteases), protein-translocating transporter, and hypothetical proteins by using rice straw as sole carbon sources. Quantitative SWATH and protein network interaction analysis results showed that ADP ribose pyrophosphatase may be the critical factors to effect the metabolic rate of *T. guizhouence* NJAU4742 during the biodegradation process at different temperature. Therefore, we discuss here the possible biological mechanism how temperature effect the lignocellulose decomposition by *T. guizhouence* NJAU4742 and the optimization of lignocellulosic biomass hydrolysis by *T. guizhouence* NJAU4742.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

Understanding the halophile pathways of a lignocellulolytic fungus: *Aspergillus sydowii*

María del Rayo Sánchez-Carbente[1], Yordanis Pérez-Llano[2], Eya Caridad Rodríguez-Pupo[2], Ramón Alberto Batista-García[2], Jorge Luis Folch-Mallol[1]
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The molecular characterization of halophilic fungi with lignocellulolytic activity is attractive due to potential biotechnological use and the contribution to a better understanding of the molecular mechanisms responsible for fungal physiology in such conditions. We have isolated from a solid-state fermentation of sugarcane bagasse, a moderate halophile strain that has been identified as *Aspergillus sydowii* Interestingly, this strain can grow on diverse lignocellulosic materials,as well as on polyciclyc aromatic hydrocarbons, both on halophile conditions. To our knowledge, there is none information about the strategies that the fungus use to contend with high salinity. Therefore, we aimed to characterize the transcriptomic profiles of this strain to salinity when growing in wheat straw as sole carbon source. For this purpose, we characterized the growth of *A. sydowii* in a semi-solid fermentation of wheat straw in the presence of 0.5M and 2.0M of NaCl or in its absence. The analysis of the mechanisms of halophilia in this fungus showed that hydrophobins (a protein family that occurs exclusively in fungi) have a very strong differential expression. This behavior has been described previously for a basidiomycete (*Wallemia ichthiophaga*), but the role of the "halophilic hydrophobins" has not been confirmed. This would represent a novel mechanism of halophilia in fungi when compared to other halophilic microorganisms. Also, other strategies that are regulated in microorganism on halophile conditions include the High-Osmolarity Glycerol (HOG) pathway, in the transcriptomic analysis there is no differential expression of the putative HOG1 orthologue of *Saccharomyces cerevisae,* however HOG is post-translational regulated and we have found the presence of osmolytes, such as glycerol and manitol, among others. Interestingly, the osmolyte identity change in dependance of the salinity and the stage of growth. This is the first approach to understand the halophile pathways of a fungus growing on a lignocellulosic substrate.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

Carbon regulation of metabolic processes contributing to pathogenicity by postharvest pathogens

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Fruit pathogens can contribute to acidification or alkalization of the fruit host environment. This capability has been used to divide fungal pathogens into acidifying and/or alkalizing classes. 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. In *C. gloeosporioides* we have showed that limited or excess of carbon may lead to increase or decrease, respectively, of environmental pH resulting in differential mechanism of fungal pathogenicity. In *P. expansum* increase in sucrose culture amendment from 15 to 175 mM decreased the accumulation of the mycotoxin patulin suggesting a negative regulation of the global regulator *laeA*. However functional analysis of CreA, the global carbon catabolite regulator suggested that LaeA and CreA appear to independently regulate secondary metabolism as patulin synthesis as *laeA* expression was restored in *creA* deletion in sucrose media, while patulin production was not. Our present results indicate that host sugar changes during fruit ripening in the postharvest life, may modulate environmental pH to enhance fungal pathogenicity by activation of pathogenicity factors and secondary metabolism production.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

The *Aspergillus nidulans* pyruvate dehydrogenase kinases are essential to integrate carbon source metabolism

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The pyruvate dehydrogenase complex (PDH), that converts acety-coA to pyruvate, is regulated by a consortium of pyruvate dehydrogenase kinases (PDHK) and phosphatases (PDHP) that have been shown to be important for morphology, pathogenicity and carbon source utilisation in different fungal species. The aim of this study was to investigate the role played by the three PDHKs PkpA, PkpB and PkpC in glucose, cellulose and acetate utilisation in the reference filamentous fungus *Aspergillus nidulans*, in order to unravel regulatory mechanisms which could prove useful for fungal biotechnological and biomedical applications. All three PDHKs were shown to be mitochondrial with PkpA positively regulating PDH activity. In the presence of glucose, PkpA and PkpC function in the same pathway and deletion of the respective genes resulted in reduced glucose utilisation, which affected carbon catabolite repression (CCR) and hydrolytic enzyme secretion, due to de-regulated glycolysis and TCA cycle enzyme activities. Furthermore, PkpC was shown to be required for the correct metabolic utilisation of cellulose and acetate. PkpC negatively regulated the activity of the glyoxylate cycle enzyme isocitrate lyase (ICL), required for acetate metabolism. In summary, this study identified PDHKs important for the regulation of central carbon metabolism in the presence of different carbon sources, with effects on the secretion of biotechnologically important enzymes and carbon source-related growth. This work demonstrates how central carbon metabolism can affect a variety of fungal traits and lays a basis for further investigation into these characteristics with potential interest for different applications.

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POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

Changes in phosphorylation states of the NDR kinase COT1 alter lipid composition in *Neurospora crassa*

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The *Neurospora crassa* NDR kinase COT1 is involved in the regulation of hyphal elongation and branching. COT1 dysfunction results in ultrastructural irregularities, including thickened cell walls, the presence of many cytoplasmic vacuoles, some with vesicle-like inclusions, as well as abnormally shaped mitochondria and nuclei. COT1 has been shown to be associated with the cytoplasmic membrane. This localization, along with COT1 phosphorylation, are required for its proper Ser/Thr protein kinase activity.

In order to investigate the changes that occur in the cell membrane composition, which are dependent on COT1 activity, we applied a liquid chromatography-mass spectrometry (LC-MS)-based lipidomics approach to a set of *cot-1* strains, mutated in three conserved phosphorylation sites of the kinase, mimicking non-phosphorylated or constitutively phosphorylated residues of the protein. A mutation in COT1 Thr589, which markedly affects COT1 activity and hyphal morphology, was accompanied with a significantly altered lipid composition that was characterized by an increase in unsaturated glycerolipids, namely diacylglyceols (DAG), triacylglyceols (TAG), phosphatidylethanolamines (PE) and phosphatidylcholines (PC). Furthermore, the phosphomimetic state of COT1 Thr589 strongly affected the saturation profile of the glycosylceramides (GSL) fatty acid components, as well as methylation of their long chain bases (LCBs). GSLs are key components of the plasma membrane and their presence is linked with membrane fluidity and stability, as well as cell signaling and protein sorting. The involvement of GSLs in COT1 dependent morphogenesis was further demonstrated by differential sensitivity of the *cot-1* strains to the serine-palmitoyl transferase inhibitor Myriocin and the UDP-glucose:Cer glucosyltransferase inhibitor PDMP.

Only minor changes in transcription of genes involved in GSL biosynthesis were observed in a *cot-1* background, suggesting that most of the regulation of COT1-mediated GSL biosynthesis is post transcriptional. The data presented here provide first evidence for the involvement of an NDR kinase in fungal lipid metabolism.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Physiology and metabolism

Uncovering the molecular bases for chilling tolerance of phytopathogenic fungi

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Fungal pathogens are considered the main cause of postharvest losses of fresh fruits and vegetables, which are estimated at about 30% product loss globally. Low temperature storage is an efficient practice to prolong the postharvest performance of crops with minimal negative impact to human health and the environment. However, some phytopathogenic fungi like *Botrytis* and *Alternaria* spp. are highly tolerant to cold-storage conditions and can survive as a quiescent infection or even grow and cause rotting and decay of fruit during cold storage.

We apply molecular biology techniques combined with basic biochemistry and Liquid chromatography–mass spectrometry (LC/MS) based lipidomics approach to study the molecular basis of fungal tolerance to cold stress. Specifically we investigate the involvement and cross talk between reactive oxygen species (ROS) signaling and lipid metabolism in determining fungal morphogenesis and pathogenicity at low temperatures. A special focus is given to the involvement of sphingolipids in regulating ROS production that control cell death and host-pathogen interactions.

New insights from our work will facilitate the development of environment-friendly treatments to control postharvest fungal rotting of fruits stored at low temperatures.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Symbiosis & endophytes

Genetic approach to root colonization by Trichoderma virens

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Trichoderma virens colonizes roots of a wide range of plants in a generally beneficial interaction, which can systemically prime plant defenses against infection by pathogens. The extent and outcome of the interaction varies on the Trichoderma-plant pair chosen for study. Nevertheless, comparison of different studies suggests that there is a core set of Trichoderma genes induced upon interaction with roots, encoding CAZymes and some small secreted proteins. To define the time course of colonization, we followed growth and ingress of *T. virens* in maize and tomato roots by staining with wheat germ agglutinin (WGA) Alexa-Fluor and live imaging by confocal microscopy. Image analysis of maize roots shows spore adhesion and germination within 24 hours post inoculation (hpi), followed by hyphal growth over the next 24 hpi. At 72 hpi there was massive hyphal development surrounding the root, with first indication of penetration. At 96 h and 120 hpi, *T. virens* colonized the maize epidermal and first cortical layers. Image analysis of tomato at 96 hpi shows the same colonization profile as in maize. In both experiments, Trichoderma hyphae appear to grow between the cells. A second approach is based on the hypothesis that ingress of Trichoderma requires increased extensibility of the plant cell wall. This hypothesis will be tested by constructing knockout mutants of genes encoding the expansin-like swollenin gene, and three polygalacturonidases, guided by studies showing colonization phenotypes by mutants in other Trichoderma species deleted for some of these genes.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Symbiosis & endophytes

Postharvest microbiota dynamics of mango fruit stem-end in response to light, temperature and during storage

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Stem-end rots (SER) develop in harvested mangos during fruit ripening and cause significant losses. SERs are caused by pathogenic fungi (e.g. *Colletotrichum gloeosporioides, Alternaria alternata, Lasiodiplodia theobromae, Neofusicoccum, Dothiorella, Phomopsis mangiferae*, and others) that endophytically colonize fruit stems during fruit development in the orchard and remain quiescent until the onset of fruit ripening. This work was conducted to characterize the endophytic microbiota in mango fruit stem-end tissue and study the effect of different postharvest treatments on the composition of bacterial and fungal communities. Microscopic observations showed that during the quiescent stage various fungi colonize the phloem of fruit stem-end, and after switching to the pathogenic stage they expand to the fruit parenchyma surrounding the stem, causing SER. Interestingly, fruits that were subjected to high light in the orchard developed less SER after storage. These fruits accumulated anthocyanins leading to red color peel, which was correlated with resistance to both anthracnose and SER. The bacterial and fungal microbiomes in stem-end of red and green mango fruit stored at different temperatures were examined using universal bacterial and fungal primers (16S and ITS respectively). Bioinformatic data analysis showed that the community compositions of the fungi and bacteria in the mango stem-end were significantly modified during storage, in response to different storage temperatures and in response to high light in the orchard. For example, Pleosporaceae (*Alternaria*) was the most abundant fungi in green (susceptive fruit) that was not exposed to sunlight or during storage (fruit ripening). This change in fungal composition was accompanied with increased occurrence of SER. Soon before the development of SER, the increased amount of fungi was correlated with the increase in abundance of chitin degrading Chitinophagaceae bacteria. Collectively, our results show that pre and post-harvest treatments modify microbial community in the stem-end and could be associated with reducing postharvest SERs.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Symbiosis & endophytes

Mechanisms of interspecific interactions in two model brown rot fungi, *Postia placenta* and *Gloeophyllum trabeum*

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Wood degrading basidiomycetes live in complex communities of microbes and interspecific interactions are vital processes for their survival. In this study, we used model interaction microcosms to pair two model decay fungi, *Postia placenta* and *Gloeophyllum trabeum*, against one another and investigate the mechanisms of interspecific interactions using transcriptomics and proteomics. We found 20 proteins, mostly of unknown function, that were secreted from exclusively at the interaction zone, 10 from *P. placenta* and 10 from *G. trabeum*. Pectinase activity was highest at the interaction zone compared to ingle species cultures or non-interacting hyphae. Most genes 4-fold or more upregulated at the interaction zone in both fungi had no known function, but the second largest group of genes upregulated were oxidoreductases. Known lignocellulose-degrading genes such as glycoside hydrolases were mostly not upregulated at the interaction zone, suggesting greater investment in biosynthesis than decay. Several secondary metabolite-synthesizing genes in *G. trabeum* were upregulated during the interaction, whereas this was not seen in *P. placenta*, suggesting the two fungi differ in competitive tactics. This work identifies several secondary metabolites synthesizing genes that could help mediate interspecific combat and identifies *G. trabeum* as a useful model fungus for studying the interactions of wood degrading basidiomycetes.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Symbiosis & endophytes

Bad fungi gone good: how to control *Fusarium* wilt disease with *Fusarium* endophytes

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Fusarium oxysporum (*Fo*) is known to cause vascular wilt disease in over 100 different hosts. Although most studies focus on its ability to cause disease, *Fusarium* is also capable of colonizing plants without triggering disease symptoms. Among these non-pathogenic strains, the endophytic strain *Fo*47 strain has repetitively been shown to confer protection against wilt disease on tomato caused by *Fo* f.sp. *lycopersici* (*Fol*).

In order to assess how widespread, the capacity is to suppress *Fusarium* wilt disease, we have acquired a collection of 80 *Fo* strains isolated from non-cultivated soil or non-symptomatic plants in three different continents (America, Australia and Europe). We found that all the strains tested have the ability to protect to varying degrees against *Fusarium* wilt disease in tomato in 1:1 co-inoculation assays. Heat killed spores of the biocontrol strain shows no disease suppression. Moreover, we found that pathogen abundance is reduced in the presence of *Fo*47 in root and stem tissue. Surprisingly, the colonization of *Fo*47 is increased in tomato stems when co-inoculated with *Fol*. The plant hormones jasmonic acid, salicylic acid and ethylene do not appear to be required for *Fo*47-induced suppression of disease. Understanding the mechanisms behind diseases suppression may help us to increase compatibility with *Fo* endophytic strains and resistance to *Fusarium* wilt.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Symbiosis & endophytes

Fungal diversity in citrus fruit at different ripening stage

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Orange is one of the main fruits in the world. Postharvest fruit decay is very common at the later stage of citrus growth, transportation and storage and the decay rate usually is 20%-30%, even up to 50%, which causes great losses. Fungi are the main causes of decay including *Penicillium italicum*, *P. digitatum*, *Alternaria citrus*, *Collectotrichum gloeosporioides*, *Diplodianata lensis*, *Phomopsis cytosporella* and *Oosporacitri aurantii* etc. There is fungal resource even in the healthy orange. In order to clarify the fungal community in fruit, the fungal diversity in the rind and the flesh of Wanmi No 1 citrus fruit samples at growth, ripening and storage stage was examined by Illumina MiSeq sequencing technique. Through analyses of OTUs with abundance, diversity index, and community structure at the order and genus level, we found that there was higher extend of fungal diversity in rind than in flesh. The dominant genera were different at different stage, with *Medicopsis* and *Collectotrichum* for the rind at the growth stage, *Collectotrichum* for the ripening stage, and *Botrytis*, *Erythrobasidium* and *Strelitziana* for the storage stage, while they were *Penicillium* and *Cladosporium* at the growth stage, *Botrytis* at the ripening stage, *Penicillium* and *Alternaria* at the storage stage in the flesh. The population of plant pathogenic fungi *Cladosporium*, *Magnaporthe*, *Sclerotinia*, *Botrytis*, *Erysiphe*, *Penicillium*, *Alternaria* and *Fusarium* in the rind were larger than in the flesh. The large population of fungi and the various pattern suggest that the postharvest fruit decay should be a result of interaction of the endopytic fungi.

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Symbiosis & endophytes

Carbohydrate metabolism and production of phytohormones of *Serendipita indica* and *Serendipita herbamans* in interactions with their plant partner

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Carbohydrate allocation in plants plays a primary role in economy and human food consumption. The storage of sugars in specific organs is an important driver to yield plant products with high nutritional value. *Serendipita indica* (former *Piriformospora indica*) and *Serendipita herbamans* are root endophytic fungi of the order Sebacinales; they can live in symbiosis with different species of plants without negative interference with plant health. *S. indica* possess growth promoting effects by changing the host metabolism probably through the secretion of particular proteins and metabolites including phytohormones. The resulting holosymbiont shows metabolic behaviours different from both partners alone. The current project is aimed to understand the relation between carbohydrate metabolism and phytohormone balance in order to reveal the mechanisms underlying the plant growth-promoting effects.

S. indica and *S. herbamans* show particular carbon source preferences and modify the carbohydrate metabolism accordingly. Genes for invertase and phosphoglucose-isomerase are present in their genomes and the corresponding protein activities have been detected in liquid culture of both fungi. In addition, they secrete different enzymes into liquid medium apparently related with the sugars used to feed the culture. Sugars seem to be a modulator of gene expression of carbohydrate related enzymes. Molecular data concerning expression of invertase gene will be presented.

On the plant side, secondary metabolites of both fungi can boost carbohydrate metabolism in tomato not-transformed root-organ culture, with a significant increase of cell-wall and cytosolic invertase activities.

The presence of fungal genes for auxin biosynthesis have been already shown; in addition, putative genes encoding enzymes involved in the biosynthesis of cytokinins, gibberellins, brassinosteroids and ABA have been detected in *S. indica* and *S. herbamans* genomes.

Interaction with plant mutants show that the fungi can produce compounds which complement phytohormone deficiencies of their plant partner. Level of cytokinins and biosynthetic genes (tRNA-IPT) data will be presented.

POSTER SESSION 1
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Symbiosis & endophytes

Association of the mycorrhiza-like fungus *Serendipita indica* with bacteria to enhance plant resistance against fungal pathogens

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Serendipita indica (syn. *Piriformospora indica*) is a root-colonizing endophytic fungus that boosts plant vigor and confers resistance against plant pathogens. This fungus is further known as having a bacterial endosymbiont living inside its hyphae. Similarly, some bacterial endophytes promote plant growth and enhance plant resistance. The application of this fungus as a biostimulant often leads to irregular performance in the field, perhaps due to antagonisms with other microbes. We aimed at boosting effects of the fungus and its bacterial symbiont on plants by combining the fungus and its symbiont with selected bacterial helpers.

A collection of bacteria from roots of potato and tomato plants were isolated and combined with the beneficial fungus *Serendipita indica* in order to study the type of interaction. Some bacterial endophytes stimulate *Serendipita* growth and colonize its hyphae. Some combinations of the beneficial fungus with endophytic bacteria can further help to reduce tomato wilt disease caused by *Fusarium oxysporum,* while others do not. Genomes of selected isolates have been sequenced and annotated to understand how the bacteria interact positively with the fungus.

The future transcriptomics analyses of tomato plants inoculated with only *Serendipita* or combinations of the fungus and bacteria will unravel the mechanisms behind these interactions, identifying genes up- and down-regulated during the interplay. The mechanisms of the bacterial helpers on the fungus and its symbiont will be further elucidated to understand better the multi-partite interactions between a fungus, bacterial symbiont, the helpers, and the plant.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 676480.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Symbiosis & endophytes

A draft genome sequence of the arbuscular mycorrhizal *Gigaspora margarita*: investigating cross-kingdom interactions

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Arbuscular mycorrhizal fungi (AMF) are members of the plant microbiota, being associated with the roots of most land plants, and enhancing the hostâ€™s ability to acquire nutrients. On the other hand, they host their own microbiota (Desiro et al, 2014), since some of them possess obligate endobacteria. AMF represent thereforeÂ the hub for an interkingdom interaction between plants, fungi and bacteria (Bonfante and DesirÃ², 2017). The fungal endobacteria show reduced genomes and nutritional dependence on the fungal host (Ghignone et al., 2012; Torres-CortÃ¨s et al., 2015), but functional OMICs analyses suggested that their presence deeply impacts AMF host physiology (Salvioli et al., 2016; Vannini et al., 2016). While genome-scale comparisonsÂ have become a powerful tool for the reconstruction of the fungal lineage (Spatafora et al., 2017), genomics data for AMF are available only for *Rhizophagus irregularis* (Tisserant et al., 2013; Lin et al., 2014), that does not possess endobacteria. Here, we present a draft genome sequence of *Gigaspora margarita* BEG34, which hosts the endobacterium *Candidatus Glomeribacter gigasporarum*. The nuclear genome sequence of *G. margarita* is ~775Mb, almost 6 times bigger than *R. irregularis.* While scanning for transposable elements and centromeric/telomeric regions, we discovered that ~80% of *G. margarita* genome mainly contains repeated sequences ascribable to transposable elements. Preliminary approaches for gene prediction revealed a low gene density (~4 genes/100Kb on average), and allowed the identification of genomic regions with homology to known bacterial sequences. On one hand, the genome ofÂ *G. margarita* has size and composition features which make its description a challenging task, but on the other hand it will provide interesting clues to understand how AMF evolved, allowing some fungal isolates to harbor endosymbionts, and in the mean time to interact with the host plant.

POSTER SESSION 1
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Symbiosis & endophytes

Ménage à trois: how do *Fusarium verticillioides* and *Sarocladium zeae* compete and interact with each other in maize kernels?

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Fusarium verticillioides (*Fv*) is a prevalent seed-borne maize endophyte capable of causing severe kernel rot and fumonisin mycotoxin contamination. Within maize kernels, *Fv* is primarily confined to the pedicel, while another co-occurring seed-borne fungal endophyte, *Sarocladium zeae* (*Sz*), is generally isolated in the endosperm and embryo. *In vitro* competition assays have indicated *Sz* can inhibit the growth of *Fv*. The two lactam-containing antibiotics produced by *Sz*, named pyrrocidine A and B, are associated with this inhibition of *Fv*. To explore the mechanism of antagonism, RNA-seq experiments were conducted by challenging the *Fv* with pyrrocidine B at subinhibitory concentrations. Among the most differentially expressed genes was FVEG_11089 (up-regulated 470-fold) that codes for an ABC transporter. Deletion of FVEG_11089 caused increased sensitivity of *Fv* to pyrrocidine B. The expression of FVEG_11089 is independent of its adjacent and similarly-induced transcription factor. Additionally, deletion of a PB-induced zinc-binding dehydrogenase gene in *Fv* led to 10-fold increase in fumonisin production compared to wild type. Hence, we theorize that FVEG_11089 functions in pyrrocidine B resistance by transporting the antibiotic out of the *Fv* cells. Exposure to pyrrocidine B may suppress fumonisin production and affect the pathogenicity of *Fv*. Further exploration of the antifungal resistance mechanisms addresses the overall competitive relationships of the two maize seed endophytes colonizing the same ecological niche and how they cope with xenobiotic challenges.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Symbiosis & endophytes

Antimicrobial and mycofumigation potential of novel Indian *Muscodor* species at enhancing the shelf life of fruits and vegetables

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Muscodor is a genus of sterile, volatile organic compounds (VOCs) producing endophytic fungi with antimicrobial and mycofumigation properties. In the present study, seven novel *Muscodor* species (*M. kashayum, M. strobelii, M. tigerii, M. darjeelingensis, M. ghoomensis, M. indica* and *M. camphora*) were isolated from *A. marmelos, C. zeylanicum*, and *C. camphora,* respectively growing in Western Ghats and North eastern Himalayan region of India. When tested for their antimicrobial properties, *M. kasahyum* emerged most lethal to the battery of plant and human pathogens. It exhibited complete inhibition of 26 pathogenic microorganisms whereas growth of rest of the isolates was reduced to 50-70%. Further, *Muscodor strobelii*, *M. darjeelingensis, M. camphora* also exhibited strong antibacterial and antifungal activity whereas *M. tigerii* only showed antifungal activity. The volatiles produced by *M. kashayum* successfully preserved grapes, jamun, cherry, black gram and wheat from *Botrytis cinerea, Rhizoctonia solani, Collectrotrichum gloeosporioides* and *cercospora beticola* infection till 15 days of infection. All the isolates produced fruity smell which is attributable to a mixture of volatile compounds predominantly producing 3-cyclohexen-1-ol,1-(1,5-dimethyl-4-hexenyl)-4-methyl; 1,6-dioxacyclododecane- 7,12-dione; 4-octadecylmorpholine, 2, 6-bis (1, 1-dimethylethyl)-4-(1-oxopropyl) phenol, aspidofractinine-3-methanol, tetracontane etc. *Muscodor* species produced sterile ropy mycelia with coiling and non-descript structures and lacks sexual stage. Their ITS sequence also showed high similarity with other *Muscodor* species. Phylogenetic, distance and haplotype analysis confirms their identity as novel *Muscodor* species. Thus, these *Muscodor* isolates can be taken into account to be developed as a myco/biofumigant that act as a biopreservative for fruits, vegetable and grains and help to reduce post harvest losses.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Symbiosis & endophytes

Plant-mycorrhizal fungi symbiosis: from metabarcoding data to diversity analysis

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Next Generation Sequencing (NGS) approaches are currently used as a tool to study communities of plant-associated mycorrhizal symbionts such as arbuscular mycorrhizal fungi (AMF). They enable fungal identification in roots without the need of assessing morphological features and provide sufficient depth and magnitude to get insights into fungal community ecology. DNA metabarcoding infers the species composition of environmental samples by amplifying, sequencing and analysing target genomic regions.

Relatively little is known about the effect of habitat fragmentation on belowground mycorrhizal communities. Gypsum ecosystems are usually characterized by fragmentation phenomena in such a way that they can be considered as a model system to understand the mycorrhizal interaction-area size relationships.

In our study, we assess AMF community composition in plant roots from a fragmented gypsum landscape located in Spain. We sampled 225 individuals corresponding to 28 plant species, along a 15 sized-fragment gradient. DNA was extracted form root samples. Amplicons libraries were prepared using region specific primer NS31 and AML2 to target 18s r-DNA V4 region of Glomeromycota and sequencing performed on Illumina MiSeq 2*300v3. Bioinformatic analysis included demultiplexing, quality filtering, artefacts removal and Operational Taxonomic Unit (OTUs) clustering. MaarjAM data base of Glomeromycota was used to assign OTUs to taxa.

We observed a marked AMF richness and diversity loss when habitat size decreases. Plant individuals have less Glomeromycota OTUs in their roots and number of OTUS in the whole plant community is reduced in small fragments. Plant-AMF interactions loss is nested, with smaller fragments harbouring those interactions that more recurrently appears across fragments. Concerning robustness, fungal community seems more vulnerable to partner loss in small fragments.

Habitat fragmentation may constitute a risk for a loss of plant-mycorrhizal fungi interactions and ultimately for AMF extinction. The implications on nutrient cycle and ecosystem functioning cannot be underestimated.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Symbiosis & endophytes

Induction of tomato defense against pseudomonas mediated by Endophytes isolated from wild wheat

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In previous work, we isolated two fungal endophytes from *Triticum diccocoides* and *Aegilops sharonensis*, two ancestors of bread wheat *Triticum aestivum.* The two isolates were identified as *Acremonium sclerotigenum* and *Sarocladium implicatum*, and were able to induce resistance against drought in *Triticum aestivum*. In this work, we tested whether these fungal endophytes were able to induce resistance in plants of different class from the ones that were isolated from the beginning. Tomato plants (*Solanum lycopersicum* cv. Ailsa Craig) were inoculated with *Acremonium sclerotigenum* or *Sarocladium implicatum* and inoculated with the pathogenic bacterium *Pseudomonas syringae*. The endophytes-inoculated plants had significantly lower symptoms of bacterial infection as well as lower levels of colony forming units per leaf compared with endophytes-free control plants. Moreover, plants inoculated with endophytes and infected with *P. syringae* showed an altered balance of Jasmonic acid and Salicylic acid compared with endophyte free plants. In contrast to the decrease of bacterial infection, both endophytes had no effect on infection of plants with the grey mold fungus *Botrytis cinerea.*

In conclusion, our results show that endophyte induced resistance is effective across plants from different species and classes. *A. sclerotigenum* and *S. implicatum* are able to induce resistance in tomato against the biotrophic bacterium *P. syringae*, which was associated with changes in phytohormones levels, but they did not affect infection by the necrotrophic fungus *B. cinerea*.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Symbiosis & endophytes

The influence of management strategy on plant organ microbiomes in a corn-soybean-wheat rotation

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Manipulating plant-associated microbes to reduce disease or improve crop yields requires a thorough understanding of interactions within the phytobiome. Michigan State University's Kellogg Biological Station Long Term Ecological Research site harbors a set of plots in a three year wheat-maize-soybean rotation, providing an ideal location to conduct long-term characterization of these economically important row crops. The site has been maintained since 1989 with six replicate hectare plots for each of four management styles: conventional, no till, reduced inputs, and organic. Plants were sampled from three years of one rotation cycle. We analyzed the fungal and bacterial communities of leaves, stems, and roots throughout the growing season using fungal ITS2 and bacterial 16S rRNA gene amplicon sequencing. The most prevalent operational taxonomic units (OTUs) were shared across all management styles for each crop. We identified core OTUs for each crop, and used network analysis to identify microbial hub taxa. Our results suggest that microbial communities were strongly affected by plant organ and plant age, and that management strategy was less influential on community composition.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Applied and industrial mycology

DERMADYN-Dermatophytes detection test

Inbal Binsky[1], Marie-Jeanne Carp[1], Keren Ben-Zion[1], Maya Goshen[1], Noam Maisler[1], Rina Segal[2]

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Overview-

A real time PCR- based multiplex application for fast, reliable, ready to use and high throughput in vitro diagnostic of dermatophytes. This multiplex PCR system enables 7 different dermatophytes types detection within 3 hours. The test contains two ready to use mixes for detection of 7 most common dermatopytes types:

[1] Trichophyton tonsurans
[2] Trichophyton violaceum
[3] Trichophyton rubrum complex
[4] Trichophyton mentagrophytes complex
[5] Microsporum canis
[6] Microsporum gypseum
[7] Epidermophyton floccosum and Internal Control.

Specimen - This test is for use with extracted DNA from skin scale specimens, plucked hair, nails, culture and swabs of human origin.

Workflow - Sample collection-sample lysis in lab-DNA purification manually or automated-add DNA and running Real time PCR-results automatic analysis.

Clinical validation results - A total of 220 samples were examined. Acorrelation of 86.3% (190/220) was found between the methods.

Cross reactivity detection - reference species from CBS 100% detection of 7 dermatophytes types. Additional detection of Microsporum audouinii Gruby Test detection

[1] CBS 358.93 Epidermophyton floccosum
[2] CBS 161.69 Arthroderma gypseum
[3] CBS 289.86 Trichophyton rubrum
[4] CBS 319.31 Trichophyton violaceum
[5] CBS 132.88 Microsporum canis
[6] CBS 572.75 Trichophyton mentagrophytes
[7] CBS 219.32 Trichophyton tonsurans
[8] CBS 134.66 Trichophyton verrucosum
[9] CBS 335.32 Trichophyton schoenleinii
[10] CBS 404.61 Microsporum audouinii Gruby
[11] CBS 511.73 Trichophyton erinacei
[12] CBS 318.56 Trichophyton mentagrophytes
[13] CBS 135.33 Acremonium strictum
[14] CBS 287.95 Aspergillus fumigatus Fresen
[15] CBS 467.48 Scopulariopsis brevicaulis
[16] CBS 562 Candida albicans

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Applied and industrial mycology

Functional analysis of gene expression signals in basidiomycetous fungi using transfection, and random or targeted integration on the chromosome

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Gene expression signals such as promoter and terminator sequences play an essential role in controlling timing and efficiency of each gene's expression. In basidiomycetes, basic requirements for gene expression signals are obscure because of the lack of proper experimental approaches. In this report, a functional analysis of promoter sequence was conducted in white-rot basidiomycete, *Ceriporiopsis subvermispora*, using transfection phenomena with recombinant hygromycin phosphotransferase (*hph*) genes as a reporter. We found that a 14-bp region (BCE) plays a critical role in expression of the recombinant *hph* driven by the promoter sequence of *ß-tubulin* gene in *C. subvermispora*. *In silico* analysis demonstrated that other basidiomycetes also harboured BCE-like sequence in *ß-tubulin* promoter, suggesting its functional conservation among species of the class. To address this hypothesis, we investigated the effects of deletion of the BCE-like sequence in *ß-tubulin* promoter on expression levels of the reporter genes, *hph* or *NanoLuci*, in *Pleurotus ostreatus* and *Coprinopsis cinerea*. The reporter genes driven by either deletion mutant of BCE-like sequence or intact *ß-tubulin* promoter from each species was integrated into host chromosome either randomly or targeted site by homologous recombination. The results demonstrated its essential function in transcription from these promoters. Our findings provide new insights into the vital role of BCE region in the *ß-tubulin* promoter among basidiomycetes. The transfection system was also used for characterizing sequence involved in 3' end formation of mRNA of glyceraldehyde-3-phosphate dehydrogenase (*gpd*) terminator in *C. subvermispora*. Our results showed that an AT-rich region located at 13 nt upstream of the polyA site played a crucial role in the function of *gpd* terminator. It is conceivable that the assay system developed in this work is available for analysis of other functional sequences like translation controlling signals etc.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Applied and industrial mycology

Fungal strain development for screening and production of enzymes: development of a suite of tailored *Aspergillus* host strains with improved characteristics regarding proteolytic degradation, enzyme screening and fermentation characteristics

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., Dutch DNA Biotech, Utrecht, Netherlands

Fungal host strains such as *Aspergillus niger*, *Aspergillus sojae* and *Trichoderma reesei* are used for the production of a wide variety of industrially relevant enzymes. About 80% of all industrial enzymes are derived from filamentous fungi, making these organisms also the hosts of choice for the production of new enzymes and proteins.

To develop suitable strain platforms to exploit the unique characteristics of fungi several strain improvement topics are being addressed in our research. These include development of protease deficient host strains using different classical genetic and molecular genetic approaches, followed up by systems biology approaches to further explore the details of the regulation of protease production in different filamentous host strains. From our research it has become clearÂ that various different pathways may operate in different fungi.

Besides protease production also strain improvement aimed at improved fermentation characteristics is highly relevant for protein production. In particular aspects of fungal morphology have been addressed in our research in line with fungal fermentation process engineering. This research has resulted in improved fermentation design and performance.

In many cases prior to producing specific proteins of interest also selection of genes and gene-designs for optimal protein secretion is an important step in fungal strain development. For this purpose we have developed various fungal host strains suitable for this screening phase. An example of this is a line of host strains unable to use specific polymeric carbon sources and their use to develop selective biological screens for specific protein activities.

POSTER SESSION 1
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Applied and industrial mycology

Organic acid production in *Aspergillus niger*: Rewiring endogenous metabolic pathways by introducing and modifying the itaconic acid pathway from *Aspergillus terreus*

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Rising carbon emissions due to increased industrialization and its effect on the global climate are raising awareness to move from a fossil fuel-based economy to a bio-based economy. Organic acids have huge potential as alternative for petrochemicals and concomitantly its derivatives as commodities [1]. Filamentous fungi are widely known as efficient organic acid producers, in particular members of the genus Aspergillus.

Itaconic acid (IA), a C5-dicarboxylic acid, has been identified as one of the top twelve building block chemicals that can be produced by biotechnological means. The potential applications of IA in green chemistry are numerous and IA is already naturally produced by *Aspergillus terreus*. However, for several reasons heterologous production in the related species *Aspergillus niger* has been proposed. Previously we have shown that rewiring of a non-canonicla citrate synthase gene (*citB*) derived from an *A. niger* secondary metabolism cluster has led to an increased yield, titer and productivity of IA, reaching to the highest levels reported for heterologous IA production.

In our research we have now performed a RNA-Seq analysis of high, medium and low IA producing strains to further improve our optimized IA pathway and understand the effect of heterologous IA production on *A. niger* metabolism. It was found that apart from *citB*, another non-canonical citrate synthase displayed a similar role in itaconic acid production upon overexpression. Further rewiring of metabolic pathways was seen by specific gene deletion of pathways involved in byproduct formation and overexpression of canonical primary metabolic pathways genes. Several of these strain modifications were found to improve production of itaconic acid. Finally, our research also showed a hitherto unknown involvement of N-metabolism on prolonged itaconic acid production to achieve higher titers and yields.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Applied and industrial mycology

Protease regulatory factors of *Trichoderma reesei* can be controlled to improve therapeutic protein production

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Protease secretion limits the production of many sensitive therapeutic proteins such as hormones and cytokines that are by nature easy to degrade. There are over 40 potential proteases secreted by *Trichoderma reesei*. We looked for transcriptional regulators of these proteases with the aim to control and reduce the expression of a wide range of proteases. Protease induction studies were set up to trigger protease activity with peptide and protein substrates in liquid cultures of *T. reesei*. Genome-wide expression data was generated and clustered to find out what genes are co-regulated after different treatments. Twelve candidate transcription factors or regulatory proteins were selected as potentially being involved in upregulating protease activity. To narrow the selection, the regulator genes were located on the scaffold to see if they were physically near any protease genes. Transiently silencing *ptf1*, *prp1*, and *ptf3* with siRNA downregulated the expression of a selection of protease genes in accordance with the co-regulation observed. Treatment with both *ptf1* and *prp1* siRNAs increased the effectiveness of the knockdown and reduced protease activity. The deletion of single, double, and triple combinations of the regulators successfully reduced protease activity and increased interferon alpha 2b production. For example, the triple deletion Δ*ptf1*Δ*prp1*Δ*ptf8* lead to a 3.7-fold improvement in interferon alpha 2b production. In conclusion, we have demonstrated that silencing or deleting protease regulatory factors can broadly reduce protease activity.

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(Monday, February 26, 2018 13:45-15:30)

Applied and industrial mycology

Erythritol from straw. A multilevel feasibility study in *Trichoderma reesei*

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Erythritol is a naturally abundant sweetener gaining more and more importance especially within the food industry. It is widely used as sweetener in calorie-reduced food, candies, or bakery products. In research focusing on sugar alternatives, erythritol is a key issue due to its, compared to other polyols, challenging production. It cannot be chemically synthesized in a commercially worthwhile way. Studies are therefore focusing on the biotechnological production of erythritol.

Using the industrial cellulase and hemicellulase production fungus *Trichoderma reesei,* we applied different approaches to increase erythritol production: substrate selection and treatment, strain improvement by metabolic engineering as well as process optimization. The organism is able to degrade lignocellulosic material and can therefore utilize renewable and cheap material, like wheat straw as starting material. The substrate can be pretreated to facilitate analysis and speeding up the degradation by *T. reesei*. We analyzed different types of straw and hydrolyzation parameters to find the most suitable starting material for the erythritol production in *T. reesei*. The key enzyme for the synthesis of erythritol is naturally present in *T. reesei*. The overexpression of this gene leads to an increase in erythritol synthesis. Further genetic modifications include deletion, overexpression and co-expression of different, not yet characterized enzymes, which may be involved in the erythritol production pathway. To further improve the production yield and facilitate the future purification, we anticipate introducing a known sugar transporter from *Saccharomyces cerevisiae.* Besides substrate and genetic modifications we performed a Design of Experiment to gain information about the influence of certain production parameters like osmotic pressure or fermentation time.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Applied and industrial mycology

Asp30 and Asp73 of *Aspergillus oryzae* cutinase CutL1 are involved in the ionic interaction with fungal hydrophobin RolA

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When the industrial fungus *Aspergillus oryzae* is grown with polyesters as a sole carbon source, the fungus co-produces a hydrophobin RolA and an esterase CutL1. RolA attached to polyesters specifically recruits CutL1 and consequently promotes hydrolysis of polyesters by CutL1. The mechanism of its recruitment is attributed to the ionic interaction between positively charged residues (H32, K34) of RolA and negatively charged residues (E31, D142, D171) of CutL1. The K_D for the interaction of RolA with the CutL1-E31S/D142S/D171S was considerably higher than that for its interaction with wild-type CutL1. In the presence of 250 mM NaCl, both K_D values were similar, suggesting that some additional charged residues in CutL1 are involved in the CutL1—RolA interaction besides E31, D142 and D171. In this study, we investigated whether D30 and D73 of CutL1 are also involved in the CutL1—RolA interaction. First, we compared amino acid sequences of CutL1 and CutL1 orthologs, and analyzed CutL1 3D-model, leading to prediction of D30 and D73 as the candidate residues involved in RolA-CutL1 interaction. Next, we purified CutL1-D30S, CutL1-D73S, CutL1-D30S/E31S/D142S/D171S and CutL1-E31S/D73S/D142S/D171S, and measured Circular Dichroism (CD) spectra of them to confirm their structures. To analyze the kinetics of binding of the CutL1 mutants to RolA, we used a Quartz Crystal Microbalance (QCM) and calculated K_D. The QCM approach revealed that the K_D values of the CutL1 single mutants to RolA were higher than that of wild-type CutL1 to RolA, and the K_D values of CutL1 quadruple mutants to RolA were higher than that of CutL1 E31S/D142S/D171S. We conclude that D30 and D73 are important for CutL1–RolA interaction. These results also imply that CutL1 3D-model is useful for prediction of amino acid residues that are involved in the interaction with RolA despite little conservation of the residues among CutL1 orthologs.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Applied and industrial mycology

Hyperbranching in the filamentous fungus *Aspergillus niger* following deletion of the GTPase RacA leads to altered glucoamylase secretion upon overexpression of the enzyme

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Filamentous fungi secrete hydrolytic enzymes to degrade polymeric substances into smaller molecules which are then taken up as to sustain growth and metabolism. The accepted paradigm in fungal biology is that the tips of fungal hyphae are the highly active regions of a fungal colony, where polarised growth and secretion are coupled processes. However, it is currently debated if the amount of growing hyphal tips in filamentous fungi correlates with an increase in secretion, with previous studies showing either a positive or no correlation. In this study, we investigated the previously described hyperbranching strainof the industrial cell factory *Aspergillus niger*, which is deleted in the GTPase RacA and builds more hyphal tips but shows otherwise identical growth rate and total protein secretion as the wildtype.

Here, we use a v-SNARE reporter strain (SncA-GFP) to show that the hyperbranching strain exhibits an increased level in secretory vesicles at the hyphal tip upon overexpression of glucoamylase driven by the Tet-on system. Thus, we establish for the first time a link between level of transcript/secretory cargo load with the gradient of secretory vesicles at hyphal tip. We show that Δ*racA* secretes altered amounts of glucoamylase upon Tet-on driven overexpression of the enzyme in comparison to the parental strain despite unaltered biomass yields, total secretory vesicles, or total protein secretion. Our results contribute to the understanding of fungal protein secretion at the hyphal tip, and have profound implications for biotechnology and applied mycology.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Applied and industrial mycology

Fungi to the rescue; the use of mycelium in the development of novel, sustainable bio-based materials

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Filamentous fungi colonize organic materials such as plant waste by means of hyphae that grow at their tips and that branch subapically. As a result, a hyphal network is formed that is called mycelium with a fabric-like appearance. Here, material properties of the mycelium of the mushroom forming fungus *Schizophyllum commune* were determined in relation to environmental growth conditions and genetic background. Mycelium of liquid standing cultures of wild-type strain 4-39 grown in the light or dark at ambient or high CO_2 showed a Young's modulus in the range of 438 - 913 MPa. The maximum tensile strength ranged between 5.1 – 9.6 MPa, while elongation at breaking ranged between 1.2 and 1.4 %. Mycelium of the hydrophobin deletion strain Δ*sc3* of *S. commune* showed a higher elongation at breaking (1.7 – 2.6 %). Moreover, it was stronger than wild-type with an elasticity modulus ranging between 1237 and 2727 MPa, and a maximum tensile strength of 15.6 – 40.4 MPa. Together, it is concluded that material properties of mycelium of *S. commune* can be modulated by changing the growth conditions and the genetic background. The resulting Young's moduli and maximum strength is similar to specific thermoplastics showing the potential of fungal mycelium as a sustainable replacement of oil-based plastics.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Applied and industrial mycology

Polyporales wood-decay fungi for bioconversion of lignocelluloses: genomics, interactions, and decomposition mechanisms studied for bioeconomy and industrial applications

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Basidiomycota Agaricomycetes, order Polyporales fungi are able to depolymerize wood-lignocellulose components: white-rot species decompose all biopolymers including lignin while brown-rot species are efficient in decomposing wood polysaccharides, mainly cellulose [1]. However, exact details of the degradation processes and the genetic, biochemical and proteomic factors involved are not fully understood despite of accumulating genomic data. Our aim is to combine fungal genomics to functional transcriptomics and proteomics including testing on various lignocellulose substrates for growth and bioconversions, to identify the key genes, proteins and metabolites necessary for decomposition of plant biomasses in ecologically important fungi, also presenting applicability in industrial and environmental biotechnology processes.

Screening of CAZy and oxidoreductase enzyme activities in wood-supplemented cultures demonstrated the proficiency of white-rot Polyporales phlebioid clade species [2], which were further investigated for bioethanol production from recyclable waste lignocelluloses [3]. Genome sequencing of the best, model species *Phlebia radiata* aided in description of the species' functional transcriptome and proteome on spruce wood [4], and more recently, transcriptome under fermentative, ethanol producing conditions. Differentially expressed key genes were identified to elucidate the principal metabolic pathways and putative transcription factors operating under fermentative *versus* respirative conditions, also to promote systems biology and metabolic engineering approaches in near future.

Together with phlebioid species, our concern is to elucidate brown-rot decay of wood conducted by the Polyporales species *Fomitopsis pinicola*, and its interactions with white-rot fungi [5]. In nature, fungal communities are under dynamic changes, and co-habitation of several species is common upon wood decay. Hyphal contacts and interactomes upon decomposition of lignocelluloses and plant biomasses are challenging to investigate, but may offer us new tools for fungal bioproductions and bioconversions supporting more sustainable bioeconomy.

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POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Applied and industrial mycology

Investigating the mechanism of enzyme translocation through the secretory pathway of *Neurospora crassa*

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The saprotrophic fungus, *Neurospora crassa* has evolved to be exceptionally good at secreting a large concentration of enzymes necessary to degrade complex carbohydrates. It is well established that *N. crassa* transcriptionally regulates these enzymes in order to efficiently utilize plant cell wall components as a carbon source. However, downstream regulation of the secretory pathway likely improves the efficiency of plant cell wall degradation and the mechanisms through which this occurs are still obscure. To elucidate these unknown mechanisms, we conducted a forward genetic screen to identify and characterize defective trafficking mutants that cannot translocate cellulases in the ER or that improperly retain cellulases in the ER instead of secreting them into the extracellular environment.. We generated a mutant library using random mutagenesis of a strain with a GFP tagged endoglucanase (EG-2), to screen for mutants with mis-localized EG-2 via microscopy. From the screen, we identified a particularly interesting mutant (10C2) that has EG-2-GFP mislocalization to the ER, a growth defect and temperature sensitivity. We used bulked segregant analysis to identify the putative causal mutation. The potential gene of interest is a scaffolding protein that interacts with multiple different proteins in various organisms, thus exhibiting diverse cellular functions in different organisms. However, its function in *N. crassa* has not been explored, and preliminary data suggests that this mutation causes a general secretion defect, as opposed to one specific to the cellulolytic response. We plan to assess its function by examining its localization in the cell and interacting partners and its role in ER to Golgi trafficking of cellular proteins and cellulases. Identifying unknown components that play a role in secretion in filamentous fungi, using cellulase trafficking as a tool will provide a better understanding of the regulation of the secretory pathway in filamentous fungi.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Applied and industrial mycology

Multi-trophic interactions in the entomopathogenic fungus, *Metarhizium* spp.

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Crop protection relies heavily on synthetic chemical pesticides. As a result of new legislation and the evolution of resistance in pest populations their availability is declining. One of the alternative tactics to pest management are biopesticides, agents based on living micro-organisms or natural products. Fungi in the *Metarhizium* complex (Hypocreales: Clavicipitaceae) are ubiquitous opportunistic pathogens of arthropods used worldwide as environmental friendly biopesticides. Even though *Metarhizium* play major role in natural ecosystems, the various interactions it is involved in are probably underestimated. To date, we know that the *Metarhizium* complex include both broad and narrow host-range pathogens of arthropods but also rhizosphere competent and plants endophytes. In a previous study we compared the pathogenesis process in different pathosystems including hosts that support infection (susceptible) and hosts that limit infections (resistant). We concluded that the composition of the host cuticle is a major factor determining successful fungal infection since it may support conidial germination and hyphal growth but, it may restrict penetration of hyphae into the hemoceol (host body cavity). Furthermore, the cuticle, the outer layer of the arthropod host, of a resistant host appears to contain compounds that actively suppress fungal growth and survival on the tick surface. Our current research focus on complex tri-trophic interactions occurring between EPF and plants, arthropods pests and beneficial organisms. We apply fungal transformation, live imaging and genetic approaches to reveal the high versatility displayed by EPF which mark it as a unique model organism but also as a flexible biopesticide product.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Applied and industrial mycology

Characterization of extracellular enzymatic extract obtained from *Phlebia floridensis* strain isolated from Yucatan Peninsula with a novel peroxidase and oxidase profile

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White rot fungi can degrade different types of recalcitrant and xenobiotic compounds present in the environment due to their natural ability to modify chemical bonds of various phenolic components of lignin and their derivatives[1]. Initially, twelve strains of fungi were isolated in the Yucatan Peninsula, only one of them demonstrated degradation on different dyes in preliminary screening; this strain was identified as *Phlebia floridensis* using ITS sequence analysis.

In the present work, extracellular enzymatic extract of this strain of *P. floridensis* was studied. More extensive screening assays were performed on agar minimal medium plates using phenolic and non-phenolic compounds, anthroquinone dyes and triphenyl methane dyes. As a result, *P. floridensis* strain was able to mineralize Aniline Blue, Methyl Blue, Brilliant Blue G250, Brilliant Blue R250, Malachite Green and the following substrates: 2,6-dimethoxyphenol (2,6 DMP), N,N-Dimethyl-p-phenylenediamine (DMPPDA), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and Guaiacol (GUA). Thus, *P. floridensis* strain showed extremely versatile profile of enzymatic extract.

Subsequently, we determined the presence of proteins from 20 to 70 kDa using SDS-PAGE. After this, cation exchange chromatography was carried out establishing that the fraction with maximum activity was eluted at a concentration of 0.1M NaCl. This fraction was analyzed using different phenolic and non-phenolic compounds. Currently, we are performing HPLC-MS to determine the identity of enzymes present in the extracellular extract. Based on these results we suggest that this strain could be a source of novel peroxidases and oxidases useful in different biotechnological processes.

[1]Martani, F., Beltrametti, F., Porro, D., Branduardi, P., & Lotti, M. (2017). The importance of fermentative conditions for the biotechnological production of lignin modifying enzymes from white-rot fungi. FEMS Microbiology Letters, 364(13).

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Applied and industrial mycology

Strain degeneration in *Trichoderma reesei* is triggered by high protein productivity and influenced by DNA methylation

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Since when QM6a, the wild-type *T. reesei* strain, was isolated during the Second World War, it has been used in strain development programs in order to isolate engineered derivatives for cellulase production. Nevertheless, higher productivity often comes with a cost. Hyper-productive strains (cel+) occasionally lose protein productivity after extended enzyme production fermentation and acquire a cellulase negative (cel-) phenotype. In this study we developed a lab-scale method based on extended cultivations to monitor the occurrence of the (cel-) phenotype and to evaluate the reversibility of the degeneration process. An exploratory investigation was performed with four *T. reesei* strains with different enzyme production capacities (QM6a QM6a-OE*xyr1* M4 M10). We observed that the occurrence of the (cel-) phenotype is triggered by endoplasmic reticulum stress and influenced by (1) productivity and (2) how adapted the production host is in order to deal with intense protein production. The higher the production and the slower the unfolded protein response, the higher is the frequency of the (cel-) phenotype occurrence. The loss of productivity of the (cel-) phenotype is partially related to reduced chromatin accessibility and strong down-regulation of the gene encoding the Xylanase regulator 1 (Xyr1). Moreover, the linkage of the degeneration process to DNA methylation was assumed based on hydralazine treatment. The induced DNA hypomethylation applied to strains M4 and M10 attenuates the process of degeneration and partially recovers the (cel+) phenotype in (cel-) isolates.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Applied and industrial mycology

Indoor fungal growth under changing water conditions

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Indoor fungi cause cosmetic and structural damage in a considerable part of the European dwellings. Prolonged exposure of inhabitants to allergens and mycotoxins produced by such fungi is a potential threat to human health. A relative humidity (RH) of 80 % or higher is thought to be required for fungal growth to occur. On average the RH is below 50 % in normal buildings, suggesting a crucial role of humidity dynamics for fungal growth. Xerotolerant species of *Cladosporium* are predominant in the indoor environment and they thrive on various types of surfaces including glass, gypsum, wall paper, paint and wood. In order to study the fungal response to water activity (a_w) dynamics, *Aspergillus niger* ($a_w \geq 0.80$), *Cladosporium halotolerans* ($a_w \geq 0.82$) and *Penicillium rubens* ($a_w \geq 0.82$) were dried in controlled humidity vessels to stop growth and were rehydrated under high a_w conditions after a week. The different developmental stages of these fungi were studied under these conditions using Cryo Scanning Electron Microscopy (cryoSEM). Growth of all developmental stages was halted during incubation below 75 % RH, while growth continued at 84 % RH. Swollen conidia, germlings, and micro-colonies of *A. niger* and *P. rubens* could not reinitiate growth when retransferred from a RH below 75 % to high a_w. All developmental stages of *C. halotolerans* showed growth after retransfer from 75 % RH. Dormant conidia survived retransfer to a medium with high a_w in all cases. Concluding that *C. halotolerans* is more resistant to a_w dynamics than *A. niger* and *P. rubens*, despite its limited growth, compared to these fungi, at a lowered steady state a_w.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Applied and industrial mycology

Physiology of lignocellulose-deconstructing enzyme production by white-rot basidiomycetes

Vladimir Elisashvili, Eva Kachlishvili, Mikheil D. Asatiani, Eka Metreveli, Tamar Khardziani, Aza Kobakhidze, Violeta Berikashvili, Tina Jokharidze
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White-rot basidiomycetes (WRB) break down cellulose, hemicelluloses and lignin in wood and other lignocellulosic materials by secreting powerful hydrolytic and oxidative enzyme complexes. However, these fungi show wide intra- and interspecies diversity in their response to particular environmental conditions or developmental stages. In this presentation, the state of the art of lignocellulose-deconstructing enzyme production by wood-rotting basidiomycetes will be presented focusing on their common characteristics and unique properties of individual fungi. Moreover, several approaches and strategies that aim to activate the fungi biosynthetic activity and to increase secretion and yields of lignocellulolytic enzymes will be comprehensively analyzed. Our study underlines that the maximum expression of WRB biosynthetic potential depends on the additive effect of several factors. This approach permitted to achieve the highest laccase (1450 U/ml), MnP (12 U/ml) and lignin peroxidase (0.7 U/ml) activities and as high as 140, 700, and 8 U/ml endoglucanase, xylanase, and filter paper activities, respectively. However, to fully utilize the biosynthetic potential of WRB and to develop technologies of lignocellulose-deconstructing enzyme (individual and/or cocktails) production for industrial purposes, various challenging problems associated with mechanisms of these enzymes synthesis must be solved through collaboration of microbiologists, biochemists and molecular biologists and extensive use of the fungal omics technologies, including genomics, transcriptomics, proteomics, and interactomics.

POSTER SESSION 1
(Monday, February 26, 2018 13:45-15:30)

Applied and industrial mycology

Integration of molecular biology and automation to create HTP genetic design libraries for industrial strain improvement

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Industrial fermentation by bacteria and fungi can be utilized to convert simple sugars to enzymes, pharmaceuticals, organic acids and other valuable commercial products. The efficiency in which microbes can be used to generate these products has a direct impact on cost of production and therefore strain improvement is routinely sought. Traditional strain improvement programs utilize random mutagenesis and screening to identify strains with improved characteristics. This “classical” mutagenesis approach has been used to obtain improved strains with desired phenotypes such as higher titer, yield, and productivity, as well as other valuable tolerance characteristics (i.e. heat, pH, product toxicity etc.). However, mutagenesis campaigns also accumulate deleterious changes and are limited in their ability to provide improvements in multiple traits simultaneously. To address this problem, Zymergen has built a platform for automated and high-throughput strain engineering that is focused on improving the economics of large-scale fermentation processes. This is done by generating a comprehensive library of genetic changes that is applied to production strains and systematically screened for improvements in multiple traits of interest. Genetic changes associated with desired phenotypic outcomes are then combined to generate a strain with improved fermentation characteristics. Zymergen has successfully deployed these methods for large scale industrial production hosts that have demonstrated superior performance at scale.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Fungal-human interactions

Bioluminescent *Mucor circinelloides* – a promising new tool to study mucormycosis and antifungal drug efficacy

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Invasive infections caused by members of the *Mucorales* (mucormycosis) have increased in the last years, making it the third most common invasive fungal infection after aspergillosis and candidiasis. Despite this increasing clinical relevance, little is known about the establishment of disease, its progression and successful therapy. New tools to study this disease in more detail are needed, therefore the objective of this work was to construct a luciferase expressing *Mucor circinelloides* strain, as one representative of mucormycosis causing pathogens. Here, we describe the construction and functional analysis of the strains, which will further be used as a reporter system for *in vivo* and *in vitro* models of Mucorales infections.

A leucine auxotroph *M. circinelloides* strain, R7B, was used as recipient strain to allow selection of transformants on selective medium. Firefly luciferase gene without the peroxisomal target sequence was cloned in the pMAT1477 vector. Expression of firefly luciferase under the control of a constitutive promoter was successful in *M. circinelloides* at several conditions. Light emission was detectable by imaging and with a luminometer. Data so far indicate the strain being suitable for further *in vivo* and *in vitro* studies. Phenotype, virulence potential and antifungal susceptibility are indifferent to the wild type strains

The construction of this first bioluminescent *Mucor* strain will allow for the visualization of temporal and spatial progression of infection by a non-invasive method in insect and murine models, and the testing of antifungal efficacy by other means than survival only. This will give valuable new insights in the pathogenesis of Mucorales infections.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Fungal-human interactions

SakA and MpkC interact with PkaAC1 to regulate the mobilization of carbon sources required for cell wall biosynthesis in *Aspergillus fumigatus*

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In *Aspergillus fumigatus*, sensing of cell wall-related stresses and subsequent signaling pathways involve mitogen-activated protein kinases (MAPKs). In *A. fumigatus* there are four MAPKs: MpkA, MpkB, MpkC and SakA. These kinases are involved in maintaining the normal morphology of the cell wall and in the resistance against cell wall-damaging agents. The main components of *A. fumigatus* cell wall are chitin, and glucans. Upon cell wall stress, cell wall-related sugars are synthesized from intracellular carbohydrate storage compounds. Carbohydrate sensing and stress response are regulated by protein kinase A (PKA). In *A. fumigatus*, PKA contains two catalytic subunits named PkaAC1 and PkaAC2; PkaC1 seems to be the main catalytic subunit. This work shows that glycogen, trehalose content and PKA activity were reduced in the *sakA* and *sakA/mpkC* null deletion mutants. Overexpression of pkaAC1 shows reduced levels of trehalose and glycogen and the treatment with Congo red induces glycogen degradation but not trehalose, showing that glycogen degradation is required to the response to Congo red. The mobilization of trehalose and glycogen go through phosphoglucomutase (PgmA) and hexo/glucokinases. The *sakA* and *sakA/mpkC* null mutants showed reduced PgmA activity while a *hxkA/glk*A mutant is very sensitive to cell wall-damaging agents, osmotic stress and it has attenuated virulence in neutropenic and non-neutropenic mouse models. Finally, the uptake of external glucose was reduced in *sakA* and *sakA/mpkC* null mutants and N-acetylglucosamine and glucose concentration was reduced in their cell walls. These results suggest that the reduced mobilization of simple sugars impairs the structure of the cell wall. In summary, we propose that SakA and MpkC are important for the modulation of PKA activity, therefore regulating the availability and mobilization of monosaccharides for cell wall biosynthesis during cell wall damage response.

Keywords: SakA, MpkC, pkaAC1, PKA, cell wall, HogA, glycogen, trehalose.

Financial support: FAPESP and CNPq, Brazil

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Fungal-human interactions

Metabolic characterisation of *Aspergillus fumigatus* clinical isolates

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Fungal infections caused by opportunistic pathogens such as *Aspergillus fumigatus*, are estimated to kill more people annually than malaria and tuberculosis. Nutrient acquisition and subsequent metabolic processes, such as carbon and nitrogen catabolite repression (CCR, NCR) are important for colonisation and promoting fungal survival within the human host and have profound effects on virulence traits such as cell wall composition and enzyme secretion. The aim of this work was therefore to investigate metabolic aspects in thirteen *A. fumigatus* clinical isolates and establish a relationship with virulence. In a neutropenic mouse model, all strains, except for three, presented the same degree of virulence. No correlation between virulence and CCR or NCR was observed, despite strain-specific differences in protease secretion, phenotypic growth and CCR drug resistance. Metabolome analysis in the presence of different carbon sources was carried out for the protease hyper-secretion strain Afs35 and compared to the routinely used laboratory strain CEA10. Intracellular sugars, that can serve as precursors for cell wall polysaccharides, were significantly increased in strain Afs35. This strain was more sensitive to cell wall perturbing agents and conidia were less phagocytised by macrophages when compared to strain CEA10, indicating substantial differences in cell wall organisation and structure between both strains. Despite these differences, both strains were equally virulent in a neutropenic murine model and elicited the same *in vitro* immune response. Furthermore, protease secretion was shown to be inhibited under hypoxic conditions, that are predicted to occur at defined locations within the human host, suggesting that protease secretion may not be essential for infection. In conclusion, great metabolic plasticity exists between *A. fumigatus* clinical strains and further investigation into this trait, as well as how it is affected by the underlying immune system disturbance, is required in order to develop new avenues for combatting systemic fungal infections.

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POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Fungal-human interactions

Interaction of *Curvularia lunata* with human neutrophil granulocytes

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The genera *Curvularia* and *Bipolaris* contain closely related melanin producing filamentous fungi. While *Bipolaris* species infect only plants, there are some opportunistic human pathogenic species in *Curvularia*, such as *C lunata*, *C. spicifera* or *C. hawaiiensis.* These species typically cause phaeohyphomycoses, which can manifest as local infections (e.g. keratitis, sinusitis and cutaneous lesions) in immunocompetent or invasive mycoses with frequent involvement of the central nervous system in immunocompromised patients. Although their plant-fungal interactions have been intensively studied, only little information available about the host response to these fungi in case of human infections. Aim of this study was to investigate the neutrophil' response to the hyphal forms of *Curvularia* and *Bipolaris* species in comparison with that to *Aspergillus fumigatus.*

In the study, *C. lunata* SZMC 23759 and *A. fumigatus* SZMC 23245, both isolated from human eye infections, and *B. zeicola* BRIP 19582b from plant leaf were examined. Release of $O_2^{\bullet-}$ and H_2O_2 from neutrophils were measured in the presence or the absence of the supernatant of germinating conidia and after serum treatment. Activation and survival of neutrophils were checked by measuring myeloperoxidase and LDH release, respectively.

ROS production of neutrophils in interaction with the three fungi were compared. It is already known that *Aspergillus* species induce ROS production of neutrophils only after serum treatment. Similarly, *C. lunata* and *B. zeicola* were also able to induce H_2O_2 release only after serum opsonisation. Infection with *C. lunata* caused an increment in the extracellular H_2O_2 after 30 minutes, while a decline was noticed after 60 minutes. During the interaction, an H_2O_2 specific signal could be detected from the conidia of *C. lunata*. Viability of fungi were also checked after co-incubation.

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POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Fungal-human interactions

The role of the transcription factor Stp2 in *Candida albicans* virulence

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The fungal pathogen *Candida albicans* colonizes a variety of host niches, which confront the fungus with various environmental conditions, including different nutrients. To persist in the host *C. albicans* has developed a remarkable metabolic flexibility. Utilization of amino acids, an abundant host nutrient, is initiated by sensing and uptake via amino acid permeases. When amino acids serve as the sole carbon source, the fungal cell extrudes ammonium as a by-product of their metabolism in order to prevent cytotoxicity. This leads to alkalinisation of the environmental pH and triggering of hyphal morphogenesis, which, for example, contributes to fungal escape from the macrophage phagosomes.

The transcription factor Stp2 regulates the expression of general amino acids permeases and ammonium transporters, and is essential for growth on amino acids and pH modulation. Further, Stp2 has been connected to processes such as hyphal morphogenesis and heat shock response, suggesting a more global role in *C. albicans* virulence than expected. Therefore, in order to elucidate the role of Stp2, we have performed a transcriptional profiling of *stp2Δ* cells grown in amino acid-rich conditions and compared those to the wild-type strain SC5314. Our results confirm the role of Stp2 in regulation of amino acid metabolism and demonstrate a more global role for this transcription factor, as we observe differential regulation of genes involved in stress responses and hyphal growth. Since it remains elusive if the effect of Stp2 is direct or indirect, we plan to utilize ChIP-Sequencing to determine the genomic occupancy of Stp2 and to identify the exact binding motif. Altogether, our work should contribute to our understanding about the implications of metabolic adaptation to virulence.

POSTER SESSION 2
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Fungal-human interactions

A global analysis of kinase function in *Candida albicans* hyphal morphogenesis reveals a role for the endocytosis regulator Akl1

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The human pathogenic fungus *Candida albicans* can switch between yeast and hyphal morphologies as a function of environmental conditions and cellular physiology. The yeast-to-hyphae morphogenetic switch is activated by well-established, kinase-based signal transduction pathways that are induced by extracellular stimuli. In order to identify possible inhibitory pathways of the yeast-to-hyphae transition, we interrogated a collection of *C. albicans* protein kinases and phosphatases ectopically expressed under the regulation of the TET-on promoter. Proportionately more phosphatases than kinases were identified that inhibited hyphal morphogenesis, consistent with the known role of protein phosphorylation in hyphal induction. Among the kinases, we identified *AKL1* as a gene that significantly suppressed hyphal morphogenesis in serum. Akl1 specifically affected hyphal elongation rather than initiation: overexpression of *AKL1* repressed hyphal growth, and deletion of *AKL1* resulted in acceleration of the rate of hyphal elongation. Akl1 suppressed fluid-phase endocytosis, probably *via* Pan1, a putative clathrin-mediated endocytosis scaffolding protein. In the absence of Akl1, the Pan1 patches were delocalized from the sub-apical region, and fluid-phase endocytosis was intensified. These results underscore the requirement of an active endocytic pathway for hyphal morphogenesis. Furthermore, these results suggest that under standard conditions, endocytosis is rate-limiting for hyphal elongation.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Fungi in the environment

Spore heterogeneity of food spoilage fungi; *Aspergillus niger*, *Penicillium roqueforti* and *Paecilomyces variotii*

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At the moment, a significant part of food spoilage and food waste can be attributed to fungal contamination and spoilage. Food preservation methods like sterilization and salt addition reduce spoilage enormously. However, consumers prefer minimal processing of food to maintain taste and nutritional composition, which leads to increased risk of fungal spoilage. Therefore, fungal food spoilage research is needed in order to promote new and enhanced food processing protocols.

Food production needs to increase by 70% to feed the world population in 2050. Reducing post-harvest food spoilage could significantly contribute to this challenge. At the moment, 25% of the food is spoiled, a significant part due to fungal contamination. Fungal food spoilage occurs in all food categories. For instance, *Penicillium roqueforti* and *Paecilomyces variotii* are important spoilage fungi of dairy products and pasteurized beverages, respectively, while *Aspergillus niger* is a known food spoiler of fresh fruits and vegetables.

Fungal food spoilage starts with contamination of food products with conidia. These asexual reproduction structures are abundant in the environment, making contamination inevitable. Minimal processing of these contaminated food products proves to be challenging. Experimental data strongly indicates the existence of subpopulations of conidia with different levels of resistance to preservation methods. The aim of this project is to study the extent of this heterogeneity and to study the underlying mechanisms using fungal model systems.

In these works, we will elaborate on this heterogeneity by investigating the impact of the genetic background (differences in strains), environmental conditions (differences in growth conditions), and the developmental state on preservation/stress resistance of *Penicillium roqueforti*, *Paecilomyces variotii* and *Aspergillus niger* conidia.

POSTER SESSION 2
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Fungi in the environment

Generating the mutants of *Phanerochaete chrysoporium* RP 78 resistant to wood extractives for functional characterization of the detoxification system of white rot fungi

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During the wood degradation process, wood decaying fungi develop different strategies to cope with wood extractives, which contain often antifungal compounds [1]. The aim of this work is to improve our understanding of those detoxification systems using the white rot *Phanerochaete chrysoporium* as model. For that, we have generated mutants of the monokaryotic strain *Phanerochaete chrysoporium* RP 78 by UV light exposure and then test their ability to grow in presence of wood extractives. The selected extractives were from wood of *Bagassa guianensis* Aubl., and *Andira coriacea*, species found in tropical forest (French Guiana) which are well-known for their high durability [2]. Dichloromethane extracts from wood of *Prunus avium* L. also have been selected since they contain several antifungal compounds [3].

Mutations in fungi have been generated by exposure of fungal conidia to ultraviolet radiation. Screening of mutants was carried out by selection of conidia able to grow on Malt Agar medium mixed with wood extractives.

Collections of 39 *bag*, 38 *chy* and 35 *sam* mutants which are able to germinate and grow in presence of acetonic extracts of *Bagassa guianensis* Aubl., dichloromethane extracts of *Prunus avium* L. and *Andira coriacea* respectively, were obtained. These mutants are able to germinate in the liquid medium containing a lethal concentration of extractives for the wild type RP 78.

To pursue this work, these obtained mutants will be characterized through, in particular, a scan genomic approach in order to identify the major genes involved in the detoxification mechanism in this fungus.

Key words: *Phanerochaete chrysoporium*, wood extractives, detoxification systems

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POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Fungi in the environment

Genetics of monokaryotic fruiting in *Schizophyllum commune*

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In the mushroom-forming basidiomycete *Schizophyllum commune* the fruiting bodies are generally only formed by dikaryotic individuals. However, monokaryotic fruiting does occur in some strains. We have identified a monokaryotic strain that forms mushrooms when grown in the light. Interestingly, this trait showed mendelian inheritance upon crossing with a reference strain that does not produce monokaryotic fruiting bodies, suggesting that only a single gene/locus is involved. In order to identify this gene we are planning to perform a bulked segregant analysis (BSA). Furthermore, the monokaryotic fruiting bodies were subjected to histological analysis to determine differences and similarities with normal dikaryotic fruiting bodies.

POSTER SESSION 2
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Fungi in the environment

Trametes versicolor and *Schizophyllum commune* as early sapwood colonizers on wood

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Schizophyllum commune and *Trametes versicolor* are early sapwood fungi in the decay of wood of broadleaf trees. In nature, they can be observed together on branches of living trees, on freshly fallen dead wood and also on cut stems. However, *S. commune* is a weak degrader with features in between white and brown rot and may make use of wood extractives and other easy accessible compounds. *T. versicolor* in contrast is an aggressive white rot. In decay tests with beech wood, up to 52% reduction in sapwood weight of beech wood has been recorded by *T. versicolor* and at most 5% by *S. commune.* In our studies, we address the behavior of the two fungi in single and dual culture through analyzing the proteomes of the two fungi grown on beech sapwood particles in light and dark conditions. In dual culture on wood, *S. commune* appeared to be the first invader. Later, *T. versicolor* grows over the wood with *S. commune*. In the dark, *T. versicolor* grew faster over the wood with *S. commune* as compared to in light. Light induces in *T. versicolor* production of dense surface mycelia and also aggregated structures as possible step in fruiting body development. Secretomes and intracellular proteomes are isolated from wood cultures of different age. We first analyzed the secretomes of 10 and 28 days old *T. versicolor* dark grown cultures. Typical enzymes (different types of cellulases, peroxidases; in total 77 enzymes) in wood decay were shared between the samples. However, the early decay samples contained a higher number of laccases with potential effects on lignin degradation while chitinases with possible functions in reuse of aged mycelium were unique to the older samples.

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POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Fungi in the environment

Histone acetyltransferase TGF-1 is a coactivator of blue light-responsive genes in *Trichoderma atroviride*

Sergio Casas-Flores, Edith Elena Uresti-Rivera, Mayte Guadalupe Cervantes-Badillo, Tania Muñoz-Centeno, Yazmín Hernández-Díaz, Elida Yazmín Gómez-Rodríguez
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In the filamentous fungus *Neurospora crassa*, White Collar (WC)-1 and -2 proteins regulate all known responses to blue light. WC-1 is the photoreceptor and together with WC-2 functions as transcription factors of blue light-regulated genes. The histone acetyltransferase NGF-1 is the coactivator of blue light-responsive genes, and physically interacts with WC-1. In *Trichoderma atroviride*, BLR-1 and -2 are the orthologous of WC-1 and WC-2, which regulate the blue light responses in this fungus. In this work, blue light induced acetylation of histone H3 at lysines 9 and 14 (H3K9K14), paralleling the kinetics of blue light upregulated (*blu*) genes. Trichostatin A, a histone deacetylase inhibitor, downregulated the transcription of *blu* and *bld*-2 genes (blue light downregulated -2), and increased acetylation of H3K9K14. Deletion of *tgf-1,* the orthologous to *ngf*-1, led to a pleiotropic phenotype affecting growth, development, and *blu* and *bld* gene expression. Chromatin immunoprecipitation analysis showed that TGF-1 participates in acetylation of H3K9K14 on the promoter of *phr*-1, a *blu* gene. Furthermore, our data show that BLR-1 is necessary for acetylation of H3K9K14. Our results suggest that TGF-1 interacts with the BLR complex. Interestingly, BLR-1 and -2 interact in darkness but not after a light pulse.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Fungi in the environment

Interactions of wood-decay Agaricomycetes affect hyphal growth, enzyme activity profiles and decomposition events

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Fungal communities are dynamic in nature. Fungal species and isolates may be tolerant and mutualistic, or antagonists upon interactions. *Fomitopsis pinicola* is a common brown rot Basidiomycota species of Polyporales encountered in boreal and temperate forests. The influence of *F. pinicola* on hyphal growth and enzyme production of five white rot fungal species, including the Polyporales species *Phlebia radiata* and *Trichaptum abietinum*, was studied as species combinations on e.g. wood-supplemented cultures. Production profiles of activities of CAZymes and lignin-modifying oxidoreductases (xylanase, beta-glucosidase, laccase, manganese peroxidase) were followed for eight weeks, together with activities involved in recycling of organic nitrogen and hyphal decomposition, along with oxalic acid production and Fenton chemistry indicating iron reduction capability. In fungal co-cultures on agar media, *F. pinicola* was a supreme colonizer quickly advancing over hyphae of the white rot species. Other white rot species were confronted by *P. radiata* with dense mycelial front formation. In liquid cultures, as well as in solid-state cultures, fungal produced oxalic acid was the main component that acidified the medium, and marked changes in enzyme activity patterns over time were directed by the fungal combinations. In solid-state cultures on spruce wood sawdust, apparent wood carbohydrate consumption and mass loss occurred early on in co-cultures with the brown rot species *F. pinicola*. Aggressive decomposition of wood cellulose by *F. pinicola* resulted in release of increasing amounts of reducing sugars in the spruce-wood cultures. Our results indicate that fungal species-species interactions have an outstanding role in wood-decomposition processes and carbon cycling in the forest ecosystems.

Mali T, Kuuskeri J, Shah F, Lundell TK. 2017. Interactions affect hyphal growth and enzyme profiles in combinations of coniferous wood-decaying fungi of Agaricomycetes. PLoS ONE 12(9): e0185171. DOI: http://dx.doi.org/10.1371/journal.pone.0185171

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Fungi in the environment

Bioactivity of Morchella species: genetic diversity versus phenotypic plasticity

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Recent studies report on a wide range of important bioactive metabolites produced by fungi in their fruiting bodies stage, and many mushrooms are used for nutrition and medicinal purposes. Morel mushrooms (Morchella genus, Ascomycota) are important edible mushrooms primarily obtained from natural growth in the wild. Species belonging to this genus exhibit a broad range of bioactivities, including antibacterial, antioxidant, anti-inflammatory, immunostimulatory and anti-tumor activities. The high genetic diversity of the Morchella species, as well as the highly diverse habitats, trophic states and morphologies of Morchella species may affect the production of the bioactive metabolites. The present study compared the level of phenols and anti-oxidative activity of mature mushrooms of two distinguished Morchella species, *M. esculena* (belongs to the Yellow morels group) and *M. importuna* (belongs to the Black morel s group). In addition, the levels were also determined in the mushrooms according ascocarps` phenotypic color nuances within each population of the two species. Similar levels of phenols and of anti-oxidative activity were recorded for these distinct species, while high variability of the metabolites was recorded within each species, in relation to color intensity. The results suggest that environmental factors affected the production of the bioactive metabolites more than genetic differences between the two species.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Fungi in the environment

Variation of fungal populations in water-logged ancient sites in the southern Hula valley, Israel

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Studies of ancient DNA are increasingly reported, with fungal DNA being fewer. Fungi are important integral organisms in a variety of environmental processes, especially in terrestrial sites, involved in organic soil matter recycling as well as to plant and animal health. Beside, many fungi are used by people for different purposes, such as nutrition, medicine, production of valuable compounds and even are used in mystical ceremonies. Hence, we hypothesized that their presence in ancient sites may reflect on historical climate at the site as well as on human activities. An ongoing archeological excavation in the southern Hula Valley, along the Jordan River, has provided evidence of human activities in ancient time. We have used Next Generation Sequencing analysis to study the soil fungal population of the ancient water-logged site (of 60,000 and 400,000 years old) in comparison with recent soil. Total DNA levels obtained were much lower in the ancient versus contemporary soil levels in the site. Similarly, fungal species richness and abundance in archeological samples were much lower and primarily contained water fungi, such as *Cryptomycota* species, while the current soil displayed a high variability of functional fungal groups. These results suggest that the water-logged ancient soil is relatively free of live organisms, and that DNA of dead organisms, including fungal DNA, had deteriorated over time.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

The virulence hub of *Ustilago maydis*: contributions of mitochondria and mitochondrial metabolism to filamentation, mating, melanin and extracellular matrix formation

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The ability of pathogens to exploit host nutrients to support growth is a key aspect of infectious disease. The biotrophic fungal pathogen *Ustilago maydis*, the cause of common smut on corn, forms an infectious filamentous cell type that induces tumor formation in the host. Massive proliferation and the formation of spores fulfil its lifecycle. Nutritional requirements that support hyphal growth and lead to sporulation *in planta* are poorly understood. We previously showed that a wide range of fatty acids, a non-preferred carbon source, induced filamentation for haploid cells *in vitro*. Here we show that another non-preferred carbon source, acetate, does not support filamentation upon mating, and inhibits filamentation induced by oleic acid. We used RNAseq to identify transcriptional changes of *U. maydis* on non-prefered carbon sources such as acetate and oleic acid, and compared expression patterns to those for the preferred carbon source glucose and *in planta* growth. Transcriptional changes indicated that acetate negatively influenced cellular stress resistance, induced reactive oxygen formation, interfered with mitochondrial functions and inhibited mating even under inducing conditions. Specific phenotypic assays confirmed the transcriptional conclusions. Furthermore, acetate was able to reduce the virulence of *U. maydis*. Mitochondrial functions and metabolism such as the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC) are involved in growth on non-preferred carbon sources, filamentation and mating in *U. maydis*. Furthermore, TCA cycle intermediates, but not glyoxylate, in combination with glucose, triggered phenotypes normally limited to sporulation *in planta*, including the formation of an extracellular matrix, melanin production and morphological changes. Our results suggest a complex interplay of different preferred and non-preferred carbon sources for a biotrophic pathogen to fulfil its life cycle.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Multiple effectors from oomycete pathogen *Albugo candida* are recognized by distinct paralogs of *White Rust Resistance 4* locus in *Arabidopsis thaliana*

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We study the obligate biotrophic oomycete pathogen *Albugo candida* that causes white rust disease in Brassicaceae. *A. candida* comprises many races that infect distinct host species. Some *A. candida* races can also infect various *Arabidopsis* accessions, thus facilitating the characterization of effectors and resistance genes that are involved in this obligate patho-system. *A. candida* induces a potent **immuno-compromised** state in the colonized host plants, which can enable different pathogens to grow and reproduce in the same tissue. Co-habitation of different races on the same host is therefore possible, and could be an important means of generating novel races through the exchange of effector repertoires. Our analyses of multiple *A. candida* genomes reveal the presence of a novel class of secreted CxxCxxxxxG (abbreviated as CCG) effector family. Every *A. candida* race has around ~100 CCG secreted proteins which also shows presence/ absence polymorphism. To understand if any of the CCG secreted proteins are recognized by the *Arabidopsis* TIR-NB-LRR (TNL) class, we screened *White Rust Resistance 4 (WRR4)* locus that has two paralogs (*WRR4A and WRR4B*), which confer broad spectrum resistance to various *A. candida* races. A high-throughput screening with CCG effectors and these two paralogs leads to the identification of multiple non-overlapping CCG effectors that are recognized by these R proteins. Some of these candidate CCG proteins also confer an enhanced susceptibility to other oomycete pathogens in stable transgenic *A. thaliana* lines. Current experiments aim to functionally characterize these CCG effectors especially for their potential involvement in *A. candida* mediated immune suppression. I will present an overview of the recent progress of our work on *A. candida* CCG effectorome and the role of CCG effectors.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Deciphering pathogenicity mechanisms of phytopathogenic fungi and Oomycetes using protein network analysis

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Filamentous eukaryotic pathogens (FEPs, i.e., fungi and oomycetes) cause extensive yield losses of staple crops worldwide. Yet, there is scarce amount of studies using large-scale comparative genomic analysis to identify their pathogenicity associated functions and pathways.

To study pathogenicity mechanism in FEPs, we are using systematic computational comparison, followed by functional validation on economically significant pathogens. We have constructed protein similarity network [1-2] based on complete proteomes of 82 FEPs exhibiting diverse lifestyles, and the major pathogenicity strategies: 22 necrotrophs, 20 hemibiotrophs, 18 biotrophs, and 17 saprophytes. Our analysis identified **60 core pathogenicity functions** that were found in at least 70% of the organisms representing each of the pathogenicity strategies. Enrichment analysis of KEGG orthologs and InterPro domains, revealed that these core functions contain a specific arsenal of proteins participating in carbohydrates metabolism, proteolysis, antioxidant activity, plant-toxicity, and transcription regulation. Transcription profiling of *Botrytis cinerea* infection on tomato have demonstrated that 50% of these 60 pathogenicity determinants were upregulated in the course of pathogenicity. We are currently validating the role of selected pathogenicity determinants in the infection process, using functional molecular approach, on pathogens representing pathogenicity strategies: *B. cinerea* (necrotroph), *Colletotrichum gloeosporioides* (hemibiotrophs), and *Erysiphe necator* (biotroph).

Results from this study should increase our understanding of plant pathogenicity mechanisms, and consequently open new avenues for control of these pathogens. Pathogenicity determinants discovered in this work may empower screening for resistant traits (using effectoromics), and accelerate breeding programs for resistant plants.

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Equal contribution - E. Pandaranayaka PJ and D.A. Srivastava

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Identification and characterization of genes encoding small secreted proteins of Norway spruce pathogen (*Heterobasidion parviporum*)

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Heterobasidion annosum sensu lato (*s.l.*), as a species complex containing *H. annosum sensu stricto* (*s.s.*), *H. parviporum* and *H. abietinum*, is necrotrophic pathogen responsible for root and butt rot in pine, spruce and fir respectively in Europe. In *Heterobasidion*-conifer interaction, the fungus is believed to deploy a repertoire of small secreted proteins (SSP), including known effector proteins, to promote the infection of the host and sustain the disease development. Recent analysis of the *H. parviporum* genome sequence has revealed the presence of 759 secretom genes. Since *H. parviporum* and its host are not yet amenable to functional genetic manipulation, heterologous delivery-expression system such as *Agrobacterium–Nicotiana benthamiana* have been used currently to gain insight into effector functional genomics and assess putative interaction partners. Two candidate genes encoding HpSSPs in the transient expression reveals their ability to induce cell death in *N. benthamiana*. In particular, HpSSP35.8 could induce a rapid, strong and consistent cell death at 2 day post-infiltration. Thus HpSSP35.8 is regarded as a prime candidate for future functional studies. The expression level of host-defense-related genes in *N. benthamiana* is further studied under control of HpSSP35.8. Gene expression profiling of HpSSPs is monitored by using qPCR during infection between *H. parviporum* and Norway spruce seedling roots, highlighting candidates for future function analysis.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Just how many types of filamentous phytopathogens are there? Catastrophy for the bio/hemi/necrotroph divisions

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The classification model that has dominated fungal plant pathology for the last 50 years is that diseases and pathogens can be divided into three 'trophic' classes, biotroph, hemibiotroph and necrotroph. Non-pathogen species are described as a symbiont when they live on or within a living host without causing significant damage or as saprotrophs when they extract nutrients solely from decaying biomaterials.

This model of pathogen classification leaves much to be desired. Many pathogens are placed by different authors in two or even all three of these classes. Apart from the undisputed fact that all obligate pathogens are all biotrophic, no phenotypic feature can be described as diagnostic. Other pathogens, such as the wilts and those causing root diseases do not obviously comply with the biotroph/hemi/necrotroph trichotomy.

There are now 642 fungal and oomycete species with genome sequences of which 137 are pathogens. We set out to determine whether an objective analysis of these genomic resources could provide a robust classification system with predictive power.

We developed a technique for the assessment of trophic phenotypes just using the gene content of carbohydrate active enzymes (CAZyme). We found that the existing tripartite trophic classification system for is unsustainable. Instead our analysis identified novel clusters comprising eight pathogen trophic descriptors and a saprobe trophic class. These are -1- haustorial biotroph (BH), -2- non-haustorial biotroph (BN), -3- biotroph/symbiont (BS), -4- intracellular hemibiotroph (HI), -5- extracellular hemibiotroph (HE), -6- broad host range necrotrophs (NB), -7- narrow host range necrotrophs (NN) and finally -8- the wilts, anthracnoses and rots (WAR). This study highlights some longstanding anomalies and permits the objective prediction of properties of a species based solely on its CAZyme gene content. We have used the data included in this study to develop and train a predictive tool for CAZyme-Assisted Training And Sorting of TROPHY which we call CATASTROPHY.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

A conserved Colletotrichum effector that modulates the host immune response was acquired by horizontal gene transfer from plants

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Colletotrichum graminicola is an ascomycete fungus that causes maize anthracnose, one of the most devastating maize diseases worldwide. Previously, our group discovered the a subtilisin-like serine protease encoding gene named *Colletotrichum* plant-like subtilisin (*CPLS*) that was acquired by *Colletotrichum spp*. from plants through horizontal gene transfer (HGT). An updated phylogenetic analysis revealed that *CPLS* homologs are present in all members of the genus *Colletotrichum* and in only two other fungal genera, *Diaporthe* and *Rhynchosporium*. *In vivo CPLS* expression analyses using qRT-PCR and transcriptional fusions with the *CPLS* promoter and *GFP* showed that *CPLS* is expressed during the early stages of infection, reaching a maximum gene expression at 48 hours post-infection. To understand the role of the protein in virulence, we prepared *CPLS* null mutants by gene replacement. The null mutants showed similar growth rate, sporulation rate and colony morphology as the wild-type strain. To test the effect of the absence of CPLS in the maize anthracnose process, leaf pathogenicity assays were performed and resulted in a hypervirulent phenotype, supporting a role of CPLS in fungal virulence during plant-fungus interaction and suggesting a possible linkage of CPLS to plant cell death processes. In contrast, no differences between the *CPLS* null mutant and wild-type strain were seen during root infection assays indicating that the role of CPLS in virulence is tissue specific. We also performed a genome wide transcriptional analysis to understand the plant responses to CPLS during the colonization of leaves. We found that CPLS suppresses the expression of flavonoid and phenylpropanoid biosynthesis and several other immune related genes. Thus, we confirmed that CPLS is a conserved effector in *Colletotrichum* spp. and in *C. graminicola* it contributes to virulence by modulating host defense responses to promote plant susceptibility.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

The core effector Cce1 is required for early infection of *Ustilago maydis*

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The biotrophic pathogen *Ustilago maydis* is the causative agent of corn smut disease, infecting one of the most important crops worldwide – *Zea mays*[1]. During the infection process, tumor formation is induced in plant tissues. To successfully colonize its host plant, *U. maydis* secretes proteins that suppress plant defense responses and facilitate the establishment of biotrophy. These proteins are termed effectors, and among these some belong to a set of highly conserved effectors that are ultimately necessary to establish a successful biotrophic interaction[2]. In this work, we describe the *U. maydis* effector protein Cce1 (Cysteine-rich core effector 1). This protein is essential for the virulence of the fungus. Cce1 is upregulated during infection and is secreted from the fungal hyphae. Δcce1 mutant strains are blocked at early stages of infection and induce callose deposition as a result of plant defense responses. In the genome of other smut fungi highly conserved orthologs of Cce1 can be found. Complementation experiments using an ortholog derived from *U. bromivora* was successful and could re-establish the virulence of the SG200Δcce1 deletion strain.

These data indicate that it is likely that Cce1 belongs to the set of core effectors, which are absolutely needed for establishment of a successful infection.

References:

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POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Transcription profiling of the infection process of *Botrytis cinerea* on whole tomato plant leaves

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Botrytis cinerea is a foliar necrotrophic fungal-pathogen which is capable of infecting over 1,400 plant species [1] and was ranked second worldwide for its scientific and economic importance [2]. In spite of the importance of this pathogen, a transcription profiling of its infection process on a whole plant (as opposed to detached tissue) of widely used crop (except for cucumber and lettuce) was not studied. We analyzed the transcriptome of *B. cinerea* (strain B05.10) infection on one of the most important vegetable crops in the world, tomato (*Solanum lycopersicum*, cv. M82). We sampled six-week-old infected leaf tissues at 0, 16, 23, 40, and 48 hours post infection. Approximately 35, or 45% of *B. cineria* or *S. lycopersicum* genes were differentially expressed during pathogenicity, respectively, demonstrating the global effect of this process. Preliminary KEGG enrichment analysis of *B. cinerea* transcriptome illustrated over-expression of genes involved in regulation of transcription, translation, DNA repair, and recombination, in early stages of the infection. While genes involved in interaction with the environment, and plant secondary metabolite synthesis pathways (e.g., phenylpropanoid and isoquinoline alkaloid biosynthesis) were upregulated in the later part of the infection (i.e., establishment). The latter could illustrate how *B. cinerea* manipulates plant growth/defense systems to accomplish establishment. Altogether, our analysis and its future validation may increase our understanding of plant-fungal interactions which are essential for successful infection.

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Equal contribution - D.A. Srivastava and E. Pandaranayaka PJ

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

The core effectome of smut fungi and its functional analysis in *Ustilago maydis*

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Plant pathogenic smut fungi establish a biotrophic interaction with their respective host plants, and this relationship is governed by secretion of fungal effector proteins. Such effector proteins suppress plant immune responses, facilitate nutrition and regulate the progress of the infection process. The release of the *Ustilago maydis* genome led to uncovering its effector repertoire, clustering of effector genes in the genome and the demonstration that these molecules are crucial determinants for virulence. By now the genomes of the smut fungi *U. maydis*, *U. hordei*, *U. bromivora*, *Sporisorium reilianum* f. sp. *zeae*, *S. reilianum* f. sp. *reilianum*, *S. scitamineum* and *Melanopsichium pennsylvanicum* parasitizing six different plant species are available. We have used comparative genomic studies of the secretomes of these species to provide evolutionary and functional information on the effector repertoires. We are particularly interested in effectors without predicted functional domains that are conserved (core) among all abovementioned species. We present the *in silico* definition of the core effectome of smut fungi, analysis of the expression pattern of the core effectors in *U. maydis* and preliminary results on the assessment of virulence of single core effector mutants and core effector family mutants via reverse genetics.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Characterization and functional roles of the flavohemoglobin genes of the plant pathogen *Fusarium oxysporum*

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Nitric oxide (NO) is a highy reactive molecule playing important roles in essential developmental processes and stress responses. Phytopathogenic fungi use NO as a signalling molecule to modulate development but, concurrently, they have to counteract the plant defensive responses primed by NO. Fungi have evolved several mechanisms against nitrosative stress, among them the detoxification of NO radicals by the enzymes flavohemoglobins is a prominent one.

Genome analysis of *Fusarium oxysporum* showed that this fungus *present four genes encoding flavohemoglobins. FHG1, FHG2,* and *FHG3 encode fungal cytoplasmatic flavohemoglobins, while FHG4 encodes a flavohemoglobin of a probable bacterial origin, as indicated by the clustering in phylogenetic analyses and the putative mitochondrial localization. Gene expression analyses performed with F. oxysporum f. sp. phaseoli strains grown in several nitrogen sources and exposed to NO donors, showed that FHG1 and FHG2 increased their transcript levels during spore germination. FHG3 showed a complex regulation while FHG4 did not show any transcriptional response in the assayed conditions. Functional analysis of mutants deleted in the* FHG2 gene and silenced in the *FHG1* gene in both strains, confirmed that *FHG1* plays the major role and *FHG2* a minor role in NO detoxification, which confers WV and HV virulent strains resistance to nitrosative stress and prevents the delay in spore germination produced by high levels of NO.

Transcript levels of the four genes were also determined in common bean plants (*Phaseolus vulgaris* L.) inoculated with WV and HV strains. The *FHG1* transcript acumulation increased after infection and reached maximum levels by the first week of colonization, while the three other genes did not show changes in expression. However, inoculation assays performed either with single mutants deleted in FHG2 or double mutants deleted in FHG2 and silenced in FHG1, did not evidence that these genes are pathogenicity or virulence factors.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Partial deletion of the small chromosome produces loss of pathogenicity in *Fusarium oxysporum*

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Chromosome 14 is the smallest chromosome of the standard genome of *Fusarium oxysporum* (*F. oxysporum* f. sp. *lycopersici* strain 4287) and has been described as a 'pathogenicity chromosome'. It contains *loci* that encode virulence/pathogenicity factors and confers pathogenicity to non-pathogenic strains after its transfer from a pathogenic strain. Also, it has been recently shown that complete loss of this chromosome results in the loss of pathogenicity, although partial deletions that affect only supercontig 22 do not reduce virulence (Vlaardingerbroek *et al*., 2016). This chromosome is likely equivalent to the smallest chromosome of *F. oxysporum* f. sp. *phaseoli* (FOP).

The *FTF* gene family is composed of two pathogenicity factors: *FTF1*, with multiple paralogues all located in the small chromosome of highly virulent strains of FOP, and *FTF2*, a single copy factor located in the core genome. Both factors are involved in virulence/pathogenicity (Niño-Sánchez *et al*., 2016). We describe here the isolation and characterization of some strains carrying a partial deletion of the small chromosome (FOP-SP1sChr-pΔ). The region deleted includes all the paralogues of *FTF1*. FOP-SP1sChr-pΔ mutants show a complete loss of pathogenicity on common bean plants, suggesting that the deleted fragment harbours the relevant set of genes required to produce disease in this forma specialis. Although no Fusarium wilt symptoms were observed in common bean plants inoculated with FOP-SP1sChr-pΔ mutants, confocal laser microscopic analysis revealed the ability of these strains to colonize the host, indicating a behaviour similar to that shown by endophytic strains

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POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Small RNA bidirectional crosstalk during the interaction between *Zymoseptoria tritici* and wheat

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Eukaryotic sRNAs are short regulatory non-coding RNAs that induce silencing of target genes at the transcriptional or post-transcriptional level. This conserved eukaryotic mechanism is known as RNA interference (RNAi), and plays important roles in maintaining RNA stability, RNA processing, the response to biotic stresses and the regulation of morphological and developmental processes. The RNAi mechanism is present in all fungal families, although most of our knowledge about RNAi pathways in fungi comes from studies on model fungal species, in which sRNAs play important roles in the regulation of developmental processes. However, the effects of sRNAs are not limited to the organism where they are produced and they can sometimes extend to interacting species, including species that come from different kingdoms yet are able to alter each other`s gene expression, a phenomenon known as cross-kingdom RNAi. In this study we explored the small RNAs produced during the interaction between wheat and the important wheat pathogen *Zymoseptoria tritici*. We combined small RNA sequencing, degradome sequencing, and dual RNA sequencing of both pathogen and host at different stages of the infection cycle to identify small RNAs from both pathogen and host as well as their gene targets in both organisms. Our results suggest that a small RNA bidirectional crosstalk is taking place during the interaction between *Z. tritici* and wheat, therefore contributing to our understanding of this complex interaction.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Determining how *Magnaporthe oryzae* senses turgor pressure to trigger rice blast disease

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Plant pathogenic fungi cause many of the world's most devastating crop diseases, and pose a significant threat to global food security. With up to 30% of the global harvest lost each year to plant disease, it is paramount to identify affordable and durable solutions to increase plant productivity in a sustainable way. Rice blast disease caused by the fungus *Magnaporthe oryzae* is found in all rice-growing regions of the world, and is responsible for 10-30% loss of the global rice harvest annually. *M. oryzae* develops a specialised infection cell called an appressorium, which is a unicellular dome-shaped cell that forms soon after spore germination on the leaf surface, and is characteristic of many cereal pathogens. This together with the genetic tractability makes *M. oryzae* a model organism for studying host-pathogen interactions. To cause plant disease, the appressorium develops enormous turgor of up to 8.0MPa, by accumulating high concentrations of glycerol. The appressorium has a differentiated cell wall rich in melanin, which is essential for turgor generation. This is rapidly translated into mechanical force allowing a narrow penetration hypha to emerge from the base of the appressorium, rupturing the rice leaf cuticle and cause disease. Development and re-polarisation of the appressorium requires four septin GTPases, Sep3, Sep4, Sep5 and Sep6 which form a toroidal, heterooligomeric complex at the base of the appressorium that re-models and re-organises the F-actin cytoskeleton to the precise point at which plant infection occurs. Septin-mediated plant infection is controlled by NADPH oxidase activity. A specialised Nox2-NoxR NADPH oxidase is necessary for septin-mediated control of actin dynamics. Here, we present a model that shows how the fungus is able to monitor the turgor pressure within the appressorium and determine the optimal point which triggers its puncturing of the rice leaf cuticle to cause rice blast disease.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Functional diversification of orthologous effector shapes pathogenic lifestyle in smut fungi

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The smut fungi *Ustilago maydis* and *Sporisorium reilianum* both parasitize maize. *U. maydis* causes tumor formation and anthocyanin induction on all above ground organs of the maize plant, while *S. reilianum* causes disease symptoms only in the floral organs without tumor formation and anthocyanin induction. These two pathogens share highly similar genome structures. It is yet unclear what drives their diversity in pathogenic lifestyles. Here we show that the functional diversification of orthologous effector proteins contributes to the distinct pathogenic lifestyles. The secreted Tin2 effector of *U. maydis* contributes to virulence and is responsible for anthocyanin induction in maize, and this is achieved by stabilizing the maize kinase ZmTTK1. The genome of *S. reilianum* encodes a Tin2 effector orthologue at a genomic location syntenic to *U. maydis tin2*. When *U. maydis tin2* is expressed in *S. reilianum*, anthocyanin is induced in infected leaf tissue. On the other hand, *tin2* from *S. reilianum* (*Srtin2*) is unable to induce anthocyanin induction in the *U. maydis tin2* mutant. We demonstrate that *Srtin2* in *S. reilianum* is also required for virulence. SrTin2 fails to interact with ZmTTK1 but interacts with ZmTTK2 and ZmTTK3, two paralogues of ZmTTK1. Furthermore, recombinant SrTin2 protein negatively affects the kinase activity of ZmTTK2 and ZmTTK3 *in vitro*. A resurrected ancestral Tin2 protein does not complement anthocyanin induction of the *U. maydis tin2* mutant. This suggests that the *U. maydis* Tin2 effector has evolved unique features, which are not shared by Tin2 effectors of other smut fungi.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Partial resistance in wheat is triggered upon recognition of an avirulence gene

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Adaptation of fungal pathogens to colonize a plant often involves escape from host surveillance. This can be mediated by sequence polymorphism of avirulence genes that evolve to prevent recognition by host resistance genes. Despite the ubiquity and importance of avirulence genes for the infection outcome, the mechanisms behind their evolution remain undetermined. The causal agent of Septoria Tritici Blotch on wheat, *Zymoseptoria tritici*, is a necrotrophic pathogen that is globally distributed. Resistance is mediated by 21 mapped major resistance genes, of which many lead to partial resistance. It still remains unknown what components are recognized by these particular resistance genes. In order to elucidate the genetic basis of quantitative virulence, a genetic mapping approach was undertaken. Differences between two isolates were mapped to a transposable element-rich and highly dynamic genomic region that included a cluster of four genes encoding putative effectors. We confirmed that one of the genes, *Avr3D1*, encodes an avirulence protein that is specifically recognized by some wheat cultivars. Disruption of *Avr3D1* in the avirulent isolate led to an increase in virulence on the resistant hosts. Complementation experiments demonstrated that polymorphism in the coding sequence is responsible for the differences in virulence between the two isolates. Population genetics analyses showed that *Avr3D1* is present in all of 132 investigated isolates from around the world and that it has evolved under diversifying selection. In contrast, the transposable elements and the putative effector genes surrounding *Avr3D1* are under presence/absence polymorphism. Genes in the cluster were shown to be silenced in vitro but highly up-regulated during infection. Thus, we identified a novel avirulence gene whose recognition leads to partial resistance. Its high controlled gene expression regulation, its clear signs of diversifying selection and its localization in a highly dynamic genomic environment provide us with evidences of the evolution of this avirulence gene to escape recognition.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Two genes in a gene cluster encoding secreted proteins are required for appressorial penetration of the maize anthracnose fungus *Colletotrichum graminicola*

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The contribution to virulence of 58 effector candidates of the hemibiotrophic maize pathogen *Colletotrichum graminicola* were analysed by gene deletion. In search of gene clusters encoding secreted proteins we identified a cluster of co-linear genes (*CLU5a* to *CLU5e*) in the genome of *C. graminicola* that is needed for pathogenicity. Analyses of the individual genes in that cluster showed that *CLU5a* deletion mutants are severely and *CLU5e* mutants are moderately impaired in virulence. Highly resolved TEM images and quantitative assessment of development of infection structures by LM showed that both mutants were impaired in appressorial penetration. Virulence deficiencies exist in the mutants independently from the host genotype and host organ inoculated. Papillae formed by *CLU5a* mutants are smaller and less structured than those elicited by the wild type, suggesting reduced PAMP exposure during arrested infection-related development. In contrast, *CLU5e* mutants elicited WT-like papillae, though at increased frequencies. Vegetative growth, sporulation and assays testing several types of stress conditions failed to exhibit significant differences between the mutants and the wild type reference highlighting the importance of the two proteins in the establishment of biotrophy. Possibly, defects of the appressorial cell wall may cause the phenotypes observed. Interestingly, genes in cluster 5 are highly syntenic only in the genomes of *Colletotrichum* spp. infecting grasses but not in *Colletotrichum* spp. infecting dicotyledonous plants.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

A pathogenicity cluster for exploiting maize kernels defenses in *A. Flavus*

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Aspergillus flavus is a saprophytic cosmopolitan fungus, capable of infecting crops in pre- and post-harvest exploiting different secondary metabolites, including aflatoxins. The latter are held in high regard as carcinogenic and genotoxic in animals and humans, even though they have no effect on host plants. In mining the genome of *A. flavus* for identifying secondary metabolite clusters putatively involved in the pathogenesis process, our attention has turned to the cluster 32 containing some fungal effectors such as salicylate hydroxylase, quercetinases and necrosis/ethylene inducing proteins (NepA). In order to understand how this cluster works during the disease, we conducted histological and histochemical experiments in *A. flavus* pin bar-infected maize caryopses.

The same samples were analyzed for (i) the expression of specific genes inside the cluster (e.g. *salOH*, *NepA*), (ii) the production of salicylate and the presence of its dehydroxylated form, *i.e.* cathecol, by LC-MS/MS.

Within this frame, several mutants of *A. flavus* impaired or enhanced in specific functions (*e.g*. cluster 32 overexpression, NepA KO and OE strains) were checked for their ability to cause disease in maize caryopses. A scenario emerged in which fungal progression through living tissues (*e.g*. aleuron) is accompanied by a significant rise in the level of fungal effectors, such as SalOH and NepA, and by a degradation of SA that, in turn, appears strategic for the fungus to bypass caryopses defences and attenuate programmed cell death phenomena naturally occurring in the aleurone layer of maturating kernels

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

The *Arabidopsis thaliana* sRNAs-mediated gene silencing machinery and its role on the establishment of a beneficial relationship with *Trichoderma atroviride*

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Plants interact with a plethora of microbes to establish a pathogenic or beneficial relationship. To establish a relationship with plants, pathogens and beneficial microbes use effector molecules to suppress plant immunity. To defend from pathogens, plants have developed the induced systemic resistance, which is effective against necrotrophic pathogens, whereas the systemic acquired resistance is effective against hemibiotrophic and biotrophic microbes. In plants, the components of small RNAs (sRNAs) silencing-machinery, including Dicer-like (DCL), Argonaute (AGO), RNA-dependent RNA polymerase (RDR) and DNA-dependent RNA polymerase (POL) are involved in plant-microbe interaction. We evaluated a set of *Arabidopsis thaliana* sRNAs silencing-machinery mutants, to test whether they are involved in the beneficial interaction with *Trichoderma atroviride*. The *Arabidopsis* mutant *ago2-1*, *ago6-2* pretreated with *Trichoderma* showed a wild-type (wt) growing phenotype compared to Col-0 treated plants, whereas the treatment of *ago8-1*, *rdr2-1* and *pol V* seedlings with *T. atroviride*, affected the fungal plant growth promotion effect. Protection assays against phytopathogens *Botrytis cinerea* and *Pseudomonas syringae Pst* DC3000 respectively, showed that *ago2-1* untreated seedlings with *T. atroviride* were more resistant to *B. cinerea*, and presented susceptibility to *Pst* DC3000. The *pol V* lines showed a susceptible phenotype to *B. cinerea*, but resistant to *Pst* DC3000. pol V plants treated with *T. atroviride* and challenged with *B. cinerea* presented a wt phenotype, but showed more susceptibility to *Pst* DC3000. The pretreatment of *ago2-1* plants with *Trichoderma* turned them more susceptible to *B. cinerea* and did not respond to the bacterium. Furthermore, *rdr2-1* mutants showed a wt phenotype to *B. cinerea* and *Pst* DC3000, however, the root treatment of these plants with *T. atroviride* presented a similar phenotype as compared to the wt with both pathogens, whereas *ago7* showed the same phenotype to *B. cinerea* than the wt plants, and the pretreatment with *Trichoderma* showed less damage compared to Col-0.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Role of gliotoxin in plant root colonization and induction of systemic resistance by *Trichoderma virens*

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Trichoderma virens colonizes the outer root tissues of a wide range of plants. This interaction is generally beneficial, often promoting growth and systemically priming plant defenses against infection by pathogens. Q strains of *T. virens* produce the epipolythiodioxopiperazine (ETP) metabolite gliotoxin, while P strains produce, instead, a different ETP, gliovirin (Sherkane et al. 2017, ChemistrySelect). Mutants in the GliP locus encoding the non-ribosomal peptide synthetase of the gliotoxin biosynthetic gene cluster of the reference Q strain Gv29-8 were constructed by homologous recombination: these strains produced no gliotoxin (Vargas et al. 2014, Microbiology). While characterizing the ability of Q and P strains to colonize Arabidopsis roots, we noted that several Q strains did not promote growth in soil, and in plate assays even destroyed the seedlings. To answer the question of whether gliotoxin is a major factor in this phytotoxic effect of Trichoderma, we compared the interaction of wild type Gv29-8 and its *gliP* mutants (Vargas et al. 2014) with Arabidopsis and tomato seedlings. Apparently, seedlings treated with Trichoderma mutants that do not biosynthesize gliotoxin survive much better than seedlings exposed to their corresponding wild-type strain. Moreover, both mutants and wild-type strains activate the plant's induced systemic resistance. We are following up an initial observation that only the wild-type activates systemic acquired resistance (SAR) - related genes. Secondary metabolite production is thus an important factor to take into account in development of biocontrol strains of *T. virens*. The ETP biosynthetic clusters are not the only differences between the genomes of P and Q strains, and we are beginning to characterize other strain-specific differences in genomic content and gene expression.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Identifying genes induced by ferulic acid and dependent on transcription factor ChAP1 in *Cochliobolus heterostrophus*

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Ferulic acid (FA) belongs to a group of phenolics widespread in plants, which are perceived by the necrotrophic maize pathogen *C. heterostrophus* as a stress signal. The mechanisms of signaling and toxicity are unknown. We used RNAseq to follow the transcriptome in response to short (30 min) exposure to 0.5-2 mM FA. Differentially expressed genes related to metabolism of phenolics, major facilitator family transporters and acetyl CoA metabolism were prominent among the many differential transcripts. Since FA promotes nuclear retention of the redox-sensitive transcription factor ChAP1 without up-regulating genes for oxidant tolerance, we are identifying specific ChAP1-dependent FA targets. Comparing the transcriptome of *Δchap1* and WT showed 819 genes that were significantly differentially expressed, at a pvice-versa. Interestingly, the annotation of some of these genes suggests that they might participate in the regulation of the PCD, like ankyrin domain containing protein and a NACHT nucleoside triphosphatase domain protein. In contrast, others like a major facilitator superfamily member and a gene annotated as "AMP-dependent synthesis" are positively regulated by ChAP1. In response to FA, the fungus apparently operates two mechanisms to cope with this stress. Transcriptomics indicate that there is a balance or competition between them. One is a defense pathway in which the fungal cell can overcome the stress by removing and metabolizing FA, and the other is a PCD pathway. The transcription factor ChAP1 has an important role in determining the balance between defense and PCD.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Involvement of effector proteins in *Penicillium expansum*-apple fruit interactions

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Penicillium expansum regarded as one of the most important postharvest rots of apple fruit and of great concern to fruit processing industries due to secretion of potent mycotoxins. Elucidating the pathogenicity mechanisms of this pathogen is of utmost importance for the development of effective and safe management strategies. We found that *P. expansum* secretes proteins during apple infection that suppress resistance related ROS production in the apple wounds. These findings suggest the possibility that effector-proteins may play an important role in the *P. expansum* interaction with the host. In the current study, bioinformatic tools were used to develop a pipeline to predict potential effector genes in *P. expansum* and study their role in pathogenicity on apples. Features such as secretion, differential expression *in planta* during infection, small size, and large number of cysteines as well as information available in databases on known effectors were used to predict genes relevant to processes associated with the infection of apples by *P. expansum*. Applying the effector-prediction pipeline to the secretome of *P. expansum* revealed 103 enzymes that degrade cuticle-building polymers, carbohydrates, peptides, lipids and phospholipids, as well as 106 potential effector proteins. Among the predicted effectors that were identified, 47 of them have homology to known effectors. LysM proteins, NEP-1-like proteins, small-cystein rich (SCR) proteins with unknown function and subtilisin-related Peptidase S8 were characterized and functionaly analyzed. We found several putative effectors that have an effect on the pathogenicity and virulence of *P. expansum* on apples as well as proteins with pleiotropic effect affecting growth rate, morphology and sporulation.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Conserved effectors with a ribonuclease domain are involved in virulence of phytopathogenic *Colletotrichum* fungi

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Members of the genus *Colletotrichum* infect many commercially important crops. *Colletotrichum* fungi secrete small effector proteins, which are thought to be important for successful plant invasion. By comparing *Colletotrichum* genomes, we selected 37 highly conserved genes encoding small-secreted proteins that are expressed during infection in both *Colletotrichum higginsianum* and *Colletotrichum orbiculare*. Using the transient gene expression system in *Nicotiana benthamiana*, we expressed these candidates to investigate their cell death-inducing activity. We identified a cell death-inducing gene that encodes a protein with a ribonucleases domain, designated as secreted ribonuclease (SRN). Phylogenetic tree analysis revealed that SRN homologs are conserved in all tested Pezizomycotina species, but not in Taphrinomycotina and Saccharomycotina, implying that SRNs in fungi originated after the diversification of Pezizomycotina from these groups. In particular, SRN homologs are significantly expanded in *Blumeria graminis* (Pedersen *et al*., 2012, BMC Genomics). In *C. orbiculare*, there are four members of the SRN family, SRN1 to SRN4. To characterize these further, we established knockout mutants of SRN genes in *C. orbiculare* and found that they are required for full virulence of the pathogen. Quadruple mutants of SRNs, but not triple mutants, showed reduced virulence on plants, indicating that all SRNs have a redundant function. In *N. benthamiana*, transiently expressed SRN1, 2, and 4 induced cell death. A SRN2 variant that has a mutation in the ribonuclease catalytic residue did not induce cell death, indicating that the ribonuclease activity is required for cell death. Interestingly, *B. graminis* has 29 SRN homologs, however all of them lack the ribonuclease catalytic residue required for induction of cell death by C. orbiculare SRN2. In summary, *SRNs* encode virulent effectors in *C. orbiculare* but their function may be distinct from those in *B. graminis*.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Elucidating the interactions between *Fusarium fujikuroi and* rice

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Fusarium fujikuroi, causal agent of Bakanae disease, is the main seedborne pathogen on rice. Profiles of defense-related phytohormones and phytoalexins were investigated on two rice cultivars, inoculated or not with *F. fujikuroi*. In the resistant genotype Selenio, the 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. A RNA-seq transcriptome study was performed. The basic rice resistance machinery against *F. fujikuroi* involved PR genes, glucanases and peroxidases, since they were upregulated in both the resistant and susceptible cultivars. The specialized and evolved resistance mechanisms in the resistant cultivar included WRKY transcriptional factors, MAPK cascades, and some cytochrome P450 genes. These mechanisms were further confirmed by KEGG identification of Ca^{2+}-dependent protein kinase gene, JASMONATE ZIM-DOMAIN-like genes, *CEBiP*, *CERK1*, and *MYC2* genes, found only in 'Selenio'. These genes participate in one of the molecular patterns: response to chitin, jasmonic acid biosynthesis, and plant hypersensitive response. When the gibberellin production was controlled, the 'Selenio' plants activated the jasmonic acid metabolic pathway. The fungal pathogen in the resistant cultivar acts locally, at lower concentrations, and probably it causes a rice hypersensitive response without any further damage to the plants. In order to gain insight into secondary metabolites (SM) synthesis in *F. fujikuroi,* we sequenced the genome of three Italian strains, identified the allelic variants in the genes responsible for some SM production, and compared the pathogenicity and SM production *in vitro* and on rice. *F. fujikuroi* strains can vary deeply both in the symptoms they induce and in mycotoxins production. The significance of the findings in the genomics of *F. fujikuroi* will be discussed.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Metschnikowia fructicola, a biocontrol yeast against postharvest diseases: genome sequence, assembly and characterization

Edoardo Piombo[1], Noa Sela[2], Michael Wisniewski[3], Maria Hoffmann[4], Maria Lodovica Gullino[1], Marc Allard[4], Elena Levin[5], **Davide Spadaro**[1], Samir Droby[5]
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The yeast *Metschnikowia fructicola* has been reported as an efficient biocontrol agent of postharvest diseases of fruit and vegetables. Several mechanisms of action by which *M. fructicola* inhibit postharvest pathogens were suggested, including iron-binding compounds, induction of defence signalling genes, such as PRP and MAPK cascade genes, production of fungal cell wall degrading enzymes and relatively high amounts of superoxide anions. *M. fructicola* also exhibits chitinase activity and the chitinase gene, *MfChi,* was highly induced in response to fungal pathogen cell walls.. Several studies have examined differential gene expression during the interaction of the yeast, *M. fructicola*, with host fruit or with a postharvest pathogen. In the current work, we awe report the assembly of the whole genome sequence of two strains of *M. fructicola* using PacBio and Illumina shotgun sequencing technologies. Using the PacBio, a high-quality draft genome consisting of 93 scaffolds, with an estimated genome size of approximately 26 Mb, was obtained. Comparative analysis of *M. fructicola* proteins with three available closely-related genomes revealed a shared core of homologous proteins coded by 5,776 genes. Comparing the genomes of the two *M. fructicola* strains using a SNP calling approach resulted in the identification of 564,302 SNPs/indels with a total of 2,004 predicted high impact mutations. Based on the assembled genome, sequences were annotated with a gene description and gene ontology (GO term) and clustered in functional groups. Analysis of CAZyme family genes revealed 1,145 putative genes. Transcriptomic analysis of CAZyme expression levels in *M. fructicola* during its interaction with either grapefruit peel tissue or *Penicillium digitatum* revealed a high level of CAZyme gene expression when the yeast was placed in wounded fruit tissue. The significance of the findings in biocontrol capabilities of *M. fructicola* will be discussed.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Dimorphic conidia exhibit different life styles during early colony development in the corn pathogen *Colletotrichum graminicola*

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Since the late 1980s the formation of dimorphic conidia by the hemibiotrophic corn pathogen *Colleotrichum graminicola* is known [1]. Nevertheless, research is focusing exclusively on the investigation of deep colored, falcate conidia produced on solid media and leaf surfaces in light. Here we describe that the smaller, oval conidia are generated under deviant conditions and furthermore show highly divergent behavior in early colony development. Among others, we report for the first time the formation of conidial anastomosis tubes (CAT) in *C. graminicola*, a process conducted exclusively by oval conidia and strictly absent from falcate spores. Outgoing from these results, we are investigating a putative aberrant role of falcate and oval conidia in the infection of different plant tissues.

[1] Pannacione DG, Vaillancourt LJ and Hanau RM (1989) Conidial Dimorphism in *Colletotrichum graminicola*. Mycologia (81): 876-883

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Evolutionary history of *AvrRvi6*, the first avirulence gene identified in the apple scab fungus *Venturia inaequalis*

Collemare Jérome[1], Lemaire Christophe[1], Sannier Mélanie[1], Leroy Thibault[1], Schouten Henk[2], Bijsterbosh Gerard[2], Caffier Valérie[1], **Bruno Le Cam**[1], Shiller Jason[1]
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The management of fungal diseases of apple depends largely on the use of chemical. In apple orchards an average of 20 treatments are required each year to combat its main pathogen, the apple scab fungus *Venturia inaequalis*. The use of cultivars carrying resistance (*R*) genes, which rely on the recognition of the product of avirulence (*AVR*) genes from the pathogen, known as gene-for-gene interactions is an environmentally friendly alternative to chemicals. Although 17 gene-for-gene relationships have been reported in this pathosystem, only two resistance genes, *Rvi6* and *Rvi15*, have been cloned, and nothing is known about *V. inaequalis AVR* genes. Only one major apple *R* gene, *Rvi6*, has been extensively deployed in orchards. *Rvi6* was introgressed into domesticated apple (*Malus x domestica*) from the related ornamental crabapple *Malus floribunda*. Recently, a population genetics analysis of *V. inaequalis* european populations indicated that *VIR* alleles that have circumvented the *Rvi6*-mediated resistance in cultivated orchards originate from standing genetic variation in a wild population. Here we report the identification of the first *V. inaequalis AVR* gene, *AvrRvi6*, by means of genetics, genomics and functional approaches. Transient expression assays in *Nicotiana benthamiana* allowed the functional characterization of the recognition of *AvrRvi6* by *Rvi6*, and population genomics of *Avr* locus polymorphism approaches revealed the exact evolutionary history of *Rvi6* circumvention at the European scale.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

ATMT mutagenesis of *Colletotrichum acutatum* identified a nitrite utilization mutant caused by the deletion of a 19-kb DNA fragment

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Chili pepper is one of the most important food additives used in spicy cuisines worldwide. However, the yield and quality can be highly destructed by anthracnose disease caused by *Colletotrichum* species. In this study, we identified a nitrate utilization mutant through T-DNA insertional mutagenesis. T-DNA inserted in the upstream of two genes (Ca-02433-HP and Ca-02434-GPI-HP) with no known function. Ca-02434-GPI-HP is a glycosylphosphatidyl-inisotol-anchored protein and could be delivered to cell membrane or cell wall; therefore, it may have function involved in nutrient uptake. Analysis with GFP-fusion and cell-wall-degrading enzyme digestions reveals that Ca-02434-GPI-HP is a cell-wall associated protein. Functional analysis of the two genes with gene disrupted mutants,indicating that they are not involved in nitrate utilization defect of B7, neither involved in the function of pathogenicity, growth, development and tolerance to various environmental stresses. After whole genome sequencing and PCR analysis, T-DNA insertion was found to cause large DNA fragment (19-kb) deletion in B7. Three genes are deleted including Ca-02435-NPT which encodes a Nitrite/Nitrite transporter. Gene disruption and complementation analysis demonstrated that Ca-02435-NTP is the factor causing nitrate utilization defect of B7.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Molecular basis for differential host response of sorghum against different *formae speciales* of *Sporisorium reilianum*

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Sporisorium reilianum is a biotrophic fungus causing head smut in maize and sorghum. The fungus exists in two *formae speciales*, *S. reilianum* f. sp. z*eae* (SRZ) and *S. reilianum* f. sp. *reilianum* (SRS), which can infect maize and sorghum, respectively. SRZ is not able to systemically spread in sorghum, presumably because it is challenged by several plant defense responses. The strongest visual defense response of sorghum against SRZ is the production of phytoalexins that lead to a red coloration of infected leaf tissues. We hypothesize that phytoalexin biosynthesis is induced by SRZ-specific secreted effector proteins. Effectors are classified as apoplastic or cytoplasmic based on their localization in the extracellular space or inside the plant cells, respectively. Identification of factors from SRZ that induce phytoalexin biosynthesis will lead to the identification of host-specificity factors of *Sporisorium reilianum*. To find out which factor of SRZ induces phytoalexin formation in sorghum, 77 predicted SRZ-specific effectors were cloned in an expression vector under control of a strong constitutive plant promoter. Of these, 40 are high-priority effector candidates as predicted by EffectorP. Potential apoplastic effectors with high cysteine content were cloned with signal peptide and potential cytoplasmic effectors were cloned without signal peptide to ensure proper localization after expression in plant cells. The candidate effectors are being tested for their ability to induce phytoalexin formation by particle bombardment of sorghum leaves. This analysis will show whether any of the cloned candidate effectors is able to induce phytoalexin formation in sorghum and thus determines host selection of *S. reilianum*.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

A two-pronged forward genetics approach to identify *Magnaporthe oryzae* genes that activate reactive oxygen species in rice

Nicole Donofrio[1], Jessica Cooper Pancake[1], Danielle Mikolajewski[1], Jonathon Neifert[1], Timothy Chaya[2], James Sweigard[3], Jeffrey Caplan[4]
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Rice Blast disease, caused by the fungus *Magnaporthe oryzae,* results in devastating loss to crops worldwide, including economically important rice and barley. Research from other labs repeatedly demonstrates this pathogen's ability to overcome single gene resistance, as well as fungicide applications. Using forward genetics, we seek to further understand and harness host plant basal immunity for better protection against virulent pathogens. Our aim is to identify *M. oryzae* genes involved in sensing or detoxifying reactive oxygen species (ROS) from the plant. Previous work from our lab characterized the *HYR1* gene, which in the deletion mutant triggers a strong, plant-produced ROS burst and lowered susceptibility. Our hypothesis is that a two-pronged forward genetic approach will identify additional fungal genes with this phenotype. We are currently screening ~50,000 random insertion mutant library generated in the early 2000's (FGSC-m), and a library we are developing (HyPer-m). The HyPer-m library is being developed in a fungal strain that contains a live ROS sensor gene, HyPer-sensor (HS). The HS gene fluoresces in the presence of ROS, thus providing a quantifiable measurement of this basal defense response. Our insertion construct consists of a gene for resistance to the drug Hygromycin, surrounded by two outward facing fungal promoters, to increase chances of maximum gene disruption. We have currently screened about 4,000 and 600 mutants from the FGSC-m and HyPer-m libraries, respectively, and results will be shared.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Towards understanding host specificity in the smut fungus *Sporisorium reilianum* - probable role of a diversity cluster

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The two host-adapted varieties of the smut fungus *Sporisorium reilianum* (*S. reilianum* f. sp. *zeae*, SRZ, and *S. reilianum* f. sp. *reilianum*, SRS) produce spores either on maize or on sorghum. For plant infection, mating compatible haploid sporidia need to fuse and form infectious dikaryotic filaments that infect at seedling stage and form spore in inflorescences of the plant. Despite both formae speciales being very closely related on the genomic level why they differ in terms of host selection remains elusive. To know the host determining factors we generated sexual spores by crossing the compatible SRZ strain SRZ1_5-2 (mating type *a1b1*) with the SRS strain SRS2_H2-7 (mating type *a2b6*). Meiotic progeny (SRSZ) with the mating type *a1b1* were selected and tested for virulence on sorghum after mating with SRS_H2-7. Virulence assays showed that the progeny were either non-virulent (107 offspring) or showed various degrees of disease phenotype.We selected 107 non-virulent, 40 full-virulent and 41 offspring with intermediate virulence for genotype analysis. 190 strains (188 offspring and 2 parental strains) were sequenced using Illumina technology. Mapping of the reads against the two assembled parental genomes showed that parental origin of chromosomal regions could be unambiguously assigned for all SRSZ strains. Interestingly, many strains contained partially duplicated genomic regions, i.e. carrying the same chromosomal fragments from both parents. Correlation of phenotype with parental origin of genomic loci revealed a region of 51 genes in the left arm of chromosome 7 potentially associated with the virulence phenotype on sorghum. Analysis of this region revealed a cluster of 9 genes that were more highly expressed in planta, showed the least amount of sequence conservation and coded for proteins carrying predicted secretion signal peptides. Deletion of the gene cluster in SRS strains is under way to show whether this gene cluster is necessary for full virulence on sorghum.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Genetics underpinning host range of the dothideomycete *Corynespora cassiicola*

Marcio Zaccaron, Burt Bluhm
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The dothideomycete *Corynespora cassiicola* is a major fungal pathogen of several economically important crops, including soybean, cotton, papaya, rubber tree, and cucumber. *C. cassiicola* hosts can be found in over 50 different plant families making it one of the dothideomycetes with the widest host range. No studies thus far have examined the genetics behind *C. cassiicola* ability to infect such a myriad of hosts. The overarching goal of this work is to develop *C. cassiicola* into a tractable system for molecular genetics in order to identify genetic components involved in its host range regulation. We have identified isolates that segregate for virulence in cotton and soybean. To elucidate C. cassiicola host range regulation, current work is focused on genome resequencing, forward genetics, and *in-planta* transcriptomics.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Adaptation of the scab fungus *Venturia inaequalis* to different host plants and ecosystems

Jason Shiller[1], Christophe Lemaire[1,2], Mélanie Sannier[2], Pascale Expert[2], Marie Noëlle Bellanger[2], Valérie Caffier[2], Bruno Le Cam[2], Jérôme Collemare[2]
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Venturia inaequalis is a species of biotrophic fungi best known for the disease it causes on apple, apple scab. However, *V. inaequalis* isolates have been categorised into two *formae speciales, V. inaequalis f. sp. Pyracantha,* that can infect *Eriobotrya japonica* (loquat) and *Pyracantha* sp. but not *Malus ;* and *V. inaequalis f.sp. pomi* that can infect *Malus* and *E. japonica* but not *Pyracantha sp*. Host range has been further categorised within *V. inaequalis f.sp. pomi.* In this group, seventeen races of *V. inaequalis* have been identified based on the corresponding resistance responses observed on a panel of differential hosts (*Malus spp*.). Furthermore, studies on the population genetics of *V. inaequalis f.sp. pomi* isolates from wild Malus species (*Malus sieversii*) in Central Asia, and those from modern domestic apple (*M.× domestica*) have revealed distinct population structures and differences in aggressiveness and host range depending on the populations from which they originate. Isolates from wild *Malus sieversii* Kazakh forests are less aggressive than those from domestic apple and while isolates from domestic apple are able to infect *M. sieversii*, isolates from *M. sieversii* are unable to infect domesticated apple. To understand the genic basis of these differences in host range and aggressiveness we have used traditional genetic analysis (crosses), comparative genomics and comparative transcriptomics of isolates from different populations and formae speciales. This approach has enabled us to identify candidate genes which may be involved in enabling or restricting growth of different isolates on specific hosts. More broadly, this analysis may help us to understand how pathogens can adapt to different host plants and from wild to domestic ecosystems.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

An imaging-based approach to aid in the identification of fungal genes regulating the Reactive Oxygen Species infection response in rice and maize

Timothy Chaya[1,3], Jessica Cooper[2], Jonathan Neifert[2], Danielle Mikolajewski[2], Nicole Donofrio[2], Jeffrey Caplan[1,3]

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Reactive oxygen species (ROS) have been shown to be vital for plant responses to fungal infection as well as plant developmental signals. The ability of a fungus to withstand ROS that is produced both internally and externally by a plant's defense response is key to a successful invasion. In collaboration with the Horwitz lab, we aim to explore the mechanisms of ROS creation and responses in the hemi-biotroph *Magnaporthe oryzae* and the necrotroph *Cochilobolus heterostrophus*, which cause rice blast and Southern Leaf Blight, respectively. The infection cycle of both is being analyzed with advanced, three-dimensional (3D) confocal microscopy and image analysis to characterize the progression of these pathogens. Our preliminary 3D data was acquired by using the Sca*l*eP technique to clear barley and rice leaves inoculated with *M.* oryzae. We captured data over a fourth dimension (4D), time, to show pathogenesis progression from germination to full infection into the host tissue. We also will utilize a genetically-encoded ROS sensor, called HyPer, in both *M. oryzae* and *C. heterostrophus* coupled with various ROS staining techniques in the host tissue. A time course of the HyPer sensor in *M. oryzae* (MoHyPer) shows the increase in ROS production through the early infection stages on the leaf surface. These tools will allow us to quantify the ROS response from both perspectives and make a comparative analysis between ROS levels and time of infection in these two distinct pathosystems. From these data we will determine the optimal time in pathogenesis to conduct a forward genetic screen to generate a mutant library of ROS-related mutants. These mutants will then be sequenced to determine the genes that have altered the ROS response. The identified genes will further elucidate the ROS infection response and could be potential targets for novel crop defense strategies or antifungals.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Analysis of VELVET mutants reveals important roles of host tissue acidification and protein secretion for pathogenesis of *Botrytis cinerea*

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The necrotroph *Botrytis cinerea* kills host tissue by secretion of lytic enzymes and necrosis-inducing metabolites and proteins. Loss of BcVEL1, BcVEL2 or BcLAE1 from the VELVET regulatory complex results in disturbed light-dependent development and impaired virulence. The mutants were unable to acidify the host tissue, and showed strongly reduced release of citrate, the major acid secreted by the wild type. In contrast to previous reports, no significant secretion of oxalic acid was observed in the first days of lesion formation, and an oxalic acid deficient *BcoahA* mutant showed no major reduction in virulence. Keeping infection sites of VELVET mutants artificially low at pH 3 resulted in increased lesion formation, whereas alkalinizing wild type infection sites to pH 6-7 strongly suppressed lesion expansion, which confirmed that acidification is required for optimal infection.

VELVET mutants showed coordinate changes in transcriptome profiles *in planta* compared to the wild type. A common set of genes encoding secreted proteins were underexpressed in all VELVET mutants during infection, and many of these genes were induced *in planta* in the wild type strain. Quantitative analysis of *in planta* secretomes using ^{15}N metabolic labeling revealed a high correlation of changes in mRNA and protein levels in the mutants, indicating that *B. cinerea* regulates infection-related protein secretion mainly by transcript abundance. The VELVET mutants showed drastically reduced secretion of proteases, and significantly impaired degradation of proteins released from killed host cells. Furthermore, the phytotoxic activity of the mutant secretomes was strongly reduced, due to lower expression of phytotoxic proteins and increased pH.

Taken together, VELVET controls major aspects of pathogenic development of *B. cinerea* by mediating acidification of the host tissue and activating secretion of virulence-related proteins. Current studies are focusing at functional and mutational dissection of *B. cinerea* secretome proteins and their effects on host killing and defence.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Chemical talk, warfare and aid: a case of an ectomycorrhiza-forming fungi, *Tricholoma vaccinum*, and its interacting partners

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Different chemicals have been implicated in communication occurring in biological system. A plethora of chemicals and enzymes have been shown to be involved in these communications occurring between organisms of the same species, different species and even evolutionarily distant ones. There is a need to properly understand the role of these chemicals and the involvement of different proteins/enzymes in communication and interaction between organisms. Recent researches had however infer functions and explains evolutionary deterministic purposes for the biosynthesis of different chemicals in biological systems. There are numerous examples of such chemicals that has been shown to infer antimicrobial activities against a host of interacting partners and/or organisms that might be competing for common goods with the producer in the environment. Some other chemicals on the other hand has been shown to serve as an aid for the support of cooperating partners. While some are merely signal molecules or chemical cues for interacting partners or simply the community members. We therefore set out to understand the biosynthesis of some of these chemicals in our working strain of *Tricholoma vaccinum*, an ectomycorrhiza-forming fungi. We try to understand the role of these chemicals in the interaction of the mycorrhiza fungi and its plant host and other interacting partners. Also, we seek out biochemical and morphogenetic effects observable during such interactions.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

sRNA analysis during Botrytis infection in tomato plants

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The fungal pathogen botrytis is able to infect a wide range of plants. This fungus contains an arsenal of degradative enzymes that destroy the plant tissue as the infection progresses. This mechanism of infection is characteristic for necrotrophic pathogens. However Botrytis also possesses subtle specific tools to modulate plant defenses in its benefit. Two regions of the Botrytis genome act as factories producing high numbers of sRNA. These regions account for 45% of the total sRNA isolated from Botrytis during the infection of tomato plants. Several tomato genes were predicted to be targeted for silencing by these sRNA. Three different target prediction tools were used; Miranda, targetfinder and psRNAtarget. For the predicted targets the expression of the corresponding tomato gene was correlated with the expression of the corresponding sRNA pair. Only genes showing down-regulation during the early infection (early than 24hpi) were used for further analysis. GO enrichment analysis of target tomato genes revealed multiple enriched GO terms including, carbohydrate metabolism, post-translational protein modification and defense response. Using string database a network has been created where enrichment in signaling kinases was observed. This subnet of tomato kinases were divided in kinases related to defense and development. Key components of the tomato defense signaling pathway seems to be targeted by the sRNA produced by botrytis during the early stages of the infection.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Identification of genes from *Trichoderma virens* involved in the colonization of maize roots, and the relation of colonization to induced systemic resistance

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As ubiquitously distributed plant beneficial symbionts, members of the genus *Trichoderma* provide experimental models to understand the molecular mechanisms underpinning the delivery of plant benefits by root-associated symbionts. A critical process that initiates this symbiotic relationship is the colonization of host roots. We propose a model of root colonization by *T. virens* that partitions the process into discrete units that are experimentally tractable: Recognition, contact/attachment, penetration, and ingress. Based on microarray and proteomic data, we have targeted genes encoding hydrophobins, expansins, glycoside hydrolases, and small secreted cysteine-rich proteins, putatively involved in individual components of the model, for characterization. As a proof of concept that individual genes involved in these components can be identified, we initiated studies with the gene (*TvHyd1*) encoding a hydrophobin to test the hypothesis that hydrophobins are necessary for attachment of hyphae to the root cell wall. Δ*TvHyd1* mutants showed a significant decrease in colonization ability compared to the wild type strain. We also hypothesized that reduction of colonization at the early phases (recognition and contact/attachment) would influence the induction of beneficial effects in the plant. Using induced systemic resistance (ISR) as a model of host benefits, we demonstrated that Δ*TvHyd1* mutants were also impaired in their ability to induce ISR in maize plants, exhibiting more protection than the untreated plants, but less protection than the wild type treated plants. These proof of concept experiments provide support for our model and help us to better understand how components of colonization allow the fungus to convey beneficial attributes to the plant host.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Using pathogen genomics to decipher the plant-fungal interaction of *Pyrenophora teres* f. *teres* and barley

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Pyrenophora teres f. *teres*, a fungal pathogen of barley and causal agent of net form net blotch, interacts with the host in a complex manner, exhibiting hallmarks of gene-for-gene and inverse gene-for-gene interactions, indicated by dominant resistance and susceptibility, respectively. Previously, using the 15A × 6A population, four loci (*VR1*, *VR2*, *VK1*, *VK2*) were identified corresponding to virulence (dominant susceptibility) on barley lines Rika and Kombar. Using the FGOH04Ptt-21 × BB25 population, a major locus (*Tif1*) was identified corresponding to avirulence (dominant resistance) on Tifang barley and a gene-for-gene interaction was identified in barley line CI5791 effective against a broad range of *P. teres* f. *teres* isolates. However, the CI5791 dominant resistance was recently defeated by a population of Moroccan isolates. The specific pathogen molecules eliciting most of these responses have yet to be identified. To mediate effector identification, reference quality genomes of isolates 15A, 6A, FGOH04Ptt-21, and BB25 were developed via PacBio sequencing and RNAseq was conducted to facilitate gene annotation. A *VR2* candidate encoding a 422 aa secreted protein was disrupted in a 15A × 6A progeny isolate, resulting in a loss of virulence, functionally validating the *VR2* gene. Underlying the *Tif1* avirulence locus in isolate BB25, an ~100 kb expansion containing 23 genes was found lacking homology to *P. teres* f. *teres* genome sequences, but conserved in *P. tritici-repentis*, providing several candidate genes for validation. Additionally, a natural population of 146 *P. teres* f. *teres* isolates were sequenced at ~25× coverage, resulting in the identification of 83,043 SNPs/INDELs used for association mapping. A highly significant association was detected corresponding to avirulence on CI5791, underlying which are two candidate genes in close proximity. The validation of *VR2* and the continued investigation of other effector loci will allow us to understand the intricate host-pathogen molecular interactions governing this pathosystem.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Blumeria metallo-protease like effector: a zinc scavenging universal virulence factor in fungi?

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Blumeria metallo-protease like effector (BEC1019) is a virulence factor for the obligate biotrophic barley powdery mildew (PM) pathogen, *Blumeria graminis* f. sp. *hordei* (*Bgh*)[1]. Silencing of BEC1019 reduced barley PM disease development and dispersion[1]. BEC1019 has homologues in a third of sequenced fungal genomes, including known zinc scavenging virulence factors – Aspf2 in *Aspergillus fumigatus* and PRA1 in *Candida albicans*[2,3,4]. These homologues share a zinc binding HRXXH domain, similar to M35 superfamily of metallo-proteases, but lack any known protease activity[2,3,4]. Therefore, BEC1019 homologues are potential zinc sequesters or "zincophores" with a universal virulence role in fungi.

This project aims to study the role of BEC1019 homologues in zinc sequestration and virulence of economically important wheat fungal pathogens.

We can show that the BEC1019 homologue acts as a virulence factor in *Blumeria graminis* f. sp. *tritici (Bgt)* (causing PM in wheat) but not in *Fusarium graminearum* (*Fg*) (causing Fusarium head blight). BEC1019 deletion mutants are being created in *Zymoseptoria tritici* (*Zt*), to evaluate its role in Septoria tritici blotch disease development. Also, our findings indicate that BEC1019 expression is regulated by zinc availability in *Zt* and by a homologue of the zinc sensitive transcription factor ZAP1 in *Bgh.* Therefore, the role of BEC1019 and other proteins involved in zinc sequestration and homeostasis in these fungal pathogens will be studied. We will also evaluate the role of BEC1019 in countering nutrional immunity in plants. Our ultimate goal is to discover new targets for crop protection.

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POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Role of gluconic acid in pathogenicity of *Aspergillus carbonarius* in grapes

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Many fungi are epiphytic on grapes in the vineyard, but the main concern is for the black mycotoxigenic *Aspergillus* species that causes severe losses. *Aspergillus carbonarius* causes severe postharvest decay of grapes in the orchard, and is considered as the major source of ochratoxin A (OTA) contamination of grapes and derived products. Our previous findings indicated that production of organic acids, such as D-gluconic acid (GLA), by *A. carbonarius* in the growth medium or in the decayed fruit tissue was directly related to ambient pH reduction. Under these conditions, induced transcript expression of genes involved in OTA biosynthesis concurrently with the mycotoxin accumulation takes place. The contribution of GLA secretion to the pathogenicity of *A. carbonarius* in grapes and its involvement in OTA biosynthesis remain unclear. Deletion of the gene encoding glucose oxidase (*gox*) in *A. carbonarius* was carried out to suppress the conversion of glucose to GLA with the aim to investigate the roles of GLA and OTA accumulation in *A. carbonarius* pathogenicity. The obtained results showed that the GLA accumulation was completely inhibited in grape berries infected with *Δgox* knockout mutant, which was accompanied by a concomitant reduction in decay development compared to the wild-type strain, suggesting that tissue acidification significantly contribute to *A. carbonarius* pathogenicity. Interestingly, however an increased production of OTA was detected by *Δgox* mutant both *in vitro* and *in vivo*, suggesting that not GLA production, but other environmental factors, such as high sugar content, may determine the accumulation of OTA. The present data suggest that GLA, but not OTA, is contributing to *Aspergillus* colonization in grapes.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

The functional analysis of late effectors in the maize pathogen *Ustilago maydis*

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The biotrophic basidiomycete fungus *Ustilago maydis* causes smut disease in maize. Hallmarks of the disease are large tumors that develop on all aerial parts of the host in which dark pigmented teliospores are formed. Ros1, a member of the WOPR family of transcription factors, is a central regulator of the late infection stages in *U. maydis* and is essential for karyogamy, hyphal aggregation, formation of a mucilaginous polysaccharide matrix and spore production. Ros1 also triggers a major switch in the effector repertoire and a set of 70 late effectors is upregulated by Ros1. We are speculating that late effectors could be used as defense against other microbes which colonize mature tumor tissue when it ruptures. To analyze this, single gene deletion mutants of the eight of candidate late effector genes regulated by Ros1 were created in compatible haploid strains. Seven of these mutants showed no significant defects in virulence and spore formation. One of the mutants showed reduced virulence and formed hyphal aggregates, but produced fewer spores. We are currently isolating plant proteins interacting with this late effector and discuss our results. For the effectors which do not contribute to virulence, we are testing a possible antimicrobial function towards microbes isolated from corn plants grown outside of the laboratory. In addition, we are determining the microbiome composition of uninfected plants and plants infected with wild type and *ros1* mutant strains to investigate whether they are difference in community structure. This would indicate an involvement of late effectors in controlling microbial colonization of *U. maydis* infected plants.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

CBM18 containing lectin-like protein of *Verticillium nonalfalfae* binds chitin and protects hyphae from degradation by xylem hydrolases

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Verticillium nonalfalfae is a soil-borne hemibiotrophic ascomycete infecting various plant species, including hop (*Humulus lupulus* L.). During the infection of hop, the fungus produces a 38.6 kDa cysteine rich lectin-like protein (*Vna*CBP8.213) harboring six carbohydrate binding CBM18 domains. Its gene expression increases with disease progression, reaching the highest abundance in stems of susceptible hop. We examined here genetic diversity of *VnaCBP8.213* in various isolates of *Verticillium* spp. and biochemically characterized recombinant *Vna*CBP8.213 to determine its biological role.

The *VnaCBP8.213* gene was PCR-amplified and its sequence variation was studied. It was detected in all *V. nonalfalfae* and *V. alfalfae* isolates, but was not present in *V. dahliae, V. tricorpus* and *V. isaacii.* Deletion of the *VnaCBP8.213* gene did not affect the fungal growth, conidiation or pathogenicity of *V. nonalfalfae* in susceptible hop.

E. coli expressed and purified *Vna*CBP8.213, infiltrated into *Nicotiana benthamiana* leaves, did not trigger a visible HR response. The nonspecific subcellular localization pattern of mRFP fusion protein in *N. benthamiana* leaves led to the hypothesis that *Vna*CBP8.213 could function as an apoplastic effector involved in the evasion of chitin-triggered plant immune responses. A carbohydrate sedimentation test confirmed that the recombinant protein *Vna*CBP8.213 specifically binds to chitin beads and crab shell chitin, but not to cellulose and xylan. Carbohydrate binding specificity and affinity will be determined by surface plasmon resonance (SPR). Furthermore, the addition of 3 µM *Vna*CBP8.213 caused an aggregation of fungal hyphae, shielding them from degradation by xylem sap hydrolases. To confirm the immunosuppressive activity of *Vna*CBP8.213, the inhibition of a chitin oligomer triggered immune response in tobacco BY-2 suspension cells will be examined.

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POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Epigenetics: Role of methyltransferases in the pathogenesis of *Alternaria brassicicola*

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Alternaria brassicicola is the causing agent of black spot disease in *Brassicaceae*. This pathogenic fungus is able to transmit itself on and by seeds. Then it has developed resistance mechanisms against hydric, osmotic and osmolar stresses.

Epigenetic mechanisms are hereditary modifications of gene activities without mutations in the DNA sequence and show an implication in the virulence and plant defense molecules response of pathogen fungi. In *A. brassicicola*, 2 methyltransferases (abDIM-2, abDIM-5) have been identified.

In this study, we investigated the implication of epigenetic mechanisms on the virulence and the seed transmission of *A. brassicicola*.

Phenotyping of abDIMs deleted mutants didn’t show involvement of epigenetic mechanisms in their growth, sporulation, virulence or susceptibility against plant defense molecules. However, abDIM mutants are able to transmit better to seeds due to an increased resistance to hydric and osmotic stresses. Expression study of known *A. brassicicola* genes involved in these stresses resistance showed different expression pattern between abDIM mutants and the wild-type strain and also according to the exposure time to osmotic stress.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Diverse selective sweeps in the genome of the fungal phytopathogen *Sclerotinia sclerotiorum*

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The phytopathogenic fungus *Sclerotinia sclerotiorum* causes disease in more than 400 host species, including several economically important crops. We used single nucleotide polymorphism (SNP) data from 25 re-sequenced isolates of *S. sclerotiorum* to identify genetically distinct global populations. We then used an approach not commonly applied to plant pathogenic fungi to detect recent selective sweeps based on SNP frequency spectra. In addition, we characterised numerous presence / absence polymorphisms among gene models of the genome of the reference isolate 1980 (1).

We found that among the isolates there were two major global clades of *S. sclerotiorum,* corresponding to individuals from Europe and North America (population 1), and Australia and Africa (population 2). We found 41 putative selective sweeps in population 1 and 21 in population 2. By considering non-synonymous SNPs in these regions, we identified several candidate genes that may have undergone recent adaptive evolution. We highlight a methyltransferase, two secreted proteins, and a putative secondary metabolite biosynthesis cluster. The latter three of these were significantly up-regulated during infection of *Brassica napus* based on previously published RNA sequencing data (2).

We also found numerous presence / absence polymorphisms among reference genes and highlight a 200 Kb stretch of chromosome 11 that has been deleted in several isolates. This region contains genes with domains that have previously been associated with fungal vegetative incompatibility. We hypothesise that it may be involved in prevention of hyphal fusion between unrelated isolates of *S. sclerotiorum*.

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POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Transcriptomic sequencing of the wheat pathogen *Puccinia striiformis* f. sp. *tritici* isolated from infected wheat across the United States reveals key effectors

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Stripe rust, caused by the fungal pathogen *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most devastating diseases of wheat and is present in all major wheat-growing regions of the world. Within the U.S., stripe rust has traditionally affected cooler regions with higher moisture levels, such as the Pacific Northwest, but in the last 15 years its geographic footprint has expanded. This expansion is associated with suspected incursions of novel *Pst* races that have unique and broader virulence profiles, are better adapted to warmer environments, and are more aggressive than previously characterized races. Effectors, a group of virulence proteins deployed by the pathogen to manipulate plant cell structures and functions, might contribute to the aforementioned expansion. Furthermore, recent evidence has demonstrated that the pathogen effectors are under strong selective pressure to adapt in order to evade detection by the host resistance mechanisms. Towards the ultimate goal of understanding the diversity, distribution and function of *Pst* effectors in the pathogen population, we collected 15 field *Pst* samples from several regions across the U.S. and sequenced their transcriptomes. We further assess the transcriptomes for genome-wide effector diversity and differential expression to identify key effectors that likely contribute to the virulence of *Pst*. These key effectors could be targets for further molecular characterization as well as be used to devise novel sequence-based tools for the efficient and high-resolution surveillance of outbreaks.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Extracted metabolites from the biocontrol agent *Pseudozyma aphidis* inhibit phytopathogenic fungi and bacteria using a dual mode of action: antibiosis and induced resistance

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Natural product-based pesticides may serve as an alternative for traditional synthetic pesticides. Microorganisms are a prospective source of such biological pesticides.

A unique strain of *P. aphidis* (designated isolate L12, Israel 2004) that was isolated in our laboratory was previously established as a potent biocontrol agent against diverse phytopathogens. This work demonstrates that metabolites extracted from the biocontrol agent *P. aphidis* (isolate L12) can inhibit fungal and bacterial phytopathogens, and in addition can activate an induced resistance in plants.

Biologically active metabolites were extracted from *P. aphidis* biomass, and the antimicrobial activity of the extract was demonstrated for diverse plant pathogens. Growth inhibition was demonstrated *in vitro* on bacterial phytopathogens such as: *Pseudomonas syringae pv. tomato*, *Xanthomonas campestris pv. vesicatoria*, *Clavibacter michiganensis subsp. michiganensis*, *Erwinia amylovora* and *Agrobacterium tumefaciens*. Strong inhibition of fungal mycelial growth was also demonstrated *in vitro* on phytopathogenic fungi such as: *Botrytis cinerea*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum f. sp. lycopersici* and *Alternaria alternata* and on the oomycete *Pythium spp*.

Additionally, *in planta* experiments demonstrated a dose-dependent inhibition of *B. cinerea* infection on tomato plants. Significant reduction of 70-95% in *B. cinerea* infection was obtained when the spore suspension was pre-treated with extract concentrations higher than 4.2mg ml^{-1}. Similar results were obtained in preventative assays. Spraying the plants with 5mg ml^{-1} of the extract two hours prior-inoculation with *B. cinerea*, reduced the disease symptoms by 68%.

Furthermore, preliminary results demonstrated that application of *P. aphidis* crude extract can systemically decrease *B. cinerea* infection by 20%, along with up-regulation of pathogenesis related genes such as: *PR1a* (2-fold), *LOX* (13-fold), *GlucA* (12-fold), *Chi3* (14-fold), *Chi9* (5-fold), *PIN1* (3-fold) and *AOS* (2-fold).

These results suggest that the extracted metabolites from *P. aphidis* L12 may serve as natural pesticides using a dual mode of action: antibiosis and induced resistance.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

A novel Major Facilitator Superfamily (MFS) transporter from *Botrytis cinerea* provides tolerance towards glucosinolate breakdown products and required for pathogenicity

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Glucosinolates are a diverse class of secondary metabolites found mainly in members of the Brassicaceae (Cruciferae) and a few other unrelated families of dicotyledonous angiosperms. The importance of these metabolites has increased in the last few decades, upon discovery of their potential function in crop defense, agricultural biofumigation and cancer prevention. Upon plant injury or pathogen attack, glucosinolates are rapidly released and hydrolyzed to a multitude of physiologically active products so-called isothiocyanates. They have been described as promising molecules with antimicrobial activities against plant pathogens such as the causative agent of grey mold, *Botrytis cinerea*, this is a necrotrophic plant pathogen, which causes devastating losses on a number of crops. In the present study we studied the mechanisms of tolerance to glucosinolates in the fungus *B. cinerea*. Exposure of *B. cinerea* to glucosinolate breakdown products induces expression of the *BcMFSI* transporter that functions in the efflux of fungitoxic compounds. *B. cinerea* inoculated on wild-type *A. thaliana* plants activate the *BcMFSI* transporter to higher levels than on glucosinolate-deficient *A. thaliana* mutants. *B. cinerea* strains lacking functional *BcMFSI* are more sensitive to glucosinolate breakdown products *in vitro* and less virulent on plants. We demonstrate that a *BcMFSI* transporter is a virulence factor that increases tolerance of the pathogen towards glucosinolates. We also demonstrate that the *BcMFSI* gene can provide the yeast *Saccharomyces cerevisiae* tolerance towards glucosinolate hydrolysis products.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

New insights into pathogenicity of emerging tropical plant pathogens: genomics and transcriptomics of *Phytophthora colocasiae* on taro

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Phytophthora colocasiae is a phytopathogenic oomycete that causes leaf blight, and corm rot, on taro (*Colocasia esculenta*). Taro is an important staple crop in the tropics, and the emergence of *P. colocasiae* as a devastating pathogen is a matter of serious concern for food security in the region, since many of the Asian and Oceanic cultures heavily rely on taro to maintain their daily calorific intake. *P. colocasiae* strain 7290 was isolated from a diseased taro plant in Vietnam in 2010 and is primarily triploid. The draft genome of *P. colocasiae* strain 7290 is 56.59 Mb. It contains 19,984 predicted genes. *Phytophthora* species secrete a large arsenal of effectors during infection of their host plants and genome mining along with transcriptome analysis of taro-*P. colocasiae* interactions has identified a number of putative Carbohydrate active enzymes, Peptidases, secretory proteins (1782) including cytoplasmic effectors such as RxLR (337) and CRN family members (203), as well as novel effectors that specifically target taro. The genome and transcriptome analysis of *P. colocasiae* is a first step towards understanding pathogenicity determinants, in this pathogen and to design specific management strategies to control the disease. Tropical plant pathogenic oomycetes are not well studied, and our data provides insights into the development of disease within such a climate A detailed comparative genomics analysis of the genomes of this and other closely or distantly related plant pathogenic oomycetes and fungi will allow us to elucidate the precise genetic components of pathogenicity in *P. colocaisae,* and uncover the evolution of pathogenicity in this unique tropical species. Our latest analysis of the genome and transcriptome data, including discovery of putative novel effector proteins will be discussed.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Plant-fungal interactions

Evolution of plant penetration strategies in pathogenic fungi

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Fungal spores are responsible for initiation and propagation of the majority of biotic plant diseases. Yet the core and species-specific genetics of spore germination have not been comparatively analyzed in multiple fungal lineages. We performed comparative transcriptomics of spore germination among six fungi to determine how expression of orthologous genes has changed during evolution, and to predict genes whose knockouts will exhibit phenotypic differences in the spore germination and host penetration processes. We chose fungi representing different approaches to plant penetration, including penetration using melanized appressoria, penetration through natural openings, and direct penetration without melanization, and included *Neurospora crassa* as a comparison. To provide a uniform basis for comparison among species, we assayed transcriptional profiles during germination on a single defined medium as well as during germination on hosts. We estimated ancestral gene expression for orthologous genes, identifying genes that undergo species- or clade-specific transcriptional shifts during the spore germination process, as well as transcriptional shifts that are unique to different mechanisms of plant penetration. Functional assays of a subset of genes exhibiting species-specific and infection-type specific up-regulation were performed to determine the roles of these genes in conidial germination of these fungi. These assays revealed genes, not previously identified, which have important roles in conidial phenotypes, germination, and hyphal growth. These experiments contribute to our understanding of how shifts in gene expression drive the evolution of conidial germination in a wide range of fungi.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

High resolution QTL mapping of ethanol tolerance in *Saccharomyces cerevisiae* using an integrative genomics approach

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Ethanol, produced by *Saccharomyces cerevisiae* fermentations, is the main biofuel used worldwide. The toxicity effect of ethanol on yeast, which inhibits fermentation and industrial productivity, calls for better understanding of the genetic basis of ethanol tolerance. However, until now genomic elements affecting ethanol tolerance have only been mapped at low resolution, hindering their identification. Here, we used an Advanced Intercross Line design, to perform high resolution mapping of QTLs affecting ethanol tolerance in yeast. Selective DNA Pooling and whole-genome sequencing were carried out in the F6 of a cross between two widely separated *S. cerevisiae* strains. Fifty-one and 96 QTL regions (QTLR) respectively affecting growth and survival under ethanol stress, were identified by applying a unique statistical pipeline based on LOESS smoothing to identify QTLs and Log drop to determine QTLR boundaries. We identified a larger number of QTLs than in any previous single mapping study in yeast, with a high resolution; in some cases, down to single genes. Some QTL overlap observed between the growth and survival traits, suggesting partially shared mechanisms. Finally, enrichment analysis highlighted biological processes important for ethanol tolerance in *S. cerevisiae*. The presented integrative genomics approach can be applied with any sexual model organism for high resolution mapping.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

Genomic comparison of five early diverging fungi encounter in different environments and belonging to the *Mucor* genus

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The fungal genus *Mucor* belongs to the Mucoromycota phylum, one of the five groups of the early diverging fungi. *Mucor* species are ubiquitous, they show diverse lifestyles and may have contrasting impacts on human activities. Indeed, some opportunistic pathogenic *Mucor* species represent a threat for human health, some others can be involved in food spoilage whereas some few others are commonly used for Asian and African fermented food manufacturing or cheese ripening. Despite these impacts on human activities, little is known on the genus *Mucor.* Here, we are investigating on specificities linked to *Mucor* species lifestyles. We engaged a genomic comparison focused on five species: *M. fuscus* and *M. lanceolatus*, two technological species used in cheese ripening, *M. racemosus*, a recurrent cheese spoiler, *M. circinelloides*, a pathogenic species and *M. endophyticus,* a plant endophyte.

Strains of *M. fuscus* UBOCC 1.09.160, *M. lanceolatus* UBOCC 1.09.153, *M. racemosus* UBOCC 1.09.155 and *M. endophyticus* CBS 385-95 genomes were sequenced and assembled. Genes were structurally and functionally annotated following a standard pipeline including *ab initio* prediction and RNAseq data support, transposable elements were annotated using the REPET pipeline that includes a *de novo* prediction. These data were integrated in an instance of the genome viewer Apollo allowing experts to validate the gene prediction quality.

Investigating these four genomes along with genome of the reference species *M. circinelloides* (strain CBS 277.49), core and pan genomes were established. Genome structures were analyzed and compared between species showing, among others, a lack of synteny among species and questioning the existence of gene cluster organization. Gene functions were compared among *Mucor* species and among species with different lifestyles. Evolution of gene families was also studied in order to give new hints regarding adaptation to specific habitat and/or lifestyle.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

Comparative genomics of DNA repair in the Fusarium species complex

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Fungal plant pathogens are continuously exposed to DNA damage originating from the environment and their host. Even though DNA repair is a fundamental process in all organisms, very little is known about fungal plant pathogen DNA repair machinery. In this research, we focus on DNA damage repair pathways in *Fusarium oxysporum* (FOL) and *Fusarium mangiferae* (FMN) which are closely related fungal plant pathogen species that occupy very different ecological niches. To our surprise, we found that FMN, a foliar plant pathogen, is more sensitive to UV than FOL, a soil-borne plant pathogen, while the opposite was true for alkylating agents. Moreover, a few hours exposure to sunlight seem potentially very lethal for FMN spores. However, there was no difference in sensitivity to 4-NQO between the two fungi indicating that nucleotide excision repair is not defected in FMN. The UV-repair specific genes and MMS repair genes are highly conserved between FOL and FMN therefore we hypothesize that the difference stems from gene expression. RNAseq analysis revealed a clear and typical transcriptional response of both FOL and FMN to MMS. Many NER-associated transcripts were induced under MMS in both fungi suggesting that NER might play an important role in protecting FOL and FMN genome from methylating agents. Interestingly, both fungi did not respond to UV-C as expected; there was a significant down regulation of many DNA repair genes including UV-related *phr1* and *uvde*. In conclusion, we aim to identify species-specific DNA damage response signature and suggest that this is a fundamental component in a set of factors that dictate fungal plant pathogen agroecology niche and disease cycle.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

Does a trans-species polymorphism affect meiotic silencing by unpaired DNA in Neurospora?

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Meiotic silencing by unpaired DNA (MSUD) was discovered in crosses made in the *Neurospora crassa* standard OR genetic background. We have found that crosses in the novel *N. crassa* B/S1 and the *N. tetrasperma* 85 backgrounds show a relatively less efficient silencing of unpaired genes and increase in inappropriate silencing of paired genes. Crosses in the OR, B/S1, and 85 backgrounds heterozygous for an *::r*^{ec} transgene produced, respectively, 97%, 24%, and 7% MSUD-induced round ascospores, whereas the corresponding *::r*^{ec}-homozygous crosses produced 1%, 6%, and 1.4%. Additionally, *Dp*-heterozygous crosses in 85 were non-barren whereas those in OR were barren. Analysis of the f1 progeny from the OR x B/S1 crosses suggested that as few as three unlinked loci might determine these differences between the parental strains.

Results of Novak and Srb (Can. J. Genet. Cytol. 15: 685-693, 1973) suggest that MSUD efficiency in the *N. tetrasperma* T-220 strain might be similar to that in *N. crassa* OR. If such is the case, the difference between OR and B/S1 in *N. crassa* would be paralleled by that between T-220 and 85 in *N. tetrasperma*, and suggest a trans-species polymorphism (TSP) in MSUD efficiency. TSPs in Neurospora are associated with self/non-self recognition systems. Allorecognition protects against the spread of mycoviruses, and MSUD might protect against the spread of transposable elements, and it is not inconceivable that alternative alleles of one or more allorecognition system have differential effects on MSUD efficiency.

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POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

High efficient genome-editing system for a valuable plant-beneficial strain, *Trichoderma harzianum* NJAU4742

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The specie of *Trichoderma harzianum* has valuable functions in agricultural applications, such as plant growth-promotion and anti-pathogens of plant. However, the deeper studies were strictly limited by genetic manipulation, especially in multiple genes editing. In this study, we developed a high efficient marker-free recycling gene-deletion system in *T. harzianum* strain NJAU4742, based on a strategy of two stepped homologous recombination (HR). Firstly, hygromycin B resistance gene, encoding aminoglycoside phosphotransferase, was used as a marker but to construct a marker-free auxotrophic mutant, NJAU4742*Δura3*, based on which the native *ura3* gene was then used to screen the mutants of target genes with a destroyed expression box after the first HR. The final mutant, just deleting the whole sequence of target gene, would be screened out after the second HR using the plate with uridine and 5-Fluoroorotic acid (5-FOA). Meanwhile, screening steps were optimized to be able to keep the rate of HR in 10%-20% for strain NJAU4742 even without the contribution of *ku70*-deleted mutant, which was largely increased than the general value of 1% in filamentous fungi. Surely, no quantitatively restricted target genes could be knocked out one by one through this system in a quite short period. It is the first time to report the method of multiple gene editing in plant-beneficial *T. harzianum* strain, and this study will largely promote the scientific researches involved in the mechanisms of interactions among *Trichoderma*, plant and plant pathogens.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

Genome sequencing of four *Pyricularia s*pecies provides insights into the evolution of Blast-Disease Fungi

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Pyricuaria oryzae is the well-known fungal pathogen of blast disease that destroys rice, millet, wheat, oat, and other crops. Several related species of the fungus also cause blast disease in different hosts. To study the evolution of blast disease pathogens, we sequenced and obtained high-quality *de novo* genome assemblies of four species from the *Pyricularia* genus. In particular, we obtained a chromosome-level assembly, using SMRT and Illumina sequencing, of a *P. oryzae* strain isolated from finger millet. This genome showed overall synteny with the *P. oryzae* 70-15 reference genome, but we found major intra- and interchromosomal rearrangements and significant differences in gene content. The rearrangements include a partial fusion of two chromosomes, large inversions and insertions. Furthermore, the 500 kb unassigned region of the reference genome is located within a chromosome of the finger millet isolate. Genomes of the four *Pyricularia* species have undergone notable changes. Their sizes differ by more than 8 Mb, mainly because the proportion of repeats goes from 7.8 to 25.10 %. We predicted effector genes and found a surprising high proportion of effectors comprising gene families. Here we report the genome structure evolution and the repertoire of predicted effector genes and other putative genes involved in adaptation of four *Pyricularia* species to their hosts, and discuss the implications for blast-disease pathogen evolution.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

Differential expression of transposable elements through the course of a *Zymoseptoria tritici* infection

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Transposable elements (TEs) are dynamic genetic entities that affect the expression landscape of the genome. In addition to transcription related to their own propagation, TEs can modulate the transcription of nearby genes. Genomic defences of the host genome limit the activity of TE by epigenetic silencing of TE-rich regions in the genome. In many pathogenic fungi TEs are located in close proximity to pathogenicity-related genes. Therefore, epigenetic silencing of TEs by the host genome can sometimes influence the expression of neighbouring genes through leakage. A pathogen infecting a host plant is undergoing high levels of cellular stress that is conducive to the reactivation of TEs. To study the interaction between host infection and TE mobilization, we used *Zymoseptoria tritici* as a model system. *Z. tritici* is an important pathogen of wheat with a highly dynamic genome, consisting of 18% TEs of which many are transcriptionally active. We investigated the differential expression of transposable elements in an infection of four isolates of *Zymoseptoria tritici,* with different infection rates and virulence profiles to characterize TE regulation. TE transcriptomes were compared at 4 different infection stages. The strains did not differ significantly in TE composition, but the TE expression profiles varied between isolates and over the course of the infection. We found a correlation between the evolutionary age of a TE and levels of derepression during infection, where younger TEs were more expressed. The reactivation of TEs and neighboring genes plays an important role in shaping the expression landscape of *Z. tritici* during infection.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

Pathogenicity chromosomes in *Fusarium oxysporum*

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Tomato-infecting strains *Fusarium oxysporum* f.sp. *lycopersici* (Fol) contain pathogenicity chromosomes. These chromosomes determine host range and can be transferred to a non-pathogenic strain, turning the recipient strain into a pathogen [1]. Surprisingly, loss of a big part of the pathogenicity chromosome in a strain of Fol does not affect virulence [2]. To investigate which parts of the chromosome in Fol are required for pathogenicity to tomato plants and which parts can be transferred, we labeled several positions of the pathogenicity chromosome in Fol with different marker genes (encoding green fluorescence and red fluorescence). We then used fluorescence-assisted cell sorting (FACS) to select spores that have lost green fluorescence or red fluorescence and thus obtained several lines with a variety of deletions in this chromosome. We are currently testing virulence of these lines and aim to perform horizontal chromosome transfer experiments to assess whether these partial chromosomes can be transferred. Finally, we aim to characterize the pathogenicity chromosome of a strain that is specific to melon -*Fusarium oxysporum* f.sp. *melonis* (Fom)- and compare this to the previously identified pathogenicity chromosome of *Fusarium oxysporum* f.sp. *radicis-cucumerinum* (Forc), a strain that has a broad host range (cucurbits). We want to identify the regions or genes in Forc and Fom that are responsible for the difference in host range.

[1] Ma, L. J., et al. (2010). "Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*." Nature 464(7287): 367-373.

[2] Vlaardingerbroek, I., et al. (2016). "Dispensable chromosomes in Fusarium oxysporum f. sp. lycopersici." Mol Plant Pathol **17**(9): 1455-1466.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

Phylogenetic analysis of leucine aminopeptidase (LAP) in the Ascomycota

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Leucine aminopeptidase (LAP, EC 3.4.11.1) is an exopeptidase, which removes the N-terminal L-leucine from peptide substrates. LAPs of aspergilli are of interest in the soybean fermentation industry because of their debittering activity. Through genomic analysis of *Aspergillus sojae* SMF 134 isolated from the Korean traditional soybean brick, *meju*, we identified three LAPs in *A. sojae*. By using these identified LAP genes as the query, we analyzed the published Ascomycota genomes for *lap* genes. Most of Ascomycota including Pezizomycotina and Saccharomycotina have one or two LAPs, however, *Aspergillus* spp. have three LAPs. Based on the amino acid sequences, Ascomycota LAPs can be divided into four distinct clades, with two clades in Pezizomycotina and two in Saccharomycotina, respectively. LAP1 and LAP2 are divided into separate clades in each Pezizomycotina and Saccharomycotina. LAP3 of *Aspergillus* sp. belong to the same clade as the LAP1 clade of Pezizomycotina. We find that all LAPs contain 15 highly conserved amino acids sequences in the major domain of LAP, suggesting that the conserved amino acids play an important role in LAP function. This analysis will provide the basis for biochemical studies of LAP with purpose of aspergilli application in industry.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

Friends and foes - comparative genomics of 23 *Aspergillus Flavi* species

Inge Kjaerboelling[1], Tammi C. Vesth[1], Jane L. Nybo[1], Sebastian Theobald[1], Jens C. Frisvad[1], Martin E. Kogle[1], Ellen K. Lyhne[1], Alan Kuo[2], Asaf Salamov[2], Robert Riley[2], Thomas O. Larsen[1], Uffe H. Mortensen[1], Igor V. Grigoriev[2], Scott E. Baker[3], Mikael R. Andersen[1]

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A. oryzae is widely used in food fermentation for the production of soy sauce, sake and miso in addition to enzyme production and it has GRAS status. A close relative *A. flavus* on the other hand produces some highly toxic compounds such as aflatoxin and is an opportunistic pathogen. Both species belong to section *Flavi* consisting of at least 29 species[1].

In this study, we have whole genome-sequenced 19 novel *Flavi* species to examine the core of this section and the differences based on comparative genomics. The genomes reveal a highly diverse section with the number of predicted genes ranging from 9,078 to 14,216 in *A. coremiiformis* and *A. transmontanensi* respectively. We have identified 1,119 *Flavi* specific core protein families corresponding to approximately 9% of the proteome while the number of species specific protein families ranges from 395 for *A. nomius* NRRL 13137 to 2,219 for *A. leporis*.

Of particular interest is enzymes for degradation of carbohydrates, due to their essentiality both for food fermentation, plant pathogenicity, and biotechnology. Thus, the Carbohydrate-Active enZYmes (CAZY) potential was investigated ranging from 353 to 617 identified proteins belonging to a CAZy family for *A. coremiiformis* and *A. novoparasiticus* respectively.

In addition, we have investigated the secondary metabolite (SM) potential of this section since it is vital for food safety but also represents potential useful bioactive compounds. The total number of predicted SM clusters in the *Flavi* section is 1,527 constituting 283 cluster families with an average of 73 clusters per species. No SM gene cluster family is shared between the all the *Flavi* species however 106 unique cluster families are only found in one species. Overall this investigation paints a picture of a highly diverse section encompassing friends and foes.

[1] Varga *et al.* 2011 Studies in Mycology 69:57-80

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

Genome sequencing and genomic comparison of the conifer pathogen *Heterobasidion parviporum*

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In this study, 15 isolates of the causative agent of root and butt rot disease *Heterobasidion parviporum* from all across Finland were screened for pathogenicity and virulence. All isolates were pathogenic and displayed varied virulence. A draft genome assembly of the most virulent isolate as a reference was constructed and described for the rest of 14 isolates, of which whole genomes were also sequenced. Genome-wide alignments and variant callings showed that these isolates exhibited overall high genomic similarity with an average of at least 96% nucleotide identity when compared to the reference, yet had remarkable intra-specific level of polymorphism with a bias for CpG to TpG mutations, probably attributed to repeat-induced point mutation (RIP)-like activity or DNA methylation as one way of epigenetic regulation of gene expression. Reads mapping coverage analysis classified all predicted genes into 5 groups and secreted proteins in each group were more closely explored. Two genomic regions found exclusively in the reference may contribute to its virulence. Genes enriched for copy number variations (deletions and duplications) and nucleotide polymorphisms were found to be involved in oxidation-reduction process and encoding domains relevant to transcription factors. However, gene number variation cannot be directly correlated to varied virulence and not a single gene could be pinpointed as a determinant for the virulence in this fungus. Instead, it is likely the regulatory networks play more important roles in compensation of modification of gene number and genetic variations.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

A RID-like Cytosine Methyltransferase Homologue is essential for sexual development in *Podospora anserina*

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In filamentous fungi, the Repeat Induced Point mutation (RIP) system, which is active during sexual reproduction, introduces G/C to A/T mutations within the repeats thus creating AT-rich regions. Originally described in *Neurospora crassa*, the presence of active RIP has then been evidenced in various other filamentous fungi, including *P. anserina*. To date the RID gene (RIP deficient), encoding a DNA methyltransferase-like protein, is the only one that has been demonstrated to be essential for RIP. But while RIP is conserved among filamentous fungi, DNA methylation does not seem so. Indeed, no significant levels of methylcytosines have been detected so far in the genomes of *P. anserina* and the chromatin status of their RIPped loci is still unknown.

To gain insight into PaRID function, we constructed a knocked-out Δ*PaRid* defective mutant. Remarkably, in contrast to *N. crassa* RID defective mutant, crosses involving *P. anserina* Δ*PaRid* mutants are sterile. We have shown that although gametes are readily formed and fertilization occurs in a Δ*PaRid* mutant background, the sexual development is blocked just before the individualization of dikaryotic cells. Conversely, knockout of the *PaDim2* gene, encoding a second putative DNA methyltransferase related to *N. crassa* DIM-2, had no effect on Podospora's complete life cycle. Complementation of the Δ*PaRid* mutant with ectopic alleles of *PaRid*, including GFP-tagged, point-mutated, inter-specific and chimeric alleles, demonstrated that the catalytic motif of the putative PaRID methyltransferase is essential to ensure proper sexual development and that the expression of *PaRid* is tightly regulated in both space and time. Finally, a microarray transcriptomic analysis of the Δ*PaRid* mutant highlighted the genetic network differentially regulated in the absence of this putative DNA methyltransferase. Altogether, this study sheds a new light on sexual development regulation in a heterothallic model ascomycete.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

Insights into chromosome structure, transposable elements and secondary metabolite gene clusters from the complete genome sequence of *Colletotrichum higginsianum*

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The ascomycete fungus *Colletotrichum higginsianum* causes anthracnose disease on many cruciferous plants, including the model plant *Arabidopsis thaliana*. Previous versions of the genome sequence based on short-read data from 454 and Illumina sequencing were highly fragmented, causing errors in gene prediction and preventing the analysis of repeats and genome architecture. By combining the long reads from single-molecule real-time sequencing together with optical mapping, we obtained a highly contiguous assembly where all 12 chromosomes are sequenced telomere to telomere without gaps except for one region containing the rDNA repeats. The more accurate gene annotation based on this new assembly provided a comprehensive inventory of secondary metabolism-related genes corresponding to 77 putative biosynthetic pathways, suggesting a large capacity for chemical diversity. Similar to the conditionally dispensable chromosomes of other plant pathogenic fungi, the two mini-chromosomes differed markedly from the core genome in being repeat- and AT-rich and gene-poor but were significantly enriched with genes encoding putative secreted effector proteins. Annotation of transposable elements (TEs) revealed that certain TE families showed a statistically significant association with effector genes and secondary metabolism gene clusters and were transcriptionally active at particular stages of fungal development. The complete genome assembly also enabled us to identify chromosome segmental duplications. Four of these were associated with highly-conserved subtelomeric repeats, which may provide sites for homologous recombination. Repeat-mediated segmental duplication may thus be a mechanism for generating genetic diversity in this asexual fungus.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

Analysis of the *Alternaria solani* genome reveals effector protein candidates that trigger cell death in potato

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The fungal genus *Alternaria* consists of saprophytes as well as important necrotrophic plant pathogens. Early blight is a serious disease of potato that is caused by *Alternaria solani* and closely related *Alternaria* species. In *A. alternata*, host specific toxins have been identified that enable it to infect specific hosts, but, as of now, it is still unknown which genes from *A. solani* are involved in causing early blight. We used Single Molecule, Real-Time sequencing technology to sequence the *A. solani* genome to identify genes that contribute to the infection of potato. The finished genome sequence of *A. solani* was compared with the genomes of related *Alternaria* that are not pathogenic on potato, leading to the identification of proteinaceous effector candidates. Transient expression of these putative effectors in potato plants showed that some of them can trigger cell death in susceptible hosts, suggesting a role for these genes in disease development. In parallel, we have performed a wide screen of wild potato accessions for resistance to *A. solani*, which resulted in the identification of *Solanum* genotypes with high levels of resistance. Thus, we provide a promising starting point to explore the roles of proteinaceous effectors, and the underlying molecular mechanisms, in causing susceptibility or resistance of potato plants to early blight. By screening for responses to the effector candidates that were identified in our study, the mapping of host susceptibility factors or resistance genes will be facilitated.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

Bioinformatic as a tool to highlight and characterize extragenomic sequences within *Fusarium verticillioides* strains isolated from Italian *Zea mays* kernels

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Fusarium Link is a genus including ubiquitous plant-pathogenic fungi that may cause severe crop losses. The *Fusarium* genus is divided in species complexes; the species are grouped by physiological, biological, ecological and genetic similarity. The *Fusarium fujikuroi* species complex (FFSC) is one of the largest complexes. Although species within the FFSC complex are closely related, they may have distinct phenotypic traits like mycotoxin production and pathogenicity. In addition to a set of species-specific core chromosomes, some FFSC species may have extra, or supernumerary chromosomes (SCs) that may even differ between isolates in presence/absence or length. SCs in other *Fusarium* have been shown to have important roles in the biology and virulence of these fungi. In a previous study, bioinformatics analysis pinpointed the presence of two additional mega base (Mb) of sequence data in the genome of an “Italian” strain 10027 of *Fusarium verticillioides (Sacc.) Nirenberg (Fv) as compared to* the reference strain (*Fv* 7600) sequenced by the Broad Institute. This additional *Fv* sequence data shares significant similarity to sequence present in the genome of *F. fujikuroi* (Sawada) Wollenw as well as other species within the FFSC. We used sequences within the two Mb of sequence as markers for classifying about 200 *Fv* strains isolated from *Zea mays* L. kernels collected between 2013 to 2016 from northern Italy. A representative subset of 24 of these strains were sequenced using Next Generation Sequencing technology. The sequence data was used to develop a pipeline for highlighting the *inter-intra* specific differences present in the additional two Mb, potential SCs. Moreover, this pipeline identified interesting set of genes belonging to Ascomycota genus with a different Gene Onthology enrichment among the various *Fv* strains isolated across northern Italy.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

Functionality of *Fusarium oxysporum* chromosome telomeric regions in genome plasticity

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The genome of *Fusarium oxysporum* is highly dynamic and contains lineage specific (LS) chromosomes, named 3, 6, 14 and 15, which are rich in transposable elements and are involved in the pathogenic behaviour of the species (Ma *et al.,* 2010). Recent results, using Southern blot hybridisation and sequence analysis have revealed a high degree of structural and physical conservation in the telomeric and subtelomeric regions of the wild type strain (4287), extending approximately 10 kilobases from the chromosome ends. The evolutionary origin of this highly conserved region is currently unknown. In order to analyse the role of these conserved regions in the maintenance and plasticity of chromosome structure, we are studying their functionality in *Saccharomyces cerevisiae* as a heterologous model system.

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Genomes, chromosomes & epigenetics

HFB8, the orphan hydrophobin of *Trichoderma guizhouense*, is involved in mycoparasitism, surface growth and protects hyphae from fungicides

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Hydrophobins (HFBs) are surface-active proteins secreted by filamentous fungi. They can self-assembly in single molecular layers at hydrophobic-hydrophilic interfaces and can, therefore, be directly involved in the establishment of fungi in their habitat. In this work, we have studied the function of hydrophobin 8 (HFB8) that is encoded in genomes of *T. guizhouense* and *T. afroharzianum* from the Harzianum clade of *Trichoderma* (Hypocreales, Ascomycota). *hfb8*-overexpressing mutants were constructed for *T. guizhouense* under a promoter of a constitutively expressed *cdna1* gene of *T. reesei* with an mRFP reporter. Results showed that HFB8 was predominantly secreted and can be readily found in the fungal culture filtrates and also on the fungal cell walls especially on germinated spore surfaces, growing hyphal tips, and near septae. The *hfb8*-overexpressing mutants had substantially increased capacity to colonize the hydrophilic glass surface in water, but their ability to colonize tomato roots was reduced compared to the wild-type strain. Confrontation experiments confirmed the involvement of HFB8 in the interactions between *Trichoderma* and other fungi, which shows reduced antagonism ability of the *hfb8*-expressing mutants. More remarkable, overexpression of *hfb8* showed improved growth of *Trichoderma* cells in the cultivations containing ROS-producing compounds (i.e., menadion) or fungicides (i.e., amphotericin B) demonstrating a “raincoat” effect of this particular HFBs. We conclude that HFB8 plays a role in *Trichoderma* mycoparasitism, attachment to hydrophilic surfaces and it is also required for the physical protection of the hyphal surface against fungicides.

POSTER SESSION 2
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Genomes, chromosomes & epigenetics

Development of artificial fungal chromosomes

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Artificial chromosomes are potentially a valuable tool in the development of stable cell factories. The genetic information is stored in the native manner and propagates in the same way as the original chromosomes. Since chromosomes store a large amount of genetic information it is possible to reconstitute large metabolic pathways. Although there are clear fields of application for artificial chromosomes, to this date there are no vectors in filamentous fungi established that contain the three functional units of chromosomes: A centromere, telomeres and origins of replications. The main problem in the creation of artificial chromosomes in filamentous fungi is, that the structure of the centromere is poorly understood. This makes the *de novo* assembly of chromosomes difficult; another strategy for artificial chromosome creation is to make large scale deletion in existing chromosomes. In order to facilitate these large chromosomal deletion we use a bipartite marker integrated close to the centromere and telomere, and CRISPR-Cas9 to induce double strand breaks. This allows to delete large parts of chromosomes in a controlled manner with strong selection pressure.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

Experimental evolution of the fungal pathogen *Fusarium oxysporum*

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Natural selection is a fundamental evolutionary process that acts on changes in a genome and results in the adaptation of an organism. Genome changes range from single nucleotide polymorphism (SNPs), small insertion/deletion, segmental duplication, chromosomal rearrangement, to whole genome duplication. How do these different changes influence the evolutionary processes under different selection pressure? To explore answers to these questions, this study takes an experimental approach to observe evolving processes using a model organism *Fusarium oxysporum*, a highly adaptive species complex containing the mobile and lineage-specific (LS) chromosomes that are rich for transposons and determines host-specific pathogenicity. The same starting population (*F. oxysporum f. sp. lycopersici* strain 4287, a tomato pathogenic isolate) was passaged ten times through three distinct serial transfers in: its host, on rich media plates, or on minimal media plates. We have sequenced 5 evolved populations from each serial transfer at the end of the experiment. Comparative genomics revealed the presence of segmental duplications and deletions on parts of LS regions, confirming the highly dynamic nature of the *F. oxysporum* genome. Interestingly, the populations that evolved in rich media tended to lose parts of or entire LS chromosomes, suggesting the dispensable nature and energy cost associated with these chromosomes when growing on rich medium. We observed few SNPs which can explain most of the phenotypic changes. These results highlight that different evolutionary constraints determine the outcome when populations are adapting to different environments.

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(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

The mutational spectrum in *Marasmius oreades* fairy rings

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The sources of new haploid genotypes in natural populations are mutation and recombination. In fungi, having no separated germline, variation introduced during vegetative growth is likely to be carried on to the next generation. Still little is known about the genetic mechanisms that produce variation in these organisms under natural conditions. Indications exist of very low rates of spontaneous substitution in some basidiomycetes, suggesting a highly efficient mechanism working to minimize the number of mutations during vegetative growth. In addition, somatic recombination has been suggested to occur in several basidiomycetes, where haplotypes are mixed outside of meiosis, something that could carry great implications to how basidiomycetes evolve in nature. A well-suited system to study genetic variation gained during vegetative growth is so-called fairy rings; fungal mycelia that grow in circular patterns. The initial genotype that gives rise to a fairy ring is the shared throughout the ring, while different sectors have had their own evolutionary trajectories since the initial split. Here we studied genetic variation within six individual fairy rings of *Marasmius oreades*. By sequencing whole genomes of 5-8 fruiting bodies from different sectors of each ring, the numbers and types of new mutations were determined. The results point to a strikingly low number of mutations with substitution rates in the order of magnitude of 10^{-12} per cell division and base pair. This suggests either a higher-than-expected selection pressure keeping mutations to a minimum, or that the mutation rate is indeed low in these organisms. Current work also includes investigating the occurrence of somatic recombination using protoplast isolation and long-read sequencing to generate full genome sequences of the two haploid nuclei in the dikaryon.

POSTER SESSION 2
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Genomes, chromosomes & epigenetics

Impala-based transposon mutagenesis is influenced by chromatin modifications in the fungal plant pathogen *Zymoseptoria tritici*

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Transposition of *TC1-mariner impala* from *F. oxysporum* was tested in *Zymoseptoria tritici*. We used an existing excision assay in which *impala* is inserted in *A. nidulans* nitrate reductase gene promoter. This vector was introduced in a *Z. tritici* nitrate reductase mutant unable to grow on nitrate minimal medium (MM). Inoculating these transgenic strains onto MM allowed recovering revertants (10-30/plate). Almost all revertants corresponded to excisions of *impala* from *niaD* (95%). Inoculum culture conditions had a significant effect on *impala* excision rate, the optimum being obtained with yeast-like cells grown on YPD for 5 days at 18°C. Varying culture conditions during reversion assay (carbon starvation, light, darkness, heat or cold shocks, and copper stress) had no effect on *impala* excision rate. However, adding histone deacetylase inhibitor trichostatin at a sub-inhibitory concentration (0.1 microM) to MM during reversion assays, significantly increased *impala* excision rate. This unexpected result suggests that modifying histone acetylation level has an effect on *impala* excision either as a consequence of modifying chromatin status at vector integration site, or through a direct effect on *impala* machinery. In most revertants (90%), *impala* was inserted in *Z. tritici* genome. 60 *impala* re-insertion loci were characterized by Ligation-mediated PCR amplification and sequencing. *Impala* was inserted at random locations in core chromosomes (no hot spots), but not in accessory chromosomes (0/8 expected insertions). Since accessory chromosomes mostly carry repressed chromatin, this result suggests that *impala* preferentially inserts in regions with open chromatin. *Impala* also inserts at lower rate in native transposons (7%) compared to random (30%), suggesting that it avoids these regions. Overall, *impala* was preferentially inserted either at the 5' end of transcriptionally active genes (5'UTR, promoters; 68%) or in genes (14%). These results show that *impala* is active in *Z. tritici*. Its integration patterns make it particularly suitable for insertional mutagenesis.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

A long noncoding RNA promotes cellulase expression in *Trichoderma reesei*

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Trichoderma reesei is a saprotrophic fungus that is used in industry for production of carbohydrate-active enzymes such as cellulases. We discovered an intergenic region as a regulatory factor that influences the expression of these enzymes. It turned out that this region actually codes for a long non-coding RNA (*HAX1*). Interestingly, the length and consequently the length of *HAX1* seem to have evolved during the strain selection process done for industrial purposes. We observed a *HAX1*-dependent phenotype in differently good enzyme producing strains. The essential transactivator of the cellulases (i.e. the Xylanase regulator 1) and *HAX1* do not only share a palindromic sequence, *HAX1* also bears an unusual number of binding sites for the transactivator. We wished to learn if and how those two interact and how exactly the regulation of gene expression works on the molecular level. Based on our recent findings we are able to present a model on this regulatory mechanism.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

Meiotic Drive vs. Meiotic Silencing. Intragenomic conflict in *Neurospora*

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In order to prevent the uncontrolled expansion of selfish elements within their genomes, organisms have developed numerous mechanisms to monitor genome integrity. An important mechanism in fungi is meiotic silencing of unpaired DNA (MSUD). MSUD uses RNAi machinery to transcriptionally silence genomic regions that do not pair during meiosis. This mechanism has been studied thoroughly in the model fungus *Neurospora crassa*. In the closely related species *N. sitophila*, a type of meiotic drive known as spore killing has been observed. Crosses between strains containing the driving allele (Sk-1) and strains without the allele (sensitive), will result in the death of spores that do not possess Sk-1 with 90% efficiency. We have identified the Sk-1 locus, which contains ~1kb of novel sequence not found at the sensitive allele. Additoinally, analysis of the locus suggests that it was introduced from a closely related species. Based on studies in *N.* crassa, MSUD should silence Sk-1 in these crosses. We have observed that F1 strains from crosses between killer strains and sensitive strains from Tahiti show a reduced killing efficiency, as low as 20%, when backcrossed to the sensitive parent. To determine if this is due to the action of MSUD, we have generated a Sad-2 deletion mutant, which does not have a functional MSUD system. In the deletion strain, killing efficiency of the F1 strains is restored to nearly 100%. These results demonstrate that *N. sitophila* possesses an active MSUD defence system that is ineffective against Sk-1 in outcrosses, but effective in inbred lines. Furthermore, these results could not be replicated with strains from Europe, suggesting that Sk-1 is able to completely evade MSUD in these strains. This work highlights the interplay between genome defence and selfish elements and provides a powerful system in which to study MSUD and meiosis in general.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

Comparative genomics to assess myco-parasitism and pathogenicity in oomycetes with potential as biocontrol agents

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The oomycetes are a lineage of filamentous Eukaryotes most closely related to the heterokont (brown) algae. Many oomycetes are plant pathogens, however, some Pythium species are parasitic to fungi and other oomycetes (*Pythium oligandrum* and *Pythium periplocum*) whilst others such as *Lagenidium giganteum* are parasites of insects including mosquitos that vector Zika virus and Dengu fever. These three species have great potential as biocontrol agents. Molecular, genetic and genomic data from these species provides new insights into pathogenicity and the fundamental biology of oomycetes with diverse lifestyles beyond the well-studied plant pathogens. We have carried out *de novo* genome sequencing and genome-wide transcriptomics (RNA-seq) of these three understudied oomycetes, using both paired end and mate pair Illumina Hiseq sequencing. We are currently investigating the genetic and molecular determinants of host specificity using this data. Crinkler (CRN), and elicitin effector proteins are present in the predicted secretomes of all three oomycetes. Novel effector families are also present in the mosquito pathogen. RNA-seq data from *P. oligandrum* colonising the potato late blight oomycete *Phytophthora infestans* reveals that secondary metabolite biosynthesis is important for mycoparasitism. This is the first time secondary metabolites have been shown to play a role in oomycete pathogenicity. Novel transcripts, including putative R-genes from *P. infestans* reveal how this oomycete defends itself against mycoparasitic attack, and may provide vital clues for the sustainable control of potato late blight in the future. Traits important for successful mycoparasitism are being revealed by comparative analyses of the hyper-aggressive mycoparasite *P. oligandrum* versus the weaker mycoparasite *P. periplocum*. Our latest comparative genomics and transcriptomics data including the identification of novel effector gene families will be discussed. These data will pave the way for a better understanding of pathogenicity in diverse hosts and facilitate the development of new biological control agents.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

The *Colletotrichum orchidophilum* genome sequence: an example of how closely related species improve pan-genome analyses in fungal plant pathogens

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Many species belonging to the genus *Colletotrichum* are associated with plant diseases, commonly referred to as anthracnose. *Colletotrichum* spp. can affect a wide range of hosts and these pathogens are characterized by a global distribution. In addition to its economic impact, *Colletotrichum* is one of the most studied genera of plant pathogenic fungi. *Colletotrichum orchidophilum* is a plant pathogenic fungus infecting a wide range of plant species belonging to the family Orchidaceae. *C. orchidophilum* has been used in recent years in evolutionary studies to study evolution and host specialization in plant pathogens, as it represents the closest related species to the *C. acutatum* (CA) species complex. Here we present the first draft whole-genome sequence of *C. orchidophilum* IMI 309357, providing a platform for future research on anthracnose of Orchidaceae and for evolutionary analyses of other *Colletotrichum* spp. The *C. orchidophilum* genome assembly consists of 321 scaffolds and encodes 14,496 proteins. We compared the newly sequenced genome with all *Colletotrichum* genomes publically available investigating the need for higher resolution taxonomic sampling for comparative genomics. Our results show that *C. orchidophilum* shares 85,4 % of the proteins with other CA species and the number of shared proteins is strongly correlated with phylogenetic distance. Further analyses revealed that 1564 have a predicted secretion signal peptide and of those 86 are *Colletotrichum* specific and 76 are unique to *C. orchidophilum*. Phylogenetic analyses of specific gene families (such the GH43) show that gene loss in *C. orchidophilum* is an important force driving the evolution of gene family size. This study also shows that major phenotypic changes are associated with comparatively recent changes in gene content.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

Designing genome-wide mutagenesis approaches for non-model organisms by leveraging an artificial transposon, deep sequencing, machine learning and a stable haploid isolate

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The human pathogen *Candida albicans* was recently found to have a viable haploid state (Hickman et al. 2013). We used a stable haploid isolate as the basis for an *in vivo* transposon screen by exploiting the heterologous mini-*AcDs* transposon system (Weil and Kunze 2000). Together with high throughput TnSeq deep sequencing (van Opijnen, Bodi, and Camilli 2009) and analysis, we generated a large-scale pooled library of mutants carrying insertions that can be screened for many functions to identify the mutants that are enriched or depleted under a given condition.

We first used the collection to determine gene essentiality comprehensively across the complete *C. albicans* genome. We applied Random Forrest machine learning algorithms trained on sets of genes reported in the literature to be non-essential in *C. albicans*, together with *C. albicans* orthologs of genes essential in both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*.

As expected, the essentiality of genes that are orthologous between *C.albicans and/or S. cerevisiae and S.pombe* is well conserved. We identified “core” essential genes necessary for viability in all three organisms. Outliers include genes duplicated in specific genomes and not in others. Furthermore, we found that some genes listed as essential can tolerate insertions outside of specific domains, highlighting the importance of specific domains in gene function and the ability of transposon mutagenesis to detect them. The identification of genes essential in fungi and not in humans also highlights potential targets for the development of new antifungal therapeutics.

In summary, in vivo transposon mutagenesis is a facile method for studying gene essentiality in non-model eukaryotic pathogens. This library has much potential to identify essential genes, and new genes or genomic regions involved in a broad range of measurable functions.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

The Aspmine - comparative genomics analysis of 9 new species of Aspergillus section Sparsi, Ochraceorosei, Tanneri and Rubusti

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In this work, we present the new whole genome sequences, functional annotation and comparative analysis of 6 filamentous fungi of the Aspergillus genus. The new species belong to the sections of Sparsi (3), Ochraceorosei (1), Tanneri (1) and Rubustii (1).

Species of section Sparsi, *A.* funiculosus, *A.* implicatus and *A.* biplanus are found in warm soil climates and produce antimicrobial compounds and toxins such as kojic acid, auraglaucin, gregatins, funicin and sidins. The section Ochraceorosei (suggested 2009) consists of *A.* ochraceoroseus and A. rambelii. *A.* ochraceoroseus, these species produce the mycotoxin aflatoxin B1.

The analysis presented here include genome sequence quality, secondary metabolism potential, carbohydrate degradation potential, shared proteomes and species as well as section specific genes. Comparisons were made to other filamentous fungi, Penicillium (3), Neurospora (1) and Aspergillus (49 species, 32 from section Nigri)

The species of Ochraceorosei have a much smaller number of predicted genes than the other species in the set (7.800-8.200). This is in comparison to some of the Nigri species with up to 18.000 genes. Section Sparsi species have a very wide range of predicted genes (9.000-15.000) while *A.* tanneri falls in the midrange (13.000). The large range of predicted genes illustrates the large diversity within these species.

Analyzing the CaZyme distribution of the 6 species revealed a diversity comparable to that of section Nigri. In the analysis of secondary metabolism, we find shared and conserved clusters within some sections while other sections have not associated clusters. Unique gene clusters are found in all the newly sequenced genomes, to the same extent as found in the Aspergilli in general.

The six new species provide additional information to the comparative genomics studies of Aspergillus and illustrate the large diversity and application of species in this genus.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

Patterns of genome evolution across the fungal kingdom

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A comparative genomics analysis of over 200 fungal genomes has revealed large differences in genome evolution across the fungal kingdom. These differences are caused by variable rates of interchromosomal rearrangements, variable inversion lengths in intrachromosomal rearrangements, and variable rates of tandem duplications. Each class of fungi follows its own pattern of genome evolution. An important driving force behind these diffences are repetitive elements. Moreover, these various patterns of genome evolution have important implications for gene localization.

For example, it was previously shown in the class Dothideomycetes (phylum Ascomycota) that a low rate of interchromosomal rearrangements and high rate of intrachromosomal rearrangements leads to mesosynteny, where gene content but not gene order is conserved. In the class Saccharomycetes (phyum Ascomycota), in contrast, interchromosomal rearrangements occur frequently, quickly degrading conserved syntheny. A similar pattern of genome evolution as in Dothideomycetes occurs in the class Agaricomycetes (phylum Basidiomycota), with the important difference that the intrachromosomal inversion length is much smaller. This results in a distinct pattern only found in this class. Moreover, in Agaricomycetes several conserved ancestral chromosomes could be identified with varying patterns of genome evolution, likely due to their difference in sequence composition.

On gene level, conserved gene clusters were identified despite the observed intrachromosomal inversions. Notably, certain transcription factors were over-represented in these conserved gene clusters.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

How to handle big data in fungal genomics?

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Genomics has a transformational effect on biology. The genome of Saccharomyces cerevisiae was the first sequenced fungal genome and played a critical role in developing a variety of molecular tools to move biology forward. With additional sequenced species, comparing unique genes, gene family expansions and contractions, for dozens of diverse fungal species shed light on genetic toolkits of mycorrhizae, pathogens, and saprobes.

Genomes of over 1000 of fungal species have been sequenced and this number continues to grow in the large scale projects like the 1000 fungal genomes project, which samples diversity across the entire Fungal Tree of Life, or the 300 Aspergillus genomes, focused on a single genus. In addition, large-scale functional genomics studies employing transcriptomics, proteomics and other omics approaches like fungal ENCODE add new dimensions to the genomic data.

Genomics Big Data has a huge unexplored potential but we can no longer efficiently explore hundreds millions of data points staring at the tables of gene counts. How to visualize Big Data in fungal genomics? What are the determinants of fungal lifestyles? Can we predict them from genomics sequences? These questions and new approaches to answer them for specific groups of fungi will be discussed.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

FungiDB: an integrated functional genomics database for fungi and oomycetes

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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 searches and create in-silico experiments via an intuitive web-based graphical system. FungiDB contains genome sequence and annotation for over 75 species spanning the oomycetes and fungi, the latter including genomes of Agaricomycetes, Chytridiomycetes, Eurotiomycetes, Leotiomycetes, Pneumocystidomycetes, Pucciniomycetes, Saccharomycetes, Schizosaccharomycetes, Sordariomycetes, Tremellomycetes, Ustilaginomycetes, and Zygomycetes. In addition to genomic sequence data and annotation, users can explore functional data (eg. transcriptomics or proteomics), whole genome polymorphism data, metabolic pathways and results from genome wide analyses such as InterPro scan, signal peptide and transmembrane domain predictions, GO term and EC number associations and orthology profiles. FungiDB supports the capture of community knowledge in the form of user comments. Such comments may include free text updates and information, images, files, PubMed records, etc., and once submitted to FungiDB records (ie. gene pages) they 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. In addition, user datasets can be analyzed in the EuPathDB Galaxy instance, which offers preloaded genomes and several sample workflows for RNASeq and SNP analysis pipelines. Analysis results can be further explored and visualized in the FungiDB genome browser.
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POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

Dynamic gene duplication/loss history marks the unique evolutionary route to fungal multicellularity

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Multicellularity has evolved numerous times during eukaryote evolution, yet the genetic prerequisites for these transitions are hardly known. In contrast to other organisms fungi used their own unique evolutionary route to achieve multicellularity with different physiological bases. This raises the question whether the genetic-mechanistic principles of the evolution of multicellularity are common to both fungi and animals and how fungal multicellularity-related gene families evolved during the history of life. Here we reconstruct the evolution of the genetic background of fungal multicellularity based on both known multicellularity-related genes from the literature and genome-wide identification of gene families that evolve in a correlated fashion with multicellularity. Based on literature surveys, we identified 875 genes involved in the establishment and maintenance of cell polarity, vesicular transport and cytoskeletal rearrangement. The evolutionary origins of these genes were examined using complete genomes of 76 unicellular and multicellular eukaryotes. We implemented phylostratigraphic analyses using a custom pipeline, which uncovered the evolutionary origins of multicellularity-related genes, and reconstructed gene duplication and loss histories by COMPARE analysis. These yielded a high-resolution view of the dynamics of these multicellularity-related gene families. Further we could identify 316 gene families, including certain cytochrome P450 families, monocarboxylate permeases and vacuolar aspartyl proteases that show strong correlated evolution with multicellularity, providing candidates for future functional studies. Our results demonstrate that part of the genetic toolkit behind fungal multicellularity was already present in ancestral unicellulars and that some of the hyphal morphogenesis related gene families diversified before the emergence of the first filamentous fungi. This suggests that beside gene duplications and *de novo* gene family birth, the rewiring of gene regulatory networks could have had a crucial role in the evolution of multicellular fungi.

POSTER SESSION 2
(Tuesday, February 27, 2018 13:45-15:30)

Genomes, chromosomes & epigenetics

Multivariate analysis of Diversity in *Botrytis cinerea* Isolates from Israel

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The necrotrophic fungus *Botrytis cinerea,* known as the causal agent of gray mold, is ranked second for its phytopathological global-impact. The disease is controlled by agrotechnical means and fungicides, however, these techniques have a variable effect on different fungal isolates. Several studies have scanned for resistance in wild-type tomato lines, however, these works utilized one or few fungal isolates, and plant organs. Here we studied and characterized *B. cinerea* isolates from different sources and conditions.

We characterized 31 *B. cinerea* isolates, collected from six hosts in different locations of Israel. Based on the initial screening on leaves and stems, ten isolates that exhibited significant phytopathogenic variability were selected for further study. Two genetic markers (microsatellite) Bc-1 and Bc-7, were able to differentiate between these isolates. Characterization of these isolates showed 178, 68, 142, and 62% (maximal) differences in the rate of necrosis on detached leaves or stems, infection-rate on plants, and saprophytic growth-rate on the solid substrate, respectively. When necrosis-rate was normalized by saprophytic growth-rate, we found 156 and 86% differences among isolates inoculated on leaves and stems, respectively, which could not be related to host-free growth capacity. These isolates also show wide variation in resistance to oxidizing paraquat previously correlated with virulence. Based on these results hierarchical clustering enabled highlighting isolates with higher virulence and paraquat resistance. Further analysis demonstrated that these isolates had higher expression of superoxide dismutase (SOD1), which was previously shown to play an important role in pathogenicity, then an isolate demonstrating lower virulence and paraquat resistance.

In line with reported plasticity for *B. cinerea*, isolates from different sources showed genetic, physiologic and phytopathogenic variability. Selected isolates containing significant variability will facilitate future research aimed to identify and subsequently breed for sustainable resistance in cultivated tomato.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Evolution & taxonomy

Understanding the genomic and transcriptomic changes leading to morphological simplification in fruiting body forming Basidiomycete *Schizophyllum commune*

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The evolution of complex multicellular development is affected by a range of genetic innovations, among which changes to the regulatory repertoire are generally considered to have great significance. Throughout evolution we can observe a definite tendency of morphological complexity being coupled with the size of the gene regulatory apparatus – mainly with the number of transcription factors (TFs). Our study focuses on placing morphologically simplified organisms in this chart: do we see a decreased number of TFs in simplified organisms compared to their more complex relatives? In order to find out we performed comparative genomic analysis on 41 fruiting body forming Basidiomycete fungi and comparative transcriptome analysis during five developmental stages of four species of the Agaricales (*Armillaria ostoyae*, *Coprinopsis cinerea*, *Schizophyllum commune*, *Auriculariopsis ampla*) – two complex and two simplified fruiting body forming ones. Surprisingly the morphologically simplified organisms contain a higher number of TFs than most of the species with a more complex morphology. Examining the age distribution of TFs we discovered that genomes of simplified organisms contain the highest proportion of family or species specific regulator genes. Furthermore RNA-Seq data shows that these newly evolved genes play a crucial role during fruiting body formation – so their development is more dependent on evolutionarily young regulators. Our results contribute to a more comprehensive understanding of the development of mushroom fruiting bodies and to that of secondarily simplified species. The species we observed might be interesting examples of simplified morphology as an evolutionarily favorable attribute.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Evolution & taxonomy

The genetic background and transcriptome level changes during fruiting body development of fungi

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Fruiting bodies are complex, three-dimensional reproductive structures, which emerged independently in many distantly related groups of Fungi. The roles of several regulators and structural genes in fruiting body development have been uncovered; yet, the general principles of fruiting body formation remain largely unknown. We set out to identified the conserved gene set underlying fruiting body initiation and development of altogether six species (*Armillaria ostoyae* C18*, Coprinopsis cinerea* AmutBmut*, Lentinus tigrinus* RLG-9953-Sp*, Phanerochaete chrysosporium* RP-8*, Rickenella mellea* SZMC22713, *Schizophyllum commune* H4-8*)* of distantly related groups of Agaricomycetes using comparative transcriptomics and genomics. We performed paired-end RNA sequencing on five developmental stages (vegetative mycelium, early and late primordium, young and mature fruiting body) and three tissue types (cap, lamellae, stipe). Our results demonstrate that 10-40% of the expressed genes of each species are developmentally regulated, most of which showed differential expression during fruiting body initiation. We identified numerous genes that were shared among species and showed upregulation at the early primordium stage. The molecular functions of the encoded proteins were associated with cell wall component synthesis, mRNA stability, cell growth and regulation of transcription. In addition to shared gene families, similarity was observed at the global transcriptome level, as inferred by Pearson correlation coefficients across each pairs of transcriptomes. In general, transcriptomes showed highest similarity within species, suggesting that phylogenetic affinity predicts gene expression patterns better than tissue or stage identity. Nevertheless, shared expression patterns across primordium stages of different species suggest a conserved early developmental program conserved across the Polyporales and Agaricales. This pattern was more pronounced in expression patterns of transcription factor genes, suggesting that regulators constitute a major part of this conserved developmental program. Our results contribute to understanding the general principles of fungal fruiting body development and its initiation, and represent a step towards identifying conserved and species-specific elements of fruiting body development.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Evolution & taxonomy

Insight by proxy – investigating the evolutionary history of beauvericin biosynthesis through ketoisovalerate reductase accessory gene

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Study of core fungal biosynthetic genes is frequently complicated due to multi-modular structure and tangled history of key synthase families. In this case, relatively simpler accessory genes responsible for early parts of biosynthetic process (e.g. substrate synthesis) can provide additional insight as proxies sharing common history after recruitment into the biosynthetic cluster. We have investigated the origins of key accessory enzyme in beauvericin (cyclic oligodepsipeptide) biosynthesis – ketoisovalerate reductase (KIVR) – from its emergence in a subfamily of NADP-dependent reductases involved in alternate pyrimidine biosynthetic route, throughout its role as provider of hydroxyisovaleric acid metabolite, until its strong genetic linkage as part of cyclic oligodepsipeptide beauvericin cluster.

Our studies pinpoint the recruitment of ketoisovalerate reductase to a common hypocrealean ancestor and its spread via postulated duplications and horizontal gene transfer throughout key genera in *Cordycipitaceae* and *Nectriaceae*. The existence of beauvericin cluster within divergent complexes of phytopathogenic *Fusaria* is shown to be paraphyletic, with independent acquisition of cluster by species in *F. oxysporum* and *F. tricinctum* complexes. The remarkable mechanistic conservation of reductase structural template point to the crucial role of regulatory rewiring of KIVR during its emergence as key substrate (D-Hiv) provider for beauvericin biosynthetic process. Prospects for use of KIVR as a marker of toxigenicity, as well as possibility of KIVR complementation in low producing strains of pathogenic/biocontrol *Hypocreales* are discussed as well.

Keywords: cyclodepsipeptides, beauvericin, bassianolide, *Hypocreales*

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POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Cell regulation & signaling

Calcium singling genes are critical for growth, development and circadian clock in *Neurospora crassa*

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We found that the calcium (Ca^{2+}) signaling genes *cna-1*, *cnb-1*, *ncs-1*, *plc-1*, *splA2*, *cpe-1*, *cam*, *camk-1*, and *camk-2* are critical for the growth and development in the model filamentous fungus *Neurospora crassa*. The *cna-1* and *cnb-1* genes encodes the calcineurin catalytic and the regulatory subunits, respectively, and we isolated their dominant negative mutants that affected growth and circadian rhythm in *N. crassa*. Additionally, we found that the calcineurin interacts with the calmodulin (CaM), and transcription factor CRZ-1. The CRZ-1 is localized in nucleus after its dephosphorylation by the calcineurin to upregulate the expression of *ncs-1* in response to Ca^{2+} stress. We propose a model that the calcineurin dephosphorylates CRZ-1 promoting its nuclear localization to upregulate the NCS-1 expression for survival under cellular stress condition. Thus, we showed that the Ca^{2+} signaling genes play a critical role in regulating growth, development, and circadian clock in *N. crassa*.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Cell regulation & signaling

The chitin synthase regulator CSR-3 and the SO protein function together in cell‑cell fusion and stress-induced cell wall remodeling in *Neurospora crassa*

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Chitin is an important component of the fungal cell wall, whose synthesis is mediated by chitin synthases and chitin synthase regulators with defined tasks. For instance, response to cell wall stress usually requires chitin synthesis which is controlled by the cell wall integrity (CWI) pathway. In the ascomycete *Neurospora crassa* the MAP kinase MAK-1 and the potential scaffold protein SO are part of CWI signaling. These proteins also play an essential role in cell-cell fusion of germinated spores. This process results in an interconnected, supracelluar network, the mycelium. During fusion the cell walls of the interacting partners are partially degraded before the opposing plasma membranes merge. This cell wall remodeling bears the risk of cell lysis and probably requires a fine-tuned equilibrium between chitin synthesis and depletion.

A Y2H-screen revealed that the SO protein physically interacts with the two upstream MAP kinases of three-tiered MAK-1 MAP kinase module and the chitin synthase regulator 3 (CSR-3). When fusing germlings achieve physical contact, CSR-3 and SO together with MAK-1 and it upstream kinase MEK-1 co-localize at the fusion point until cell merger has been completed. A *csr-3* knockout mutant tends to lyse during this process, suggesting a supporting role of CSR-3 during fusion pore formation. In addition, exposing cells to cell wall stress results in recruitment of those factors to the cell surface. CSR-3 and its potential target chitin synthase 2 also seem to play a role in septation in germlings and in hyphae.

Based on these observations, we hypothesize that shared molecular factors are involved in cell-cell fusion and stress induced cell wall remodeling.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Cell regulation & signaling

Role of regulated proteolysis in the response to cation stress and alkaline pH tolerance of *Aspergillus nidulans*

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Three regulatory systems coexist in *Aspergillus nidulans* and other filamentous fungi to provide with adequate response to ambient alkaline pH and excess of cations. The calcineurin-dependent transcription factor CrzA is required for growth at alkaline pH and in conditions of high extracellular calcium concentrations. PacC is the well known regulator of ambient pH signaling and SltA is required for tolerance to high alkali-cation concentrations and to alkalinity. CrzA and PacC homologues are present in almost all known fungal genomes, however SltA homologues can only be found in species of *Pezizomycotina* subphylum. All these transcription factors are subjected to posttranslational modifications for modulating their activities. Particularly PacC and SltA are subjected an irreversible posttranslational modification, egulated proteolysis. Here we present our analysis of the Slt regulatory system. SltA is synthesized in a 78 kDa form which is proteolysed to a functional 32 kDa form. SltB is the second element identified in this pathway. SltB is probably a bi-functional protein, with a pseudo-kinase domain and a chymotrypsin-like domain. Our studies identified SltB as the protease involved in SltA78kDa to SltA32kDa proteolysis. Notably, SltB is also subjected to a proteolytic step to become active. Interestingly, suppressors of poor growth phenotype derived of deletion of *vps* genes provided us with a large battery of mutations affecting either *sltA* or *sltB*. Characterization of these mutations is allowing us to decipher the mechanism of SltA signaling. In this presentation we will show our current knowledge of the Slt pathway.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Cell regulation & signaling

Identification and characterization of PDC1, a novel protein involved in the epigenetic cell degeneration Crippled Growth in *Podospora anserina*

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Many fungi display epigenetic instability. In *Podospora anserina*, the epigenetic cell degeneration "Crippled Growth" (CG) is characterized by slow growth, alteration of pigmentation and inability to differentiate fructifications and aerial hyphae (Silar,1999). CG is due to a cytoplasmic and infectious element called *C* produced during stationary phase. Two types of CG mutants were isolated: *IDC* mutants impaired in the development of CG and *PDC* mutants promoting the development of CG (Haedens *et al*., 2005). The former have been well studied and all mutants are affected in the MAPK1 pathway (Kicka & Silar, 2004 ; Kicka *et al*., 2006), the MAPK2 pathway (Lalucque *et al*., 2012), the Nox1 NADPH oxidase complex (Malagnac *et al*., 2004 ; Lacaze *et al*., 2014), or in small cysteine-containing proteins probably involved in signal transduction (Lalucque *et al*., 2017). Here, we present for the first time an analysis of a *PDC* mutant, the PDC^{2205} strain mutated in the *pdc1* gene coding the PDC1 protein. This 290 amino acids protein is present in most Ascomycota. All the proteins homologous to PDC1 show a conservation of six amino acids: four cysteines, one histidine and one tryptophan. Site directed mutagenesis revealed that each of these amino acids plays an important role in PDC1 activity. Moreover, epistasis analyses have been conducted with available *IDC* mutants to place PDC1 with respect to the other actors of CG. Cytological observations, in progress, should reveal the cellular localization of PDC1.

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POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Cell regulation & signaling

A role for the velvet protein VE-1 on transcription in *Neurospora crassa*?

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The molecular mechanisms of the perception of light have been investigated in detail in *Neurospora crassa*. Light causes many effects on behaviour and development in *N. crassa*, including the formation of asexual and sexual structures, and the regulation of carotenoids biosynthesis and the circadian clock. The velvet family of regulatory proteins is highly conserved among fungi. All the velvet proteins contain a velvet domain with a DNA binding domain, presumably for gene regulation. The *Aspergillus nidulans* velvet protein VeA plays a key role in coordinating secondary metabolism and developmental regulation, and its localization in the cell is regulated by light. The genome of *N. crassa* contains four genes with the velvet domain. A strain with a deletion of *ve-1* (orthologous of *veA*) has defects in aerial hyphal growth and increased conidiation. In order to characterize the potential transcriptional regulatory role of VE-1 we have performed RNAseq analysis of the *N. crassa* wild type and *Δve*-1 mutant grown in the dark or exposed to light. In addition, we have analysed the transcriptome of the two strains as they progress from vegetative growth to conidiation. Our results will allow us to identify genes regulated by VE-1 during the transcriptional response to light and during conidiation. We observed a higher number of genes regulated during the conidiation stage in the wild type (2653) compared to the mutant (916). These significant differences suggest an important role of VE-1 as a transcriptional regulator of conidiation.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Cell regulation & signaling

Cross-talk between osmosensing and cAMP signalling in *Botrytis cinerea*

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Fungi rapidly adapt to their environment involving to signalling pathways like those of mitogen activated protein kinases (MAPKs). The osmotic signal transduction (ST) cascade in the grey mold agent *Botrytis cinerea* is controlled by the sensor histidine-kinase Bos1 and the Hog1-like MAPK Sak1. It is involved in the adaptation to various stresses (osmotic, oxidative, cell-wall) including some fungicides, but also in fungal development and pathogenicity. Since previous studies highlighted links of the osmotic signalling pathway to other ST cascades we undertook a comparative proteomics study between the *bos1, sak1* mutants and the parental wild-type grown under *in vitro* conditions.

Applying shot-gun proteomics to mycelial extracts of the three strains, we detected 2425 proteins out of which 638 showed significant differences in abundance among the strains. While 275 proteins were clearly co-regulated by Bos1 and Sak1, 150 were under control of Bos1 solely and 213 under Sak1 control. Among this last category of proteins we saw enrichment in ST proteins. Particularly, proteins of the G-protein, cAMP and calcium ST pathways were affected by Sak1.

Additional data from partial phosphoproteomics after fungicide exposure corroborate these interactions through the identification of a differentially phosphorylated phosducin-like protein and the calcineurin responsive transcription factor Crz1.

Phosducin A (PhnA) is involved in G-protein signalling and hence cAMP pathway. Functional analysis of *phnA* revealed its involvement in development, pathogenicity and adaptation to cell wall stress, some phenotypes common to Δ*sak1*. We did not observe any influence of PhnA on Sak1 phosphorylation, but increased cAMP-levels in the *Δsak1* mutant. This result is in agreement with our proteomic data.

In conclusion, our study showed unexpected interaction in *B. cinerea* between the osmotic ST MAPK and the cAMP pathway that may explain several phenotypes of the *Δsak1* mutant. How this interaction operates remains to be established.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Cell regulation & signaling

RNA-seq analysis of the biological roles for the WcoA and CryD photoreceptors of *Fusarium fujikuroi*

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Light plays a major role as an environmental signal for fungi. They use it to control a large diversity of biological processes, such as development and sporulation, adaptation to stress conditions, circadian rhythmicity and secondary metabolism. The outstanding chemical diversity of the secondary metabolites produced by many *Fusarium* species makes these fungi attractive research models. The rice pathogen *Fusarium fujikuroi* is well known in the study of the synthesis of some metabolites, as bikaverin, gibberellins, and carotenoids. As in *Neurospora crassa*, this fungus accumulates the xanthophyll neurosporaxanthin in response to light through the transcriptional induction of the structural genes of the carotenoid pathway. In contrast to *N. crassa*, the mutation of the gene for the only white-collar protein of *F. fujikuroi*, WcoA, slows down the synthesis of carotenoids under light, but does not impede its accumulation. Moreover, a *wcoA* mutant exhibits a more complex phenotype, that includes a reduced synthesis of carotenoids in the dark, as well as alterations in growth, mycelial hydrophobicity and production of other secondary metabolites, as bikaverin. To gain more insights on the global regulatory roles of WcoA both in the dark and as a photoreceptor, RNA-seq analysis of a wild type strain and a *wcoA* mutant have been performed in darkness and after different times of illumination. To test former observations that point to DASH cryptochrome CryD as an accessory photoreceptor in carotenogenesis, the data, currently under analysis, have been extended to the effect of light in a mutant of this cryptochrome gene.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Cell regulation & signaling

Intracellular pH as a new mechanism for MAPK signaling in fungi

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pH is a key player in the control of fungal pathogenicity. We previously found that extracellular pH governs pathogenicity in the plant pathogen *Fusarium oxysporum* by reprogramming phosphorylation levels of mitogen-activated protein kinases (MAPKs). The molecular events underlying the pH response are currently unknown. Here we identify intracellular pH (pH*) as a key signal regulating MAPK activity in F. oxysporum*. Using the ratiometric GFP-based pH sensor pHluorin, we found that *F. oxysporum* responds to extracellular alkalinisation and acidification with a transitory shift in pH*. Exogenous application of diethylstilbestrol (DES), a specific inhibitor of the plasma membrane H^+-ATPase Pma1, induced a rapid and sustained decrease of pH accompanied by rapid and transitory changes in MAPK phosphorylation, supporting the idea that pH acts as a key switch controlling MAPK activity. To search for fungal proteins involved in pH-mediated MAPK regulation, we screened a subset of acid-sensitive mutants from the yeast deletion library for loss of DES-triggered MAPK phosphorylation. This identified a number of candidates functioning in conserved cellular processes such as lipid metabolism, endocytosis or V-ATPase function, many of which have predicted orthologs in Fusarium*. Understanding how pH *regulates MAPK signaling may reveal new ways to control fungal growth, development and pathogenicity.*

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Cell regulation & signaling

The role of homeodomain transcription factors in fungal development

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Homeodomain (HD) transcription factors are well established as regulators of development in animals since the identification of homeobox gene clusters in Drosophila melanogaster. However, in fungi this class of transcription factors remains poorly characterized. In fungi genes with HD domains were first identified in mating type loci, which regulate sexual compatibility and development. In both Ascomycota and Basidiomycota several HD transcription factors have previously been shown to play an important role in multicellular development and fructification, including *hom1* and *hom2* in *S. commune*. Furthermore, our preliminary results suggest *hom3* in *S. commune* is also involved in fructification. We used a phylogenetic analysis of 222 previously published genomes to provide an evolutionary framework for HD transcription factor function in fungi. On average fungi with a yeast-like lifestyle carry fewer HD transcription factors than multicellular fungi. Particularly HD genes related to complex multicellular development and fructification are lacking in yeast-like fungi. Ascomycota and Basidiomycota both feature distinct HD gene groups, with Basidiomycota carrying more genes and featuring broader diversification. However, a single group of conserved HD genes across both Basidiomycota and Ascomycota has a role in fructification in both Phyla, suggesting shared regulatory pathways. Most HD transcription factor groups we identified do not have any characterized members and as such potential functions cannot be predicted. This emphasizes the necessity of powerful molecular tools in fungi to reliably combine *in silico* functional prediction with *in vivo* characterization.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Cell regulation & signaling

MpkB MAP Kinase is dispendible for production of mycotoxin in *Aspergillus nidulans* and *Aspergillus flavus*

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Mitogen-activated protein kinase (MAP Kinase) pathways regulates the growth, development and stress responses in most of eukaryotes. *Aspergillus nidulans* MAP Kinase encoded by *mpkB* was known to coordinate sexual development as well as secondary metabolism. Also, it had been reported that the *mpkB* gene could regulate sterigmatocystin (ST) gene expression and produce mycotoxin at low levels. However, the results of the TLC investigation in this study shows that *mpkB* gene did not affect the ST production and ST related gene expression. In the *veA*$^{+}$ background, ST production of Δ*mpkB*, Δ*mkkB* and Δ*mpkB*Δ*mkkB* mutants were similar with wild type. Furthermore, whether MpkB constitutively activated or not in the mutants, the result 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 MAP Kinase module. ST production of *mpkB* and *mkkB* mutants was remarkably delayed in the *veA1* background as well, suggesting that the ST production is affected by the *veA* gene but not *mpkB*. Similarly, in *Aspergillus flavus*, MpkB ortholog *Afl_mpkB* mutant couldn't produce any sclerotia, but it produced aflatoxin B1 normally. So *mpkB* gene does not affect the expression of genes involved in mycotoxin production such as ST in *A. nidulans* or aflatoxin B1 in *A. flavus*. This study suggests that the signal pathway of MpkB MAP Kinase and mycotoxin production were independent.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Cell regulation & signaling

Ascospore-specific gene expression analysis in *Aspergillus nidulans*

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Developmental process and spore formation in a homothallic model filamentous fungus *Aspergillus nidulans* is environmentally and genetically regulated. Although asexual spores or conidia differentiation is well-characterized by analyzing important genes for controlling orchestrated developmental pathways, including the *brlA* gene-mediated conidiophore and conidia morphogenesis, a few genes have been elucidated for playing an important role in sexual development and ascospore formation. The *nsdD*, *nsdC* and *veA* genes are responsible for regulation of sexual and asexual developmental processes. Unlike conidia, however, physiological and genetic studies of ascospores are remained to be characterized. To know more about the ascospores biology, we performed RNA-seq analysis from from *A. nidulans* conidia and ascospores RNA samples. Comparative analysis of transcription profiles of conidia and ascospores revealed many genes that are expressed differentially in both spores. Detailed investigation of the differentially expressed genes is in progress.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Cell regulation & signaling

Iron sensing is governed by mitochondrial, but not by cytosolic iron-sulfur cluster biogenesis in *Aspergillus fumigatus*

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For optimal growth, microorganisms have to adapt their iron metabolism to the requirements of their ecological niche to avoid iron shortage as well as iron toxicity. Therefore, mechanisms have been evolved to tightly regulate iron uptake, consumption and detoxification, respectively, which depend on sensing the cellular iron status. In the facultative anaerobic yeast *Saccharomyces cerevisiae,* iron sensing has been shown to depend on mitochondrial (MIA) but not cytosolic iron-sulfur cluster assembly (CIA), while in mammals the cellular iron state is sensed via cytosolically synthesized iron-sulfur clusters. To address the question how the obligatory aerobic mold *Aspergillus fumigatus* senses the cellular iron state, mutant strains allowing down-regulation of MIA and CIA were generated. These studies revealed that: (i) Af‑Nfs1 (Afu3g14240) and Af‑Nbp35 (Afu2g15960), which are required for MIA and CIA, respectively, are essential for growth; (ii) inactivation of the Frataxin homolog Af‑FxnA (Afu4g10510), which is involved in MIA, is not lethal, but results in a severe growth defect; (iii) a decrease in MIA (Af-Nfs1 depletion, Af‑FxnA-deficiency) but not CIA (Af-Nbp35 depletion) results in an iron starvation response accompanied by increased iron toxicity; and, likewise, (iv) a decrease in mitochondrial iron import results in an iron starvation response. Taken together, these data underline that iron sensing in *A. fumigatus* depends on the mitochondrial, but not the cytosolic iron-sulfur cluster machinery. Moreover, depletion of the glutathione pool caused an iron starvation response underlining a crucial role of glutathione in iron sensing in *A. fumigatus*.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Cell regulation & signaling

The bZIP transcription factor HapX is regulated at multiple levels to control iron homeostasis in *Aspergillus fumigatus*

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HapX is a bZIP transcription factor required for maintenance of iron homeostasis in filamentous fungi. HapX-mediated adaptation to iron-limiting conditions was shown to be crucial for virulence of several fungal species in animal and plant hosts, including the pathogenicity of the opportunistic human pathogen *Aspergillus fumigatus* in animal models for aspergillosis. The differences in iron homeostasis-maintaining mechanisms between fungi and mammals might serve to improve therapy and diagnosis of fungal infections. Previously, expression of *hapX* was shown to be repressed by iron at the transcriptional level, partially dependent on the GATA factor SreA. Here we show that HapX protein stability is very low during iron starvation and decreases dramatically during a shift from iron depleted to iron-replete conditions, while siderophore biosynthetic enzymes appear to be quite stable. Immunoprecipitation followed by MS/MS analysis identified several phosphorylation sites in HapX and site-directed mutagenesis identified one phosphorylation site that determined the HapX protein stability. Additionally, our studies demonstrate that iron also controls the stability of *hapX* mRNA. Taken together, our results show that expression of hapX is subject to indicate that regulation of HapX is subject to multiple levels of control in *Aspergillus fumigatus*.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Cell regulation & signaling

The wheat pathogen *Zymoseptoria tritici* can sense and respond to different wavelengths of light

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The ascomycete fungus *Zymoseptoria tritici* (synonym: *Mycosphaerella graminicola*) is a major pathogen of wheat that causes the economically important foliar disease Septoria tritici blotch. Despite its importance as a pathogen, nothing is known about the reaction of this fungus to light. To test for light responses, cultures of *Z. tritici* were grown *in vitro* under white, blue or red light, and their transcriptomes were compared to those obtained from control cultures grown in the dark. There were huge differences in gene expression with over 3400 genes upregulated in one or more of the light conditions compared to dark, and from 1909 to 2573 genes specifically upregulated in the dark compared to the individual light treatments. Differences between light treatments were lower, ranging from only 79 differentially expressed genes in the red versus blue comparison to 585 between white light and red. Many of the differentially expressed genes had no useful annotation. For those that did, analysis of the Gene Ontology (GO) terms showed that those related to metabolism were enriched in all three light treatments, while those related to growth and communication were more prevalent in the dark. Interestingly, genes for LysM effectors that have been shown previously to be involved in pathogenicity also were upregulated in one or more of the light treatments, suggesting a possible role of light for infection. This analysis shows that *Z. tritici* can sense and respond to light with a huge effect on transcript abundance.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Cell regulation & signaling

Investigating how the DOC proteins mediate intercellular communication discrimination in *Neurospora crassa*

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Germinated asexual spores of *Neurospora crassa* detect the presence of genetically identical partners, communicate with and grow towards them, and fuse to form a syncytium. We previously found that genetically distinct isolates from the same population fall into exclusive communication groups (CGs) and we used bulk segregant analysis to identify a single locus containing one to three paralogous genes associated with CG. Although the two genes at the CG locus in our lab strain are not essential for self-communication, we demonstrated that they are necessary and sufficient to specify CG (Heller et al. 2016). We named these genes ***d****eterminant* ***o****f* ***c****ommunication* (*doc*) 1 and 2. *doc-1* and *doc-2* have no characterized homologs, nor have we identified any protein domains within them. We developed a flow cytometry-based communcation quantification system to assay *doc* mutants, but the mechanism by which the *doc* genes control CG remains elusive.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Fungal GAPDHs: role beyond glycolysis

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Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is an enzyme of ~37kDa that catalyzes the sixth step (glyceraldehyde 3-phosphate to D-glycerate 1,3-bisphosphate) of glycolysis. GAPDH is a multifunctional enzyme that exhibit several non glycolytic functions. Non glycolytic activities include DNA repair, receptor mediated cell signaling, the cellular response to oxidative stress and to apoptosis etc. While animal cells contain only one isoform, plants contain several isoforms of GAPDH. In contrast with the well established multifunctional properties of animal GAPDH, little is known on the multifunctional roles of plant cytoplasmic GAPDH. Fungal genomes usually harbours a single gene for GAPDH, with no additional moonlighting effect of this glycolytic GAPDH having been reported. Earlier we reported the association of an extra GAPDH gene with a secondary metabolism related gene cluster in *Trichoderma virens* and *Aspergillus spp*. A survey of the fungal genome database revealed that *T. virens* and *T. harzianum* genomes have two genes for GAPDH, one being associated with a terpene biosynthesis gene cluster. However, *Aspergillus* genomes harbour multiple isoforms of GAPDH, some being associated with secondary metabolism related gene clusters. We have established the role of a GAPDH in biosynthesis of volatile sesquiterpenes in *T. virens*. The two GAPDHs of *T. virens* are having more than 80% similarity in amino acid sequences, and the active site is also conserved. Molecular modeling also revealed that these two GAPDHs are identical in 3D conformation. Interestingly, we find a conserved indel (1 residue) in all the GAPDH proteins which are associated with the of VIR cluster. The deletion (aspartate) can be expected to be localized on the surface loop preceding active site residues. We have expressed and purified the secondary-metabolism related GAPDH in *E. coli* with an aim to study the biophysical properties and structural basis of this unique catalytic property.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Reduction of fumonisin production in *Fusarium verticillioides* by RNA interference

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The fungus *Fusarium verticillioides* can produce fumonisin mycotoxins in ears under certain environmental conditions. Because fumonisins are unhealthy for humans and livestock, control strategies with nominal risk to the environment are needed to reduce fumonisin exposure. Host-induced gene silencing is a molecular engineered technique in which double-stranded RNA expressed in the plant host is absorbed by an invading fungus; subsequently, the expression of targeted genes is reduced in the fungus. A vital preliminary step of this technique is identification of DNA segments within the gene target that can effectively silence gene expression when expressed in the fungus. Here, we used segments of the fumonisin biosynthetic gene *FUM1* to synthesize double-stranded RNA in *F. verticillioides*. Several of the resulting transformants had reduced *FUM1* gene expression and fumonisin production. Similar losses in fumonisin production resulted from double-stranded RNA constructs with segments of *FUM8*, another fumonisin biosynthetic gene. *FUM1* or *FUM8* silencing constructs were transformed into three isolates of *F. verticillioides* originating from different regions of the United States. Whole genome sequence analysis of seven transformants indicated that reductions in fumonisin production were not due to mutation of the fumonisin biosynthetic gene cluster and showed a complex pattern of plasmid integration. These results indicate the cloned *FUM1* or *FUM8* gene segments could be transgenically expressed in maize for host-induced gene silencing of fumonisin production.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

TRI17 gene encodes a PKS involved in the synthesis of the C-4 lateral side chain of fungal trichothecenes

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Harzianum A and trichodermin are trichothecene compounds which have aroused great interest in last years because they are produced by several *Trichoderma spp* species. Many of these *Trichoderma* strains have the ability to act as important biocontrol agents against phytopathogenic fungi. In addition, some of them are biofertilizers, increasing plant growth and inducing plant defense response and even tolerance to abiotic stresses. However, trichothecenes have been studied in last decades due to their toxigenic properties against plants and all the animals analyzed, as well as for human beings. Additionally, many of the trichothecenes have antimicrobial activities. Most of the producer fungi are indeed important phytopathogens causing important losses in economically relevant crops. Thus, they are among the mycotoxins of greatest concern to food and feed safety.

Biosynthesis of *Trichoderma* trichothecenes has been almost totally characterized in recent years. Furthermore, in the present work a genome comparative strategy was used to identify the gene involved in the synthesis of the 2,4,6 octatriendioyl side chain of harzianum A. Thus, by comparison of Ta37 genome with genomes of other trichothecene producers, a PKS encoding gene (tentatively named *TRI17*), ortholog to *PKS* genes located in the *TRI* clusters of other fungi which produce trichothecenes with lateral side chains of polyketide nature (i.e. *Stachybotrys, Myrothecium, Spicellum , Trichothecium*), was selected as candidate to be involved in this step. A *TRI17* knock-out (Dtri17) mutant was isolated which lacks production of HA, but with increased production of trichodermol, the intermediate where the 2,4,5 octatriendioyl side chain would be transferred, further supporting the hypothesis that Tri17 catalyzes its biosynthesis. In addition, Dtri17 mutation was also complemented with *Myrothecium roridum TRI17*, which indicates that synthesis of a part of the macrocyclic side chain moiety of the trichothecenes produced by this fungus is also catalyzed by this enzyme.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Fine-tuning gene expression: pantothenic acid inducible promoters in *Trichoderma reesei*

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T. reesei is the most widely employed producer of cellulases and hemicellulases. Inducible recombinant protein expression in this organism is currently hampered by the relatively small repertoire of suitable promoters, which are derived from (hemi)cellulase encoding genes. Therefore there is an urgent demand for novel tunable promoters acting in a (hemi)cellulase independent fashion. Addition of pantothenic acid to *T. reesei* did not alter growth or the expression profile of (hemi)cellulase. Using whole transcriptome analysis, five *T. reesei* genes were found that were strongly induced by the addition of pantothenic acid. One of these genes showed strong sequence similarity to a characterized pantothenic acid permease. The promoter regions of two of those pantothenic acid induced genes were fused to the green fluorescent protein gene (*gfp*) as reporter. Transcription analysis showed that expression of *gfp* was pantothenic acid inducible and surpassed the expression of the reference strain with the strong *tef1* promoter in one case. This system is inexpensive and can therefore be used as valuable tool for strain engineering of industrial *T. reesei* strains.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Elucidation of the regulatory network of *Trichoderma reesei* operating under cellulose-degrading conditions

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The ascomycete *Trichoderma reesei* is widely employed for the production of CAZymes (carbohydrate-active enzymes) which are of high importance for the production of second generation biofuels by hydrolysis of plant biomass. Hence, research of their production mechanisms as well as the investigation of their regulatory mechanisms on a cellular level is relevant from a scientific point of view and becomes even more important as the demand for plant derived biofuels is constantly growing.

By using available transcriptome data, we selected transcription factors and Gcn5-related acetylases that show an increased expression on wheat straw (inducing conditions) compared to glucose (repressing conditions) as we expect them to be directly or indirectly involved in the expression of CAZyme genes. We selected 13 candidate genes and their function was assessed by gene knock-out. Furthermore these deletion strains were characterized in terms of their phenotype, radial growth and cellulase production on different carbon sources as well as their behavior in the presence of different stressors.

We hope that this study will lead to a better understanding of the regulatory network under cellulose degrading conditions, thereby enabling a more fine-tuned manipulation of enzyme production in industrial settings of *T. reesei*.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

A Synthetic Expression System for protein production in *Trichoderma reesei*

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Trichoderma reesei is a filamentous fungus that is used world-wide as a host for industrial enzyme production. The enzymes produced are used, for example, in pulp and paper production, in the food and feed industries, and in the textile industry. *T. reesei* enzymes are also increasingly important because of their ability to turn lignocellulosic biomass into sugars that can be used to produce biofuels and chemicals. The strongest promoters used in this system typically require the use of inducing molecules, which create limitations and generate extra cost in the process. To simplify and improve the production process we have employed a novel orthogonal expression system based upon a synthetic transcription factor (sTF) that regulates expression of the target gene via a sTF-dependent promoter. The great benefit is that new system allows for high level, constitutive expression in diverse growth conditions and growth stages. Multiple sTF-binding sites can be designed to enable selection of a wide range of target gene expression levels, from very low to extremely high. We have taken advantage of this unique expression system to improve native and heterologous protein production in the industrial microbe *T. reesei*. We have been able to produce high levels of secreted proteins without the use of inducing molecules, which are typically used for this production organism. For instance, with *T. reesei* we could produce the native CBH1 cellulase, *Candida antarctica* CALB lipase, and bovine beta-lactoglobulin at high levels and high purity when grown in glucose medium. The new universal expression system allows for simpler media and bioprocesses to be used for industrial protein production.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Multi-omic characterization of *Aspergillus fumigatus* isolated from air and surfaces of the International Space Station

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The on-going Microbial Observatory Experiments on the International Space Station (ISS) revealed the presence of various microorganisms that may be affected by the distinct environment of the ISS. The low-nutrient environment combined with enhanced radiation and microgravity may trigger changes in the molecular, genetic and biochemical suit of microbes and lead to increased virulence and resistance of originally harmless microbes. Additionally, such an environment may trigger the production of bioactive compounds. So far, the majority of carried out studies have focused on bacteria, and filamentous fungi aboard the ISS have been generally understudied.

Two ISS-isolates were identified as *Aspergillus fumigatus*, which is known to be an opportunistic pathogen, and therefore may be potentially harmful for astronauts' health whose immune systems are reported to be compromised under microgravity. Whole genome sequence analysis of both isolates revealed 54,960 and 52,129 single nucleotide polymorphisms (SNPs) when compared to the clinical reference strain, Af293, which is consistent with the genetic heterogeneity amongst sequenced *A. fumigatus* strains from an array of clinical and environmental sources. Secondary metabolite (SM) profiles of both ISS isolates were compared to the reference (Af293). Additionally, ISS-isolated *A. fumigatus* strains showed enhanced UVC resistance and increased virulence, possibly in response to the unique environment of the ISS (enhanced radiation, microgravity, low-nutrients), when compared to clinical isolates. In-depth analysis of proteome changes between clinical and ISS-isolated strains revealed differential expression of unique proteins involved in stress response, carbohydrate metabolic processes, pathogenesis and secondary metabolism.

Complex analyses of possible molecular alterations triggered by microgravity and enhanced radiation will be pertinent to the future long-term manned space flights, as such an understanding is crucial for astronauts' health and maintenance of the closed habitat.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Characterization of *Aspergillus niger* isolated from the International Space Station

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As strives are made toward human interplanetary exploration, a thorough understanding of how fungi respond and adapt to the various stimuli encountered during spaceflight is imperative for the health of crew. In the current study, we used a combination of genomics, proteomics, and metabolomics to characterize the molecular phenotype of a strain of *Aspergillus niger* isolated from the International Space Station (ISS). As a predominant ISS isolate that is frequently detected in other built environments, current and future studies of *A. niger* strains that have inhabited spacecraft environments will become increasingly important as the duration of manned space missions increase. The ISS isolate exhibited an increased rate of growth compared to a terrestrial strain. Whole-genome sequencing revealed increased genetic variance when compared to several other genome sequenced strains *A. niger*. Additionally, a distinct molecular phenotype of the ISS isolate was observed that suggests increased resistance to irradiation and oxidative stress, and an enhanced ability to acquire nutrients. Increased abundance was also observed for numerous secondary metabolites, including naphtho-gamma-pyrones, which are involved in melanin production, and pyranonigrin A, an antioxidant. These findings provide insight into the adaptive evolutionary mechanism of melanized fungal species and demonstrate the need for more studies on the biological alterations of microbes adapted to extreme spaceflight environments.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Elucidating the biosynthetic pathway of Felinone A in *Aspergillus nidulans* through serial promoter replacement

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Fungal secondary metabolites (SMs) are an important source for drug discovery. Thousands of biosynthetic pathways of SMs are revealed by several approaches and classified based on their characteristics. Felinone A, recently discovered in marine fungus, belongs to azaphilones – a set of SMs that contains many compounds with potent biological activities. In our previous study, we replaced the promoter of nonreducing polyketides synthase (NR-PKS) AN7901 and isolated 2,4-dihydroxy-3-methyl-6-(2-oxopropyl)-benzaldehyde (compound **1**)[1]. However, since we did not activate the surrounding genes that may modify compound **1**, the final product of this gene cluster remains unknown. By homolog comparison, we propose that AN7901 is involved in the biosynthetic pathway of Felinone A, which isthe final product, and that compound **1** is an intermediate[2]. We conducted BLAST analysis to determine the putative biosynthesis genes involved, and will replace their promoters serially. The data will allow us to confirm our proposed biosynthetic pathway and determine the boundary of Felinone A cluster.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

A genomics approach for improving production of Itaconic Acid by *Aspergillus terreus*

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Itaconic acid is an unsaturated dicarboxylic acid produced industrially by fermentative processes using the fungus *Aspergillus terreus* (1,2). It is in widespread use around the world as a building block compound for polymer production, and can be used widely as a sustainable replacement for petroleum-derived acrylic and methacrylic acids (2). While global use and production of itaconic acid are already very high at 30 and 50 kt a year, respectively, itaconic acid is still too expensive to compete directly with petroleum derivatives for many applications (1). As a result, great interest exists in lowering the costs of production through process-based methods or genetic improvement of the strains of fungus that secrete it. While these efforts have to some extent been successful, further improvement is required to lower costs below that of petroleum. We have instituted a new approach involving three gene targets identified from transcriptomic analysis of a related fungus, *Aspergillus niger*. Industrially *A. niger* is used for the production of citric acid, a direct precursor of itaconic acid. In a high-producing strain of *A. niger* three transcription factors are significantly downregulated relative to the wildtype strain. Our approach centers on using Cas9-based methods to induce inactivating double-stranded DNA breaks in the corresponding three genes in *Aspergillus terreus.* Our end goal is to produce a novel strain of *A. terreus* which produces greater yields of itaconic acid in a shorter period of time.

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POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Activation of a silent gene cluster in *Aspergillus nidulans* through the development of a hybrid transcription factor

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Many efforts have focused on activating silent gene clusters in fungal species since they are a rich source of structurally diverse compounds with medicinal and agricultural value. Fungal genome analyses have revealed that many species contain a surprising large number of secondary metabolite (SM) genes, the products of which are mostly unknown. In this investigation, we use a genetically engineered host strain of *Aspergillus nidulans,* developed in earlier work, which allows genes to be deleted or have their promoters replaced with relative ease. This genetic system has allowed us to express cryptic SM pathways in *A. nidulans* through promoter replacement, and through deletion analysis, define the genes responsible for the synthesis of each SM. While most studies have focused on nonreducing polyketide synthase (NR-PKS) and non-ribosomal peptide synthetase pathways, this investigation highlights the discovery of a novel metabolite produced by one of the less-studied, highly reducing (HR)-PKS pathways. Preliminary work focused on generating an overexpression strain of the HR-PKS, AN11191, and subsequent experiments determined the product released by AN11191 to be octatrienoic acid (OTA). Efforts to overexpress the entire AN11191 pathway through overexpression of the cluster-specific transcription factor (TF), AN9221, proved to be unsuccessful, and prompted the design of a highly activated hybrid TF. This chimeric TF is characterized by the native DNA binding domain of AN9221 fused to the TF activation domain from the highly expressed pathway for asperfuranone. Heterologous expression of this hybrid TF in place of AN9221 resulted in the successful production of the SM derived from the AN11191 cluster. Gene deletion studies have allowed us to elucidate the biosynthetic pathway for this compound.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Mining the fungal biodiversity for novel natural product discovery

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The fungal kingdom is a well-known source of useful compounds called natural products or secondary metabolites (SMs). Their diverse biological activities are of great interest for medicine, industry and agriculture. Analyses of fungal genomes have revealed a discrepancy between the few known SMs produced by a given species relative to the many biosynthetic pathways encoded by their genomes. Despite the pivotal role in modern medicine of fungal antibacterials (e.g. penicillin) and immunosuppressive compounds (cyclosporine), the chemical diversity within the fungal kingdom has remained underexploited. This can partly be explained by the difficulty in finding conditions conducive to SM production. The Westerdijk Fungal Biodiversity Institute maintains the world largest collection of fungal species and represent a unique resource for the discovery of novel SMs. The newly established Fungal Natural Products group is harnessing this biodiversity using microbiological, computational and synthetic biological approaches. By integrating these complementary methods, we are able to discover novel SMs and identify their corresponding biosynthetic gene clusters, the first step towards engineering of biosynthetic pathways. This research is performed within the Utrecht Fungal Network, a collaborative effort between the Westerdijk Institute, Utrecht University, University Medical Centre Utrecht and the Hubrecht Institute to mine the fungal biodiversity.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Pathway-specific regulation of the botcinic acid biosynthetic gene cluster in the grey mould fungus *Botrytis cinerea*

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Botcinic acid (BOA) is a non-host specific phytotoxin produced by *Botrytis cinerea*. Its biosynthesis relies on the two PolyKetide Synthase encoding genes *Bcboa6* and *Bcboa9* which are clustered together with co-regulated genes putatively also involved in the pathway. In order to understand how BOA biosynthesis is regulated, we investigated the genomic environment of the BOA cluster and searched for putative regulator encoding genes.

Amongst the clustered genes, *Bcboa13* was predicted to encode a $Zn(II)_2Cys_6$ transcription factor (TF). Inactivation of the *BcBoa13* gene resulted in a drastic diminution of the expression of the *Bcboa* genes and in the absence of BOA. These data revealed a major positive role of BOA13 in the regulation of the cluster. Fusion of BcBoa13 with GFP further indicated that it localizes into nuclear foci. Heterochromatin and telomeric markers are currently developed to investigate the nature of these foci.

In addition to *Bcboa13*, another gene (*Bcboa1*) encodes a putative regulator: the predicted protein has a NmrA-like domain that may be involved in protein-protein interactions. The impact of BcBoa1 on BOA production is investigated by gene inactivation, while its possible interaction with BcBoa13 is tested by Bimolecular Fluorescence Complementation (BiFC).

Finally, the BOA cluster is localized in a subtelomeric region in which the A+T/G+C-equilibrated regions that contain *Bcboa* genes alternate with A+T-rich regions (85%) made of relics of transposable elements that have undergone repeat-induced point (RIP) mutations. The occurrence of RIP raises questions about possible chromatin-based regulation of BOA synthesis. Several histone methyl transferases are under studies to test this hypothesis.

Identification of BcBoa13 as the major regulator of BOA synthesis is the first step toward a comprehensive understanding of the regulation network of toxin synthesis in *B. cinerea.* Ongoing work may point out the respective role of pathway-specific transcriptional regulators and chromatin structure modifications.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Biosynthesis of acurin A and B, two novel isomeric fusarin C-like compounds from *Aspergillus aculeatus*

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This study presents the identification and proposed biosynthetic pathway for two stereoisomeric compounds of mixed polyketide-nonribosomal peptide origin that we named acurin A and acurin B. The compounds were discovered in extracts from *Aspergillus aculeatus*, a filamentous fungus known for the commercial utilization in the production of several enzymes. The structures of acurin A and B highly resemble the mycotoxin fusarin C produced by several *Fusarium* species. In our work, we used CRISPR-Cas9 to construct a non-homologous end-joining deficient strain of *A. aculeatus*, which enabled efficient gene deletions in the acurin gene cluster. Using gene-expression analysis in combination with metabolite profiling of gene-deletion strains, the gene cluster responsible for acurin production was delineated, which allowed us to propose a biosynthetic pathway for formation of acurin. Our results show that acurin is biosynthesized by an individual polyketide synthase and non-ribosomal synthetase. Moreover, at least six other enzymatic activities are required to complete the biosynthesis of acurin. This study shows how we exploit the CRISPR-Cas9 system in filamentous fungi for the rapid construction of fungal host strains that can be readily engineered to elucidate biosynthetic pathways.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Novel regulator induces biosynthesis of cryptic natural products in the fungus *Aspergillus sydowii*

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Natural products are low-molecular mass compounds with diverse chemical structures and important pharmacological activities, ranging from antibiotics to cholesterol-lowering agents (1). Filamentous fungi are well-known producers of such molecules, and recent advances in sequencing and genome mining have revealed that the biosynthetic potential of fungi is far greater than the number of currently identified compounds (2). This finding prompted the development of new methods to activate the biosynthesis of cryptic natural products. One successful approach is to simulate natural environmental conditions through co-cultivation of microorganisms (3). We previously showed that in a mixed fermentation, the bacterium *Streptomyces rapamycinicus* leads to the activation of the silent orsellinic acid (*ors*) gene cluster of the fungus *Aspergillus nidulans* (4). The metabolite production was further shown to be dependent on a novel regulator termed BasR (5), which is induced during the bacterial-fungal interaction. Here, we show that this transcription factor is responsible for the activation of the *ors* cluster in the related fungus *Aspergillus sydowii*, where its induced expression is able to activate the fungal secondary metabolism and triggers the biosynthesis of cryptic compounds.

(1) Brakhage (2013) Nature, (2) Macheleidt et al., (2016) Annu Rev Genet, (3) Netzker et al., (2015) Front Microbiol, (4) Schroeckh et al., (2009) PNAS, (6) Fischer et al., in preparation

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

CRISPR-mediated expression platform for multi-species Aspergilli

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The recent sequencing survey on many fungal species revealed a large repertoire of industrially or medically relevant enzymes and secondary metabolites. However, the industrial potential of these species is often hindered by the difficulties with the cultivation at laboratory conditions or in bioreactors. It is therefore beneficial to create heterologous expression platforms that facilitate the screening process of fungal genes for production of relevant enzymatic activities or secondary metabolites. Unfortunately, in many cases it is not possible to predict whether a given host possesses a physiology and metabolism compatible for formation of these products, resulting in an inefficient heterologous production. To increase the chance of successful heterologous production, we have created a flexible expression platform in various *Aspergillus* species. The system is based on the insertion of a reporter gene into defined locus in each of the different expression hosts. The reporter allows to assess the strength of expression from the various defined loci, as well as it can be replaced by a gene-expression cassette containing your favourite gene or pathway via marker-free homologous recombination mediated by CRISPR-Cas9 technology. Importantly, our setup allows different species to be transformed by the same gene-expression cassette to reduce DNA construction work. As a proof-of-concept, we chose three representatives of *Aspergillus*: the model fungus *A. nidulans*, and the fungal industrial workhorses *A. niger* and *A. oryzae*. We have used red fluorescent protein (RFP) as a reporter gene and inserted it into several defined integration sites in all three species. RFP production was confirmed through fluorescence microscopy and the three different strains constitute our versatile *Aspergillus* expression platform. We have tested the platform and replaced RFP in the different species with genes allowing for production of relevant enzymes and secondary metabolites.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Genus wide analysis of the secondary metabolite clusters in *Botrytis ssp.*

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The fungal genus *Botrytis* comprises around 30 species, which are pathogens with a necrotrophic infection behavior. The most extensively studied species with a necrotrophic lifestyle, *Botrytis cinerea*, causes grey mould on a broad range (>1000) of host plant species. Most other *Botrytis* species have a narrow host range, only infecting a single host or few related hosts. In *B. cinerea* more than 40 secondary metabolite clusters have been identified. Two well-studied clusters are involved in the production of the sesquiterpene botrydial and the polyketide botcinic acid, which are important but not essential for virulence. In order to understand the mechanisms of infection of host-specific *Botrytis* species, as compared to the broad host range pathogen *B. cinerea,* we sequenced more than 15 *Botrytis* species. We examined these genomes for the occurrence of secondary metabolite clusters present in *B. cinerea* by homology to the *B. cinerea* reference genome, and identified additional gene clusters using antiSMASH. Overall, we observe a complex evolutionary history that includes many losses in the secondary metabolite clusters among members of the genus. For instance, for the gene cluster of botcinic acid, which includes two key enzymes (Bcboa6 and Bcboa9), some species have lost one of the key enzymes, while others lost both of them. These findings are discussed in the context of other related plant pathogenic species.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Fluorescent based screening of heterologously-expressed polycistronic genes in eukaryotes

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Filamentous fungi produce an inestimable number of high-value natural products (NPs). NPs are synthetized by metabolic pathways, whose genes are normally clustered in the genome. These clusters usually contain one or several central biosynthetic genes encoding large multidomain proteins belonging to the polyketide synthase and/or non-ribosomal peptide synthetase protein families. These enzymes represent a sort of signature that can be exploited to identify putative gene clusters in every sequenced genome. However, the majority of the computationally identified clusters are not expressed under standard laboratory conditions, requiring molecular engineering to induce the production of unknown NPs.

The heterologous expression of entire biosynthetic clusters can be a feasible solution to decrypt unknown fungal NPs. The parallel expression of eukaryotic genes is normally limited by the presence of regulatory elements (promoters and terminators); consequently, the expression of clustered genes can happen only when all promoters are activated together. This problem can be avoided by expressing a group of genes as a polycistron under the control of a single inducible promoter. This method was implemented in the last years, obtaining a toolbox to study eukaryotic gene clusters. In particular, one of the major problems encountered when working with large polycistronic genes is the screening of positive transformants. Plasmid vectors are ectopically integrated in the genome; thus, the majority of transformants (95%) present a fragmented polycistronic construct unable to express the entire biosynthetic pathway. In order to have a faster and reliable screening method, we developed a fluorescence based selection system using a split-gfp marker. This methodology permits the fast selection of those transformants having complete clusters correctly expressed.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Cell free production of polyketide building blocks

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Due to the more and more increasing impact of infectious diseases and multi-resistant organisms the need of new active compounds is dramatically increasing, too. Polyketides are a group of natural products that are already used as cancer therapeutics and antibiotics. They are produced in many different organisms such bacteria, fungi and plants. Genome mining approaches showed that filamentous fungi comprise the genes for the production of a high number of these molecules. But because the majority of these organisms are recalcitrant to cultivation, so far, only few of the naturally available polyketides have been fully characterized.

Polyketides are synthesized by highly complex multimodular enzymes called polyketide synthases (PKSs). Fungal PKSs predominantly use small building blocks to assemble their products. In most cases acetyl-CoA is used as starter unit which is afterwards extended by adding malonyl-CoA units or its derivatives. Since the amount of those building blocks is limiting the synthesis of the polyketides also in *in vitro* experiments, additionally to the PKS itself, it is necessary to build up a system for the production of those units in high amount.

Here we show the recombinant expression in *E.coli* and purification of a malonyl-CoA-synthetase (matB) and a malonyl-CoA decarboxylase (matA). With these highly pure and stable enzymes we are able to produce malonyl-CoA out of free CoA and malonate using matB activity and acetyl-CoA by decarboxylation of malonyl-CoA *via* matA activity [1]. Adding a third, commercially available enzyme, citrate synthase, we were able to set up a whole cycle of CoA consumption and recycling.

- An, J.H. and Y.S. Kim, *A gene cluster encoding malonyl-CoA decarboxylase (MatA), malonyl-CoA synthetase (MatB) and a putative dicarboxylate carrier protein (MatC) in Rhizobium trifolii--cloning, sequencing, and expression of the enzymes in Escherichia coli.* Eur J Biochem, 1998. **257**(2): p. 395-402.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

High-throughput format for the phenotyping of fungi on solid substrates

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Filamentous fungi naturally grow on solid surfaces, yet most genetic and biochemical analyses are still performed in liquid cultures. Here, we report a multiplexing platform using high-throughput photometric continuous reading that allows parallel quantification of hyphal growth and reporter gene expression directly on solid medium, thereby mimicking natural environmental conditions. Using this system, we have quantified fungal growth and expression of secondary metabolite GFP-based reporter genes in saprophytic *Aspergillus* and phytopathogenic *Fusarium* species in response to different nutrients, stress conditions and epigenetic modifiers. With this method, we provide not only novel insights into the characteristic of fungal growth but also into the metabolic and time-dependent regulation of secondary metabolite gene expression.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Heterologous expression system of *Aspergillus oryzae* strain isolated from Korean traditional fermented foods

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It is important to find an efficient way to amplify the expression of heterologous gene(s) of interest in a fungal expression system for giving the high potential of fungi as genetic resources. Also, mass production systems of foreign protein is important for bio-industry, including pharmaceutical engineering and functional foods production. In addition, for ensuring the safety of the products, it is highly suggested to use GRAS (Generally Recognized As Safe) strain as a host. Here, we constructed a heterologous gene expression system for producing foreign gene product including bacterial origin one such as bacterial β-glucosidase. The produced β-glucosidase is a hydrolytic enzyme and the expression of the gene was stimulated by placing it under the control of the constitutively activated *gpdA* gene promoter or threonine-inducing *alcA* gene promoter of *Aspergillus nidulans*. The pyrithiamine-resistant gene, *ptrA*, was used as the selection marker for *Aspergillus* transformation. The signal peptide of *A. oryzae* α-amylase AmyB was linked to the N-terminus of the bacterial β-glucosidase protein, and 3X FLAG was tagged at the C-terminus. The fungal transformants successfully overexpressed the β-glucosidase gene and expression level was monitored by western blot analysis with anti-FLAG antibody. The functional activity of the protein was detected by esculin converting test. The expression system of *A. oryzae* could be beneficial for industrial applications.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Exploring nature`s silent pharmacy

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The medicinal properties of fungi have been known about for millennia and were exploited for the benefit of humans by many ancient civilisations, including those in Ancient China, Eastern Europe, Mesoamerica and Africa. Moving forward in time to the 20th century, scientific advances allowed the health benefits of fungi to be utilised in a more complex and sophisticated manner, with fungal natural products being developed into life-changing medicines such as the penicillins and cephalosporins.

Even more recent developments, particularly in the fields of synthetic biology and genomics, have further opened up the field of natural product research. In particular, sequencing of fungal genomes has revealed that fungi house a surprisingly large number of biosynthetic gene clusters, many of which appear to be silent or inactive under typical culturing conditions. This suggests that there are many beneficial products yet to be discovered and exploited. Of particular interest would be novel antibacterial compounds, which could contribute to the fight against growing antibiotic resistance.

The ongoing research project, 'Exploring Nature`s Silent Pharmacy', aims to design a production pipeline that will allow high throughput investigation of uncharacterised biosynthetic gene clusters and analysis of novel bioactive molecules. The fungal isolates selected for this study have been chosen to span a range of differing lifestyles, including insect, fungal and plant pathogens, marine fungi and soil fungi, in an attempt to discover as much chemical diversity as possible.

We will show progress towards automating this discovery pipeline, including the use of advanced *in silico* tools, liquid-handling robotics coupled with yeast-based recombination to build refactored gene clusters for expression in *Aspergillus oryzae*, and subsequent chemical analysis for product characterisation.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Induction of apopotosis and ganoderic acid biosythesis by cAMP signaling in *Ganoderma lucidum*

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Apoptosis is an essential physiological process that controls many important biological functions. However, apoptosis signaling in relation to secondary metabolite biosynthesis in plants and fungi remains a mystery. The fungus *Ganoderma lucidum* is a popular herbal medicine worldwide, but the biosynthetic regulation of its active ingredients (ganoderic acids, GAs) is poorly understood. We investigated the role of 3`,5`-cyclic adenosine monophosphate (cAMP) signaling in fungal apoptosis and GA biosynthesis in *G. lucidum*. Two phosphodiesterase inhibitors (caffeine and 3-isobutyl-1-methylxanthine, IBMX) and an adenylate cyclase activator (sodium fluoride, NaF) were used to increase intracellular cAMP levels. Fungal apoptosis was identified by terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) assay and a condensed nuclear morphology. Our results showed that GA production and fungal apoptosis were induced when the mycelium was treated with NaF, caffeine, or cAMP/IBMX. Downregulation of squalene synthase and lanosterol synthase gene expression by cAMP was detected in the presence of these chemicals, which indicates that these two genes are not critical for GA induction. Transcriptome analysis indicated that mitochondria might play an important role in cAMP-induced apoptosis and GA biosynthesis. To the best of our knowledge, this is the first report to reveal that cAMP signaling induces apoptosis and secondary metabolite production in fungi.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

A cross-talk between harzianolide and harzianic acid, the secondary metabolites of plant beneficial *Trichoderma guizhouense* NJAU 4742

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Harzianolide, a secondary metabolite from a group of butenolides, is secreted by *Trichoderma* species (Hypocreales, Ascomycota) from the *Harzianum* Clade. Previous studies suggest that this compound is involved in plant growth promotion and it also triggers plant defense responses. In this research, we have used HPLC/MSMS and NMR to isolate and identify harzianolide from the cultivation broth of *Trichoderma guizhouense* NJAU 4742 (*Harzianum* Clade) grown in potato dextrose broth. We also used the RNA deep sequencing and gene deletion experiment to predict and verify the cluster of secondary metabolite producing genes that are involved in harzianolide synthesis. Interestingly, the Δ*pks7* mutant of *T. guizhouense* NJAU 4742 lacking the gene encoding polyketide synthase orthologous to the reducing PKS7 (fumonisins) sensu Baker *et al*. (2012), resuscitates the biosynthesis of the two isomers of harzianic acid, another plant growth promoting metabolite of *Trichoderma* that also has its antifungal activity. Consequently, the mutant is able to suppress the growth of several other fungi including pathogens of plants. To investigate the synthetic pathway for harzianolide, we used *Saccharomyces cerevisiae* BJ5464-npgA as a host for the heterologous expression of this PKS7. Thus, the combination of metabolomics and transcriptomic data points to the possible involvement of harzianolide in the regulation of secondary metabolite production in *T. guizhouense*.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Genetic engineering of the red yeast *Xanthophyllomyces dendrorhous* for high-yield synthesis of carotenoids such as astaxanthin, zeaxanthin and phytoene

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Economic chemical synthesis of carotenoid as colorants, food and feed additives is restricted to a few compounds, whereas exploitation of natural sources may be restricted by low concentrations. To increase carotenoid production in fungi, genetic pathway engineering is a method of choice. Additionally, pathways can be extended to novel carotenoid products.

The red basidiomycetous yeast *Xanthophyllomyces dendrorhous* (with *Phaffia rhodozyma* as its anamorphic state) was successfully developed as a carotenoid production system. Plasmids for gene integration into the genome have been developed. Knock-out of genes and knock-in was successful. The genetic modification strategies involved generation and selection of suitable mutants, pathway enhancement and pathway extension. This combinatorial approaches led to high-yield prototypes for astaxanthin (1), zeaxanthin (2) and phytoene (3). Furthermore, novel carotenoids such as multi-hydroxy β-carotene derivatives could be generated in *X. dendrorhous* (4).

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POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Molecular characterisation of BasR, a novel regulator of natural product biosynthesis in *Aspergillus nidulans*

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In environmental microbial consortia natural products, also known as secondary metabolites (SMs), play a pivotal role acting as infochemicals or as antimicrobials for the defense against competitors (1). Several secondary metabolites exhibit pharmacological properties revealing their particular importance in the medical field (2). Recently, we discovered that the model organism *Aspergillus nidulans* specifically interacts with the soil-dwelling bacterium *Streptomyces rapamycinicus and its closely related Streptomyces iranensis (3).* As a consequence, the silent orsellinic acid *(ors)* gene cluster is activated in the fungus, leading to the formation of orsellinic acid, lecanoric acid and derivatives thereof. Continuation of this work led to the discovery that the co-cultivation with *S. rapamycinicus* leads to a re-programming of the histone acetyltransferase GcnE-containing SAGA/ADA complex of *A. nidulans* (4). Consequently, the histone H3 amino acids K9 and K14 were specifically acetylated at distinct natural product genes (5). The transcription factor BasR was identified as a novel regulator of secondary metabolism during the bacterial-fungal interaction by performing a genome-wide chromatin immunoprecipitation (ChIP) analysis (6). Here, we present a molecular characterisation of the novel regulator BasR in *Aspergillus nidulans* and its impact on the regulation of secondary metabolism during the interaction with the streptomycete.

(1) Spiteller, (2015) Nat. Prod. Rep., (2) Brakhage, (2013) Nature, (3) Schroeckh et al., (2009) PNAS, (4) Nützmann et al., (2011) PNAS, (5) Nützmann et al., (2013) AEM, (6) Fischer et al., in preparation

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Towards new molecular scaffolds of fungal secondary metabolites

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The Pilot Unit of CNRS, France, developed new tools and strategies for the investigation of microbial and especially fungal secondary metabolites from isolation and structural elucidation to biosynthetic pathways. Recent achievement in the field will be illustrated through diverse examples (sporochartines, geralcins, SCH-Sch-642305 ...) highlighting the involvement of the pilot unit in national, European and international projects dedicated to the fungal metabolite valorization.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Sporochartines A-E, A New Family of Natural Products from the Marine Fungus *Hypoxylon monticulosum*

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The fungal Xylariaceae family includes more than 16 genera and 130 species and has been extensively investigated for the chemo diversity and biological activity of their metabolites. Among the 16 genera reported, *Hypoxylon* with 14 species is largely distributed in various marine and terrestrial habitats, and producing a large variety of bioactive compounds. We recently isolated Sporochartines A-E as a new family of natural compounds from the marine fungus *Hypoxylon monticulosum* CLL-205, isolated from a *Sphaerocladina* sponge collected in Tahiti coast. Based on the structure of the previously reported sporothriolide and trienylfuranol A, we suggested a hypothetic biosynthetic pathway of sporochartines, involving a "spiro" Diels-Alderase reaction. We are currently achieving the hemisynthesis of sporochartines and investigating the biosynthetic pathway of this new class of fungal natural compounds.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Synthetic biology and secondary metabolites

Genome sequence and secondary metabolites of *Cordyceps militari*s, an insect pathogenic fungus and traditional medicine

Glenna Kramer, Jing Li, Stefanie Mak, Justin Nodwell
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Cordyceps are a genera of insect pathogenic fungi which are renowned for their utility in traditional medicine. These fungi follow a fascinating lifecycle in which they infect their host and use the host as a nutrient reservoir for mycelial growth. During this process, the fungus often exhibits a behaviour modifying effect on the host, causing it to perish in a location ideal for fungal fruiting body growth and spore dispersal, a process that is potentially small molecule mediated. We have assembled the first chromosome level genome sequence of a fungus from this genus, *Cordyceps militaris,* which reveals the potential for a large number of uncharacterized secondary metabolites. In our work moving forward, we are applying traditional bioactivity screening and small molecule isolation techniques along with genome mining and heterologous expression to identify and characterize specific natural products from this and other fungi in the *Cordyceps* genera.

POSTER SESSION 3
(Wednesday, February 28, 2018 13:45-15:00)

Antifungals and fungicides

Contribution of active drug efflux to enhanced tolerance to new fungicides in the wheat pathogen *Zymoseptoria tritici*

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Active drug efflux is a widespread resistance mechanism in the wheat pathogen *Zymoseptoria tritici* as in other plant pathogenic fungi, but also in human pathogens. Active drug efflux has been demonstrated using radioactively labeled fungicides or fluorescent compounds. However, both technologies do not easily allow testing efflux of molecules that are neither radioactive nor fluorescent. We therefore designed a miniaturized cellular test to quantify active drug efflux in *Z. tritici* that will permit rapid analyses in restricted reaction volumes of unlabeled molecules. We incubated isogenic strains of IPO323 with normal, enhanced or reduced efflux in 24-well-micro-titer plates along with a succinate dehydrogenase inhibitor (SDHI) fungicide known to be affected by active drug efflux, under previously determined conditions of temperature, light exposure and shaking. After a week of growth, we extracted the fungicide from the growth medium (extracellular), from the fungal cells (intracellular) and from both to determine the extracellular, intracellular and total fungicide concentrations respectively by LC/MS/MS analyses. We showed that the ratios between the intracellular quantity and the total quantity of the tested SDHI perfectly correlated to the determined EC50. This approach will allow to easily and rapidly assay molecules for active drug efflux.

POSTER SESSION 3
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Antifungals and fungicides

Involvement of an *Aspergillus fumigatus* putative sphingolipid-synthesis related protein OrmA in antifungal azole stress responses

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Previous studies have identified that the sphingolipid, which is a major structural component of eukaryotic cytoplasmic membrane, is involved in many important biological processes, including cell metabolism and stress tolerance response, etc. It has been reported that there are two sphingolipid-synthesis related protein Orm1p and Orm2p in *Saccharomyces cerevisiae*s, which negatively regulate activities of the serine palmitoyl transferase that is the first speed-limit enzyme of sphingolipid synthesis.

In this study, through genome-wide homolog search analysis, we found that the *A. fumigatus* genome only contains one Orm homolog, referred as OrmA. Deletion of *ormA* causes hypersensitivity to azole while overexpression of OrmA shows the azole resistance. Moreover, Western blotting results indicate that OrmA protein expression could be induced by azole antifungals in a dose dependent way. These data suggest that OrmA in *A. fumigatus* is required for responding to the azole stress. In addition, rescued phenotypes for susceptibility to azole drugs by adding an sphingolipid synthesis inhibitor-myriocin in *ormA*-defective strains combined with data for deletion of *ormA* leads to significant increased main aacomponents of sphingolipid ceramide, suggesting that OrmA may work as a negative regulator for the sphingolipid synthesis.

Further mechanism analysis verified that OrmA is involved in drug susceptibility through affecting endoplasmic reticulum stress responses in an unfolded protein response pathway (UPR) HacA-dependent way. Our data suggest that endoplasmic reticulum stress caused by azole drugs could stimulate HacA activation accompanied with inducing the increased expression of OrmA so that affecting the sensitivity of azole drugs by regulating the synthesis of sphingolipid. Most importantly, virulence tests demonstrated that OrmA deletion caused a significantly decreased virulence in immunosuppressive mice model. Our findings suggest that the unexplored sphingolipid metabolism pathway in *A. fumigatus* plays important roles for fungal virulence and azole susceptibilities and it may be used as new antifungal drug targets.

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Antifungals and fungicides

Chemical protection using drip irrigation and seed coating against maize late wilt disease in the field

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Late wilt, a disease severely affecting maize fields throughout Israel, is characterized by relatively rapid wilting of maize plants before tasseling and until shortly before maturity. The disease's causal agent is the soil-born and seed-born fungus *Harpophora maydis*. The pathogen is currently controlled using maize cultivars having reduced sensitivity. In an earlier study, we showed that Azoxystrobin (AS), injected into a drip irrigation line assigned for each row, prevent the disease symptoms in the field. Here, we examine an economically practical treatment using the AS fungicide in a mixture with difenoconazole (DC), or other new fungicide mixtures, in a combined treatment of seed coating and drip irrigation protection, for two coupling rows (row spacing was 50 cm instead of 96 cm). A recently developed Real-Time PCR method enables us accurate and sensitive tracking of the fungal DNA inside the host tissues during pathogenesis. AS-DC seed coating alone, managed to delay the pathogen spread in the maize tissues up to the age of 50 days (near the appearance of the first symptoms and the fertilization at day 55-57), but was not sufficient to protect the crops later (at the age of 70 days). Drip protection with AS-DC was the most successful treatment and in the double-line cultivation reduced the fungal DNA in the root and shoot host tissues to near zero levels. This treatment inhibited the development of wilt symptoms by 41% and recovered cob yield by 36%. Moreover, the yield classified as A class (cob weight of more than 250 gr) increased from 27% to 63% in this treatment. No residuals of this fungicide were identified in the maize cobs. This successful economical treatment to prevent maize late wilt disease in infested fields can now be applied in vast areas to protect sensitive maize cultivars against the pathogen.

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Antifungals and fungicides

Evolution of azole resistance and thermal adaptation in global populations of *Parastagonospora nodorum*

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Organisms become adapted to their local environment through natural selection. This process is easily observed in agroecosystems where human activities result in rapid evolution of fungal plant pathogens towards higher levels of virulence and reduced sensitivity to fungicides. Different pathogen populations may respond differently to the same human-imposed selection. A sustainable management of crop diseases requires improved knowledge of the genetic basis of the observed phenotypes in pathogen populations. A first step to obtain this knowledge is to differentiate the evolutionary forces acting on quantitative traits by using a Q_{ST}/F_{ST} comparison. Q_{ST} describes the distribution of the phenotypic variation in quantitative traits within and among populations while F_{ST} describes the distribution of neutral genetic variation within and among populations. The joint comparison of Q_{ST} and F_{ST} indexes enables evaluation of the causes of adaptive divergence in quantitative traits that reflect local adaptation of the organism. *Parastagonospora nodorum* is a globally distributed necrotrophic pathogen causing the Stagonospora nodorum leaf and glume blotch (SNB) disease on wheat. We conducted a Q_{ST}/F_{ST} analysis using 176 isolates sampled from field populations in Switzerland, South Africa, Australia, China, Oregon, New York, Texas and Iran. Q_{ST} values were obtained by phenotyping all isolates for azole sensitivity (EC_{50}) and thermal adaptation (growth rate at different temperatures). Full genome sequences of all isolates were used to calculate F_{ST} based on neutral single nucleotide polymorphisms (SNPs). We also conducted a genome-wide association study (GWAS) for the same isolates to identify genomic regions and candidate genes associated with the observed variation for fungicide sensitivity and thermal adaptation.

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Antifungals and fungicides

Diversity of polyphenols interacting with omega glutathione transferases of *Trametes versicolor*

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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. 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…) are potentially toxic. Indeed wood extractives have several ways to cause fungal damages. They could disrupt the fungal cell wall and plasma membrane, or alter ion homeostasis in the fungal cell. They can also inhibit enzymes involved in detoxification processes (laccases …), in wood degradation (cellulases, cellobiohydrolases …) by chelating metals, by scavenging reactive oxygen species and by fixing directly inside the protein.

To cope with this potential harmful environment, fungi have developed detoxification system involving multigenic families such as cytochrome P450 monooxygenases (involved in the first oxidation step of detoxification) and glutathione transferases (acting in the second conjugaison step). Concerning the fungal glutathione transferase family, it has been showed that several members are able to interact with extracts of various wood species. However, their physiological roles remain mysterious and their substrates and ligands are still unknown.We have particularly worked on glutathione transferases from the white-rot *Trametes versicolor* focusing on isoforms belonging to the omega class (TvGSTO) which is the most important GST class in this fungus. Among the sixteen TvGSTOs identified, we performed the biochemical and structural characterization of two isoforms: TvGSTO3S and TvGSTO6S. After that, we focused our attention on the research of potential ligands by using high-throughput screening and co-crystallization methods especially by affinity crystallization. This method allowed us to isolate a natural ligand (initially containing in a wood extract) retrieved in the active site of TvGSTO3S.

Here, we report that flavonoids and benzophenones are recognized by glutathione transferases.

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Antifungals and fungicides

Versatility of mycoparasitic mechanisms employed by *Trichoderma guizhouense* against *Fusarium oxysporum* and other plant pathogenic fungi

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The mycoparasitic fungus *Trichoderma guizhouense* NJAU 4742 (*Harzianum* clade, Hypocreales, Ascomycota) can suppress the causative agent of banana wild disease *Fusarium oxysporum* f. sp. *cubense* 4 (Foc4, Hypocreales, Ascomycota) and kill a broad range of plant pathogens.

A neutral metalloprotease gene (encoding NMP1) is required in mycotrophic interactions with Athelia rolfsii, Alternaria alternata, Foc4 and some other fungi. However, the NMP1 activity was not the only mechanism employed by NJAU 4742 against Foc4. We have observed the bursting of H_2O_2 production during interactions with some fungi. It possibly implicates the involvement of diffusible oxidant generated via Fenton-like chemistry in NJAU 4742 preying Foc4. A dual RNA-Seq approach was applied for a better deciphering if the interaction between the two fungi. Consistent with an important role for Fenton chemistry in cellulose depolymerization, high transcript levels and upregulation were observed for genes involved in iron homeostasis, iron reduction, and H_2O_2 generation. Moreover, the genes caused cell death or putative apoptotic processes in Foc4 suggested their role on mycoparasitism. NADPH oxidase (NOX) complex is vital for cellular differentiation and signalling in fungi. Combining the morphology of Δ*nox1* and overexpressing *nox1* mutant, transcriptome strengthened the main pattern of Fenton chemistry attacking in NJAU 4742 preying other fungi. The mechanism of detoxification of H_2O_2 in NJAU 4742 were explored by transcriptome and analysed in several other *Trichoderma* strains.

The analysis of the transcriptomic data also revealed that several polyketide synthase (pks) genes cluster for polypeptides were induced in NJAU 4742 preying Foc4. In vitro fermentation and HPLC-MS and Nuclear Magnetic Resonance allowed us to identify harzianolide, the secondary metabolite from the group of butenolides. The ascertainment of genes cluster responsible for the synthesis of harzianolide possibly extended the application of *Trichoderma* sp. in agriculture as this component triggers defense mechanisms in plants.

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Antifungals and fungicides

Large-scale phylogenomic roadmapping – sources of diversity in factors of resistance to fungicidal substances

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Broad inquiries into evolution of gene families in multiple, both closely related and divergent taxa resolve naturally into “phylogenomic roadmaps” — annotated resources documenting sources of extant diversity in a way that reconciles species and gene histories explicitly. These, by definition, will not provide a conclusive proof in favor of one or the other evolutionary scenarios. However, a large-scale reconciliation can show how some parts of the family`s history are better explained with or without recourse to horizontal transfer versus duplication, or determine which parts best reflect the common functional traits betrayed by tight genetic linkage into process-related clusters.

In an effort to facilitate the ongoing characterisation of sources of resistance to fungicidal substances in both “higher” and “lower” fungi, we analysed relationships between members of several gene families involved in either efflux (families of ATP-binding casette pumps) or detoxification (hydrolases involved in resorcylic acid lactone breakdown). Owing to a large-scale analysis of genomic neighborhoods of family members, we were able to document how inferred patterns of synteny reflect ties between the biosynthetic process of toxins and the likely capability to detoxify the compounds (lactonases). In an opposite vein, our findings trace both how ABCC family proteins of higher fungi evolved in conjunction with specific biosynthetic activities and how extensively duplicated ABCG family pumps remained coupled to the different primary metabolic or housekeeping activities that they protected in the past. For the first time, our analysis integrates over 20 available genomes of divergent “lower” fungi lineages, showing ancestral groups of diverging transporters in *Dikarya*, as well as documenting adaptation of transporter repertoire towards different ecological niches.

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Antifungals and fungicides

Comparative infection and sensitivity studies of *Microdochium nivale, Microdochium majus* and *Zymoseptoria tritici* for a better understanding of the field performance of fungicides

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In recent years outbreaks of Microdochium leaf blotch have hit wheat farmers in Germany and other European regions unexpectedly. Symptoms were frequently following cool and wet weather periods usually associated with *Zymoseptoria tritici* and commonly observed on upper leaves of fungicide treated fields. Little is known on the epidemiology of Microdochium leaf blotch, caused by the *Microdochium nivale* and *Microdochium majus*. The species are usually associated with pink snow mold and are part of the Fusarium Head blight complex. The leaf symptoms are easily confused with those of *Z. tritici.* The species *M. nivale and M majus* typically occur as a complex with a dominance of one or the other and reliable quantification is possible by molecular detection (qPCR).

Since the outbreaks are observed rather in fungicide treated fields than in untreated a first association is with loss of fungicide sensitivity. To untangle the complex of species and their role for symptom development comparative infection studies were carried out with isolates of *Z. tritici, M. nivale and M majus.* Starting with similar favorable conditions the microscopic studies revealed fundamental differences between *Z. tritici* and the *Microdochium* species in in the time for symptom development and considerable differences during early infection between *M. nivale* and *M majus.* Different systems for sensitivity testing were established to investigate the role of fungicide resistance for the observed outbreak of Microdochium leaf blotch. The focus was on the two DMI fungicides Prothioconazol and Prochloraz.

In summary, despite differences in fungicide sensitivity the outbreaks in the field rather relate to a different infection biology than fungicide resistance. Still the development of sensitivity towards DMI fungicides must be monitores attentively and the epidemiology *M. nivale* and *M majus* and the role of leaf infection in the life cycle needs further investigation.

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Antifungals and fungicides

RNA interference in *Fusarium graminearum* – opportunities and challenges as a biological toolkit

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RNA interference (RNAi) mediated by small RNA (sRNAs) is a conserved eukaryotic molecular mechanism involved in a plethora of cellular processes, ranging from developmental control to immune responses. Recent studies highlight the fact that the sRNA-orchestrated gene expression regulation is playing a considerable role in specific plant-pathogen interactions. For instance, *Botrytis cinerea* was demonstrated to secrete sRNAs hijacking the plant immune system, and *B. cinerea* spores were sensitive to exogenously applied inhibitory sRNAs (exoRNAi). Similarly, *Fusarium graminearum* was demonstrated to be susceptible to exoRNAi targeting *CYP51* genes. These reports rise the question whether exoRNAi presents a conserved molecular toolkit applicable for gene function explorations and fungal control, or whether it confines a peculiar signaling situation within a specific environmental situation.

We report an assessment of exoRNAi for gene silencing in *F. graminearum*. Novel Fusarium lines expressing a destabilized nanoLuciferase (nanoLuc) enzyme were generated, allowing the ultrasensitive quantification of silencing of the *nanoLuc* reporter gene *in vivo*. Using these reporter lines, the silencing potency of stably expressed hairpin sRNA constructs was validated and compared to exoRNAi triggered by treatment of axenic cultures with different sRNA triggers. Systematic approaches to evaluate the potential and tentatively improve exoRNAi efficiency in *F. graminearum* were undertaken, i) the screening of a large sRNA library targeting genes essential for growth, ii) the genetic engineering of lines with increased sRNA uptake and iii) of lines overexpressing components of the RNAi machinery. Collectively, our data point towards a transient and limited exoRNAi phenotype in *F. graminearum in vitro*. In view of the previously reported *in planta* RNAi efficacy against selected plant pathogens, our findings advocate a contrasting *in vitro* situation, suggesting distinct sRNA uptake, processing and amplification mechanisms during plant-pathogen interactions.

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Antifungals and fungicides

Cryptococcus neoformans Titanisation is an inducible and regulated morphotype underlying pathogenesis and drug resistance

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Changes in fungal cell shape in response to environmental stimuli drive pathogenesis and niche adaptation. The basidiomycete *Cryptococcus neoformans* undergoes an unusual morphogenetic transition in the host lung from haploid yeast to large, highly polyploid cells termed Titan cells. Titan cells influence fungal interaction with host cells, including through increased drug resistance, altered cell size, and altered Pathogen Associated Molecular Pattern exposure. We recently described an *in vitro* induction system for these host-specific cells that provides new insight into the molecular mechanisms underlying Titan cell biology. *In vitro* Titan cells exhibit all the properties of *in vivo*-generated Titan cells, the current gold standard, including altered capsule, cell wall, size, high mother cell ploidy, and aneuploid progeny. In the current work, we investigate the role of Titanisation in transient antifungal resistance among clinical and environmental isolates. Specifically, we test the hypothesis that Titanisation is an intrinsic property of *Cryptococcus neoformans* var. *grubbii* isolates underlying pathogenesis and fluconazole resistance. We show that there is wide variation in the Titanisation capacity of *C. neoformans* var. *grubbii* isolates and link this to host response. Further, we test the role of Titanisation in drug resistance and virulence (capsule, melanin, thermotolerance) via experimental *in vitro* evolution. Finally, we begin to dissect the molecular mechanisms underlying Titanisation by investigating the role of the master regulator of pathogenesis, Usv101, in the yeast-to-Titan transition.

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Antifungals and fungicides

Development of a system in *Fusarium oxysporum* for the search of new antifungals

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Fusarium species are well-known plant pathogens that produce a big array of secondary metabolites including mycotoxins that produce diseases in animals fed with contaminated cereal grains. Superficial human infections such as keratitis and onychomycosis are commonly provoked by *Fusarium*. *Fusarium* invasive infections are mainly found in immunocompromised patients. A molecular strategy originally designed to screen cell wall mutants of *Aspergillus niger* has been used to detect induction of cell wall integrity (CWI) pathway in *Fusarium oxysporum*. The method consists in the detection of expression of a reporter gene under control of a CWI pathway-responding promoter. *F. oxysporum* was transformed with a *mluc* reporter gene under control of *Aspergillus agsA* promoter (*PagsA*::*mluc*). The promoter was previously engineered with 3 boxes for the transcription factor RlmA and luminescence was measured in transformant SX76 with luciferin after addition of calcofluor white (CFW). Although results showed that it is functional in *F. oxysporum*, the system should be optimized. Promoter sequences were analyzed to find canonical Rlm1 boxes that could be used for an improved cassette. Promoters were selected based on data of upregulated genes detected in RNA-seq of wild-type *A. fumigatus* treated with CFW or detected in microarray of samples treated with Congo red in comparison to non-treated *Aspergillus* (Rocha *et al,* 2008). The up-regulated candidate genes should also be uninduced in deltaRlmA mutants. Expression of three selected genes was studied by RT-PCR in *F. oxysporum* treated with CFW at different times and compared to non-treated mycelia. Our current data are very promising for optimization of this reporter system in *Fusarium.*

Reference

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Antifungals and fungicides

Antifungal activity of stingless bee *Melipona beecheii* honey against *Candida albicans* and its effects on virulence gene expression

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Infections related to Candida genus have increased around the world, this is caused by development of antifungal resistance in clinical strains, complications are arising due to changes on human microbiota and immunosuppressive regimes related to organ transplantations1. The use of natural compounds present in plants and bacteria is an attractive alternative to commercial antifungals. In this sense, honey has been used since ancient times to treat skin disorders and infections2. The objective of this work was to determine the antifungal effect of *M. beecheii* honey on *Candida albicans* ATCC 10231 strain. This honey has a long tradition in the folklore medicine against several human diseases such as gastrointestinal and skin disorders. We determined that 20 % (v/v) of honey is able to reduce more than 80 % of growth culture in liquid media and notably, concentrations of 35 % (v/v) and above are able to inhibit completely the growth of this pathogen. Based on these results, we concluded that Melipona honey has antifungal effects against *C. albicans*. Subsequently, we performed qRT-PCR in order to determine the effect of sub-lethal concentrations on genetic expression of virulence factors, as a result we demonstrated that relatively low concentrations of 12 % (v/v) of stingless bee honey reduced the levels of expression of two virulence genes *sap6* (secreted aspartyl protease) and *hwp1* (hyphal wall protein) using *act1* (actin) gene as reference. Hence, results indicated that honey from *M. beecheii* could be a natural source of novel antifungal compounds.

1Morschhäuser, J. (2016). The development of fluconazole resistance in *Candida albicans*–an example of microevolution of a fungal pathogen. Journal of Microbiology, 54(3), 192-201.

2Ortiz-Vazquez, E., Cuevas-Glory, L., Zapata-Baas, G., Martinez-Guevara, J., & Ramon-Sierra, J. (2013). Which bee honey components contribute to its antimicrobial activity? A review. African Journal of Microbiology Research, 7(51), 5758-5765.

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Antifungals and fungicides

Biological control of plant pathogens: from soil bacteria to bio-pesticide

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In recent years, there is increasing awareness regarding the potential detrimental environmental and public health effects of pesticides. Biological control, a potential alternative to chemical pesticides, applies microorganisms to inhibit plant pathogens by either direct antagonism through antimicrobial activity, or ecological exclusion or through stimulation of plant resistance. Although this approach is a safer and more environmentally sustainable method of plant protection, the efficacy of applying these microorganisms is often very low, and therefore, it is generally not a realistic alternative to chemical pesticides.

Within the framework of this project, we are applying an interdisciplinary approach aimed at developing new and effective biocontrol agents that antagonize soil borne fungal pathogens. The novelty of this project is that it integrates state-of-the-art chemical analyses with whole genome sequencing. Specifically, active metabolites from selected biocontrol agents that antagonize soil-borne pathogens are isolated, purified and characterized; and concomitantly, the expression of secondary metabolite-encoding genes are monitored to determine optimal conditions for production of antagonistic metabolites in selected bacteria. Our efforts specifically focus on *Rhizoctonia solani* (Basidiomycetes), *Pythium aphanidermatum* (Oomycetes) and *Fusarium oxysporum* (Ascomycetes). To date, approximately 500 bacteria were isolated from various soil niches. We conducted *in-vitro* assays against the three pathogens, and selected approximately 100 antagonistic bacteria for *in-planta* tests. In tandem, we extracted secondary metabolites from 22 bacteria that displayed significant antagonistic activity. Crude extracellular bacterial extracts and specific fractions, separated by polarity, were tested *in-vitro* for antagonistic effects. We are currently purifying the most active fractions using a sephadex LH-20 column, and will subsequently characterize active purified molecules using NMR. Future work will focus on *in-planta* experiments using both purified metabolites and combinations of antagonistic bacteria. We hope that this project will inevitably lead to the development of commercial biological products for treatment of soil-borne plant pathogens.

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Antifungals and fungicides

Perseverance: a mechanism of proliferation in fungistatic drugs

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Candida albicans infections obey the 90-60 rule: susceptible isolates respond to therapy 90% of the time; resistant isolates respond 60% of the time. Thus, susceptibility cannot account for all therapeutic responses. Indeed, in disk diffusion assays with azoles, *C.albicans* sub-populations often grow within the zone of inhibition (ZOI) via a non-genetic mechanism that we term perseverance, which provides a quantitative measure of trailing growth or tolerance. Here, we quantified growth parameters that affect the ability of subpopulations of cells to grow, albeit slowly, at supra-MIC drug concentrations for a broad range of clinical isolates using *diskImageR* (Gerstein AC et al, Microbiology 2016), an image analysis pipeline that resistance as the radius of the ZOI (RAD) and perseverance as the Fraction of Growth (FoG), the degree to which a subpopulation of cells grows within the ZOI. RAD corresponds to MIC, and as expected, RAD is heritable, concentration-dependent and time-independent. FoG is due to non-genetic heterogeneity in the population, is independent of drug concentration and of RAD and the degree of heterogeneity is heritable for a given strain. Interestingly, *C.albicans* strains with higher FoG levels exhibit shorter lag phase length than those with lower FoG levels, a feature very different from tolerance in bacteria. The size of the subpopulation of cells that form colonies on supraMIC drug concentration correlated with FoG levels, while colony growth rates did not. Importantly, several adjuvant drugs, including inhibitors of Hsp90 and calcineurin, eliminate perseverance without altering the MIC. We also find that perseverance and resistance are sensitive to mutations in different drug resistance and stress response pathways, underscoring the distinct nature of the two phenomena. Importantly, clinical persistence was associated with higher FoG levels, suggesting that quantitative measurements of perseverance may provide important prognostic information for clinical considerations.

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Antifungals and fungicides

Characterizing molecular mechanisms of heteroresistance in *Candida glabrata*

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Heteroresistance (HetR) is the ability of a small subpopulation to grow within a drug-susceptible majority population at high drug concentrations. We recently described HetR in *Candida glabrata*, the second most common cause of *Candida* infections, which is increasing in prevalence. HetR is an important phenomenon in clinical C. *glabrata* isolates and it appears to contribute to the recurrence of infections. Quantitative PAP (population analysis profiling) assays and more practical penetrance analysis both indicated that levels of HetR are found in *C. glabrata* as a continuum ranging from 1/1000 to 1/100,000 cells in the population. The dynamics of growth of different subpopulations within a genetically identical isolate was measured using ScanLag, which showed that HetR levels correlate with final colony size. Low magnification MSM 400 time-lapse microscopy (MSM) revealed that highly HetR isolates included at least two distinct subpopulations of cells that grew faster and slower in the presence of high drug concentrations, while the majority of the population did not exhibit detectable growth. These two sub-populations expressed different resistance genes, especially efflux pump genes CDR1 and PDH1, to different degrees as determined by RT-PCR. HetR is epigenetic: the rare colonies that appear on drug reproducibly gave rise to mixed populations of progeny with some forming colonies as did the parent (COL) – and others forming a lawn of cells that grow very slowly in the presence or absence of drug (tiny dense- TD). RNA-seq of the different progeny showed that when comparing COL to TD, TD cells repress genes in the ergosterol biosynthetic pathway, which is the target of the fluconazole, while COL express more genes involved in respiration -We propose that HetR is due to epigenetic or transient mechanisms that facilitate the ability of small numbers of cells to grow in drug.

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Antifungals and fungicides

Biosynthesis of histidine, arginine, riboflavin and pantothenic acid, but not siroheme, is crucial for virulence of *Aspergillus fumigatus*

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Aspergillus fumigatus is the most prevalent airborne fungal pathogen causing invasive fungal infections in immunosuppressed individuals. Limitations in antifungal therapy arise from non-specific symptoms of infection, poor diagnostics and comparatively few options for treatment. The aim of this study is to explore the metabolism of *A. fumigatus* on a comprehensive scale as essential virulence determinant to generate a collection of *A. fumigatus* strains with a focus on primary metabolism to target fungal pathways that are absent in mammals. Based on the annotated genome of *A. fumigatus*, metabolic network reconstruction served to identify fungal-specific pathways and key reactions. Predictions for unique enzymes resulted in a candidate list of genes, the inactivation of which is likely to result in an auxotrophic phenotype. The virulence potential of the generated auxotrophic mutant strains was then analyzed in various host niches. We identified five *A. fumigatus* pathways that are essential for growth in minimal medium: biosynthesis of the amino acids histidine and arginine, the vitamins riboflavin and pantothenic acid, and the heme-like prosthetic group siroheme, which is essential for sulfate and nitrate assimilation as well as nitric oxide detoxification. Inactivation of biosynthesis of histidine, arginine, riboflavin and pantothenic acid, but not siroheme, resulted in attenuated virulence of *A. fumigatus* in murine models for invasive aspergillosis with intranasal and systemic infection. The results characterize the availability of nutrients in the host niche and reveal targets for development of novel antifungal therapeutic approaches.

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