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ROME • ITALY
2020

Fungal genetics, host pathogen interaction and evolutionary ecology

FEBRUARY 17-20, 2020 ROME - SAPIENZA UNIVERSITY OF ROME

15TH EUROPEAN CONFERENCE ON FUNGAL GENETICS

PROGRAM & ABSTRACTS

Program & Abstracts

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SUMMARY

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WELCOME

Welcome to ECFG15!

Under the mainstream of fungal genetics several themes will be considered: cell signaling and dynamics, gene expression, genomics, bioinformatic, multiple interactions with hosts and symbiotic partners, molecular evolution, ecology, synthetic biology, environmental and applied mycology.
Sapienza University and Centro Congressi Frentani host this conference, with its interdisciplinary combination of fundamental and technological topics. Alongside the main conference, 7 Satellite workshops will update our current knowledge on specific fungal genera, important as model organisms, as beneficial microorganisms or pathogens.
Roma and its main historical University, Sapienza (established in the 14th century), the largest atheneum in the whole of Europe, are enthusiastic to provide the background for the 15th edition of European Conference . This university is vocated for the study of fungal genetics: the basis for RNA silencing and several other aspects of fungal genetics & biology were here discovered using *Neurospora crassa* as model organism.

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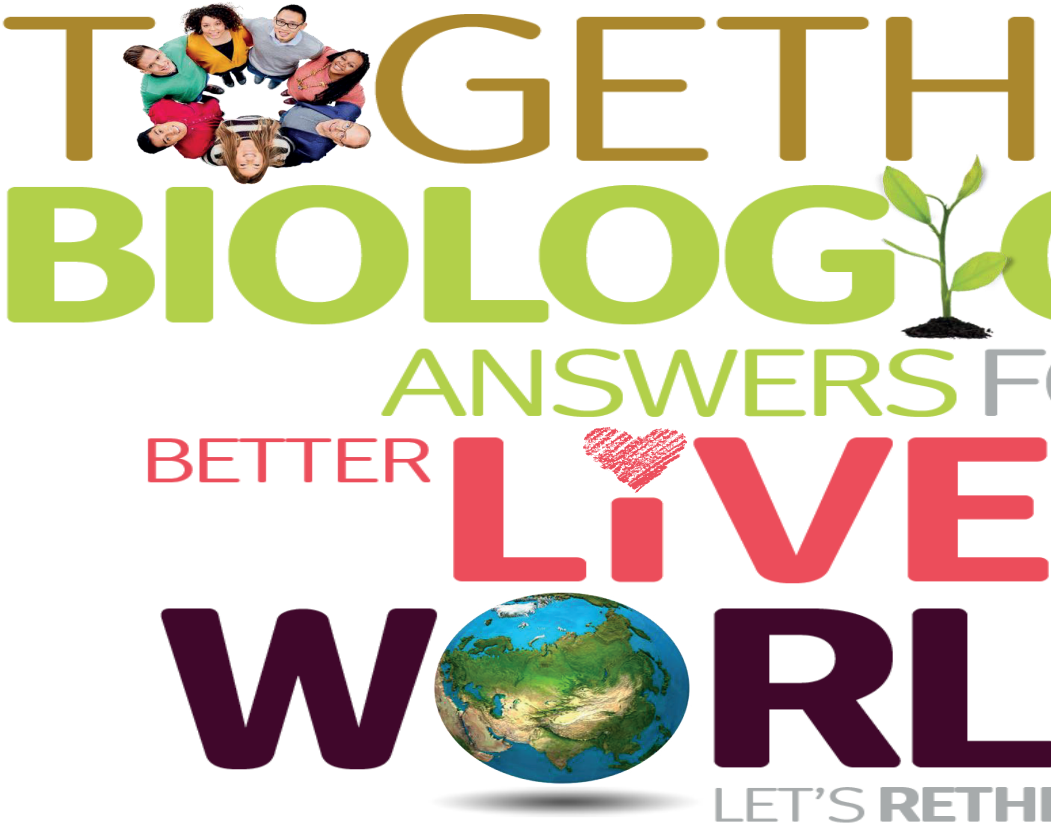
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GENERAL SCIENTIFIC PROGRAM

MONDAY, FEBRUARY 17

08:00 - 09:00	**Satellite Workshops Registration and Welcome Coffee**	***Gipsoteca*** *Sapienza Art Museum*
09:00 - 17:30	SATELLITE WORKSHOP 1 ***ASPERFEST***	*Room:* ***Montalenti*** *- Building:* ***CU022*** *- Side:* ***Genetica***
	SATELLITE WORKSHOP 2 ***FUSARIUM***	*Room:* ***Giacomini*** *- Building:* ***CU022*** *- Side:* ***Botanica*** *- Floor:* ***Ground***
	SATELLITE WORKSHOP 3 ***TRICHODERMA***	*Room:* ***D*** *- Building:* ***CU022*** *- Side:* ***Botanica*** *- Floor:* ***Ground***
	SATELLITE WORKSHOP 4 ***COLLETOTRICHUM***	*Room:* ***C*** *- Building:* ***CU022*** *- Side:* ***Botanica*** *- Floor:* ***Ground***
	SATELLITE WORKSHOP 5 ***NEUROSPORA***	*Room:* ***Marini Bettolo*** *- Building:* ***CU022*** *- Side:* ***Botanica*** *- Floor:* ***First***
	SATELLITE WORKSHOP 6 ***MAGNAFEST***	*Room:* ***E*** *- Building:* ***CU022*** *- Side:* ***Botanica*** *- Floor:* ***Ground***
12:45 - 14:00	**Lunch Break**	***Gipsoteca*** *Sapienza Art Museum*
14:00 - 18:00	**ECFG15 Conference Registration**	*Sapienza Main Campus*
18:00 - 19:30	**Welcome Reception**	*Room:* ***Tarantelli*** *Building:* ***RM019*** *Faculty:* ***Economics*** *Sapienza University of Rome*
19:30 - 20:00	CONFERENCE OPENING SESSION **Massimo Reverberi & Matteo Lorito** | Congress chairs **Marc-Henri Lebrun** | President of ISC	*Room:* ***Tarantelli*** *Building:* ***RM019*** *Faculty:* ***Economics*** *Sapienza University of Rome*
20:00 - 20:45	KEYNOTE 1 **Control of gene expression** CHAIRS: ***Claudio Scazzocchio*** | Imperial College SPEAKER: ***Giuseppe Macino*** | Sapienza University of Rome	*Room:* ***Tarantelli*** *Building:* ***RM019*** *Faculty:* ***Economics*** *Sapienza University of Rome*

TUESDAY, FEBRUARY 18

09:00 - 09:45	KEYNOTE 2 **Language and neighborhood arrangements of BFFs (Bacterial-Fungal Frenemies)** SPEAKER: **Nancy Keller** | University of Wisconsin	*Frentani Convention Center*
09:45 - 12:45	PLENARY SESSION 1 **Cell Biology & Genetic** CHAIRS: ***Andrea Genre*** | Università di Torino ***Nancy Keller*** | University of Wisconsin	*Room:* ***Auditorium*** *Frentani Convention Center*
10:45 - 11:15	**Coffee Break**	*Frentani Convention Center*
12:45 - 14:00	**Lunch Break**	*Frentani Convention Center*
14:00 - 18:00	CS 1.1 **Development and Morphogenesis** CS 1.2 **Cell Regulation and Signaling**	*Room:* ***Latini*** *Frentani Convention Center*
	CS 2.1 **Animal - Fungi Interactions** CS 2.2 **Plant - Fungi Interactions**	*Room:* ***Auditorium*** *Frentani Convention Center*
	CS 3.1 **Evolution** CS 3.2 **Molecular Taxonomy & Phylogenomics**	*Room:* ***Accademia*** *Frentani Convention Center*
15:45 - 16:15	**Coffee Break**	*Frentani Convention Center*
18:00 - 19:30	POSTER SESSION 1 & FLASH TALKS **& Drinks And Appetizers**	*Frentani Convention Center*

WEDNESDAY, FEBRUARY 19

09:00 - 09:45	KEYNOTE 3 **EMBO LECTURE** **Investigating the cell biology of invasive growth by the rice blast fungus *Magnaporthe oryzae*** SPEAKER: **Nick Talbot** | The Sainsbury Laboratory	*Frentani Convention Center*
09:45 - 12:45	PLENARY SESSION 2 **Fungal - Host Interactions** CHAIRS: ***Matteo Lorito*** | University Federico II di Napoli ***Antonio Di Pietro*** | University of Córdoba	*Room: **Auditorium*** *Frentani Convention Center*
10:45 - 11:15	**Coffee Break**	*Frentani Convention Center*
12:45 - 14:00	**Lunch Break**	*Frentani Convention Center*
14:00 - 18:00	CS 1.3 **Primary and secondary metabolism** CS 1.4 **Genome, chromatin and epigenetics**	*Room: **Auditorium*** *Frentani Convention Center*
14:00 - 15:45	CS 1.5 **Omics & bioinformatic**	*Room: **Accademia*** *Frentani Convention Center*
15:45 - 16:15	**Coffee Break**	*Frentani Convention Center*
16:15 - 18:00	CS 2.3 **Antifungal and fungicides**	*Room: **Accademia*** *Frentani Convention Center*
14:00 - 18:00	CS 3.3 **Applied and Environmental Microbiology** CS 3.4 **Synthetic biology and biotechnology**	*Room: **Latini*** *Frentani Convention Center*
18:00 - 19:30	POSTER SESSION 2 & FLASH TALKS **& Drinks And Appetizers**	*Frentani Convention Center*

THURSDAY, FEBRUARY 20

09:00 - 09:45	KEYNOTE 4 **Rapid evolution of Multicellular Snowflake Yeast using Experimental Evolution** **Michael Travisano** | University of Minnesota	*Frentani Convention Center*
09:45 - 12:45	PLENARY SESSION 3 **Evolution and Molecular Ecology** CHAIRS: ***Eva H Stukenbrock*** | Max Planck Institute for Evolutionary Biology ***Silvano Onofri*** | University of Tuscia	*Room:* ***Auditorium*** *Frentani Convention Center*
10:45 - 11:15	**Coffee Break**	*Frentani Convention Center*
12:45 - 14:00	**Lunch Break**	*Frentani Convention Center*
14:00 - 15:30	POSTER SESSION 3 & FLASH TALKS	*Frentani Convention Center*
15:30 - 17:45	WORKSHOP **Perspectives and Potentialities of the JGI Doe Fungal - Omics Resource**	*Frentani Convention Center*
15:45 - 16:15	**Coffee Break**	*Frentani Convention Center*
17:45 - 19:00	**Poster Prizes** Concluding Remarks Announcement of the next meeting: **Susanne Zeilinger**	***Main Hall*** *Frentani Convention Center*
19:30	**Conference Dinner**	***Gipsoteca*** *Sapienza Art Museum*

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KEYNOTE LECTURE ABSTRACTS

MONDAY, FEBRUARY 17

20:00 - 20:45 | Location: **Sapienza University of Rome** | Room: **Tarantelli** | Location: **Faculty of Economics**
FEMS Chair: ***Claudio Scazzocchio*** | Imperial College

KEYNOTE 1

Control of gene expression

Giuseppe Macino

Sapienza University of Rome, Department of Cellular Biotechnology and Hematology BCE, Rome, Italy
Corresponding author: giuseppe.macino@uniroma1.it

The post transcriptional gene silencing mechanisms appeared unexpectedly out of the blue almost thirty years ago.

The first evidence of the phenomenon came from plants and fungi followed later on from animals. The impact of those early discoveries it has been enormous having since changed our way to study biology as recognized by the 2006 Nobel Price to Fire and Mello.

Since then gene silencing has been found in almost every organism playing extremely important roles from genome defense against transposons and viruses, to mRNA stability, to chromatin conformation, to space-temporal gene regulation, to trans-generational inheritance of complex behavioral traits in animals.

An overview will be discussed.

TUESDAY, FEBRUARY 18

09:00 - 09:45 | Location: **Frentani Convention Center**

KEYNOTE 2

Language and neighborhood arrangements of BFFs (Bacterial-Fungal Frenemies)

Nancy Keller

University of Wisconsin, Medical Microbiology and Immunology, Madison, WI, USA
Corresponding author: npkeller@wisc.edu

Bacteria and fungi reside together in diverse environmental niches ranging from soil matrices to communities within and on the host body. We find that small extracellular signaling molecules are the key coinage for inter-kingdom microbial communications. I present three disparate microbial playgrounds where secondary metabolite production influences bacterial and fungal community composition and survival. First, I consider the role of ralsolamycin, a bacterial lipopeptide, in mediating antibacterial secondary metabolite production in fungal colonized by the producing bacterium *Ralstonia solanacearum* (1, 2, 3). Ralsolamycin provides 'housing' for *Ralstonia* by inducing fungal chlamydospores that the bacterium can enter (1, 2) and facilitates 'hitchhiking', allowing other bacteria to invade the chlamydospores. Next, I present a case of nutrient acquisition where copper chelation by *Aspergillus fumigatus* isocyanides (4) robs copper accessibility from other fungi and bacteria. Finally, I show that secondary metabolism of a cheese rind *Pencillium* sp. steers the composition of cheese rind bacteria. Deletion of *Penicillium* LaeA, a global regulator of secondary metabolism, affects bacterial communities due to altered production of fungal natural products with antibacterial properties.

WEDNESDAY, FEBRUARY 19

09:00 - 09:45 | Location: **Frentani Convention Center**

KEYNOTE 3

Investigating the cell biology of invasive growth by the rice blast fungus *Magnaporthe oryzae*

Nicholas J. Talbot

The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, NR4 7UH, United Kingdom
Corresponding author: nick.talbot@tsl.ac.uk

Magnaporthe oryzae is the causal agent of rice blast, one of the most serious diseases affecting rice cultivation around the world. During plant infection, *M. oryzae* forms a specialised infection structure called an appressorium, which generates enormous turgor, applied as mechanical force to breach the rice cuticle. We are studying the mechanism by which appressoria function. Re-polarisation of the appressorium requires a hetero-oligomeric septin GTPase complex to organise a toroidal F-actin network at the base of the infection cell. Formation of the septin complex requires a turgor-dependent sensor kinase, Sln1, required for development of a rigid penetration hypha to rupture the leaf cuticle. Once the fungus invades plant tissue it secretes a large set of effector proteins to suppress host immunity. The fungus also manipulates pit field sites, containing plasmodesmata, to facilitate its spread from cell-to-cell in plant tissue. I will discuss recent progress into understanding the mechanisms of invasive growth by this devastating pathogen.

THURSDAY, FEBRUARY 20

09:00 - 09:45 | Location: **Frentani Convention Center**

KEYNOTE 4

Rapid evolution of Multicellular Snowflake Yeast using Experimental Evolution

Michael Travisano

University of Minnesota Twin Cities, Dept. of Ecology, Evolution & Behavior, BioTechnology Institute, Minneapolis, MN, United States
Corresponding author: travisan@umn.edu

Morphological complexity is one of the most extravagant aspects of Life. Multicellularity facilitates this diversity, in which developmental mechanisms afford tremendous evolutionary possibilities. Understanding the evolution of multicellular complexity is extremely challenging, largely because the first steps in this process occurred in the deep past. The evolutionary origins of most complex multicellular life occurred over 500 million years ago. To address this challenge, we carried out experimental evolution with populations of two species of yeast, *Saccharomyces cerevisiae* and *Kluyveromyces lactis.* We had three major questions: 1. Can multicellularity rapidly evolve under the appropriate selective conditions?, 2. Are evolutionary responses to selection similar across replicates and genetic ancestors, and 3. What are the processes by which cooperation among cells is mediated?

In every replicate and regardless of the species, convergent evolution of "snowflake" multicellular phenotypes rapidly evolved within 8 weeks. The degree of phenotypic convergence was striking, given the over 100 million years of independent evolution between the species. We also observed substantial convergence for the genetic mechanisms of snowflake multicellularity, as mutations in ace2, a transcriptional activator, were causal in several of the *S. cerevisiae* and all of the *K. lactis* replicates. Despite these similarities in selective responses, we also observed striking divergence among the species for mechanisms of cooperation. In all *S. cerevisiae* replicates, cellular cooperation is mediated by clonal reproduction: every cell in a snowflake multicellular individual is identical except for mutations arising during cellular replication. The *K. lactis* snowflakes had the same pattern of genetic identity, but each replicate also contained additional distinct genotypes that aggregated on the multicellular snowflakes. Our results indicate that multicellularity can rapidly arise, and that extraordinary diversity in evolutionary responses is possible regardless of strong genetic convergence.

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PLENARY SESSIONS

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PLENARY SESSION 1

Cell Biology & Genetic

TUESDAY, FEBRUARY 18

09:45 - 12:45 | Location: **Frentani Convention Center** | Room: **Auditorium**
CHAIR: ***Andrea Genre*** | University of Turin & ***Nancy Keller*** | University of Wisconsin

The genetics of Arbuscular Mycorrhizal Fungi
Nicolas Corradi | University of Ottawa

A Toxic Arrangement: Subcellular Compartmentalization of Sesquiterpene Mycotoxin Synthesis
Corby Kistler | University of Minnesota

***Aspergillus fumigatus* signal transduction mechanisms for secondary metabolism production and self-protection**
Gustavo Henrique Goldman | University of São Paulo

Kind recognition regulates social behavior in fungi
Louise Glass | University of California

Cell Wall dynamics in the control of cell growth and morphogenesis
Nicolas Minc | Institut Jacques Monod

The genetics of Arbuscular Mycorrhizal fungi

Nicolas Corradi

University of Ottawa, Dept. Biology, Ottawa, Ontario, Canada
Corresponding author: ncorradi@uottawa.ca

The genetics of arbuscular mycorrhizal fungi (AMF) have been notoriously elusive. In particular, their perpetual multinucleated state, with thousands of nuclei floating in the same cytoplasm, and their obligate biotrophy, has made it difficult to perform experiments with these organisms and isolate good quality DNA and single nuclei to better understand their nuclear complexity and overall biology. Here, I will present recent work based on AMF genomics and single cell/nuclei analysis, as well as new laboratory experiments, and discuss how these are now reshaping our understanding of the genetics and (para) sexual potential of arbuscular mycorrhizal fungi.

A Toxic Arrangement: Subcellular Compartmentalization of Sesquiterpene Mycotoxin Synthesis

Corby Kistler

USDA ARS Cereal Disease Laboratory, University of Minnesota, St. Paul, MN, USA
Corresponding author: hckist@umn.edu

Terpenes are one of the major classes of bioactive fungal secondary metabolites. While knowledge of the enzymology and genetics of the fungal terpenome has advanced greatly in recent years, scant information is available on the cell biology of terpene biosynthesis. Where are terpenes assembled within the cell and how are they exported efficiently? Since other pathways for fungal primary and secondary metabolism also draw upon terpene precursor molecules, how do cells channel and apportion the supply of shared precursors to the different pathways? The answer to these questions will require greater understanding of the subcellular and developmental processes involved in global terpene metabolism. To be discussed is the synthesis of the sesquiterpene mycotoxins deoxynivalenol and culmorin in the fungus *Fusarium graminearum*. Knowledge of subcellular compartmentalization may be essential for understanding the efficient and high level production of these mycotoxins and other terpenes by filamentous fungi.

Aspergillus fumigatus signal transduction mechanisms for secondary metabolism production and self-protection

Laure Nicolas Annick Ries[1], Michael Bromley[2], Sean Doyle[3], Monica Tallarico

Pupo[4], Antonis Rokas[5], Flavio Vieira Loures[6], Koon Ho Wong[7,8], Gustavo H. Goldman[4]

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[7] *Faculty of Health Sciences, University of Macau, Macau SAR, China*
[8] *Genomics and Bioinformatics Core, Faculty of Health Sciences, University of Macau, Macau SAR, China*
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Aspergillus fumigatus is an opportunistic fungal pathogen that secretes an array of immune-modulatory molecules, including secondary metabolites (SMs), which contribute to enhancing fungal fitness and growth within the mammalian host. Gliotoxin (GT) is a SM that interferes with the function and recruitment of innate immune cells, which are essential for eliminating *A. fumigatus* during invasive infections. We identified a C6 Zn cluster-type transcription factor (TF), subsequently named RglT, important for *A. fumigatus* oxidative stress resistance, GT biosynthesis and self-protection. RglT regulates the expression of several gli genes of the GT biosynthetic gene cluster, including the oxidoreductase-encoding gene gliT, by directly binding to their respective promoter regions. Subsequently, RglT was shown to be important for immunomodulation and virulence in an immunocompetent murine model of invasive pulmonary aspergillosis. Homologues of RglT and GliT are present in eurotiomycete and sordariomycete fungi, including the non-GT-producing fungus *A. nidulans*, where a conservation of function was described. Phylogenetically informed model testing led to an evolutionary scenario in which the GliT-based resistance mechanism is ancestral and RglT-mediated regulation of GliT occurred subsequently. In conclusion, this work describes the function of a previously uncharacterised TF in GT biosynthesis and self-protection in both GT-producing and non-producing *Aspergillus* species.

Financial support: FAPESP and CNPq, Brazil

Kind recognition regulates social behavior in fungi

N. Louise Glass, Pedro Goncalves, Jens Heller, Asen Daskalov and Adriana Rico-Ramirez

The Plant and Microbial Biology Department, The University of California, Berkeley, CA, USA
Corresponding author: lglass@berkeley.edu

Kind recognition in microbes is regulated by genetic differences at nonself recognition loci and modulates cooperative versus antagonistic interactions. During vegetative growth in filamentous fungi, somatic cell fusion and conspecific cooperation are social traits that affect colony expansion, substrate utilization and asexual spore production. However, indiscriminate fusion between genetically different asexual spores (germlings) and/or colonies carries the risk of transfer of parasitic elements. Thus, genetic differences at nonself (allorecognition) loci regulate somatic cell fusion in filamentous fungi, thereby restricting cell fusion to individual cells/colonies that are genetically identical at nonself recognition loci. Using *Neurospora crassa* as a model organism, we identified three checkpoints during interactions between cells/colonies that regulate the outcome of somatic cell fusion. The first checkpoint, mediated by differences at the determinant of communication (*doc*) loci, regulates chemotropic interactions between cells/hyphae. The second checkpoint is mediated by genetic differences at the cell wall remodeling (*cwr*) loci and regulates cell wall dissolution following physical contact between germlings/hyphae. The third checkpoint regulates the outcome of somatic cell fusion events, which induces programmed cell death (PCD) when cells carry genetic differences at *het* loci. We identified two loci that mediate PCD in germlings when cells carry different alleles at *sec-9/plp-1* or *rcd-1* loci. PLP-1 shows similarities to NOD-like factors that regulate innate immunity in plants/animals and RCD-1 is a homolog of Gasdermin, which causes pyroptosis in mammalian cells. These observations suggest a relationship between loci that regulate interactions during somatic cell fusion between fungal cells and loci that may regulate innate immunity in fungi.

Cell Wall Dynamics in the Control of cell growth and morphogenesis

Nicolas Minc

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The cell wall is a thin and rigid layer encasing the plasma membrane. It provides mechanical integrity to fungal cells and define their shapes and modes of growth. By developing a super-resolution imaging method to map Cell Wall thickness all around live and growing fission yeast cells, we deciphered the mechanisms controlling CW dynamics during growth and shape changes. We uncovered a homeostatic mechanism controlling wall thickness at growing cell tips, which impinges on cell viability and growth regulation. This mechanism implicates mechanosensing activities of the cell wall integrity. Using novel devices to apply local mechanical stresses on the cell wall, we further establish how cell wall mechanosensor act to probe cell wall mechanics. Those data impact our current understanding of the mechanobiology of cell growth and morphogenesis.

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PLENARY SESSION 2

Fungal - Host interactions

WEDNESDAY, FEBRUARY 19

09:45 - 12:45 | Location: **Frentani Convention Center** | Room: **Auditorium**
CHAIR: ***Matteo Lorito*** | Università di Napoli Federico II & ***Antonio Di Pietro*** | Universidad de Cordoba

Structure, evolution, and functions of the *Arabidopsis* root mycobiota
Stéphane Hacquard | Max Planck Institute

Pathogen-secreted effectors: host subversion and …. what else
Bart P.H.J. Thomma | Wageningen University & Research

Rhizosphere interactions: the plant, the pathogen .... and the hitchhiker
David Turrà | Università di Napoli Federico II

Cross kingdom small RNA trafficking between plants and fungal pathogens
Hailing Jin | University of California

Natural diversity in the predatory behavior facilitates the establishment of a new robust model strain for nematode-trapping fungi
Yen-Ping Hsueh | Academia Sinica

Structure, evolution, and functions of the *Arabidopsis* root mycobiota

Stéphane Hacquard

Max Planck Institute for Plant Breeding Research, Cologne, Germany
Corresponding author: hacquard@mpipz.mpg.de

Roots of healthy plants are colonized by a rich diversity of microbes that include mainly bacteria and fungi. A continental-scale survey of the *A. thaliana* root microbiota revealed that several bacterial and few fungal taxa consistently colonize plant roots across Europe, irrespective of differences in soil conditions and climate. Reciprocal transplant experiments further demonstrated that, across large spatial scales, climate had more effect than soil conditions on the composition of root-associated fungal communities, consistent with the hypothesis that climate strongly influences fungal biogeography worldwide. Extensive isolation of fungi from roots of healthy *A. thaliana,* followed by re-colonization experiments in a gnotobiotic plant system with a complex synthetic fungal community indicated a detrimental effect on *A. thaliana*, which was reverted in the presence of bacteria. Our results suggest that plant immune system and bacterial commensals act in concert to modulate abundance and pathogenic potential of fungi in plant roots. Large-scale fungal genome sequencing of 41 *A. thaliana* root-associated fungi and comparative analysis with 79 additional genomes of fungi from different ecological groups revealed an unexpectedly large repertoire of genes encoding small-secreted proteins and carbohydrate active enzymes in *A. thaliana* fungal associates. These signatures resemble those detected in genomes of pathogenic and endophytic fungi but not of symbiotic ectomycorrhizal fungi. Re-colonization experiments using each of the 41 strains in mono-association with germ-free *A. thaliana* validated the pathogenic nature of several of these isolates, but also identified few phylogenetically diverse strains that promote plant growth exclusively under phosphate-deficient conditions. We now investigate which genes are associated with beneficial and pathogenic activities in order to better understand the genetic basis driving the fine line between endophytism and parasitism in fungi.

Pathogen-secreted effectors: host subversion and …. what else?

Bart P.H.J. Thomma

Wageningen University, Wageningen, The Netherlands
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Fungal plant pathogens continuously evolve to evade host immunity. During host colonization, pathogenic fungi secrete effectors to perturb immune responses. However, these effectors may become recognized by host immune receptors in turn to reinstall immunity. To facilitate the evolution of effector repertoires, such as the elimination

of recognized effectors, effector genes often reside in genomic regions that display increased plasticity. The genome of the vascular wilt fungus *Verticillium dahliae* displays regions with extensive presence/absence polymorphisms, so-called lineage-specific regions. These hypervariable regions between *V. dahliae* and contain genes that support host colonization and adaptive traits, are associated with methylated repetitive DNA, H3 Lys-27 methylated histones (H3K27me3). Moreover, repetitive DNA within LS regions is more transcriptionally active and has increased DNA accessibility.

Functional analysis has shown that particular *V. dahliae* effectors act as host range determinants, presumably by differential effects on host physiology. Additionally, we recently hypothesized that pathogens may have evolved to manipulate host microbiomes to their advantage. Recent evidence indicates that *Verticillium dahliae* utilizes effector proteins for niche colonization through selective manipulation of host microbiomes by suppressing microbes with antagonistic activities.

Rhizosphere interactions: the plant, the pathogen .... and the hitchhiker

David Turrà

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Microbes have developed diverse strategies to successfully encounter the proper ecological niche in the extremely variable and complex matrix of the soil. Hyphae of the destructive plant pathogen *Fusarium oxysporum* (*Fo*), the causal agent of vascular wilt disease in more than a hundred different crop species, can track shallow chemical gradients released from nutrient sources, self- and fungal partner- or plant host-secreted compounds. We have used genetic, biochemical and microscopy-based approaches to understand which host- and self-generated signals function as chemoattractants and how *Fo* hyphae sense and transduce these external stimuli to re-orient their direction of growth. While different sets of conserved mitogen activated protein kinase (MAPK) cascades are used by *Fo* to discriminate between nutrient and fungal (pheromones) or plant (peroxidases) secreted cues, the perception of the latter type of stimuli requires the cell wall integrity MAPK Mpk1 and the G-protein-coupled receptors (GPCRs) Ste2 and Ste3. In *Fo* the perception of self-secreted α- and a-pheromone only requires its cognate receptor, respectively Ste2 or Ste3, to regulate conidial germination via autocrine signaling. Contrarily, the chemotropic response of *Fo* towards class III peroxidases, the major chemoattractants secreted by host plants, requires both Ste2 and Ste3, but not α-pheromone and a-pheromone, ruling out that plant cues might trigger chemotropism indirectly via autocrine activation of pheromone receptors. However, hyphal re-directioning towards the roots is not only beneficial for *Fo* to reach and infect the plant host, but also to a soilborne endophytic rhizobacterium, *Rahnella aquatilis* (*Ra*), which

establishes a symbiotic relationship with it. Indeed, *Ra* cells locate *Fo* hyphae in the soil via pH-mediated chemotaxis, then use them as highways to gain preferential access into the host plant roots by blocking *Fo* penetration and colonization. Taken together, our data suggest that *Fo* has co-opted and specialized its pheromone perception system to discriminate between self-pheromone peptides and host-derived signals, an ability that is unwittingly used by other soil-inhabiting microbes for their own benefit.

Cross kingdom small RNA trafficking between plants and fungal pathogens

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Small RNAs (sRNAs) are short non-coding RNAs that mediate gene silencing in a sequence-specific manner. We discovered that some sRNAs from eukaryotic pathogens, such as *Botrytis cinerea*, can be transported into host plant cells and suppress host immunity genes for successful infection (Weiberg *et al*., Science 2013). We further demonstrated that such cross-kingdom RNAi is bi-directional. Plants can also send sRNAs into pathogens using extracellular vesicles to silence fungal virulence genes as part of its immune responses (Cai *et al*., Science 2018). We found that plants have multiple classes of extracellular vesicles, and exosome is the major class responsible for sRNA delivery.

Furthermore, we have discovered that many fungal pathogens can take up double-stranded RNAs (dsRNAs) and sRNAs from the environment. Applying sRNAs or dsRNAs that target fungal virulence-related genes can suppress fungal diseases. For example, application of RNAs that target Botrytis *Dicer* genes on the surface of fruits, vegetables and flowers significantly inhibits grey mold disease (Wang *et al*,, Nature Plants, 2016). Such pathogen gene-targeting RNAs represent a new generation of fungicides that are durable and eco-friendly.

1. Arne Weiberg, Ming Wang, Feng-Mao Lin, Hongwei Zhao, Zhihong Zhang, Isgouhi Kaloshian, Hsien-Da Huang, Hailing Jin*: Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. Science, 2013, 342 (6154) 118-123.
2. Qiang Cai, Lulu Qiao, Ming Wang, Baoye He, Feng-Mao Lin, Jared Palmquist, Hsien-Da Huang, and Hailing Jin*: Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. Science, 2018, 360(6393)1126-1129.
3. Ming Wang, Arne Weiberg, Feng-Mao Lin, Bart P. H. J. Thomma, Hsien-Da Huang and Hailing Jin*: Bidirectional cross-kingdom RNAi and fungal uptake of external RNAs

confer plant protection. Nature Plants, 2016, 10.1038/nplants.2016.151.

Natural diversity in the predatory behavior facilitates the establishment of a new robust model strain for nematode-trapping fungi

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Nematode-trapping fungi (NTF) are a group of specialized microbial predators that consume nematodes when food sources are limited. Predation is initiated when conserved nematode ascaroside pheromones are sensed, followed by the development of complex trapping devices. To gain insights into the co-evolution of this inter-kingdom predator-prey relationship, we investigated natural populations of nematodes and NTF, that we found to be ubiquitous in soils. *Arthrobotrys* species were sympatric with various nematode species and behaved as generalist predators. The ability to sense prey amongst wild isolates of *A. oligospora* varied greatly, as determined by the number of traps after exposure to *Caenorhabditis elegans*. While some strains were highly sensitive to *C. elegans* and the nematode pheromone ascarosides, others responded only weakly. Furthermore, strains that were highly sensitive to the nematode prey also developed traps faster. The polymorphic nature of trap formation correlated with competency in prey killing, as well as with the phylogeny of *A. oligospora* natural strains, calculated after assembly and annotation of the genomes of twenty isolates. A chromosome level genome assembly and annotation was established for one of the most sensitive wild isolate, and deletion of the only G protein β subunit-encoding gene of *A. oligospora* nearly abolished trap formation, implicating G protein signaling in predation. In summary, our study establishes a highly responsive *A. oligospora* wild isolate as a novel model strain for the study of fungal-nematode interactions and demonstrates that trap formation is a fitness character in generalist predators of the NTF family.

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PLENARY SESSION 3

Evolution and Molecular Ecology

THURSDAY, FEBRUARY 20

09:45 - 12:45 | Location: **Frentani Convention Center** | Room: **Auditorium**
Chair: ***Eva H Stukenbrock*** | Max Planck Institute & ***Silvano Onofri*** | University of Tuscia

Genomics and the making of biodiversity across the budding yeast subphylum
Antonis Rokas | Vanderbilt University

The link between sexual and vegetative (in)compatibility in fungal speciation
Hanna Johannesson | Uppsala University

The evolution of mycorrhizal genomes and transcriptomes
Annegret Kohler | Institute National Recherche Agronomique

Evolution of secondary metabolite diversity in fungi
Daren W. Brown | USDA ARS NCAUR, Preoria

Soil fungal responses to global change
Matthias C. Rillig | Freie Universität Berlin

Genomics and the making of biodiversity across the budding yeast subphylum

Antonis Rokas

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Yeasts are unicellular fungi that do not form fruiting bodies. Although the yeast lifestyle has evolved multiple times, most known species belong to the subphylum Saccharomycotina (hereafter yeasts). This diverse group includes the premier eukaryotic model system, *Saccharomyces cerevisiae*; the common human commensal and opportunistic pathogen, *Candida albicans*; and over 1,000 other known species. Yeasts are found in every biome and continent and are more genetically diverse than either plants or bilaterian animals. Ease of culture, simple life cycles, and small genomes have made yeasts exceptional models for molecular genetics, biotechnology, and evolutionary genomics. As part of National Science Foundation's Dimensions of Biodiversity program, we and our collaborators have undertaken a large-scale examination to uncover the genetic basis of metabolic diversity in the entire Saccharomycotina subphylum. In my talk, I will discuss the team's latest evolutionary discoveries from organisms spanning the diversity of the subphylum and how they are revising our understanding of the major drivers of genomic and phenotypic evolution in eukaryotes.

The link between sexual and vegetative (in)compatibility in fungal speciation

Hanna Johannesson

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Allorecognition, the capacity to recognize self from conspecific non-self, is likely to influence the evolution of reproductive isolation. Pre- and post-zygotic reproductive barriers can result from an overlap between the genetic basis of sexual (in)compatibility and that of the recognition of non-self. In the model fungus *Podospora anserina*, the loci controlling vegetative incompatibility, or *het* genes, are associated with various degrees of sexual incompatibilities, including female sterility, hybrid inviability, and meiotic drive. We have used genomic data of a natural population to explore the effects of pleiotropic allorecognition genes on reproductive isolation and genetic differentiation. We show that the *het* genes in *P. anserina* display strong signatures of balancing selection, as expected from allorecognition loci, amongst a genomewide context of extremely low genetic diversity. We determine that mating success correlates to the identity of the locus *het-v*, which we characterize through positional and complementation cloning, as well as site-

directed mutagenesis. The epistatic interaction of *het-v* alleles with the unlinked *het-r* gene defines the boundaries of two mating groups. We confirmed a significant deficit of recombinant genotypes in the wild, demonstrating the lack of current mixing despite substrate co-occurrence. We conclude that the *het-r/v* system not only contributes to speciation, but it directly defines reproductively isolated groups by equating vegetative recognition with sexual compatibility.

The evolution of mycorrhizal genomes and transcriptomes

Annegret Kohler[1], Eniko Kiss[2], Emmanuelle Morin[1], Shingo Miyauchi[1], Laszlo G. Nagy[2], Igor Grigoriev[3], Francis Martin[1], Mycorrhizal Genome Initiative

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Mycorrhizal fungi have coevolved with their hosts since the emergence of land plants and the combination of calibrated phylogenies with the growing number of fungal genomes, allows new insights into the evolutionary history of mycorrhizal symbiosis.

The comparison of 135 genomes from mycorrhizal fungi with wood or litter decomposers confirmed the general trend within ectomycorrhizal fungal (ECM) genomes to reduce genes coding for plant cell wall degrading enzymes. However, ECM species seem to have retained diverse sets of enzymes depending on their ecological niches.

More genomes enable us as well to compare transcriptomes and to identify symbiosis-induced genes.

Do these mycorrhiza-induced transcripts originate from conserved genes or are they species-specific? For *Laccaria bicolor* we could show that both conserved and clade-specific genes are used to establish symbiosis with the roots of the host tree *Populus*. Using a phylostratigraphy approach we compared the «symbiosis-toolbox» of 10 ectomycorrhizal interactions.

In Ascomycota and Basidiomycota, an average of 74 and 67% of ectomycorrhiza-induced genes predated the evolution of ectomycorrhizal symbiosis, respectively. Approximately 6 and 18% of ectomycorrhiza-induced genes were already present in the most recent common ancestors of Ascomycota and Basidiomycota. These findings suggest that the origin of most genes induced during ectomycorrhiza development and functioning

predates the emergence of symbiosis in the studied fungal lineages, implying that these genes have been co-opted for ectomycorrhiza development during evolution from saprotrophic ancestors.

The set of conserved ectomycorrhiza-induced genes showed little or no overlap among the analyzed species, suggesting that independently evolved lineages recruited different ancestral gene families for symbiosis. The induced genes are coding for the same functions but without orthology, like small-secreted proteins, transporters or carbohydrate active enzymes.

Evolution of secondary metabolite diversity in fungi

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Fungi produce thousands of secondary metabolites (SMs) that are diverse in chemical structure and biological activity. SMs produced by plant pathogenic species of Fusarium include pigments, plant hormones, and some of the mycotoxins of greatest concern to agricultural production and food safety. Chemical and genetic analyses over the last four decades have provided tremendous insights into biosynthesis of Fusarium SMs, which has contributed to understanding of fungal secondary metabolism in general, particularly with respect to genetic and evolutionary bases for variation in SM production. Such variation includes: 1) production versus nonproduction of SM families; and 2) production of different structural analogs of the same SM family. The former can result from the presence versus absence of the corresponding SM biosynthetic loci, whereas the latter typically results from sequence differences within homologous biosynthetic loci. Gene loss and horizontal gene transfer (HGT) appear to be major contributors to differences in presence and absence of SM clusters. The presence of closely related homologous biosynthetic loci in the same species appears to result from HGT more often than it does from recent duplication of the loci. Sequence differences within homologous loci that affect SM structural variation can result from loss, acquisition and changes in function of individual biosynthetic genes. The evolutionary drivers of SM structural diversity likely depend on the ecological advantage(s) provided by a particular SM. Because of their role in plant pathogenesis, we propose that resistance of plants to trichothecenes can drive structural diversity of this important family of mycotoxins.

Soil fungal responses to global change

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Global environmental change is a multifactorial phenomenon, and the concurrent action of multiple factors gives rise to large uncertainty in predicting effects. For example, fungal communities in soils, and soils themselves, have to respond to a multitude of drivers of global change. This fact is currently not well reflected in the available literature, where only a tiny fraction of studies on global change effects on soils considers more than two drivers. We conducted a microcosm experiment (Rillig *et al*. 2019, Science, doi: 10.1126/science.aay2832) including ten such drivers, including abiotic factors (including temperature), resource availability, chemical toxicants and compounds (inorganic and synthetic organic), and an agent of physical change (microplastics). We did so by randomly selecting drivers from a pool of these ten factors of global change, borrowing a design from biodiversity-ecosystem functioning studies. This way, we provided a gradient of 2, 8, and 10 drivers, each replicated 10 times. Each replicate in these 'factor richness' levels had a different, randomly determined combination of factors; we also replicated all 10 individual factors. Patterns of fungal biodiversity and ecosystem processes showed a consistent directional trend along the number of factors; there was a progressive loss of fungal diversity, concomitant to decreased fungal-influenced soil process rates (decomposition, soil aggregation, respiration). Our study emphasizes the need to consider multiple factors of global change to achieve a better understanding of the pressures that fungal communities face.

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CONCURRENT SESSION ABSTRACTS

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CONCURRENT SESSION 1
Cell Biology & Genetic

Concurrent Session 1.1
DEVELOPMENT AND MORPHOGENESIS

TUESDAY, FEBRUARY 18

Location: **Frentani Convention Center** | Room: **Latini**
CHAIR: ***Monika Schmoll*** & ***Daniela Uccelletti***

14:00 - 14:15 **Appressorium: the Breakthrough in Dikarya**
Alexander Demoor | Université de Paris LIED UMR

14:15 - 14:30 **The phosphatidylserine flippase Drs2 has a unique role during *Candida albicans* invasive growth**
Miguel A. Basante-Bedoya | University Côte d'Azur CNRS, Inserm, IBV
FEMS Grant

14:30 - 14:45 **Revealing tissue-specific developmental trajectories in fruiting bodies of *Coprinopsis cinerea* by single-cell RNA sequencing**
Torda Varga | Biological Research Center Institute of Biochemistry
FEMS Grant

14:45 - 15:00 **The phosphatase ZtVh1 is required for the growth transition to a stress response morphotype in a fungal plant pathogen**
Carolina Sardinha Francisco | Swiss Federal Institute of Technology - ETHZ

15:00 - 15:15 **A-to-I mRNA editing: Diversification of the fungal proteome during sexual propagation**
Ines Teichert | Ruhr University Bochum General and Molecular Botany

15:15 - 15:30 **GUL-1 mediates cell wall remodeling via the COT-1 pathway in *Neurospora crassa***
Inbal Herold | The Hebrew University of Jerusalem, Plant Pathology and Microbiology

15:30 - 15:45 **Many-sided DHN melanin – spotlight on its function in microcolonial black fungi**
Julia Schumacher | Bundesanstalt für Materialforschung und -prüfung (BAM)

Appressorium: The Breakthrough in *Dikarya*

Alexander Demoor, Philippe Silar, Isabelle Lacaze & Sylvain Brun

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Appressoria are well known structures allowing mechanical penetration of fungal hosts. Despite their thorough study in plant pathogens and in symbiotic fungi because of their importance in the infection/life cycle, the differentiation of similar structures in saprobes has seldom been investigated. We have shown that the saprotrophic model fungus *Podospora anserina* develops appressoria [1] and that a common set of genes (*Nox2*, *NoxR*, *Pls1*, *Mpk2*, etc.) regulates appressorium development in *P. anserina* in a comparable manner to plant pathogenic species such as *Magnaporthe oryzae*. This greatly suggested that appressoria in pathogens and in saprotrophs are homologous structures. This prompted us to address the question whether appressorium development is a common feature of filamentous fungi. And indeed, we have recently shown that many saprotrophic fungi from a large range of taxa can produce appressoria, leading us to conclude that the ability of filamentous fungi to differentiate appressoria is widespread in saprotrophic *Dikarya* [2]. Moreover, we have shown that this set of genes controlling appressorium development is also required for ascospores germination [3]. In order to characterize both of these key processes, we designed a combined approach based on the study of mutants showing spontaneous ascospores germination. Six GUN (Germination UNcontrolled) mutants have been isolated and sequenced. We have started the characterization of the candidate gene for the first two of them. Interestingly, *GUN1* is orthologous to a virulence factor of *M. oryzae* required for appressorium development and *GUN2* is a transcription factor never studied in filamentous fungi. Preliminary results show that both genes are important for appressorium development.

1. Brun, S., Malagnac, F., Bidard, F., Lalucque, H. & Silar, P. Mol. Microbiol. 74, 480–496 (2009)

2. Demoor, A., Silar, P. & Brun, S. Journal of Fungi 5, 72 (2019)

3. Lambou, K. *et al*. Eukaryotic Cell 7, 1809–1818 (2008)

The phosphatidylserine flippase Drs2 has a unique role during *Candida albicans* invasive growth

Miguel A. Basante-Bedoya[1], Rocío García-Rodas[2], Óscar Zaragoza[2], Robert Alan Arkowitz[1], Martine Bassilana[1]

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Phospholipid flippases (P4-ATPases) transport lipids across the membrane bilayer to generate and maintain membrane asymmetry; these ATPases translocate lipids from the external leaflet to the cytosolic leaflet of cellular membranes. There are 5 flippases in *Candida albicans*, including Drs2, which we showed to be critical for hyphal invasive growth (1). Indeed, a drs2 deletion mutant grew similar to the wild-type strain during budding growth, yet was deficient for invasive growth and, in particular, unable to maintain hyphal growth. This mutant had an altered distribution of phosphatidylserine (PS), as assessed with a fluorescent reporter, and was additionally hypersensitive to fluconazole. Very recently, this flippase was also shown to be critical for copper sensitivity (2). We now show that Drs2 is essential for virulence in a murine model for systemic infection. To delineate the role of Drs2 during *C. albicans* hyphal growth, we investigated the dynamics of distribution of this ATPase, as well as that of different lipids and key regulators, during initiation and maintenance of hyphal growth. We also characterized a Drs2 point mutant, analogous to that shown to be altered for PS flipping in *S. cerevisiae* (3).

Furthermore, we examined the role of other flippases, such as Dnf2, in invasive hyphal growth, together with their importance in response to membrane stress. All together, our results indicate that Drs2 has a unique role during *C. albicans* hyphal growth maintenance, which appears to be particularly critical upon septum formation.

1. Labbaoui *et al*., 2017, PLoS Pathogens, 13(2):e1006205.

2. Douglas & Konopka, 2019, PLoS Genetics, 15(1):e1007911.

3. Baldridge & Graham, 2012, PNAS, 109(6):E290-8.

Revealing tissue-specific developmental trajectories in fruiting bodies of *Coprinopsis cinerea* by single-cell RNA sequencing

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The fate of cells in a developing organism has always been a central question for biologists. While elaborate cell fate maps exist in animals and plant model systems, cell trajectories during multicellular development are almost completely unknown in fungi. Here we explore tissue differentiation of *Coprinopsis cinerea* using laser-capture

microdissection (LCM) coupled with single cell RNA sequencing (scRNA-Seq) and compare temporal and spatial mRNA expression changes in different cell populations to understand fine-scale patterns of fruiting body development.

We first adapted LCM and scRNA-seq to Basidiomycetes and modified methods to obtain the following procedure. (1) Primordia are histologically fixed. (2) Samples are embedded in sucrose solution and frozen. (3) 13 µm thick cryo-sections are placed on PEN slides. (4) LCM is performed on a Zeiss PALM MicroBeam microscope. (5) RNA extraction by the PicoPure™ RNA kit. (6) Library construction using the Smart-Seq2 protocol. (7) Illumina Hiseq paired-end (150 bp) sequencing to a depth of ~40M reads per sample.

We examined six developmental stages and overall 26 tissue types: mycelium, primary hyphal knot, secondary hyphal knot (two tissue types), stage one primordium (five tissue types), stage two primordium (eight tissue types) and stage three primordium (nine tissue types). From each tissue types we isolated ~ 31 sections with a total area of 1.16 +/- 1.72 mm^2 per tissue type. Four biological replicates for each tissue samples were used for RNA extraction generating 2.3 +/- 2.6 ng/µl total RNA with sufficient quality for sequencing (RIN: 7.3 +/- 0.6).

Our analysis will provide both temporal and spatial information on gene expression of different tissue types through six developmental stages, which will give the opportunity to discover developmentally regulated genes. Furthermore, by examining co-expression modules we will interpret genes having key role in tissue formation and fruiting body development.

The phosphatase *ZtVh1* is required for the growth transition to a stress response morphotype in a fungal plant pathogen

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The important fungal wheat pathogen *Zymoseptoria tritici* can change growth morphology in response to environmental stresses, which allows this fungus to adapt to a changing environment. We recently characterized the responses of *Z. tritici* to a series of environmental stresses, leading to the discovery that *Z. tritici* can form chlamydospores. *Z. tritici* chlamydospores are better able to survive extreme cold, heat, drought and fungicides than other cell types. Here, we used natural variation in the morphological stress response occurring among *Z. tritici* strains to identify candidate

genes associated with the different morphotypes using quantitative trait locus (QTL) mapping. We phenotyped growth morphologies at 27°C for 261 offspring isolates from a cross between 1E4 (mainly hyphal growth) and 1A5 (only chlamydospore formation) strains. QTL mapping identified a 95% confidence interval with a large LOD score containing only eight genes. Two of them encoded transcription factors (*ZtAsg1* and *ZtTf2*) and another two encoded phosphatases (*ZtVh1* and *ZtPtc5*) that were highly polymorphic among the parental strains. Functional characterizations of these four genes showed that deletion of the phosphatase *ZtVh1* severely represses hyphal growth and induces earlier chlamydospore formation. *ZtVh1* orthologs in other filamentous fungi dephosphorylate the mitogen-activated protein kinase (MAPK) *ZtSlt2*, a downstream kinase of the Cell Wall Integrity (CWI) pathway. Consistent with a role of this phosphatase, *ZtVh1* mutants exhibited an increased sensitivity to cell wall-interfering compounds. We demonstrated that chlamydospore formation depends on the CWI pathway regulation in *Z. tritici*. We propose that the *ZtVh1* allele from 1A5 leads to a complete repression of the morphological transition to hyphal growth, which results in chlamydospore formation as an extreme stress response morphotype. Our results illustrate that a foliar wheat pathogen produces chlamydospores that enable the pathogen to survive under highly stressful conditions. The natural genetic variation allowed us to identify a new gene involved in the morphological stress response.

A-to-I mRNA editing: Diversification of the fungal proteome during sexual propagation

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The editing of mRNA occurs in bacteria, eukaryotic organelles, and in nuclear transcripts of metazoan and fungal species. Editing of organellar transcripts mostly leads to the restoration of open reading frames for conserved proteins, while editing of nuclear mRNA is known to cause changes in proteins that were already functional before. A-to-I editing in filamentous ascomycetes occurs specifically during the formation of fruiting bodies. We use the filamentous ascomycete Sordaria macrospora as a model organism for studying the effect of RNA editing on fruiting body and ascospore formation. By RNA-seq, we found that many editing sites are located in TAG stop codons, changing the stop codon to a tryptophan codon, thus extending the open reading frame (stop loss). We verified the C-terminal elongation of corresponding proteins by Proteomics and Proteogenomics and quantified the amount of different protein isoforms. We further analyzed the function of

genes whose transcripts undergo A-to-I editing, which we named edited in fruiting body development (efd) genes. Analysis of efd deletion strains revealed that these genes tend to function in ascospore generation. Complementation analysis with native, pre-edited, and non-editable efd versions further indicate that both, the genome-encoded short isoform as well as the C-terminally extended protein isoform, have distinct functions during this process. In silico analysis of the extended C-termini of proteins encoded by stop loss mRNAs showed that they often carry new targeting signals, small linear motifs, or functional domains. We present results from the analysis of these additional motifs for the function of different EFD proteins. It may be hypothesized that A-to-I mRNA editing enables filamentous ascomycetes to diversify their proteome to perform the massive cellular reorganization required for ascospore formation.

GUL-1 mediates cell wall remodelling via the COT-1 pathway in *Neurospora crassa*

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In *N. 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* (which encodes an mRNA-binding protein involved in translational regulation of cell wall remodeling proteins). Inactivation of *gul-1* also results in improved characteristics of the *cot-1* cell wall and septa. In fact, a 40% increase in chitin content in the cell wall of *cot-1* (when compared to the wild type) was almost abolished in the *gul-1;cot-1* mutant. Δ*gul-1* was also almost 2-fold more sensitive to a chitin synthase inhibitor (Nikkomycin Z), when compared to the wild type. RNASeq analysis revealed that GUL-1 affects transcript abundance of at least 25 genes involved in cell-wall remodeling via the COT-1 pathway. GUL-1 was also found to regulate additional pathways such as transmembrane transport as well as amino acid and nitrogen 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. Results of RNA antisense purification (RAP) and RNA immunoprecipitation (RIP) experiments demonstrate the occurrence of physical interactions between GUL-1 and different mRNAs. The GUL-1 protein is distributed within the entire hyphal cell, along with the presence of aggregates that traffic within the cytoplasm in a microtubule-dependent manner. Cellular stress resulted in a

2-3-fold increase of GUL-1 aggregate association with nuclei. GUL-1 physically interacts with protein components of the translational machinery as well as with stress granule proteins (e.g., PAB and EIF-A/B/E/F/H/I/L and 40S ribosomal subunits). Taken together, we have demonstrated that GUL-1 is a bona fide RNA-binding protein involved in cell wall remodeling and stress response.

Many-sided DHN melanin – spotlight on its function in microcolonial black fungi

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Dihydroxynaphthalene (DHN) melanin is produced by different Ascomycetes via slightly differing biosynthetic routes. The polyketide synthases (PKS) release the heptaketide YWA1, the hexaketide AT4HN or the pentaketide T4HN. The first two products are deacetylated by 'yellowish-green' hydrolases to T4HN, and T4HN is further converted by a core set of enzymes to DHN. Final polymerization steps are accomplished by multicopper oxidases. DHN melanogenesis is often regulated in a spatial and temporal fashion resulting e.g. in melanized reproduction, survival and/or infection structures. Remarkable is the regulation of the DHN melanogenesis in the foliar plant pathogen *Botrytis cinerea*: it involves two differently expressed PKSs providing the precursor in conidia and sclerotia, respectively (Schumacher 2016, *Mol Microbiol*). In contrast, a polyphyletic group of Ascomycetes (microcolonial fungi/ black yeast) dwelling in hostile habitats such as bare rock surfaces in hot and cold deserts, exhibits constitutive melanogenesis. Here, DHN melanin builds a protective layer around all vegetative cells thus contributing to the survival of diverse environmental stresses even without specialized reproduction structures. As part of our continuing research on microcolonial rock-inhabiting fungi, we chose the genetically amenable *Knufia petricola* strain A95 (Nai *et al*. 2013, *Fungal Genet Biol*; Noack-Schönmann *et al*. 2014, *AMB Express*) for detailed studies. DHN-deficient mutants generated by targeted mutation of biosynthetic genes were studied with regard to the architecture of the cell wall and the EPS (extracellular polymeric substances) matrix, attachment to and weathering of olivine, as well as the tolerance to abiotic and biotic stresses. We will discuss the critical role of the outer cell surface (DHN melanin and EPS) in adhesion to the substrate and subsequent damage of the colonized surface.

Concurrent Session 1.2
CELL REGULATION AND SIGNALING

TUESDAY, FEBRUARY 18

Location: **Frentani Convention Center** | Room: **Latini**
CHAIR: ***David Turrà*** & ***Nicolas Minc***

16:15 - 16:30 **Who regulates the regulator? Kinase mediated regulation of the molecular chaperone Hps90 and its role infungal virulence**
Stephanie Diezmann | University of Bristol Cellular and Molecular Medicine

16:30 - 16:45 **The MAK-1 and MAK-2 MAP kinase modules have related but different functions in cell-cell fusion in *Neurospora crassa***
Lucas Well | TU Braunschweig Institute for genetics

16:45 - 17:00 **Septins coordinate cell wall integrity and lipid metabolism**
Michelle Momany | University of Georgia Fungal Biology Group and Plant Biology Department

17:00 - 17:15 **Nutrient transporter translocation to the plasma membrane via Golgi bypass in *Aspergillus nidulans***
Sofia Dimou | National and Kapodistrian University of Athens

17:15 - 17:30 **Functional characterization of an atypical RNase III involved in a RNAi-related mechanism of RNA degradation in *Mucor circinelloides***
José Tomás Cánovas-Márquez | University of Murcia Genetics and Microbiology
FEMS Grant

17:30 - 17:45 **Horizontal gene transfer in the human and skin commensal Malassezia: a bacterially-derived flavohemoglobin is required for NO resistance**
Giuseppe Ianiri | Università di Campobasso

17:45 - 18:00 **A hierarchical transcriptional network controls appressorium development in the rice blast fungus in response to surface hydrophobicity**
Miriam Oses-Ruiz | The Sainsbury Laboratory

Who regulates the regulator? – Kinase mediated regulation of the molecular chaperone Hps90 and its role in fungal virulence

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Heat shock protein 90 (Hsp90) is a central regulator of cellular protein homeostasis with important implications for development and disease. In the recent past, Hsp90 has also emerged as a key regulator of fungal stress responses and virulence. In the leading fungal pathogen of humans, Candida albicans, the chaperone governs emergence of antifungal drug resistance (Cowen and Lindquist, 2005), morphogenetic diversity (Shapiro, *et al*., 2009), and biofilm drug resistance (Robbins *et al*., 2011). Yet, with the exception of the conserved transcription factor Hsf1 (Nicholls *et al*., 2011), the molecules and pathways regulating Hsp90 in pathogenic fungi remain enigmatic.

To begin to unravel these mechanisms, we mapped the first Hsp90 genetic interaction network in different environmental stress conditions using a chemical-genomic approach (Diezmann *et al*., 2012). While the vast majority of genes interact with Hsp90 only under specific conditions, four out of 226 interactors were relevant in most environments. Two 'high-connectivity' interactors are subunits of the CK2 kinase, which phosphorylates a conserved threonine residue in Hsp90 in other organisms (Mollapour *et al*., 2011). Here we investigated the relationship between this ubiquitous kinase and its stress-responsive chaperone target. Mass-spectrometric mapping of Hsp90 phosphorylation sites in CK2 subunit mutants revealed one site (S530) located in a less conserved region of Hsp90. Together with the previously described conserved T22 residue, we characterised their roles in survival of high temperature stress, cell and colony morphology, biofilm formation, drug resistance and virulence using phosphomimetic and non-phosphorylatable mutant alleles of Hsp90. Phosphorylation of S530 blocks Hsp90 function while any alteration of T22 leads to loss of Hsp90 function. Our work revealed a regulatory residue essential for Hsp90 function located in a divergent region with potential as a future drug target.

The MAK-1 and MAK-2 MAP kinase modules have related but different functions in cell-cell fusion in *Neurospora crassa*

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During colony establishment, germinating spores of *N. crassa* undergo mutual attraction and subsequent fusion. Germling fusion therefore represents a simple experimental model for studying directed growth in fungi. In recent years an intricate signaling network mediating germling interactions was identified. It comprises two MAP-kinase pathways (MAK-1 and MAK-2), NADPH oxidases, the STRIPAK complex and several fungi-specific proteins such as SO (SOFT). During the directed growth of the fusionpartners towards eachother, the MAP-Kinase MAK-2 and the SO protein accumulate at the membrane of the interacting celltips in an oscillating manner, while MAK-1 is only recruited to the membrane after cell-cell contact. In contrast to the different subcellular dynamics, the phenotypes of the *Δmak-2*, *Δmak-1* and *Δso* mutants are comparable and spores of all three mutants show no cell-cell interactions and fusion-related directed growth. To decipher the specific contributions of MAK-2 and MAK-1 to these processes, we turned to a chemical genetics approach, which allows the specific and fast inhibition of either MAK-2 or MAK-1 at any stage of the cell-cell interaction process. The obtained results revealed that both kinases are essential for directed growth, but have different molecular contributions. While MAK-1 is essential for the maintenance of an actin cluster at the growing celltips, MAK-2 is required for the correct localization of this polarity center. In addition, MAK-1 is essential for the recognition of cell-cell contact after the two fusion cells touch, while MAK-2 is dispensable for this process. Both kinases are required for proper dynamics of other fusion factors, such as SO, and the formation of a stable fusion pore. These data further indicate that both MAP kinase pathways interact in a fine-tuned manner to enable robust cell-cell communication, directed growth and cell-cell fusion.

Septins coordinate cell wall integrity and lipid metabolism

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The septin cytoskeleton plays many important roles in fungi including organizing division planes and coordinating nuclear division with growth. We found that all five of the *Aspergillus nidulans* septin null mutants (ΔaspAcdc11, ΔaspBcdc3, ΔaspCcdc12, ΔaspDCdc10, and ΔaspE) are sensitive to plasma membrane disturbing agents and that the core septin mutants (all septin null mutants except for ΔaspE) are sensitive to cell

wall disturbing agents. Combinatorial treatments with membrane and cell wall disturbing agents showed that septins impact sphingolipids in a way that is required for proper cell wall integrity. Double mutant analysis, live cell imaging, and cell wall composition studies showed that the core septins also function downstream of the final kinase of the cell wall integrity pathway. We suggest that *A. nidulans* septins are required for proper coordination of the cell wall integrity pathway and lipid metabolism, likely through sterol rich domain-associated lipids.

Nutrient transporter translocation to the plasma membrane via Golgi bypass in *Aspergillus nidulans*

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Eukaryotic nutrient transporters, being polytopic transmembrane proteins, are thought to traffic from their site of synthesis, the ER, to the plasma membrane, through the Golgi, using the conventional vesicular trafficking pathway. Notably, however, current knowledge on the mechanism of membrane cargo secretion has been obtained by using mostly proteins that are polarly localized, and to our knowledge no report has ever shown formally the involvement of Golgi specifically in the PM localization of *de novo* made transporters. Here we show that in *Aspergillus nidulans* several nutrient transporters follow an unconventional trafficking route that initiates at ER-exit sites (ERes) and requires clathrin and actin polymerization, but surprisingly, does not involve passage through the Golgi or other key effectors of conventional secretion (Rab11, AP-1, microtubules or endosomes). Our findings will be discussed relative to other studies concerning unconventional trafficking routes in other systems and within a rationale on why transporter traffic bypasses the Golgi. Last but not least, we will propose that the trafficking mechanism uncovered here in a lower eukaryote might hold true for the sorting of nutrient transporters and other house-keeping non-polar cargoes in higher organisms.

Functional characterization of an atypical RNase III involved in a RNAi-related mechanism of RNA degradation in *Mucor circinelloides*

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The filamentous fungus *Mucor circinelloides* shows an intricate RNAi mechanism with at least three pathways that control several physiological and developmental processes. We have identified three RNase III proteins that participate in the RNAi-related pathways. Two canonical Dicer-like proteins produce the small-RNAs that drive the defense against exogenous nucleic acids, the control of some genes at mRNA level, and the production of drug-resistant epimutants. Additionally, R3B2, a bacterial-like RNase III with two double-stranded RNA binding domains (dsRBDs), plays a pivotal role in a non-canonical Dicer-independent RNAi mechanism, which is proposed to degrade specific mRNAs with short stretches of dsRNA produced by the RNA-dependent RNA polymerases of the fungus. In this work, we characterized the RNA binding and cleaving properties of R3B2. We show using Electrophoretic Mobility shift Assays that R3B2 binds both ssRNA and dsRNA through its two dsRBDs, and substitutions of key amino acids or deletion of these domains caused the loss of affinity for RNA. Although it can bind both ssRNA and dsRNA, it only cleaves ssRNA and this substrate preference relies on the RNase III-like domain.

The activity and binding features of R3B2 concur with the characteristics of the ssRNAs produced by the non-canonical RNAi mechanism, confirming that this protein is the main ribonuclease of this mechanism. R3B2 is present only in Mucorales and shows low sequence identity to bacterial RNase III proteins. However, the crystal structure of the RNase III-like domain of R3B2 revealed that it adopts a homodimeric structure similar to *bona fide* RNase III proteins, which cut dsRNA. The substitution of R3B2 RNase III-like domain with the canonical domain from *Escherichia coli* RNase III restored the substrate specificity against dsRNA, indicating that the substitutions present in the R3B2 catalytic domain determines its unusual substrate specificity.

Horizontal gene transfer in the human and skin commensal *Malassezia*: a bacterially-derived flavohemoglobin is required for NO resistance

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The skin of humans and animals is colonized by commensal and pathogenic fungi and bacteria that share ecological niche and established microbial interactions. *Malassezia* are the most abundant fungal skin inhabitant of all warm-blooded animals, and have been implicated in skin diseases and systemic disorders, including Crohn's Disease and Pancreatic cancer. Flavohemoglobin is a key enzyme involved in microbial nitrosative stress resistance and nitric oxide degradation. We performed comparative genomics analysis within the *Malassezia* genus and identified two different flavohemoglobin-encoding genes *YHB1* present as single copy in different species, and further analyses revealed that both were acquired through two independent horizontal gene transfer events from different donor bacteria that are part of the mammalian microbiome. Through targeted gene deletion and functional complementation in *M. sympodialis*, we demonstrated that both bacterial flavohemoglobins are cytoplasmic proteins required for nitric oxide detoxification and nitrosative stress resistance under aerobic conditions. RNAseq analysis revealed that endogenous accumulation of nitric oxide in the *yhb1*Δ mutants resulted in upregulation of genes involved in stress responses, and downregulation of the MalaS7 allergen-encoding gene. Solution of the high resolution X-ray crystal structure of *Malassezia* flavohemoglobin revealed features conserved with bacterial flavohemoglobins. Both *Malassezia* flavohemoglobins are predicted to play a role in the pathogenic interaction of *Malassezia* with the host, suggesting that their horizontal acquisition from sympatric bacteria was the result of a selective pressure to resist o toxic concentrations of nitric oxide on mammalian hosts. Lastly, we identified additional genus- and species- specific horizontal gene transfer events that have shaped the *Malassezia* genomes and contribute to the evolutionary trajectory of this genus as the most common inhabitants of animal skin.

A hierarchical transcriptional network controls appressorium development in the rice blast fungus *Magnaporthe oryzae* in response to surface hydrophobicity

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Rice blast disease is caused by the ascomycete fungus *Magnaporthe oryzae* and is the most destructive disease of cultivated rice world-wide. In response to the contact surface *M. oryzae* has the ability to undergo through different morphogenetic programs mediated by the Pmk1 MAPK signalling cascade. The central kinase called PMK1 acts on a set of downstream transcription factors that rewire specific morphogenesis-associated gene expression programs. Here, we show a global analysis of the transcriptional reprograming

occurring in response to a hydrophilic non-inductive surface, when the fungus produces a non-infective undifferentiated germling, and in response to an inductive hydrophobic surface which leads to formation of the infection structure called appressorium. We show fundamental differences between both developmental programs and how these are linked to cell cycle regulation and type II autophagic cell death. We also show that surface recognition is tightly linked to activation of Pmk1 MAPK and a co-regulated set of 15 transcription factors that operate downstream to control appressorium development and invasive growth. From this set of transcription factors, we specifically describe how the homeobox transcription factor Hox7 is a direct target of Pmk1 to act as a regulator of autophagy, cell cycle arrest and suppression of hyphal-associated gene expression, that are necessary for appressorium development and septin-dependent plant infection.

Concurrent Session 1.3
PRIMARY AND SECONDARY METABOLISM

WEDNESDAY, FEBRUARY 19

Location: **Frentani Convention Center** | Room: **Auditorium**
CHAIR: ***Carmen Limon*** & ***Corrado Fanelli***

14:00 - 14:15 **How McrA regulates secondary metabolism**
Berl Oakley | University of Kansas Molecular Biosciences

14:15 - 14:30 **The fumonisin cluster gene *FUM18* encodes a functional ceramide synthase that confers self-protection against the produced sphingolipid inhibitor**
Slavica Janevska | Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute

14:30 - 14:45 **Functional subregions of the endoplasmic reticulum of *Fusarium graminearum* upon induced secondary metabolism**
Marike Boenisch | University of Minnesota

14:45 - 15:00 **Functional studies of the role of the RING-Finger protein CarS in *Fusarium fujikuroi***
M. Carmen Limón | University of Seville, Spain

15:00 - 15:15 **The lipoxygenase gene *lox1* is involved in light- and injury-response as well as volatile secondary metabolite production in *Trichoderma atroviride***
Susanne Zeilinger | University of innsbruck Dept. of Microbiology

15:15 - 15:30 **Fusion transcription factors as a tool for controlled gene overexpression in *Trichoderma reesei***
Irene Tomico Cuenca | Vienna University of Technology, Institute of Chemical, Environmental and Biosciences Engineering

15:30 - 15:45 **Signals in pathogen and host sensing: free fatty acid and oxylipins**
Valeria Scala | CREA DC

How McrA regulates secondary metabolism

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McrA is a transcription factor, discovered in *Aspergillus nidulans* but conserved in filamentous fungi, that negatively regulates many secondary metabolism (SM) gene clusters (Oakley *et al*., 2017, Mol. Microbiol., 103, 347-365). In our efforts to understand the mechanism by which McrA regulates SM, we have carried out ChIPseq with McrA and compared the results with RNAseq results in strains in which *mcrA* is deleted, expressed normally, or overexpressed. McrA binding sites are found upstream of many SM genes (both biosynthetic genes and transcription factors associated with SM gene clusters) and deletion of *mcrA* results in upregulation of the genes in many cases. This suggests that McrA binds to the promoters of these genes competing with transcription factors that might normally drive their expression. McrA also binds upstream of both the *veA* and *laeA* genes, which are important regulatory genes with important roles in the regulation of secondary metabolism. Deletion of *mcrA* results in a small upregulation of *laeA* and no significant upregulation of *veA*, but overexpression of *mcrA* results in a dramatic downregulation of both *laeA* and *veA*. These results indicate that while the increase in SM production seen in *mcrA* deletants is not due to effects on expression of *laeA* and *veA*, McrA is a negative regulator of these genes and may play a role in regulating their levels of expression. The *mcrA* locus is, itself, transcriptionally complex. Its transcripts have long 5' and 3' non-coding regions and there are McrA binding sites upstream of the gene suggesting that *mcrA* transcription may be regulated through a negative feedback loop. In addition, there are long non-coding RNAs near *mcrA* that have McrA binding sites and are downregulated by overexpression of McrA. It appears that in most cases McrA is a transcriptional repressor, and it exerts its effects on SM by regulating SM genes directly and by regulating other regulatory genes.

The fumonisin cluster gene *FUM18* encodes a functional ceramide synthase that confers self-protection against the produced sphingolipid inhibitor

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Sphingolipid inhibitors are promising pharmaceuticals for treatment of severe human diseases that have been correlated with a deregulation of sphingolipid metabolism, such as cancer, Alzheimer's and schizophrenia. Fumonisin (FUM) mycotoxins are produced by some species of the genus *Fusarium* and are efficient inhibitors of ceramide synthase (CS). FB1 is produced as major derivative by the conserved polyketide synthase gene cluster in *Fusarium verticillioides*.

We localized one of the early FUM biosynthetic enzymes, the aminotransferase Fum8, to intracellular vesicles derived from the endoplasmic reticulum – the cellular compartment of CS biosynthesis. Thereby, both inhibitor and target enzyme co-occur in these vesicles, which raises the question: How does the fungal producer protect itself from FUM toxicity? We identified two cluster-encoded mechanisms of self-protection.

Firstly, the ATP-binding cassette transporter Fum19 represses expression of FUM cluster genes. Thus, *FUM19* deletion and overexpression up- and downregulated, respectively, intracellular and secreted FB1. This was supported by the fact that FB1 feeding induced cluster gene expression, which was dependent on the pathway-specific transcription factor Fum21. *FUM17* and *FUM18* were an exception, as they could be triggered in the absence of Fum21.

Secondly, phylogenetic analysis indicated that Fum17 and Fum18 are two of five CS homologs in *F. verticillioides*. Two of the other homologs, *FvCS1* and *FvCS2*, as well as human *CERS2*, *CERS4* and *CERS6* could complement the yeast CS null mutant *LAG1/LAC1*. Intriguingly, *FUM18* was shown to be a functional CS homolog, fully complementing on its own, while Fum17/FvCS3 likely form a functional heterodimer. Finally, resazurin cell viability assays with complemented yeast strains and *Fusarium* deletion mutants revealed that Fum18 contributes to the fungal self-protection against FB1, and increases resistance by providing cluster-encoded CS activity.

Functional subregions of the endoplasmic reticulum of *Fusarium graminearum* upon induced secondary metabolism

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Fusarium graminearum is a phytopathogenic filamentous fungus causing disease of cereals including wheat, barley, and maize, and contaminates grains with trichothecene (TRI) mycotoxins. TRI mycotoxins are sesquiterpenoid secondary metabolites, which bind to eukaryotic ribosomes and inhibit vital cell functions. We hypothesize that elevated production of toxic secondary metabolites may require cellular and organellar adaptation to accommodate changing metabolic and physiological conditions. Recently, we discovered a highly modified smooth endoplasmic reticulum (ER), called OSER (organized smooth ER) in hyphae grown under elevated TRI biosynthesis *in vitro* and *in planta* (Boenisch *et al*., 2017, *Scientific Reports* 7:44296). Fluorescently tagged TRI biosynthetic enzymes, such as the cytochrome P450s Tri4-RFP and Tri1-GFP, strictly localized to OSER, but appear absent in other parts of the ER. In order to test whether cytochrome P450s of other pathways which also utilize mevalonate pathway intermediates show a similar localization patterns, we localized the ergosterol pathway enzyme squalene monooxygenase Erg1 under similar growth conditions. Interestingly, Erg1-GFP frequently localizes to lipid droplets (LDs) in addition to OSERs, indicating that cytochrome P450s of different pathways might be recruited to specific subregions of the ER. Lipid droplets (LDs) are essential ER derived organelles, and often physically connected to the ER network. They are the source of triacylglycerols and sterols and sequester toxic free fatty acids through a phospholipid monolayer. Manual measurement of the diameter of 92 fluorescently stained LDs in hyphae grown with or without TRI induction indicated that LDs are significantly enlarged under TRI induction ($P<0.001$). Proteomics of FACS isolated LDs formed in TRI induced and non-induced hyphae will further elucidate LD dynamics and the coordination of competing primary and secondary metabolic pathways and their enzymes during toxigenesis.

Functional studies of the role of the RING-Finger protein CarS in *Fusarium fujikuroi*

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Fusarium fujikuroi produces a large variety of secondary metabolites in response to different environmental factors. Light stimulates the synthesis of neurosporaxanthin (NX) and other carotenoids through the induction of transcription of three genes of the NX pathway: *carRA*, *carB*, and *carT*. Mutants affected in the gene *carS* exhibit deeppigmented phenotype due to deregulation of the *car* genes. Evidence of the role of CarS as a repressor was obtained by increasing *carS* mRNA using a constitutive *gpdh* promoter or the doxycycline-inducible Tet-on system. In both cases, enhanced *carS* transcription results in albino phenotypes under illumination.

Protein CarS has a LON-protease and two RING-fingers domains, typical of ubiquitin ligases. For identification of effectors of CarS, transformants expressing a CarS protein tagged with a FLAG epitope at either N- or C-termini have been generated. In the dark, transformants with a N-tagged CarS accumulated NX, probably due to altered CarS activity, while those with a C-tagged CarS have a phenotype similar to wild type.

Mutations in *carS* are pleiotropic, since affect not only carotenoid biosynthesis but also conidiation, germination, and sensitivity to antibiotics such as voriconazole and amphotericin B. In order to shed light on the role of CarS in morphogenesis, we checked the effect of antifungals on cell wall components and sterols in a *carS* mutant in comparison to wild type. Although no changes were detected for most of the components, we found that voriconazole increases NX biosynthesis in *F. fujikuroi.* This correlated with enhanced mRNA levels of the structural *car* genes and reduced levels of carS mRNA.

The lipoxygenase gene *lox1* is involved in light- and injury-response as well as volatile secondary metabolite production in *Trichoderma atroviride*

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Trichoderma atroviride is a necrotrophic mycoparasite antagonizing various fungal plant pathogens and hence is applied in agriculture as biological control agent. Secondary metabolites are among the main agents determining the strength and progress of the mycoparasitic attack. The main secondary metabolite produced by *T. atroviride* is 6-pentyl-α-pyrone (6-PP) which exhibits anti-fungal and plant growth-promoting activity. 6-PP is a volatile, unsaturated lactone derived from fatty acid metabolism, synthesized in a hitherto unknown biosynthetic pathway. Previous isotopic labelling experiments proposed the oxidation of linoleic acid by a lipoxygenase (*lox*) as the first step in 6-PP biosynthesis (Serrano-Carreon *et al*, 1993, *Applied and Environmental Microbiology* 59: 2945-50). Accordingly, 6-PP producing species such as *T. atroviride* encode a *lox* gene (*lox1*) in their genomes while non-producing relatives do not. In *T. atroviride lox1* expression as well as 6-PP levels furthermore are upregulated during the mycoparasitic interaction with fungal preys. Based on these evidences indicating a role of *lox1* in 6-PP

biosynthesis, we generated *T. atroviride lox1* gene deletion mutants. *Δlox1* mutants exhibited unaltered mycoparasitic and antifungal activities, as well as 6-PP levels similar to the wild type. However, *lox1* deletion resulted in a severely impaired conidiogenesis and a significantly reduced production of several low molecular weight metabolites upon growth in complete darkness as well as after mycelial injury. GC-IMS analysis of volatile organic compounds in the headspace of *T. atroviride* cultures revealed a strong decrease in the production of 1-octen-3-ol, 2-heptanone and 3-octanone by the *Δlox1* mutants compared to the wild type. We hence conclude that *lox1* is dispensable for 6-PP biosynthesis but affects the light- and injury-response as well as the production of other volatile secondary metabolites in *T. atroviride*.

Fusion transcription factors as a tool for controlled gene overexpression in *Trichoderma reesei*

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Transcription factors play a crucial role in biological systems due to their ability to control gene expression. For this reason, they have been also used for biotechnological purposes with the aim of enhancing or reducing expression of certain genes. For instance, in the filamentous ascomycete *Trichoderma reesei*, the Gal4-like transcription factor Xyr1 is the main activator of xylanases and cellulases. As *T. reesei* is used in the industry for the production of these enzymes, Xyr1 was over-expressed in different studies to enhance cellulase and xylanase expression. However, this strategy was only partially successful.

In an alternative approach for the enhanced expression of cellulases and xylanases, we fused the DNA-binding domain of Xyr1 to the C-termini of Ypr1 or Ypr2. Ypr1 and Ypr2 are two further Gal4-like transcription factors from *T. reesei* that regulate the expression of the sorbicillinoid gene cluster. Production of cellulases and xylanases were enhanced in the strains bearing these fusion transcription factors in a Xyr1-deficient genetic background.

We reason, that fusion transcription factors can also be used for the expression of silent biosynthetic clusters. To this end, we fused the DNA-binding domain of either of three cluster-specific transcription factors to the C-terminus of Ypr1 and the regulatory domain of the human estrogen receptor, making the system estradiol-inducible. The three chosen transcription factors are located within silent biosynthetic gene clusters.

The fusion transcription factors are proposed to activate the expression of the genes within the respective clusters. Consequently, yet undescribed natural products should be built. Subsequently, we follow an untargeted, isotope assisted metabolite profiling approach to selectively detect these compounds by comparison between induction and non-induction conditions.

Signals in pathogen and host sensing: free fatty acid and oxylipins

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Lipids play important roles at various stages of host–pathogen interactions and are crucial in determining the virulence of pathogens and modulating plant defences (1). Free Fatty Acids (FFA) may act as substrates for oxidizing enzymes [e.g., lipoxygenases (LOXs) and dioxygenases (DOXs)] forming oxylipins that have been extensively studied in plant–pathogen interaction. Free fatty acids (FFA) and oxylipins might also function as modulators of several pathways in cell-to-cell communication (2). The structural similarity of plant, fungal and bacterial oxylipins prompts the hypothesis that they are important in cross-kingdom communication. We present here, two case studies: the first regards an eukaryotic pathogen (*Fusarium verticillioides*) infecting maize (*Zea mays*); the second case study deal with a prokaryotic pathogen, *Xylella fastidiosa* subsp. *pauca* infecting *Olea europaea* and artificially *Nicotiana tabacum* (2). These two studies pin point out that the FFA and the oxylipins derived by the oleic, linoleic and linolenic acid are crucial to modulate the pathogen lifestyle and the interaction with the host. In particular, in *F. verticillioides* the oxylipins cross-talk modulate the fumonisin synthesis. The increase of linoleic acid-derived oxylipins favour the fumonisins production, driving the plant towards the PCD (plant cell death). In *X. fastidiosa*, the oxylipins modulate the planktonic and biofilm formation and within the host seem to pave the way for the Olive Quick Decline Syndrome symptoms. Thanks to similar and overlapping results in different pathosystems, we can assume that lipids, at the interface of interacting organisms, may act as signals able reshaping the lifestyle of the contenders and sometimes determining the fate of the challenge.

Concurrent Session 1.4
GENOME, CHROMATIN AND EPIGENETICS

WEDNESDAY, FEBRUARY 19

Location: **Frentani Convention Center** | Room: **Auditorium**
CHAIR: ***Riccardo Baroncelli*** & ***Luigi Faino***

16:15 - 16:30 **Extensive genome reshuffling during the parasexual cycle in the asexual plant-pathogenic fungus *Verticillium dahliae***
Ioannis Papaioannou | University of Heidelberg / Zentrum für Molekulare Biologie der Universität Heidelberg

16:30 - 16:45 **Insertional mutagenesis using TC1-mariner transposon impala in the wheat fungal pathogen *Zymoseptoria tritici***
Marc-Henri Lebrun | INRA / UMR BIOGER

16:45 - 17:00 **Genome-wide expression analysis to map regulatory polymorphisms in a major fungal pathogen**
Leen Abraham | University of Neuchatel / Laboratory of Evolutionary Genetics

17:00 - 17:15 **Distinct life histories impact dikaryotic genome evolution in the wheat stripe rust fungus *Puccinia striiformis* f. sp. *tritici***
Benjamin Schwessinger | Australian National University / RSB

17:15 - 17:30 **Meiotic silencing by unpaired DNA (MSUD) in *Neurospora crassa*: a new approach to studying recombination-independent homologous pairing**
Tinh-Suong Nguyen | Institut Pasteur / Department of Mycology
FEMS Grant

17:30 - 17:45 **Genome analyses reveal evolution and adaptation of carbohydrate utilization in the genus *Colletotrichum***
Riccardo Baroncelli | Universidad de Salamanca / Instituto Hispano-Luso de Investigaciones Agrarias (CIALE)

17:45 - 18:00 **Epigenetics, RIP and sexual development in filamentous ascomycetes**
Fabienne Malagnac | Université Paris-Sud / Institute for Integrative Biology of the Cell

Extensive genome reshuffling during the parasexual cycle in the asexual plant-pathogenic fungus *Verticillium dahliae*

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Meiosis is a key component of the sexual cycles of the vast majority of eukaryotes. A key function of meiosis is genetic recombination, which ensures the generation of genetic diversity and contributes to adaptation through the combination of beneficial alleles and purging of deleterious mutations. Nevertheless, a number of unrelated taxa, including a significant number of fungi, have secondarily adopted evolutionary strategies that do not involve sex, which is a long-standing conundrum in biology. Here we report conclusive evidence that the asexual plant-pathogenic fungus *Verticillium dahliae* can frequently and efficiently reshuffle parental genomes through the parasexual cycle, in the absence of sex. We documented its complete parasexual cycle by forcing tagged natural isolates to undergo conidial or hyphal fusion and studying the dynamics of nuclear migration through these fusions (anastomoses) using time-lapse imaging of strains tagged with nuclear fluorescent markers. The exchange of genetic material was indicated by pulsed-field gel electrophoresis profiling of parental strains and their recombinant progeny and time-lapse determination of nuclear DNA content with flow cytometry. To gain a better understanding of the mode and magnitude of genomic interactions that occur through the parasexual cycle, we applied Oxford Nanopore Technologies (ONT) sequencing of representative parental and recombinant strains, obtained high-quality genome assemblies and performed comparative genomics. We used this data to investigate the extent of genome reshuffling, as a result of random chromosome non-disjunction during haploidisation, stabilisation of aneuploid states, and intra-chromosomal mitotic crossovers. We provide here direct evidence that *V. dahliae* and presumably other fungi can derive the evolutionary benefits of meiosis without the need to indulge in a sexual cycle, which opens up new avenues in the study of the evolution and adaptation potential of fungi.

Insertional mutagenesis using *TC1-mariner* transposon *impala* in the wheat fungal pathogen *Zymoseptoria tritici*

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The fungal *TC1-mariner* transposable element *impala* was used for insertional mutagenesis in the wheat fungal pathogen *Zymoseptoria tritici*. Excision vectors containing an autonomous copy of *impala* inserted in the promoter of the *A. nidulans* nitrate reductase gene were introduced in a *Z. tritici* Nia1- mutant. *impala* excision events were selected by recovering nitrate-utilizing revertants. Most revertants (80%) had a single copy of *impala* inserted at a new genomic location. *impala* mainly inserts into genes located on the core chromosomes (84%), near transcriptional start sites (TSS, 50%) but more rarely into genes on accessory chromosomes (1%), native transposons (1%) and intergenic regions (14%). Since accessory chromosomes and transposons are enriched in repressive chromatin marks, we hypothesized that *impala* insertion preference is influenced by the chromatin landscape of the TE acceptor locus. Inhibition of *Z. tritici* histone deacetylases with trichostatin A increased the frequency of *impala* insertions in native transposons (5-fold), and changed its insertion pattern in genes on core chromosomes, but did not increase insertion frequency in genes on accessory chromosomes. These experiments suggest that *impala* inserts preferentially near TSS as a result of their specific chromatin landscape. This property was exploited for activation tagging with a chimeric transposon carrying a strong constitutive promoter (*pGpd*). The chimeric *impala:Gpd* transposon was able to excise and re-insert in the *Z. tritici* genome. Characterization of a few insertion sites showed that *impala:Gpd* was inserted mainly in 5'UTRs and promoters of *Z. tritici* genes, as with native *impala*. Overexpression of targeted genes is ongoing. We have also screened a collection of 300 *impala* insertion mutants and identified three pathogenicity mutants with quantitative defects, all corresponding to genes not previously known to be involved in infection.

Genome-wide expression analysis to map regulatory polymorphisms in a major fungal pathogen

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In agricultural ecosystems, outbreaks of diseases are frequent and pose a significant threat to food security. A successful fungal pathogen undergoes a complex and well-timed sequence of regulatory changes to avoid detection by the host immune system, hence well-tuned gene regulation is essential for survival. However, how regulatory adaptation enables pathogens to overcome host resistance and cause damage is poorly understood. Here, we used *Zymoseptoria tritici*, one of the most important pathogens of wheat, to generate a genome-wide map of regulatory polymorphism governing gene expression. We investigated the polymorphisms influencing transcription levels of 146 strains by performing expression quantitative trait loci (eQTL) mapping. We identified cis-eQTLs for 65.3% of all genes and the majority of all eQTL were within 2kb of the transcription start site. The proportion of genes with a mapped eQTL was higher for genes on core chromosomes than accessory chromosomes. We also found that indel polymorphism was more likely to act as a cis-eQTL and had on average a higher effect size than SNPs. Next, we contrasted the amount of cis-eQTL mapped across categories of pathogenicity-related genes. Effector genes were less likely to have a mapped cis-eQTL compared to genes encoding CAZymes and the genomic background. This may indicate that regulatory variation in effector genes is governed rather by epigenetic factors than genetic polymorphism. Our study identified extensive evidence that single pathogen populations segregate large-scale regulatory variation. Such regulatory polymorphism is likely to fuel rapid adaptation to resistant hosts and environmental changes.

Distinct life histories impact dikaryotic genome evolution in the rust fungus *Puccinia striiformis* causing stripe rust in wheat

Benjamin Schwessinger[6,#], Yan-Jun Chen[1,#], Richard Tien[2], Josef Korbinian Vogt[3], Jana Sperschneider[4], Ramawatar Nagar[6], Mark McMullan[7], Thomas Sicheritz-Ponten[1], Chris K. Sørensen[5], Mogens Støvring Hovmøller[5], John P. Rathjen[§] and Annemarie Fejer Justesen[5]

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Stripe rust of wheat, caused by the obligate biotrophic fungus *Puccinia striiformis* f. sp. *tritici*, is a major threat to wheat production world-wide with an estimated yearly loss of US $1 billion. The recent advances in long-read sequencing technologies and tailored-assembly algorithms enabled us to disentangle the two haploid genomes of *Pst*. This provides us with haplotype-specific information at a whole-genome level. Exploiting this novel information, we perform whole genome comparative genomics of two *P. striiformis* f. sp. *tritici* isolates with contrasting life histories. We compare one isolate of the old European lineage (PstS0), which has been asexual for over 50 years, and a Warrior isolate (PstS7 lineage) from a novel incursion into Europe in 2011 from a sexual population in the Himalayan region. This comparison provides evidence that long-term asexual evolution leads to genome expansion, accumulation of transposable elements, and increased heterozygosity at the single nucleotide, structural and allele levels. At the whole genome level, candidate effectors are not compartmentalized and do not exhibit reduced levels of synteny. Yet we were able to identify two subsets of candidate effector populations. About 70% of candidate effectors are invariant between the two isolates while 30% are hypervariable. The latter might be involved in host adaptation on wheat and explain the different phenotypes of the two isolates. Overall this detailed comparative analysis of two haplotype-aware assemblies of *P. striiformis* f. sp. *tritici* are the first steps in understanding the evolution of dikaryotic rust fungi at a whole genome level.

Meiotic silencing by unpaired DNA (MSUD) in *Neurospora crassa*: a new approach to studying recombination-independent homologous pairing

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Homologous chromosome pairing represents a critical aspect of meiosis in nearly all sexually reproducing species. While meiotic pairing relies on the formation of double-strand DNA breaks in some organisms, in many others it can proceed in the apparent absence of DNA breakage and recombination. The mechanistic nature of such recombination-independent pairing represents a fundamental question in molecular biology. Using “meiotic silencing by unpaired DNA” (MSUD) in the fungus *Neurospora crassa* as a model system, we demonstrate the existence of a principally new solution to the problem of inter-chromosomal homology recognition during meiosis. Here we take advantage of the unique ability of MSUD to efficiently detect and silence (by RNA interference) any relatively short DNA fragment lacking a homologous allelic partner. We show that MSUD does not require the function of eukaryotic RecA proteins and the type II topoisomerase-like protein Spo11. We further show that MSUD recognizes weak interspersed homology in which units of sequence identity as short as 3 base-pairs (bp)

are spaced apart with a periodicity of 11 bp, approximating double-helical DNA pitch and corresponding to an overall sequence identity of only 27%. Taken together, these results reveal the role of a recombination-independent homology-directed process in guiding the expression of small interfering RNAs and suggest that meiotic chromosomes can be evaluated for sequence homology at base-pair resolution by a mechanism that operates on intact DNA molecules.

Genome analyses reveal evolution and adaptation of carbohydrate utilization in the genus *Colletotrichum*

Riccardo Baroncelli[1], José Francisco Cobo Díaz[2], Tiziano Benocci[3], Evy Battaglia[4], Mao Peng[4], Sajeet Harids[5], William Andreopoulos[5], Kurt LaButti[5], Jasmyn Pangilinan[5], Bernard Henrissat[6], Bernard Henrissat[7,8], Igor V. Grigoriev[5], JoAnne Crouch[9], Gaetan Le Floch[2], Ronald P. De Vries[4], Serenella A. Sukno[1], Michael R. Thon[1]

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Colletotrichum spp. infect a wide diversity of monocot and eudicot hosts, causing plant diseases on many economically important crops worldwide. In addition to its economic impact, *Colletotrichum* is a suitable model for the study of gene family evolution to uncover evolutionary events in the genome that are associated with the evolution of phenotypic characters important for host interactions. We sequenced 17 *Colletotrichum* species covering the gaps in the genetic and biological diversity of the genus. The newly sequenced genomes show high genome size variation, between 46.92 Mb of *C. sublineola* to 89.65 of *C. orbiculare*. These results highlight an unexpected variability of more than 30 Mb (33%) between closely related species such as *C. cuscutae* and *C. paranaense*. Moreover, species with bigger genomes are characterized by a higher AT content. This evidence along with no significant difference in gene content suggests that genome size is mainly affected by expansion in repeats elements. A comparative genomic analysis

of 30 *Colletotrichum* species adapted to eudicot or monocot hosts revealed enrichment in specific functional categories such as polysaccharide degradation, transcription regulation, aconitase and serine peptidase activities in dicot pathogens. Evolutionary analyses of selected gene families revealed specific patterns such as gene losses in monocot associated pathogens, but also losses of functional domains within these families suggesting that gene family evolution might act at different level (genes vs functional domains). Our results also suggest that the ancestral *Colletotrichum* may have been associated with eudicot plants and certain branches progressively adapted to different monocot hosts reshaping part of the degradative and transcriptional arsenal. A comparative transcriptomic analysis on different substrates of selected species adapted to different hosts and with different evolutionary histories is ongoing to confirm the role of selected genes.

Epigenetics, RIP and sexual development in filamentous ascomycetes

Pierre Grognet[1¶], Hélène Timpano[¶], Florian Carlier[1], Jinane Aït-Benkhali, Véronique Berteaux-Lecellier[2], Robert Debuchy[1], Frédérique Bidard[3] and Fabienne Malagnac[1]

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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. The presence of active RIP has then been evidenced in various other filamentous fungi, including *Podospora anserina*. To date the RID protein (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.

To gain insight into the RID function, we constructed knocked-out Δ*PaRid* mutants in *P. anserina*. By contrast with *N. crassa* RID defective mutants, crosses involving *P. anserina* Δ*PaRid* mutants are sterile. We showed 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. Complementation of the Δ*PaRid* mutants with ectopic alleles of *PaRid* 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. A transcriptomic analysis performed on mutant crosses revealed an overlap of the PaRid-controlled genetic network with the well-known mating-types gene developmental pathway common to the filamentous fungi. Finally, as an attempt to decipher the potential functional links between the RIP process and sexual development, we developed a readout tool to assay RIP efficiency in various mutant backgrounds.

Concurrent Session 1.5
OMICS & BIOINFORMATIC

WEDNESDAY, FEBRUARY 19

Location: **Frentani Convention Center** | Room: **Accademia**
CHAIR: ***Walter Sanseverino*** & ***Igor Grigoriev***

14:00 - 14:15 **COFUN: An update on the construction of the genome wide-knockout library in *A.fumigatus***
Michael Bromley | University of Manchester

14:15 - 14:30 **A quantitative image analysis pipeline for the characterization of filamentous fungal morphologies to uncover targets for morphology engineering**
Claudia Feurstein | Technische Universität Berlin, Chair of Applied and Molecular Microbiology

14:30 - 14:45 **Chromosome-level genome assembly of fungi: A case study of the fairy-ring mushroom**
Markus Hiltunen | Uppsala University Organismal Biology

14:45 - 15:00 **Supernumerary chromosomes in Italian *Fusarium verticillioides* strains**
Alessandro Grottoli | SARA EnviMob s.r.l., Rome

15:00 - 15:15 **Harnessing transcriptomic data to predict the function of proteins in the microbial cell factory *Aspergillus niger***
Paul Schäpe | Technische Universität Berlin, Chair of Applied and Molecular Microbiology

15:15 - 15:30 **FungiDB: integrating genomic data for pathogens and model organisms and providing advanced search capabilities and large-scale data analysis**
Evelina Basenko | The University of Liverpool Functional and Comparative Genomics

15:30 - 15:45 **A secretome tailored to endure oxidative stress in wood decomposer *Postia placenta***
Jesus Castano Uruena | University of Minnesota Bioproducts & Biosystems Engineering

COFUN: An update on the construction of the genome wide-knockout library in *A. fumigatus*

Michael Bromley[1], Can Zhao[1], Narjes Alfuraiji[1], Takanori Furukawa[1], Norman Van Rhijn[1], Lauren Dineen[1], Isabelle Storer[1], Thorsten Heinekamp[2], Juliane Macheleidt[2], Danielle Weaver[1], Marcin Fraczek[1], Elaine Bignell[1], Paul Bowyer[1], Axel Brakhage[2], Daniela Delneri[1]

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Genome-wide knockout (KO) libraries have been used to great effect to establish an in depth understanding of microbial functional genomics. Despite their obvious value, no KO collection is available in a pathogenic filamentous fungus. To address this we have initiated the COFUN project to generate a genome-wide collection of KO mutants in the leading mould pathogen *Aspergillus fumigatus*. The objective of this project is to complete the resource by finalising the library which will ultimately consist of c.10,000 mutant strains encompassing all of the non-essential ORFs and c.1000 intergenic non-coding RNAs. Here we will update on our progress to date and define how the libraries can be used in competitive fitness studies to elucidate interconnected networks of regulatory genes that are critical for pathogenesis of *A. fumigatus*.

A quantitative image analysis pipeline for the characterization of filamentous fungal morphologies to uncover targets for morphology engineering

Claudia Feurstein[1], Timothy Cairns[2], Xiaomai Zheng[2], Ping Zheng[2], Jibin Sun[2], Vera Meyer[1]

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Filamentous fungi are widely used to produce enzymes, proteins, acids, and secondary metabolites. Morphology is one of the key parameters that can be optimized in submerged fermentation. Tools for objective quantification of filamentous growth are currently low throughput, require extensive experimental setup, and cannot be applied to heterogeneous cultures consisting of pelleted and dispersed morphologies.

To address this problem, a Java-based ImageJ2/Fiji plugin was developed that (i) enables capture and analysis of several hundred images per user/day, (ii) is designed to

quantitatively assess heterogeneous cultures and the degree of culture heterogeneity, (iii) automatically generates key Euclidian parameters for individual fungal structures including particle diameter, aspect ratio, area, and solidity, which are also assembled into a previously described dimensionless morphology number (MN), (iv) has an in-built quality control check which enables end-users to confirm the accuracy of the calls, and (v) is easily adaptable to user specified magnifications and macromorphological definitions. To provide proof of principle conditional expression mutants of the cell factory *Aspergillus niger* were generated. Here the native promoter of *aplD* with a titratable Tet-on cassette using CRISPR-Cas gene editing. Reduced *aplD*, which was predicted to play a role in endosome trafficking, expression caused a hyperbranched growth phenotype and diverse defects in pellet formation with a putative increase in protein secretion. This possible protein hypersecretion phenotype could be correlated with increased dispersed mycelia, and both decreased pellet diameter and MN.

The MPD image analysis pipeline is a simple, rapid, and flexible approach to quantify diverse fungal morphologies. As an exemplar, we have demonstrated that the putative endosomal transport gene *aplD* plays a crucial role in *A. niger* filamentous growth and pellet formation during submerged culture.

Chromosome-level genome assembly of fungi: A case study of the fairy-ring mushroom

Markus Hiltunen, Martin Ryberg, Hanna Johannesson

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Contiguity in genome assembly, the extent to which nucleotides are joined together into continuous sequences, is crucial for the majority of genomic studies. Specifically, in analyses of linkage between genetic variants, synteny, structural variation and repetitive genomic regions it is important to have a high-quality reference. The current state-of-the-art methodology in genome sequence assembly involves long-read technologies such as PacBio and Oxford Nanopore, which dramatically improve contiguity compared to short-read technologies because of the greater ability to sequence through repetitive genomic regions. Even with this improvement, long reads alone are, except in rare cases, not able to completely resolve all repetitive genomic regions. Additional long-range information, e.g. in the form of 10X Chromium linked reads may help in this aspect. Here, we present a method for genome assembly incorporating both long and linked reads. We developed a novel bioinformatics pipeline for genome scaffolding using 10X Chromium linked reads, tentatively named AnVIL, which is available to the user community. In short, AnVIL uses linked reads to infer which contig sequences originated from regions of the genome in close physical proximity, finds overlaps between the ends of such contigs,

and merges them into longer scaffolds. As a test case we used the genome of the fairy-ring mushroom *Marasmius oreades*. Initial assembly of PacBio and Nanopore reads resulted in 167 contigs (N50=859,216). The AnVIL pipeline with 10X Chromium linked reads was then used to improve the assembly, lowering the number of scaffolds to 67 (N50=2,623,530). The improved assembly included several scaffolds starting and ending in telomere repeat sequences; such scaffolds putatively correspond to full chromosome sequences. Incorporating 10X Chromium linked reads in genome assembly projects of fungi can thus massively improve contiguity and facilitate downstream analyses for a relatively low cost.

Supernumerary chromosomes in Italian *Fusarium verticillioides* strains

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Fusarium verticillioides (*Fv*) is considered one of the most common plant pathogenic fungi affecting *Zea mays* (maize) roots, stalk tissues and kernels causing diseases, such as stalk and ear rot. *Fv* is capable to produce mycotoxins, including fumonisins, which can accumulate into the kernels in field as well as in storage that can be dangerous for animal's or for human's health. *Fv* is a species included in the *Fusarium fujikuroi* species complex (FFSC), the largest *Fusarium* species complex. FFSC species may have in common distinct phenotypic traits like mycotoxin production, host-specificity and supernumerary chromosomes (SCs) in addition to core chromosomes. These SCs may differ among isolates in presence/absence, length and gene-abundance and often play an important role in the biology of the pathogenic species in the complex. Here we report the presence in an Italian *Fv* strain (ITEM 10027, *Fv*10027) of two putative SCs not presents in the American *F. verticillioides* isolate (*Fv*7600). We found these putative SCs by assembly obtained by exploiting sequence data from Illumina and Nanopore sequencing approaches; several similarities among *Fv* and *F. fujikuroi* were found in these putative SCs suggesting a putative horizontal gene transfer (HGT) between species; moreover a pulsed-field gel electrophoresis was conducted in order to confirm the presence of these chromosomes. The presence of SCs in *Fv* are already known not been deeply studied as others *Fusarium* species. These SCs may be responsible for HGT of genes involved in pathogenicity of different field species.

Harnessing transcriptomic data to predict the function of proteins in the microbial cell factory *Aspergillus niger*

Paul Schäpe, Timothy C. Cairns, Vera Meyer

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A significant challenge in our understanding of biological systems is the high number of genes with unknown or dubious function in many genomes. Despite intense research in the Aspergillus genus as biotechnological production hosts, pathogens, and model organisms, most genomes still have approximately 40-50% of genes which encode unknown, or hypothetical proteins.

Aspergillus niger is used in biotechnology for citric acid, enzyme production, and more recently as a platform for novel natural product discovery and expression. The first genome of *A. niger* was published in 2007; however, the function of only 5% of the 14,165 predicted genes were investigated in wet-lab experiments so far. For about 50% of the predicted ORFs, significant functional predictions based on sequence homology exist, leaving about 45 % with weak or dubious functional predictions.

To overcome this limitation we developed and experimentally validated a co-expression network analysis approach to allocate a biological process to predicted *A. niger* genes. The co-expression network was created by correlating expression data from 155 transcriptomics experiments and integrating over 1,200 gene functional analysis experiments from the genus *Aspergillus* to aid facile prediction of biological processes.

Using gene ontology enrichment of sub-networks, we could infer a biological process for 9,579 genes including 2,970 hypothetical genes for *A. niger*. Predictions of this method for known genes were in accordance with their previous experimental verified function and experimental validation of selected hypothetical genes uncovered so far unknown transcription factors involved in secondary metabolite synthesis, thus proving the validity of the presented analysis (1). Taken together, our approach drastically improves the predictive power of *A. niger* omics data and is rapidly applicable to other fungal and non-fungal systems for improved genome annotation.

FungiDB: integrating genomic data for pathogens and model organisms and providing advanced search capabilities and large-scale data analysis

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FungiDB (https://fungidb.org) is a free, online data mining resource supporting fungi and oomycetes, and providing functional analysis of omics-scale datasets. FungiDB is a component of the Vector and Eukaryotic Pathogens DataBase (VEuPathDB; https://veupathdb.org), the bioinformatics resource centre that integrates a diverse array of data types for invertebrate vectors of human pathogens, pathogenic and non-pathogenic species and provides sophisticated data mining tools.

VEuPathDB databases offer a one-stop-shop to enable:

- Browsing of genomes and gene pages in an encyclopedic manner to explore all available information and data.
- Searching using a unique search strategy system that utilizes an intuitive web-based graphical interface to facilitate mining of integrated data such as genomes, annotation, functional data (e.g. transcriptomic, proteomic, phenomic and variation data) and the results of in-house analyses (protein domains, molecular interactions, gene ontology annotations and orthology predictions, metabolic pathways and EC number associations, publication links, etc.).
- Annotating through the user comments system and Apollo (a web-based genomic annotation editing platform, in beta). Community expert knowledge about gene models, phenotypes, relevant PubMed records, etc. can be captured and immediately made visible and searchable.
- Analysis of your own data through a private Galaxy workspace that offers preloaded genomes and several sample workflows for RNASeq and variant calling analyses. Here, users can analyze their own datasets and transfer results to the private My Data Sets section in FungiDB for further data exploration using the integrated information and tools in FungiDB.

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A secretome tailored to endure oxidative stress in wood decomposer *Postia placenta*

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Brown rot fungi degrade wood carbohydrates with greater selectivity than white rot fungi, but they produce fewer types of carbohydrate-targeting enzymes. This 'more with less' capacity has been linked to small reactive oxygen species capable of loosening the lignin barrier, providing access to cellulose and avoiding high energy consumption in enzyme production. In a previous study with the brown-rot model *P. placenta*, we showed that some saccharification enzymes expressed early during wood decay tolerated this oxidative environment when tested in vitro (Castaño *et al*. 2018. doi: 10.1128/AEM.01937-18). In this research, we found that this tolerance was unique in *P. placenta* relative to a white rot fungus (*Trametes versicolor*) and a soft rot fungus (*Trichoderma reesei*). To understand the extent and nature of this tolerance mechanism, we used proteomics to investigate the oxidative modifications induced by an oxidative treatment (Fenton reaction) carried out in vitro. After exposure to oxidation, only 6% of the proteins from the secretome were degraded in *P. placenta*, compared to 16% and 22% in *T. versicolor* and *T. reesei*, respectively. Secondly, only 9% of *P. placenta* proteins were significantly oxidized, versus 52% in *T. versicolor* and 32% in *T. reesei*. The main modifications observed across the three fungi were monooxidation and kynurenine formation (mainly in Methionine and Tryptophan residues). Finally, by including spectral counts at the protein and peptide level in our analysis, we found significant differences between the untreated and treated samples only for *T. versicolor* and *T. reesei*.These results suggest that brown rot fungi have evolved proteins with an intrinsic capacity to tolerate high levels of oxidative stress. This makes sense, given their unique oxidative mechanisms relative to white rot and soft rot fungi, and it makes brown rot fungal enzymes a more interesting target for biotechnological applications.

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CONCURRENT SESSION 2

Fungal - Host Interactions

Concurrent Session 2.1
ANIMAL – FUNGI INTERACTIONS

TUESDAY, FEBRUARY 18

Location: **Frentani Convention Center** | Room: **Auditorium**
CHAIR: ***Andrea Peano*** & ***Patrick Van Dijck***

14:00 - 14:15 ***Aspergillus fumigatus*** **elicits host-derived extracellular vesicles upon infection**
Matthew Blango | Leibniz Institute for Natural Product Research and Infection Biology

14:15 - 14:30 **Differential gene expression of species from *Trichophyton benhamiae* clade**
Lenka Machova | Charles University Department of Botany

14:30 - 14:45 **Unraveling the developmental program underlying trap morphogenesis and nematophagy during a fungal-nematode predator-prey interaction**
A. Pedro Gonçalves | Academia Sinica Institute of Molecular Biology Taipei

14:45 - 15:00 ***In vivo*** **competitive fitness profiling reveals protein kinases required for adaptation of *Aspergillus fumigatus* to het murine host environment**
Can Zhao | University of Manchester Manchester Fungal Infection Group
FEMS Grant

15:00 - 15:15 **The *Candida glabrata* cAMP-PKA pathway affects the major virulence factor adhesion**
Patrick Van Dijck | VIB - KU Leuven Center for Microbiology, Laboratory of Molecular Cell Biology

15:15 - 15:30 **The factors affecing intra- and inter-species diversity in fungi from the *Metarhizium complex* infecting *Myzus persicae aphids***
Victoria Reingold | ARO The Volcani Center / The Faculty of Agriculture, Jerusalem

15:30 - 15:45 **Genome-wide association identifies genomic regions in fungu clonostachys rosea that influences antagonism**
Mudassir Iqbal | Swedish University of Agricultural Sciences
FEMS Grant

Aspergillus fumigatus elicits host-derived extracellular vesicles upon infection

Matthew G. Blango[1], Ann-Kathrin Zimmermann[1,2], Flora Rivieccio[1,2], Abdulrahman Kelani[1,2], Thomas Krüger[1], Olaf Kniemeyer[1], Axel A. Brakhage[1,2]

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Aspergillus fumigatus is a widespread saprophytic fungus capable of producing aerosol asexual spores, called conidia. Although typically cleared from healthy individuals by an efficient innate immune response, *A. fumigatus* conidia can initiate devastating invasive infections in the immunocompromised. The major problems associated with these deadly infections are the limited diagnostic and therapeutic options available for treatment. To address these challenges, we were interested in elucidating the contribution of extracellular vesicles (EVs) to host defense against fungal infection, due to their emerging potential as both diagnostics and therapeutics. Surprisingly, our understanding of the contribution of EVs to the infection of mammals by fungi remains rather limited, despite numerous studies of host-bacterial and plant-fungal interactions. To determine the contribution of EVs to *A. fumigatus* infection, we optimized a series of EV isolation approaches based on differential centrifugation and size-exclusion chromatography of supernatants from different host cell types. We isolated EVs consistent in size and protein content with known EV populations and observed an increase in production of EVs in response to infection with *A. fumigatus* in multiple different cell types. Using advanced proteomics analyses of EVs, we revealed cell-type specific responses to *A. fumigatus* challenge, and RNA-Seq experiments are now underway to elucidate the RNA content of these infection-derived EVs. Intriguingly in the case of neutrophils, host-derived EVs were antifungal against *A. fumigatus* hyphae. Unexpectedly, we did not find EVs produced by *A. fumigatus* during the infection process, suggesting that EV trafficking might be a unidirectional phenomenon from host to pathogen in this case. Ultimately, the full description of EV content will provide us with novel diagnostic targets and possible therapeutic options against fungal infections.

Differential gene expression of species from *Trichophyton benhamiae* clade

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Species from *Trichophyton benhamiae* clade (Onygenales) are common skin pathogens referred to as dermatophytes. They affect animals, mainly pet guinea pigs, and the transmission to humans is quite common. Their importance is now greater than ever since one of the taxa is epidemically spreading among young children in Central Europe. We recognize 5 taxa within the clade and some of them may differ in virulency, geographical location and main host preferences. This phenomenon might be explained through virulence factors of which the most studied are secreted proteases whose profiles already showed up to differ among different *Trichophyton* species. We therefore decided to find out whether there is also observable difference in gene expression of virulence factors between such closely related populations and possibly which virulence factors are responsible for higher infectivity. For purpose of the study, selected strains were grown on different media including newly established murine skin explants model, transcriptomes were then investigated by cDNA sequencing. The outcome of this study might provide better insight into the process of infection and into the complex problematics of host-pathogen relationship of dermatophytes.

Unraveling the developmental program underlying trap morphogenesis and nematophagy during a fungal-nematode predator-prey interaction

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Nutritional deprivation triggers a saprotrophic to predatory lifestyle switch in soil dwelling nematode-trapping fungi (NTF). In particular, *Arthrobotrys oligospora* has evolved to secrete food and sex cues to lure their prey – Nematoda animals – into an adhesive network of traps, specialized structures that originate from the vegetative mycelium. Upon capture, the nematodes are invaded and digested by the fungus, thus serving as a food source. Since many nematodes are pathogens of socially and economically relevant food crops and livestock, understanding the nematode-fungal interaction can pave the way for the application of NTF as biological control agents. We employed RNA-sequencing to examine the response of a highly competent strain of *A. oligospora* upon exposure to the model nematode *Caenorhabditis elegans*. A dynamic transcriptomic reaction that indicated a strong reliance on protein secretion was observed. A comprehensive prediction of the secretome of *A. oligospora* resulted in 1084 transcripts, 64% of which are upregulated in the presence of *C. elegans* at all tested time points. Transcripts encoding specific conserved domains were over-represented in the upregulated sets, including virulence-associated and carbohydrate-binding domains. A number of secondary metabolism-associated gene clusters showed a strong

induction in the presence of *C. elegans*, suggestive of novel organic compounds that may be relevant for predation. We subsequently predicted the putative effectors of *A. oligospora* and found that they represent approximately 19% of the secretome and that their expression peaked after 10 hours of introduction of nematodes. In summary, our reverse genetics approach has exposed a number of candidate pathways involved in a soil predator-prey interaction model. Follow-up experiments are currently underway to elucidate molecular mechanisms and gene-to-phenotype relationships at the same time as novel genetic tools to manipulate *A. oligospora* are being created.

In vivo competitive fitness profiling reveals protein kinases required for adaptation of *Aspergillus fumigatus* to the murine host environment

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Our understandings of the factors that drive pathogenicity *in Aspergillus fumigatus* are limited. In this study we provide a functional genomic analysis to describe the role of protein kinases in the pathobiology of *A. fumigatus*.

As part of the *A. fumigatus* genome-wide knockout program, we have generated a library of 90 genetically barcoded protein kinase null mutants. Using a competitive fitness profiling approach, we assessed the relative fitness of each mutant under 10 *in vitro* growth conditions, and in two host models (*Galleria mellonella* larvae and neutropenic mouse). By comparing the null mutants using their fitness scores, clusters of kinases from known signalling pathways were identified alongside other clusters of kinases that may represent functional partners. Although several mutants had fitness defects in *in vitro* this did not always correlate with loss of virulence and *vice versa*, indicating that some kinases are specifically required for adaptation to the host environment.

The *Candida glabrata* cAMP-PKA pathway affects the major virulence factor adhesion

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Candida infections are a major problem in hospitals, as they are the most prevalent

yeast infections in immunocompromised patients. *C. glabrata* is the second common cause of candidiasis. This pathogen has a high mortality rate as it is often tolerant to the frequently used azole drugs and therefore very difficult to treat clinically. Yet, *C. glabrata* is phylogenetically closely related to the non-pathogenic *S. cerevisiae*. One major difference is the presence of over 67 adhesins in the *C. glabrata* genome. These cell wall proteins are responsible for adherence to various surfaces, which is the first step in the infection process or biofilm formation and therefore a major virulence factor.

We have found that in *C. glabrata*, the cAMP-PKA pathway regulates the major virulence factor adhesion. In presence of cAMP-inducing sugars, adherence to polystyrene is reduced significantly. In addition, pathway mutants confirm that adherence is under control of the cAMP-PKA pathway, rather than direct binding of the sugars to the lectin binding domain which is present in many adhesins. To elucidate which adhesin(s) are downstream, we conducted RNA sequencing and from this analysis we are currently examining the most promising targets.

The factors affecting intra- and inter- species diversity in fungi from the *Metarhizium* complex infecting *Myzus persicae* aphids

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Entomopathogenic fungi (EPF) possess the potential to integrate within biological, chemical and integrated pest management against a wide range of pests. EPF might have wide or narrow host range, might sustain saprophytic in the plant rhizosphere on organic matter and some might be endophytic within the plants. The genus *Metarhizium* comprise of around 50 species. In this work, we examined 4 species from the *Metarhizium* complex (*M. majus, M. brunneum, M. robertsii, M. pingshaense*) against the adult stage of *Myzus persicae* aphids. The results show differences in the speed of kill, however 7 days after infection the mortality in all species was above 40%. Later, 2 isolates of *M. brunneum* (Mb7 and Mbk), constitutively expressing GFP reporter gene, were examined in their pathogenicity against nymph and adult stages of *M. persicae*. Disease progression within the aphid body was demonstrated in confocal microscopy. Comparison between the speed of kill of MbK and Mb7 in adult aphids provided LD_{50} values of 3.4±0.5 and 3.08±0.1 days, respectively. In nymphs infected with Mbk the LD_{50} was 5.7±0.8, while Mb7 did not exhibit pathogenicity at all. Possibly, the diversity in pathogenicity towards nymphal stages, occurs due to acceleration of molting process

as part of the aphids' defense mechanism, assisting in the reduction of infectious spores during aphid development. In order to understand the differences between Mbk and Mb7 in their ability to infect nymphs, Next Generation Sequencing (NGS) was conducted on both genomes. Thorough examination of the results, demonstrated extremely low genetic diversity, finding it hard to attribute to the diversity in pathogenicity. Therefore, we assume that epigenetic regulation might play a crucial role in the pathogenicity and mainly in the early infection stages. Studying the epigenetic diversity during infection, might serve as a tool for future improvement of pathogenicity and environmental adaptation of commercial isolates.

Genome-wide association identifies genomic regions in fungus *Clonostachys rosea* that influence antagonism and biocontrol of plant-parasitic nematodes

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Biological control is a promising approach to reduce plant diseases caused by nematodes to ensure high productivity in future agricultural production. As large-scale genomic sequencing becomes economically feasible, the impact of single nucleotide polymorphisms (SNPs) on biocontrol-associated phenotypes can be easily studied across entire genomes of fungal populations. In this study, we made use of 53 whole-genome re-sequenced *Clonostachys rosea* strains to perform a genome-wide association (GWA) study for in vitro antagonism against plant-parasitic nematodes. Potato dextrose broth culture filtrates from *C. rosea* was inoculated with the root-lesion nematode *Pratylenchus penetrans* and nematode mortality was determined after 24 h of incubation. *In vitro* antagonism assay against *P. penetrans* displayed a significant variation among *C. rosea* strains and suggests that GWA of the trait is possible. An empirical Bayesian multiple hypothesis testing approach identified a total of 279 SNP markers significantly (local false sign rate ≤ 10-10) associated with the trait. Two non-ribosomal peptide synthetase genes (nps4 and nps5) were present in the genomic regions associated with the nematicidal activity. Gene deletion strains of nps4 and nps5 genes were generated and showed increased growth and conidiation rates compared to the wild type. Culture filtrates from *C. rosea* Δnps4 and Δnps5 strains exhibited reduced nematicidal activity and immobilised nematodes to a significantly ($P \leq 0.05$) lower number compared to the wild type after 24 h of incubation. Furthermore, Δnps4 and Δnps5 strains showed reduced biocontrol efficacy in a naturally nematode infested soil in a pot experiment and

failed to reduce the populations of nematodes in soil or in roots of wheat as efficiently as the wild type strain. Taken together, we show that NPS4 and NPS5 are biocontrol factors in *C. rosea*, presumably by producing a hitherto unknown non-ribosomal peptide compound with nematicidal properties.

Concurrent Session 2.2
PLANT – FUNGI INTERACTIONS

TUESDAY, FEBRUARY 18

Location: **Frentani Convention Center** | Room: **Auditorium**
CHAIR: ***Ivan Baccelli*** & ***Valeria Scala***

16:15 - 16:30 **Chromatin remodeling contributes to the spatio-temporal expression pattern of virulence genes in a fungal plant pathogen**
Andrea Sánchez Vallet | ETH Zürich USYS

16:30 - 16:45 **Clathrin-mediated endocytosis facilitates internalization of *Magnaporthe oryzae* effectors into rice cells**
Ely Oliveira Garcia | Louisiana State University Plant Pathology and Crop Physiology

16:45 - 17:00 **Transcriptional plasticity underpins generalist and host-specific infection mechanisms in the plant pathogen *Sclerotinia sclerotiorum***
Sylvain Raffaele | INRA Plant-microbe interactions (LIPM)

17:00 - 17:15 ***Verticillium*-specific pore-forming effector VnaSSP4.2 targets host plant plasma membrane through interaction with phosphoinositide phosphates**
Sabina Berne | University of Ljubljana, Biotechnical Faculty Department of Agronomy

17:15 - 17:30 **The Crz1 transcription factor regulates lipid metabolism and fumonisin production in *Fusarium verticillioides***
Marzia Beccaccioli | Dipartimento di Biologia Ambientale, Università Sapienza

17:30 - 17:45 **Understanding the Pmk1 MAP kinase signalling pathway during plant colonisation by the rice blast fungus *Magnaporthe oryzae***
Neftaly Cruz-Mireles | The Sainsbury Laboratory **FEMS Grant**

17:45 - 18:00 **A protein complex essential for virulence in a fungal plant pathogen**
Nicole Ludwig | Max Planck Institute for Terrestrial Microbiology Dept. of Organismic Int

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Chromatin remodeling contributes to the spatio-temporal expression pattern of virulence genes in a fungal plant pathogen

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Dynamic changes in transcription profiles are key for the success of pathogens in colonizing their hosts. In many pathogens, genes associated with virulence, such as effectors, are located in transposable element-rich and heterochromatic regions of the genome. The contribution of chromatin modifications in profiling the expression pattern of pathogens remains largely unknown. Here, we show that the heterochromatic environment of effector genes in the fungal plant pathogen Zymoseptoria tritici provides a functional framework for their specific spatio-temporal expression pattern. Enrichment in trimethylated lysine 27 and probably also lysine 9 of histone H3 likely dictates the repression of effector genes in the absence of the host. Chromatin remodelling during host colonization, featured by the reduction of these repressive marks, highlighted the major role of epigenetics in effector gene induction. Our results illustrate that host-triggered chromatin decondensation is key for determining the specific expression pattern of effector genes in the cellular level and, hence, provide new insights into the regulation of virulence of fungal plant pathogens.

Clathrin-mediated endocytosis facilitates internalization of *Magnaporthe oryzae* effectors into rice cells

Ely Oliveira-Garcia[1,4], Magdalena Martin-Urdiroz[2], Clara Rodriguez-Herrero[2], Nicholas J. Talbot[2], Jungeun Park[3], Sunghun Park[3] & Barbara Valent[1]

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Rice blast disease caused by the fungus *Magnaporthe oryzae*, is a major threat to global food security given the disease is responsible for approximately 30% of rice production losses globally. After penetrating the host surface, *M. oryzae* secretes numerous effectors, which are involved in effective host infection. Effectors are classified by their destinations in the interaction court, which apoplastic effectors residing in the

extracellular plant compartment and cytoplasmic effectors translocating into the cytoplasm of living plant cells. Notably, cytoplasmic effectors of *M. oryzae* are associated with a specialized interfacial structure, the biotrophic interfacial complex (BIC). To date, little is known about how fungi deliver effector proteins inside plant cells to control plant processes. Here we show translocation of the cytoplasmic effectors Bas1, Bas170, Pwl1 and Pwl2 in vesicles from the BIC to the rice cytoplasm during biotrophic development. Through FM4-64 labeling and Lti6b:eGFP transgenic rice colocalization assays, we demonstrated that the vesicles containing effectors originate from plant plasma membrane. Likewise, Phalloidin labeling and LifeAct:eGFP transgenic rice colocalization assays revealed that BICs are plant actin-rich structures. Furthermore, we identified a novel plant plasma membrane-associated effector, Bas83, which is strictly BIC-localized. Using rice transgenic lines expressing CLC1:eGFP, clathrin-mediated endocytosis (CME) marker, or Flot1:eGFP, clathrin-independent endocytosis (CIE) marker, we demonstrated that BICs contain massive amounts of plant clathrin. All BICs and vesicles containing fluorescently-labeled effectors colocalized with CLC1:eGFP, revealing a major role of CDE in the internalization of cytoplasmic effectors. In contrast, few BICs colocalized with Flot1:eGFP. We also accessed the impact of CME and CIE inhibition on effector uptake by using virus-induced gene silencing (VIGS) and chemical approaches. Whereas CME inhibition led to abnormally-shaped, swollen BICs, inhibition of CIE had little impact on BICs. This study demonstrated that cytoplasmic effector translocation is BIC-localized and facilitated by CME, suggesting a potential role for *M. oryzae* effectors in manipulation of plant endocytosis.

Transcriptional plasticity underpins generalist and host-specific infection mechanisms in the plant pathogen *S. sclerotiorum*

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The range of host that a pathogen can infect is major determinant of disease epidemics. The molecular bases of host colonization and adaptation in fungal pathogens with a broad host range remains largely enigmatic. With its ability to infect hundreds of plant species, resulting in recurring epidemics on oil and vegetable crops, the Ascomycete

Sclerotinia sclerotiorum is a paradigmatic broad host range parasite. By examining the global regulation of *S. sclerotiorum* genes along invasive hyphae on multiple host species, we reveal programs supporting the colonization of multiple hosts as well as host-specific virulence in *S. sclerotiorum*. Gene expression patterns diverged markedly in cells at the center and apex of hyphae during the colonization of *Arabidopsis thaliana* plants compared to *in vitro* growth. Using multi-cell and genome-scale metabolic models, we show that gene regulation triggers metabolic heterogeneity and division of labor between hyphal cells. By comparing the fungal transcriptome during the infection of six plants from distinct botanical families. This revealed 1,657 fungal genes up-regulated *in planta*, among which 4.6% only are up-regulated in all six host species. We used whole genome sequencing by nanopore technology to produce high quality genomes for four sister species with a limited host range. Comparative genomics and pangenome analyses indicated that host range expansion in *S. sclerotiorum* involved lineage-specific genes as well as regulatory divergence in conserved genes. Progress towards the analysis of *S. sclerotiorum* virulence factors specific to *Arabidopsis* colonization will be presented.

Verticillium-specific pore-forming effector VnaSSP4.2 targets host plant plasma membrane through interaction with phosphoinositide phosphates

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The *Verticillium nonalfalfae* virulence factor VnaSSP4.2 was discovered in the xylem sap of infected hop [1]. Here, we characterized the function of this 14 kDa basic lysine-rich protein with a predominantly beta secondary structure but no known protein domains. Using bioinformatic analyses and RNA-Seq data, we found that VnaSSP4.2 is a highly conserved *Verticillium*-specific effector, which is abundantly expressed in the susceptible plants, while barely detected in the resistant ones. Confocal microscopy revealed a non-specific nucleocytosolic localization of VnaSSP4.2 as transiently expressed mRFP fusion proteins in the *Nicotiana benthamiana* leaves. Using Y2H screening of potato library, we did not identify any strong protein interactors. We could not detect any HR response to infiltration of 1 µM and 5 µM recombinant VnaSSP4.2 in the *N. benthamiana* leaves. In addition, we did not measure any release of reactive oxygen species from hop suspension cells following the exposure to 1 µM VnaSSP4.2. However, live cell imaging using Nanolive 3D Cell Explorer revealed that VnaSSP4.2-eGFP fusion proteins target the plasma membrane of hop protoplasts. Lipid overlay assay indicated that VnaSSP4.2 specifically interacts with phosphoinositide (PI) lipids. This

specific interaction was confirmed using large unilamellar vesicles (LUV) composed of dipalmytoyl phosphatidiylcholine and different PIs in a 9:1 (mol/mol) ratio, both in the flotation assay on a sucrose gradient, and using surface plasmon resonance. Finally, we found that VnaSSP4.2 permeabilizes calcein-loaded LUVs with the highest percentage of calcein release from the LUVs supplemented with 10 mol % of $PI(4,5)P_2$. Considering that PIs play very important roles in major signal transduction pathways, we suggest VnaSSP4.2 is a novel fungal PI effector, which might modulate host plant immune signaling or could participate in the delivery of other *V. nonalfalfae* effector proteins to plant host cells via pore formation.

[1] Flajšman *et al*., Molecular Plant-Microbe Interactions, 2016, 29(5): 362-373

The Crz1 transcription factor regulates lipid metabolism and fumonisin production in *Fusarium verticillioides*

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Calcineurin, a key player in calcium-dependent signal transduction pathways of eukaryotes, modulates colony growth, stress response and pathogenicity in fungi (Thewes, 2014). Here we investigated the role of the fungal protein Crz1, a downstream transcription factor of the calcineurin pathway, in the fumonisin-producing fungus *Fusarium verticillioides*.

Previous studies have shown that the production of fumonisins in *Fusarium* is related to the presence in the growth medium of some specific fatty acids (FAs) and oxylipins (i.e. oxidized FAs) which are released by both the plant and the fungus, and that play pivotal functions in the crosstalk between host and pathogens (Dall'Asta *et al*., 2012; Scala *et al*., 2014). In this study, we report the involvement of Crz1 in the regulation of both lipid metabolism and fumonisin production during the *F. verticillioides*-maize interaction. *F. verticillioides crz1Δ* strains showed higher membrane permeability and susceptibility to ionic stress when compared to the wild type or the *crz1Δ+crz1* complemented strains. Through a mass spectrometry approach, we also found that the deletion of *crz1* was consistently associated with an overall reduction in oxylipin, FA and mycotoxin content during maize infection. We postulate that Crz1 is required for the proper generation of signalling lipid molecules (e.g. FAs or oxylipins) which in turn activate fumonisin biosynthesis in *F. verticillioides*.

Understanding the Pmk1 MAP kinase signalling pathway during plant colonisation by the rice blast fungus *Magnaporthe oryzae*

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Rice blast disease is among the most devastating diseases affecting global agriculture. The disease is caused by the fungal pathogen *Magnaporthe oryzae*. This fungus gains entry to the plant by forming a dome-shaped infection structure called an appressorium. Once inside the plant cell, *M. oryzae* develops intracellular invasive hyphae that facilitate cell-to-cell movement through pit field sites where plasmodesmata are situated. The Pmk1 (MAP) kinase signalling pathway, plays a key role during appressorium formation, plant penetration as well as host colonisation. However, how Pmk1 regulates such physiological processes remains unclear. Here, we report a comparative phosphoproteomic analysis that we are using to identify direct downstream targets of the Pmk1 MAPK during all stages of infection. To understand the role of Pmk1 during rice tissue invasion we used a chemical genetics approach to generate a *M. oryzae* pmk1AS mutant in which we are able to conditionally inactivate the kinase in the presence of napthyl-PP1 (Sakulkoo, *et al*., 2018). We have identified phosphorylated candidate proteins related to cellular processes such as cytoskeleton remodelling, vesicular trafficking and cell cycle control. We are currently validating their roles and defining the signalling network that operates downstream of the Pmk1 MAPK during plant infection by the rice blast fungus.

A protein complex essential for virulence in a fungal plant pathogen

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Ustilago maydis is a biotrophic fungal pathogen, which causes smut disease in its host plant maize. For a successful infection *U. maydis* needs to suppress plant defense

responses and manipulate the host physiology for its own benefit. To accomplish this *U. maydis* secretes effector proteins, which function either in the apoplast or in the host cell. The mechanism by which they are translocated into the host cell are mostly unknown.

We systematically deleted effectors, whose expression is linked to the stage where biotrophy is established and thereby identified mutants, which failed to cause disease. Co-IP/MS experiments of plant tissue infected by *U. maydis* expressing these tagged essential effectors revealed that five unrelated effectors and two membrane proteins from *U. maydis* form a stable protein complex. All seven proteins are also present in all analyzed smut species. Single mutants penetrate the epidermis but are unable to downregulate plant defense responses and arrest in the epidermal layer. The complex is anchored in the fungal membrane and spans the plant fungus interface, interacting with pore-forming proteins in the plant plasma membrane. Complex mutants also fail to induce non-host resistance in barley, a reaction likely depending on translocated effectors. We therefore suggest that the complex is part of an effector translocation system in pathogenic fungi.

Concurrent Session 2.3
ANTIFUNGAL AND FUNGICIDES

WEDNESDAY, FEBRUARY 19

Location: **Frentani Convention Center** | Room: **Accademia**
CHAIR: ***Marina Collina*** & ***Gabriel Scalliet***

16:15 - 16:30 **Species-specific differences in the susceptibility of fungi towards the antifungal protein AFP depend on C3 saturation of glycosylceramides**
Sascha Jung | Technische Universität Berlin, Chair of Applied and Molecular Microbiology

16:30 - 16:45 **The use of genome-wide association to identify mutations associated with DMI resistance in *Cercospora beticola***
Melvin Bolton | U.S. Department of Agriculture

16:45 - 17:00 **CRISPR/Cas9 with ribonucleoprotein complexes allows highly efficient marker-free editing approaches in *Botrytis cinerea* and other fungi**
Matthias Hahn | University of Kaiserslautern | Syngenta

17:00 - 17:15 **PAFC is one of three small, cysteine-rich proteins in *Penicillium chrysogenum* and exhibits strong antifungal efficacy against *Candida albicans***
Jeanett Holzknecht | Medical University of Innsbruck

17:15 - 17:30 **Dimethomorph: an interdisciplinary approach through different plants and animal pathogenic oomycetes**
Irene Maja Nanni | Università di Bologna

17:30 - 17:45 **Parallel evolution of fungicide resistance in the barley net blotches and emergence of a resistant clonal population by interspecific hybridisation**
Wesley Mair | Curtin University

17:45 - 18:00 **Whole genome sequencing elucidates the genomic background of fungicide resistant- and susceptible *Alternaria solani* strains**
Remco Stam | Technical University of Munich

Species-specific differences in the susceptibility of fungi towards the antifungal protein AFP depend on C3 saturation of glycosylceramides

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The emergence and spread of multi-drug resistant pathogenic fungi represents a serious challenge and demands for smarter antimicrobials, which affect multiple cellular targets. Of special interest is the exploitation of the AFP family of antimicrobial peptides (AMP) including its founding member AFP which is a cysteine stabilized, small, cationic and amphipathic γ-core motif peptide from *A. giganteus*. AFP is a very potent inhibitor of fungal growth without affecting the viability of bacteria, plant or mammalian cells. It targets chitin synthesis and causes plasma membrane permeabilization in many human and plant pathogenic fungi, but its exact mode of action is not known. Recent studies suggested a relation between membrane glycosylceramides (GlyCer) and the antifungal activity of antimicrobial peptides, which were found to specifically recognize and bind to GlyCer of fungal but not of mammalian origin.

We have recently proposed adoption of the 'damage-response framework of microbial pathogenesis'. This model predicts that the cytotoxic capacity of a given AMP does not only depend on the presence/absence of its target(s) in the host and the AMP concentration applied but also on other variables, e.g. microbial survival strategies. We show here along the examples of filamentous fungi (*A. niger, A. fumigatus, F. graminearum*) and yeasts (*S. cerevisiae, P. pastoris*) that important parameters defining AFP susceptibilities of these fungi are: (i) presence/absence of GlyCer, (ii) presence/absence of D3(E)-desaturation of the fatty acid chain therein, and (iii) (dis)ability of these fungi to respond to AFP inhibitory effects with the fortification of their cell walls via increased chitin and β-(1,3)-glucan synthesis. Our data suggest a fundamental role of GlyCer in the susceptibility of fungi towards AFP. We uncovered that only a minor structural difference in these molecules - saturation of their fatty acid chain - is key to understand the inhibitory activity of AFP.

The use of genome-wide association to identify mutations associated with DMI resistance in *Cercospora beticola*

Lorena Ranquel[1], Rebecca Spanner[2], Jonathan Richards[2], Timothy Friesen[1], Melvin Bolton[1]

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Cercospora leaf spot (CLS) caused by *Cercospora beticola* is the more destructive foliar disease of sugarbeet worldwide. In the United States, losses to CLS approached $200 million in the 2018 growing season alone. Management strategies for CLS rely on timely fungicide application. The CLS management fungicide repertoire often includes the application of fungicides in the sterol demethylation inhibitor (DMI) class. However, the reliance on DMIs has led to the emergence of resistance in many *C. beticola* populations. Previously, we showed that DMI-resistant strains of *C. beticola* have high expression levels of *Cyp51*, which are induced further upon DMI exposure. However, no mutations in the *Cyp51* coding or promoter regions appeared to correlate with DMI resistance. To identify mutations responsible for DMI resistance, a genome-wide association approach was undertaken using 194 isolates harvested from local sugarbeet fields. Approximately half of the isolates were DMI-sensitive (EC50 value <1.0 μg mL^{-1}) while the other half were DMI-resistant (EC50 value ≥1.0 μg mL^{-1}). After whole genome resequencing, genome wide association identified one locus highly correlated with DMI-resistance. Since the candidate gene in this region only had a synonymous mutation, we developed gene-edited mutants to confirm that the synonymous mutation is associated with resistance. The results of this study will be reported.

CRISPR/Cas9 with ribonucleoprotein complexes allows highly efficient marker-free editing apporaches in *Botrytis cinerea* and other fungi

Matthias Hahn[1], Thomas Leisen[1], Fabian Bietzc[1], Janina Werner[1], Alexander Wegner[2], Ulrich Schaffrath[2], David Scheuring[1], Felix Willmund[1], Andreas Mosbach[3], Gabriel Scalliet[3]

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Botrytis cinerea is considered one of the most important plant pathogens, causing enormous pre- and postharvest crop crop losses of fruits, vegetables and flowers. Control by fungicides has become increasingly difficult due to the worldwide apperance of multi-resistant strains. We report on the establishment of the CRISPR/Cas9 technology for B. cinerea. Introduction of Cas9-sgRNAs ribonucleoprotein (RNP) complexes into protoplasts allows highly efficient genome editing. Using Bcbos1 as a target gene which allows positive selection of mutations, highly efficient repair via non-homologous end joining (NHEJ) was obtained. Using repair templates with 60 bp homology flanks resulted in marker-assisted gene insertions and deletions with ca. 90% efficiency. Two novel cotransformation strategies were established for marker free co-editing. Resistance marker shuttling is based on the exchange between different resistance markers in each transformation. The most effective strategy used cotransformation of a non-integrative

telomere vector for transient selection and a non-selected CRISPR/Cas editing construct, resulting in up to thousands of transformants and coediting rates of 20-80%. This approach allowed the marker-free introduction of mutations via NHEJ, GFP-tagging by knock-in of a superoxide dismutase (BcSod1), and simultaneous deletion of two necrosis-inducing proteins. Its power was further demonstrated by random mutagenesis of codon 272 of the BcsdhB gene followed by in vivo selection, leading to the identification of several amino acids not found in field isolates which conferred differential resistance to succinate dehydrogenase inhibitor fungicides. Telomere-mediated coediting worked also with Magnaporthe oryzae. The unprecedented performance and ease of use of these RNP-based tools, which don't require any cloning steps, will greatly improve molecular research with B. cinerea and can be transferred to other fungi.

PAFC is one of three small, cysteine-rich proteins in *Penicillium chrysogenum* and exhibits strong antifungal efficacy against *Candida albicans*

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Fungal diseases affect over one billion people worldwide, whereby *Candida* species represent the most common opportunistic fungal pathogens in humans. Due to the high risk of resistance development to licensed antifungal drugs, new compounds with novel modes of action are needed. *Penicillium chrysogenum* is one representative of only few fungal species with the ability to produce three different small, cysteine-rich antifungal proteins: PAFA, PAFB and PAFC , all of which possess growth inhibitory activity against *Candida spp*.

In this study, the antifungal activity of PAFC against clinically relevant *Candida* isolates, such as *C. albicans, C. glabrata, C. krusei* and *C. parapsilosis* was proven. Furthermore, the mode of action of PAFC against two *C. albicans* isolates, one of which was a fluconazole-resistant (fluR) strain derived from a vulvovaginitis was characterized in detail. Scanning electron microscopic observation indicated that PAFC induced pore formation in cells and the damage aggravated in a time and concentration dependent manner. Tracking of fluorescence-labeled PAFC showed that it readily bound to the outer layer of *Candida* cell before it was taken up, inducing production of intracellular reactive oxygen species (ROS) and triggering cell death when localizing in the cytoplasm. Cells which had

survived PAFC treatment in the applied concentrations, stored the protein in vacuoles and resumed proliferation. Interestingly, the fluR *C. albicans* isolate was more susceptible to PAFC treatment than the fluconazole-sensitive strain reflecting the acquirement of drug resistance at the expense of cell fitness. Assays to test for cytotoxicity indicated that PAFC does not exhibit any detrimental effects on primary human skin cells *in vitro* and has no hemolytic activity.

Based on these results PAFC holds great promise for antifungal drug development. Further studies on the mode of action, applicability and protein modification to improve efficacy are in progress.

Dimethomorph: an interdisciplinary approach through different plants and animal pathogenic oomycetes

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The class Oomycota gathers species that are pathogenic to plants and animals, including humans, these pathogens are able to cause economic losses in agriculture and aquaculture industry worldwide. *Phytophthora infestans* causes losses in potato and tomato crops for more than 6 billion per year, *Plasmopara viticola* is the causal agent of downy mildew, which is one of the most damaging grapevine disease. The genus *Pythium*, includes species that are pathogenic for both plants and animals, particularly, *Pythium insidiosum* was reported to cause disease in humans and in other mammals. Oomycetes of the order *Saprolegniales* are widely distributed in freshwater environments, causing infections in different taxa of aquatic animals. Among these members of the genus, *Saprolegnia* represent a severe problem in fish farms. Despite the wide distribution and the impact of the diseases caused by oomycetes on animal health and on economic activities, there are no effective available molecules against these pathogens. Dimethomorph was introduced in 1988 as a novel fungicide with high activity against oomycetes plant pathogens and authorized in Italy in 1994. Dimethomorph is still largely used to control downy mildew in Italy. In order to test the sensitivity of dimethomorph to *P. viticola* populations, a multi-year monitoring was carried out, showing a good efficacy in the control of the pathogen. Since the are limited possibilities to control oomycete infections in aquaculture, this fungicide was applied in *in vitro* assays on different species of *Saprolegnia* and *Pythium*. Preliminary *in vitro* results showed a non complete inhibition, but a significant slowdown in the mycelial growth. Additional microscopic

studies are still ongoing to investigate how the molecule interferes with the mycelium of *P. viticola* and *Saprolegnia*. These findings would allow to further investigate the biology of different species of oomycetes, thus promoting the research across interdisciplinary scientific disciplines.

Parallel evolution of fungicide resistance in the barley net blotches and emergence of a resistant clonal population by interspecific hybridisation

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Demethylase-inhibitor (DMI) fungicides are a key component of control programs for net form (NFNB, causal agent *Pyrenophora teres* f. *teres*, *Ptt*) and spot form (SFNB, *P. teres* f. *maculata*, *Ptm*) of net blotch diseases of barley. NFNB resistance to DMIs has been recently found in Western Australia (WA), but limited knowledge is available for resistance and its basis in SFNB. 288 *Ptm* isolates collected 1996-2019 from WA barley growing regions were screened for sensitivity to DMIs. Reduced sensitivity was correlated with several mutations in the promoter and coding sequence of the DMI target gene *Cyp51A*. Insertions of 134-bp were found at five sites in the *Cyp51A* promoter in highly DMI-resistant (HR) and some moderately DMI-resistant (MR1) isolates. Insertions were identified as Solo-Long Terminal Repeats derived from *Ty1/copia* retrotransposons. Insertions contained predicted promoter and transcription factor binding sites and correlated with constitutive *Cyp51A* overexpression. A phenylalanine to leucine substitution at residue 489 of CYP51A, previously reported in isolates of *Ptt* with reduced sensitivity to DMIs, was found in HR and some moderately DMI-resistant (MR2) isolates, and was associated with three different single nucleotide polymorphisms in codon 489. Molecular markers specific to *Ptt* and *Ptm* showed that certain HR isolates carried both markers, suggesting the possibility that they were hybrids between the two forms. Analysis of 9656 DArTSeq markers showed a clear distinction between *Ptm*, *Ptt* and barley grass *P. teres* but absolute lack of genetic diversity among the 40 HR *Ptm* tested. Genome sequencing of select isolates revealed evidence of recombination between *Ptt* and *Ptm*. Among 1393 intergenic regions tested, 75 suggested presence of significant ($P < 0.05$) recombination. The results suggest the recent emergence of the HR strain in WA likely arose from interspecific sexual recombination of *Ptm* with *Ptt*, followed by backcrossing to *Ptm* and clonal dispersion.

Whole genome sequencing elucidates the genomic background of fungicide resistant- and susceptible *Alternaria solani* strains

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Early blight is mainly caused by the fungal pathogen *Alternaria solani* and is an increasing problem for potato and tomato production world-wide. The primary strategy to control the disease is the application of fungicides such as succinate dehydrogenase inhibitors (SDHI) and quinone outside inhibitors (QoI).

QoI fungicides were registered in Germany as early blight-specific fungicides in potatoes in 2007 and the first resistances in *A. solani* appeared in 2009. They are thought to be caused by a single point mutation (F129L) in the target cytochrome b gene. Resistance, or reduced sensitivity to SDHI fungicides appears to be more complex. Resistant strains appeared in 2013, five years after SDHI introduction in Germany. Two main mutations in the Sdh complex have been found; a mutation in SdhB (H278Y) and a mutation in SdhC (H134R). Different *A. solani* strains are conferring different degree of resistance in vitro and in inoculation experiments. Preliminary experiments in our lab suggested that mutations in Sdh subunits could also potentially linked to fitness trade-offs. However, information on the genetic background of the tested isolates was not available.

We performed whole genome re-sequencing experiments for *A. solani* isolates with and without SDHI resistance, originating from two main potato growing areas in Germany, as well as outlier isolates from a different geographic origin.

In our samples, we can distinguish four main genotype groups, and these genotypes are not clearly associated with different Sdh subunit mutations. Here we present our latest results and discuss how they can help answer questions regarding the rise of mutations leading to SDHI resistance, migration of resistant strains and genotype-phenotype relationships, with regards to aggressiveness and other pathogen properties.

ECFG15
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CONCURRENT SESSION 3

Evolution and Molecular Ecology

Concurrent Session 3.1
EVOLUTION

TUESDAY, FEBRUARY 18

Location: **Frentani Convention Center** | Room: **Accademia**
CHAIR: ***Sabrina Sarrocco*** & ***Magnus Karlsson***

14:00 - 14:15 **A mosaic thiamine biosynthetic pathway in yeasts reconstructed by multiple horizontal gene transfers**
Carla Gonçalves | Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, UCIBIO-REQUIMTE, Departamento de Ciências da Vida
FEMS Grant

14:15 - 14:30 **Influence of cultivated biodiversity on the evolution of pathogen populations: the rice/blast interaction in the traditional YuanYang terraces (China)**
Elisabeth Fournier | INRA BGPI

14:30 - 14:45 **Incipient local adaptation to heavy metal pollution in a mycorrhizal fungus**
Anna Bazzicalupo | Montana State University Microbiology and Immunology Bozeman FEMS Grant

14:45 - 15:00 **Novel insights into population dynamics and lineage differentiation of the coffee leaf rust athogen *Hemileia vastatrix***
Andreia Loureiro | Instituto Superior de Agronomia, Universidade de Lisboa

15:00 - 15:15 **Emergence and diversification of a highly invasive tree pathogen lineage**
Lea Stauber | Swiss Federal Institute for Forest, Snow and Landscape Research (WSL)

15:15 - 15:30 **Niche differentiation underlies evolution of the wood decay machinery in the invasive fungus *Serpula lacrymans***
Inger Skrede | Helmholtz Centre for Environmental Research Soil Ecology

15:30 - 15:45 **Never shall those born to crawl, learn to fly: Evolutionary compromises between spore hydrophobicity and fitness in *Trichoderma***
Irina Druzhinina | Nanjing Agricultural University Fungal Genomics Group

A mosaic thiamine biosynthetic pathway in yeasts reconstructed by multiple horizontal gene transfers

Carla Gonçalves and Paula Gonçalves

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We recently reported on a group of yeasts, referred to as the *Wickerhamiella/Starmerella* (W/S) clade, that acquired multiple genes from bacteria by horizontal gene transfer (HGT), some of which were found to have reinstated the previously lost alcoholic fermentation capacity in this lineage (1). Recent studies confirmed that the number of bacterial genes in this clade was the highest among yeasts (2) and that some were simultaneously acquired as an operon (3). In the present work we found that part of the thiamine biosynthetic pathway was also lost in an ancestor of the W/S clade. Thiamine is an essential vitamin because its active form, thiamine pyrophosphate, is the cofactor of key enzymes in aminoacid and carbohydrate metabolism. Loss of part of the thiamine pathway implied an initial dependence on external sources of thiamine, however we showed that independent horizontal operon transfers were later involved in its reassembly. We experimentally demonstrated that additionally to entire operons, acquisition of single genes was essential to widen the range of compounds that can be used by these yeasts to produce thiamine. Adaptation of the acquired operons to an eukaryotic-like transcription probably involved the increase in the length of intergenic regions, possibly to accommodate *de novo* promoters as observed before (3). Strikingly, we showed that in three instances, generation of *de novo* promoters was probably avoided by fusion of adjacent genes. One such case involved the fusion of all the three genes composing the original operon, originating a pentafunctional protein. We showed that thiamine biosynthesis currently occurs through a pathway in which yeast and bacterial proteins from different donors act in different steps of the pathway. Taken together, our results endorse HGT as an important driver of evolution and adaption in eukaryotes.

1. C. Goncalves *et al*., *eLife* 7:e33034 (2018
2. X. X. Shen *et al.*, *Cell* 175, 1533-1545.e1520 (2018)
3. J. Kominek *et al.*, *Cell* 176, 1356-1366.e1310 (2019)

Influence of cultivated biodiversity on the evolution of pathogen populations: the rice/blast interaction in the traditional YuanYang terraces (China)

Elisabeth Fournier[1], Sajid Ali[2], Pierre Gladieux[1], Henri Adreit[3], Sandrine Cros-

Arteil[1], Baihui Jin[4], Isabelle Meusnier[1], Joelle Milazzo[3], Sébastien Ravel[3], Didier Tharreau[3], Huichuan Huang[4], Jean-Benoît Morel[1]

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In modern agrosystems, the use of a reduced number of plant varieties in homogeneous landscapes promotes the emergence of virulent pathogen genotypes that overcome rapidly plant resistances and cause significant damage. The diversification of varietal landscapes represents a lever to hinder pathogen evolution. To better understand the influence of cultivated biodiversity on the structure and evolution of populations of plant pathogens, we are studying a traditional rice agrosystem in southern China: the YuanYang Terraces. In this system, where a large number of rice varieties (mainly indica, with a few japonica varieties) have been grown in sympatry for centuries, no major health crises have been reported.

We first confirmed the very high genetic diversity of rice in the area, generating a very heterogeneous varietal landscape for pathogen populations. Taking the example of the interaction of rice with the blast fungus *Pyricularia oryzae*, we then showed that the sympathetic cultivation of indica and japonica rice, whose immune systems are highly contrasted, causes local adaptation of *P. oryzae* populations, leading to mutual protection of these two types of rice. We have also shown that within indica rice, the very high intra- and inter-varietal diversity selects generalist and possibly maladapted genotypes of P. oryzae. Finally, the recent introduction into the area of a modern variety with a reduced genetic basis is causing significant local declines in crop diversity, the consequences of which we have measured on the population structure of *P. oryzae*.

Incipient local adaptation to heavy metal pollution in a mycorrhizal fungus

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The genomes of organisms living in diverse environments can reflect adaptations to diverse environmental conditions. Anthropogenic disturbances such as soil pollution can quickly create steep environmental gradients that likely induce adaptation and imprint genomic signatures of selection. Fungi are ubiquitous in soil environments and are key players in soil systems processes, however little is known about how they evolve in face of anthropogenic soil pollution. We used population genomics to investigate incipient local adaptation to heavy metals in *Suillus luteus*, a widespread symbiotic ectomycorrhizal fungus associated with pine trees. Available phenotypic data show that *S. luteus* isolates from the same population have variable heavy metal tolerance with some isolates tolerating high metal concentrations, while others show reduced growth and death when exposed to heavy metals. Whole genome scans across isolates from both habitats revealed genomic signatures associated with colonizing polluted sites, with a strong enrichment for transmembrane transporters. Candidate genes underlying heavy metal adaptation in *S. luteus* were involved in metal exclusion, immobilization, and detoxification, and displayed both allelic and copy number variation. Results from our study revealed incipient local adaptation to heavy metals in *S. luteus* and elucidate on the evolutionary processes involved in environmental adaptation in fungi.

Novel insights into population dynamics and lineage differentiation of the coffee leaf rust pathogen *Hemileia vastatrix*

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Coffee Leaf Rust, caused by *Hemileia vastatrix* (Hv), has been the major constraint to global coffee production for more than a century. Only recently the population evolutionary history of this pathogen began to be unveiled. Silva *et al.* (2018) found for the first time the species to be structured into three divergent genetic lineages with marked host tropism (C1 and C2 infecting diploid coffee species; and C3 infecting tetraploid coffee species), and signals of introgression. Nevertheless, no significant structuring was found within the C3 lineage, which represent the most widespread and epidemiological relevant Hv group. Here, we extended the investigation to a worldwide scale sampling for obtaining a deeper insight on the dynamics and adaptive evolution of Hv populations. We used restriction site-associated DNA sequencing (RADseq) to generate around 21,520 SNPs

across 108 Hv isolates. Phylogenetic analyses corroborated the existence of the three well-diverged Hv groups, but furthermore showed a well-supported structuring within C3, with three main sub-groups: African, Asian and Timor. This pattern seems to reflect Hv geographical origin associated to the historical distribution and exchange of coffee materials. The Asian origin clade comprises the higher number of isolates and exhibits a ladder-like diversification pattern, with relatively low genetic diversity, suggesting rapid evolution and population expansion. On the contrary, the African and Timor populations appear to more restricted, revealing some degree of differentiation. In addition, our results reinforce the potential role of introgression in Hv lineage and virulence evolution. From the 7909 loci (comprising 9628 "diagnostic" SNPs) differentiating C2 and C3 groups, 2,63% mapped against NCBI nt database, with the majority of the hits corresponding to retrotransposons (82,76%) and putative secreted protein genes (9.58%)

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Emergence and diversification of a highly invasive tree pathogen lineage

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Invasive microbial species constitute a major threat to biodiversity, agricultural production and human health. Microbial invasions are often dominated by one or a small number of genotypes, yet the underlying factors driving invasions are poorly understood. A prime example for a successful invasion is the recent outbreak of the chestnut blight fungus *Cryphonectria parasitica* in Southeastern Europe. *C. parasitica* is native to East Asia and has colonized North America and Europe during the first half of the 20th century. We investigated the genetic structure of 188 European strains of the pathogen using 17'873 genome-wide single-nucleotide polymorphisms (SNPs). Genotypes showed high levels of diversity with evidence for frequent and ongoing recombination. European populations also exhibit a longitudinal gradient in genetic diversity. We found that the invasive S12 lineage dominating Southeastern Europe is genetically homogeneous. Our data strongly suggests that S12 emerged from the highly diverse pool of European genotypes rather than a secondary introduction from Asia. The S12 lineage is predominantly composed of a single mating type and the observed genetic diversity is consistent with mutation accumulation. Despite little or no evidence for recombination, we show experimentally that the lineage retained the ability to reproduce sexually. Overall, our findings show that an invasive pathogen lineage can arise through an intermediary, highly diverse bridgehead population. The combination of genetic and epidemiological evidence suggests that the invasive lineage switched the dominant reproductive mode and gained crucial adaptive

mutations favoring rapid expansion.

Niche differentiation underlies evolution of the wood decay machinery in the invasive fungus *Serpula lacrymans*

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Ecological niche breadth and the mechanisms facilitating its evolution are fundamental to understanding adaptation to changing environments, persistence of generalist and specialist lineages and the formation of new species. Yet, the genetic architectures underlying niche breadth evolution remain poorly understood. Woody substrates are highly specialized resources that only organisms with specialist decay machinery are capable of utilizing. Wood-decaying fungi represent ideal model systems to study evolution of niche breadth, as they vary greatly in their host range and preferred decay stage of the substrate. In order to dissect the genetic basis for niche specialization in the invasive brown rot fungus *Serpula lacrymans*, we have developed a comparative genomic system including wild relatives in the Serpulaceae with a range of specialist to generalist decay strategies. We used phenotyping and integrative analysis of phylogenomic and transcriptomic data to characterize the decay machinery of each fungus and map evolutionary innovations. Our results indicate that specialist species with rapid decay rates show decreased reliance on enzymatic machinery, and therefore nitrogen-intensive decay components, compared to their generalist relative. This shift may facilitate adaptation to a narrow tree line habitat and switch to a pioneer decomposer strategy, both requiring rapid colonization of a nitrogen-limited substrate. Among substrate specialists with narrow niches, we also found hints suggesting possible evolutionary pathways facilitating reversal to generalism, highlighting how evolution of decay machineries may move along different axes of niche space.

Never shall those born to crawl, learn to fly: Evolutionary compromises between spore hydrophobicity and fitness in *Trichoderma*

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Ecological genetics studies genes that are related to survival and reproduction, i.e., the fitness of organisms. In fungi, such investigations are frequently impeded by scarce fossils, irregular life cycle, immortality, and frequent asexual reproduction. However, the unique fungal traits can shed light on the evolutionary forces that shape their environmental adaptions. Filamentous fungi that disperse through aerial spores produce unique amphiphilic and surface-active proteins, hydrophobins (HFBs), which coat spores and mediate a multitude of environmental interactions. We exploited a library of hydrophobin-deficient mutants for two cryptic *Trichoderma* species and measured eight parameters of fitness that address fungal development, reproductive potential and stress resistance. HFB4 and HFB10 were found to be crucial for *Trichoderma* fitness because they hindered the adverse dispersal of spores and controlled at least seven other fitness traits. The *in-silico* analysis revealed stabilizing natural selection for all cases, except for HFB4 from *T. harzianum*, which evolved under strong directional selection. *T. harzianum* was also relatively less fit compared to *T. guizhouense* and interestingly, the deletion of the *hfb4* gene increased its fitness. Conversely, the deletion of *hfb4* in *T. guizhouense* led to the characteristic phenotype and relatively low fitness, similar to *T. harzianum*. Because natural selection operated on *hfb4* traits differently, the net contribution of this gene to fitness was found to result from the evolutionary tradeoff between different fitness-related properties. The analysis of hydrophobin-dependent fitness traits revealed an evolutionary snapshot of the otherwise obscured speciation and disruptive natural selection in fungi. We show that the manipulation of *hfb*s can virtually switch between the two *Trichoderma* species that diverged at least five million years ago.

Concurrent Session 3.2
MOLECULAR TAXONOMY & PHILOGENOMICS

TUESDAY, FEBRUARY 18

Location: **Frentani Convention Center** | Room: **Accademia**
CHAIR: ***Quirico Migheli*** & ***Michael Thon***

16:15 - 16:30 **Diversity of the *Botryosphaeriaceae* family in Guinea-Bissau (West Africa): the beginning of a tale in cashew**
Inês Diniz | Faculdade de Ciências, Universidade de Lisboa

16:30 - 16:45 **Population structure, genetic diversity and evidence for recombination in populations of the maize anthracnose fungus, *Colletotrichum graminicola***
Michael Thon | University of Salamanca Instituto Hispano-Luso de Investigaciones Agrarias

16:45 - 17:00 **Elucidating species boundaries between agents of superficial mycoses *Trichophyton interdigitale* and *T. mentagrophytes***
Michaela Švarcová | Czech Academy of Sciences Instute of Microbiology

17:00 - 17:15 **Genetic diversity and mycotoxin production among *Fusarium* head blight isolates belonging to the *Fusarium tricinctum* species complex from Italy**
Maria Teresa Senatore | Alma Mater Studiorum-University of Bologna Department of Agricultural and Food Sciences

17:15 - 17:30 **Mitochondrial genomes as phylogenetic backbone**
Balázs Brankovics | Wageningen University & Research BU Biointeractions & Plant Health

17:30 - 17:45 **Variation in secondary metabolite production potential in the *Fusarium incarnatum-equiseti* species complex revealed by comparative analysis of 13 genomes**
Alessandra Villani | Institute of Sciences of Food Production, National Research Council

17:45 - 18:00 **The endophytic mycobiome of spring and winter wheat (*Triticum aestivum*) forms cultivated in ecological, conventional and control conditions**
Sylwia Salamon | Institute of Plant Genetics, Polish Academy of Sciences; Department of Pathogen Genetics and Plant Resistance

Diversity of the *Botryosphaeriaceae* family in Guinea-Bissau (West Africa): the beginning of a tale in cashew

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Cashew (*Anacardium occidentale* L.) production is a major commodity in several tropical countries, mainly in the West Africa region, which accounts for close to 45% of world cashew production. In Guinea-Bissau, cashew is the main source of economic revenue for both government and household levels. Despite its value as a cash crop, cashew orchards are extensively planted with little agronomic management, thus posing a threat for the emergence of fungal diseases. Gummosis and dieback of *Anacardiaceae* plants have long been associated with infection by a complex of cryptic species of *Lasiodiplodia* and other genera of the *Botryosphaeriaceae*, as *Neofusicoccum* spp and more recently *Cophinforma* spp. An exhaustive field disease survey was carried out in several Guinea-Bissau regions and a total of 46 fungal isolates were sampled from cashew infected tissues (i.e. trunk, bark, leaf and apple). To uncover the diversity of *Botryosphaeriaceae* spp. sampled, a phylogenetic analysis by a three-amplicon approach (ITS, TEF1-alpha, β-tubulin) was performed. Preliminary results reveal the detection of three relevant genera, namely *Lasiodiplodia* sp. (n=32, 70%), *Neofusiccocum* sp. (n=12, 26%) and *Cophinforma* sp. (n=2, 4%). Among all taxa, *Lasiodiplodia* spp. was the most widespread across the country. In our study, *Neofusiccocum batangarum* is the most likely present species from the genus in Guinea-Bissau, while for *Lasiodiplodia* at least three species are confirmed: *L. theobromae*, *L. pseudotheobromae* and *L. caatinguensis*. Further analyses are ongoing to robustly assist species identification particularly in *Lasiodiplodia*. Also, the presence of *Cophinforma* spp. as a casual agent of dieback was found, only previously reported for cashew in Brazil. This work represents the first attempt to unveil the diversity of the *Botryosphaeriaceae* taxa associated to the diseases affecting cashew in Guinea-Bissau which is an essential milestone for sustainable production.

Funded by PTDC/AGR-PRO/5727/2014

Population structure, genetic diversity and evidence for recombination in populations of the maize *anthracnose fungus, Colletotrichum graminicola*

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Colletotrichum graminicola, causal agent of maize anthracnose, is an important maize disease worldwide. *Colletotrichum* is traditionally recognized as an asexual genus. Only the asexual state has been observed in the field and it is thought to reproduce clonally, although the sexual cycle has been reported in laboratory conditions. A better understanding the genetic structure and gene flow among populations is vital to the development of effective maize anthracnose control strategies. We sequenced 95 strains of *C. graminicola* using Restriction site associated DNA markers (RAD-Seq) and an additional 20 isolates using whole genome resequencing. The isolates were obtained from infected plants found in maize fields in Europe, Canada, USA, Brazil and Argentina with additional isolates coming from international culture collections. Genetic clustering, principal components analysis, phylogenetic analysis and a minimum spanning network all support the existence of at least three main populations associated with the continents of North America, South America and Europe. Our results also indicate a European origin of the isolates from Argentina. We found that there is little clonality among the isolates and of the few clones that were identified, most occurred in European fields. The phi-test implemented in the program splitstree found statistically significant evidence for recombination in each population. Additionally, a linkage decay plot shows that loci that are physically close to one another are often unlinked, as expected if recombination is frequent. These results support the hypothesis that recombination is common in *C. graminicola*, much more so than previously thought.

Elucidating species boundaries between agents of superficial mycoses *Trichophyton interdigitale* and *T. mentagrophytes*

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Dermatophytes are fungi which cause mycotic infections of the skin and its derivates. *Trichophyton interdigitale* is one of the most common agents of tinea pedis and tinea unguium in humans. On the other hand, closely related species *Trichophyton mentagrophytes* is predominantly the agent of superficial skin infections in rabbits, cats and dogs, but it can be also easily transmitted to humans and cause skin lesions (tinea corporis). Due to the different source of infection, the differentiation of these species is clinically and epidemiologically significant. Apart from different ecology, these species should be distinguishable based on characteristic phenotypic features, including macromorphology of colonies, presence/absence of macroconidia and spiral hyphae, etc. However, several recent studies indicated that the correlation between the clinical picture of infection, and phenotype and genotype of the pathogen are not so clear as expected. Consequently, the species boundaries between them are rather unclear and need to be re-evaluated using molecular and phenotypic methods.

The aim of this study is to find species limits between *T. interdigitale* and *T. mentagrophytes*. The alternative hypothesis is that these two species represent in fact only one species with a broad host range and variable phenotype.

A total of 120 isolates identified as *T. interdigitale / T. mentagrophytes* are obtained from Czech patients with various clinical manifestations (tinea pedis, corporis and unguium). The analysis of micro- and macromorphology, and physiology is performed together with molecular characterization of the strains by DNA sequences from three loci: ITS rDNA, β-tubulin and translation elongation factor 1-α (TEF). Two species delimitation methods are compared: genealogical concordance phylogenetic species recognition (GCPSR) and methods based on the multispecies coalescent model. Correlation between phylogeny, clinical manifestation and phenotypic data is performed.

Genetic diversity and mycotoxin production among *Fusarium* head blight isolates belonging to the *Fusarium tricinctum* species complex from Italy

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Fusarium head blight (FHB) is a worldwide cereal disease caused by a complex of *Fusarium* species resulting in high yield losses, reduction in quality and mycotoxin contamination of grain. In Europe, the principal species responsible for FHB are *F. graminearum, F. culmorum* and *F. poae.* However, members of the *F. tricinctum* species complex (FTSC) have become increasingly important contributors to FHB, likely due to changes in climatic conditions. In addition, members of the FTSC can produce mycotoxins such as moniliformin (MON), enniatins (ENNS) and beauvericin (BEA) that could compromise food safety and animal health. In order to understand genetic diversity among the FTSC and estimate the mycotoxin risk related to these species, we collected FTSC isolates from grain samples harvested in Italy. We analyzed a multilocus DNA sequence dataset (TEF1α, RPB2 and RPB1) to evaluate species diversity and phylogenetic relationships. In addition, we investigated the *in vitro* production of mycotoxins in relation to species limits within the FTSC. A total of 123 isolates were characterized via multilocus sequencing. Phylogenetic analyses and comparisons to reference isolates deposited in FUSARIUM MLST indicated that *F. avenaceum* was the most common species (46% of the isolates). However, 14% of the strains were identified as *F. acuminatum,* 11% of isolates were identified as *F. tricinctum*, and a single isolate was identified as *F. flocciferum*. In addition, two isolates were identified as FTSC 1 and FTSC 11, two undescribed and informally named species. Interestingly, 28% of isolates formed a distinct clade in the phylogenetic analysis and may represent a new species. These isolates were identified morphologically as *F. tricinctum* but were not part of a monophyletic cluster of *F. tricinctum* in the phylogenetic analysis. Mycotoxin production is being assessed in relation to the observed genetic diversity.

Mitochondrial genomes as phylogenetic backbone

Balázs Brankovics[1], **Meixin Yang**[2], **Hao Zhang**[2], **Cees Waalwijk**[1], **Theo A.J. Van Der Lee**[1], **Anne D. Van Diepeningen**[1]

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Mitochondrial genomes have several favourable characteristics for (phylo)genetics: They are present in high copy number, have a fast evolution, and have simple organizations with: few genes (16 conserved genes in most fungi) and a mostly circular morphology.

Homologous regions are easy to identify. In addition, they are neutral in the niche adaptation of the fungus, therefore their evolution is free from genetic sweeps. The combination of next generation sequencing and new assembly software tools make the efficient assembly of complete mitochondrial genomes possible. The goal of the presented work was to understand the evolutionary history of *Fusarium asiaticum* populations in Southern China, where a shift in toxin chemotype was observed during the past decades by contrasting the mitochondrial genome diversity with that of the nuclear regions.

Compared to the mitochondrial genome of *F. graminearum*, the mitogenome of *F. asisticum* shows less variation and the intronless mitogenome sequences were extremely conserved. Only strains from Sichuan showed a relatively high diversity, and are apparently recombining. These results indicate that Sichuan could be the source of the diversity sampled in the current study. The analysis of the nuclear regions and the distribution of the chemotypes fit this hypothesis. The analyses further indicate that the current population and its distribution is the result of two radiation events: one leaving Sichuan and spreading along the Yangtze river and then after a shift in the chemotype a second radiation event.

In conclusion, mitochondrial genomes can be efficiently assembled from next generation sequencing reads. Complete mitochondrial genomes offer a strong basis for phylogenetic studies.

Variation in secondary metabolite production potential in *Fusarium incarnatum-equiseti* species complex revealed by comparative analysis of 13 genomes

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Fusarium incarnatum-equiseti species complex (FIESC) comprises 33 phylogenetically distinct species recovered from diverse biological sources, but mostly agricultural plants and soils. Collectively, members of FIESC can produce diverse mycotoxins. However, since such species diversity in FIESC has been recognized recently, each species ability

of causing mycotoxin contamination of crop plants is unclear. We used comparative genomics to investigate the distribution of and variation in genes and gene clusters responsible for the synthesis of mycotoxins and other secondary metabolites (SMs) in FIESC.

We examined genomes of 13 members of FIESC, selected based primarily on their phylogenetic diversity and/or occurrence on crops. Presence and absence of SM biosynthetic gene clusters varied markedly among the genomes. For example, trichothecene mycotoxin as well as the carotenoid and fusarubin pigment clusters were present in all genomes examined, whereas enniatin, fusarin, and zearalenone mycotoxin clusters were present in only some genomes. Some clusters exhibited discontinuous patterns of distribution in that their presence and absence was not correlated with the phylogenetic relationships of species. We also found evidence that cluster loss and horizontal gene transfer have contributed to such distribution patterns. For example, a combination of multiple phylogenetic analyses suggest that five NRPS and seven PKS genes were introduced into FIESC from other *Fusarium lineages*. Our results suggest that although the portion of the genome devoted to SM biosynthesis has remained similar during the evolutionary diversification of FIESC, the ability to produce SMs could be affected by the different distribution of related functional and complete gene clusters.

The endophytic mycobiome of spring and winter wheat *(Triticum aestivum)* forms cultivated in ecological, conventional and control conditions

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Wheat remains to be a crucial food source for humans, in direct as well as indirect way as nutrition for livestock animals. Intensification of wheat production is essential for growing human population, despite of climate changes and conditions of the agricultural environment. Knowledge of the fungal endophytes and understanding their complex interactions with host can lead to adopting symbiotic microorganisms to improve wheat plant resistance for biotic and abiotic stresses.

The aim of our study was to isolate and molecular characterize the fungal communities inhabiting endosphere of different wheat organs (leaves, stems, kernels, roots) and to compare the obtained mycobiome structures of winter and spring wheat forms grew in different conditions: conventional and ecological in field and control in greenhouse. Molecular identification of the obtained isolates was performed based on DNA sequences

of regions: ITS1-5.8-ITS2 (internal transcribed spacer), SSU (small subunit), LSU (large subunit) and fragments of genes: *tub2* (*β-tubullin*), *tef1* (*translation elongation factor EF-1 alpha*), *RBP1* (*largest RNA polymerase subunit*), *act* (*actin*) and *CMD* (*calmodulin*).

The *Sarocladium sp.* and *Penicillium sp.* were occurrence more frequently in analysed groups. Observed strains were classified to following fungal types: *Antrahacocystis sp.*, *Phoma sp.*, *Dactylonectria sp.*, *Trichoderma sp.*, *Alternaria sp.*, *Fusarium sp.*, *Michrodochium sp.*, *Cladosporium sp.*, *Chrysosporium sp.*, *Acremonium sp.*, *Periconia sp.*, *Setoshaeria sp.*, *Epicoccum sp.* and others. The various differences in endosphere mycobiome structures between ecological, conventional and control cultivars were observed.

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Concurrent Session 3.3
APPLIED AND ENVIRONMENTAL MICROBIOLOGY

WEDNESDAY, FEBRUARY 19

Location: **Frentani Convention Center** | Room: **Latini**
CHAIR: ***Anna Maria Persiani*** & ***Susanne Zeilinger***

16:15 - 16:30 **Targeted induction of a silent fungal gene cluster encoding the bacteria-specific germination inhibitor fumigermin**
Maria C. Stroe | Leibniz Institute for Natural Product Research and Infection Biology Hans - Knöll Institute (HKI)

16:30 - 16:45 **The antifungal protein PAFB from *Penicillium chrysogenum* is highly expressed under nutrient excess conditions**
Anna Huber | Medical University of Innsbruck, Institute of Molecular Biology

16:45 - 17:00 **Sustainable carbon solutions by the aid of wood decay fungi: lignocellulose waste bioconversions opened with comparative genomics and ecophysiology**
Taina Lundell | University of Helsinki Department of Microbiology Helsinki

17:00 - 17:15 **LysM effectors regulate fungal development and required for hyphal protection and biocontrol traits in *Clonostachys rosea***
Mukesh Dubey | Swedish University of Agricultural Sciences, Department of Forest Mycology and Plant Pathology

17:15 - 17:30 **A metagenomics study involving two corn microbiomes following infection with aflatoxigenic and/or non-aflatoxigenic *Aspergillus flavus* strains**
Geromy Moore | Agricultural Research Service United States Department of Agriculture

17:30 - 17:45 **Exploring the cell structure and metabolism of *Yarrowia lipolytica***
Scott E. Baker | USA, Pacific Northwest National Laboratory

17:45 - 18:00 **Combined meta-omics reveal links among fungal community composition, gene expression, and chemical changes in decomposing leaf litter**
Marco Alexandre Guerreiro | Ruhr-University Bochum AG Geobotany

Targeted induction of a silent fungal gene cluster encoding the bacteria-specific germination inhibitor fumigermin

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Microorganisms produce a multitude of low molecular weight secondary metabolites (SMs) with various biological activities. Many of their encoding gene clusters are silent under standard laboratory conditions because for their activation they need the ecological context, such as the presence of other microorganisms. The true ecological function of most SMs still remains obscure. The understanding of both the activation of silent gene clusters and the ecological function of the produced compounds is of importance to reveal functional interactions in microbiomes. Therefore, we set out to identify a silent gene cluster activated by co-cultivation and to assign an ecological function to the produced compound. We discovered an as-yet uncharacterized silent polyketide gene cluster of the fungus *Aspergillus fumigatus* activated by the bacterium *Streptomyces rapamycinicus*. The product of the gene cluster is the novel fungal metabolite fumigermin, which is solely synthesized by the FgnA polyketide synthase and does not require other cluster genes. Fumigermin inhibits the germination of spores of the inducing bacterium *S. rapamycinicus* and thus helps the fungus to defend resources of the shared habitat against a bacterial competitor. Similar compounds are produced by bacteria via a different biosynthetic route, suggesting divergent evolution of the biosynthesis of α-pyrone-based germination inhibitors.

The antifungal protein PAFB from *Penicillium chrysogenum* is highly expressed under nutrient excess conditions

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The penicillin-producer *Penicillium chrysogenum* is the source of at least two small, cysteine-rich and cationic antimicrobial proteins (AMPs), PAF and PAFB, which represent promising candidates for the development of novel antifungal strategies [1]. Although studies have been pushed in the past to unravel the mode of action of these AMPs, little information exists on the expression regulation of the respective encoding genes. PAF is efficiently secreted into the culture supernatant during the stationary growth phase of *P.*

chrysogenum in minimal medium [2], which suggests PAF to be a secondary metabolite product [3]. The gene *pafB*, in contrast, is only mildly expressed under these conditions and PAFB remains under the detection level in the culture broth [1]. By searching for conditions that favor *pafB* expression, we found high amounts of PAFB in the culture broth during logarithmic growth phase of *P. chrysogenum* when cultivated under nutrient excess. The divergent expression regulation of PAF and PAFB suggest different functions for the producing host.

The possibility of *pafB* induction by nutrient excess allowed us to propose the *pafB*-promoter for efficient production of recombinant AMPs when applied in our recently established *P. chrysogenum* expression system [2].

To provide a proof-of-principle, we compared the efficiency of the *pafB*-promoter to express PAFB in high amounts with that of two already well established promoter systems derived from *P. chrysogenum*: the *paf*-promoter [2] and the xylose-inducible *xylP*-promoter [4]. The *pafB*-promoter was similarly efficient as the *paf*-promoter, but superior to the *xylP*-promoter. Thus, the *pafB*-promoter is a promising tool for recombinant protein production in *P. chrysogenum* as it guarantees strong gene expression with the advantage of inducibility.

1. Huber A. *et al*. 2018, Sci Rep,

2. Sonderegger *et al*. 2016, Microb Cell Fact

3. Meyer V. & Jung S 2018, Microorganisms

4. Zadra *et al*. 2000, Appl Environ Microb

Sustainable carbon solutions by the aid of wood decay fungi: lignocellulose waste bioconversions opened with comparative genomics and ecophysiology

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Our studies on *Basidiomycota* wood decay fungal omics and physiology aim at adding up functional knowledge to the rapidly accumulating genomic data, and in design of sustainable applications for production of biofuels, energy and added value bioproducts

by fungi. The filamentous fungi of class *Agaricomycetes*, especially in the order *Polyporales*, have the ability to inhabit dead wood and decompose all lignocellulose biopolymer components. White rot fungi secrete an array of carbohydrate-active enzymes (CAZymes) decomposing wood polysaccharides, and oxidoreductases to modify lignin, while brown rot fungi produce a strong oxidative attack by Fenton chemistry mainly destructive against wood cellulose. Comparative genomics on seven phlebioid species demonstrated a conserved core white rot secreted proteome with multiple CAZy GH classes and specific AA oxidoreductases (1). This supports our transcriptome and proteome studies with *Phlebia radiata* on the two-phase temporal processes of (i) oxidative decomposition with promoted expression of AA2 lignin-modifying peroxidases and individual AA9 LPMOs, followed by the (ii) second, enduring phase signified by enzymes against cellulose, hemicellulose and pectin (1, 2). We have successfully adopted the fungus for second-generation bioethanol production from wood-based and agricultural wastes (3), and opened fungal metabolism under anaerobic conditions. Recently, we observed enzymatic reactions and release of signature volatile organic compounds correlating with the dominating decay trait, either white or brown rot, in interactive species co-cultivated on spruce wood (4). In this context, novel metabolic properties and applications of wood decay fungi will be introduced.

1. Mäkinen M, *et al*. 2019 BMC Genomics 20:430

2. Kuuskeri J, *et al*. 2016 Biotechnol Biofuels 9:192

3. Mattila H, *et al*. 2017 Bioresour Technol 225:254-261

4. Mali T, *et al*. 2019 FEMS Microbiol Ecol 95:fiz135

LysM effectors regulate fungal development and are required for hyphal protection and biocontrol traits in *Clonostachys rosea*

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Lysin motif (LysM) modules are approximately 50 amino acids long and bind to peptidoglycan, chitin and its derivatives. Certain LysM proteins are reported as virulence factors in plant pathogenic and entomopathogenic fungi. However, their role in fungal-fungal interactions is not fully known. In this study, we investigated the biological function of LysM proteins in the mycoparasitic fungus *Clonostachys rosea*. The *C. rosea* genome contained three genes (*lysm1*, *lysm2* and *chic2*) coding for LysM-containing

proteins. Gene expression analysis revealed that *lysm1* and *lysm2* were induced during mycoparasitic interaction with *Fusarium graminearum* and during colonization of wheat roots. *Lysm1* and *lysm2* were suppressed in germinating conidia, while *lysm2* was induced during growth in chitin or peptidoglycan-containing medium. Deletion of *lysm1* and *lysm2* resulted in mutants with increased levels of conidiation and conidial germination, but reduced ability to control plant diseases caused by *F. graminearum* and *Botrytis cinerea*. The Δ*lysm2* strain showed a distinct, accelerated mycelial disintegration phenotype accompanied by reduced biomass production, suggesting a role of LYSM2 in hyphal protection against endogenously produced cell wall degrading enzymes. Furthermore, the Δ*lysm2* and Δ*lysm1*Δ*lysm2* strains displayed reduced ability to colonize wheat roots, while only double deletion strains Δ*lysm1*Δ*lysm2* failed to supress expression of the wheat defence response genes. Based on differences in gene expression, predicted modular structure and the severity of the phenotypes, we propose a role of LYSM1 as a regulator of fungal development and of LYSM2 in cell wall protection against endogenous hydrolytic enzymes, while both are required to supress plant defence responses. Our findings expand the understanding of the role of LysM proteins in fungal-fungal interactions and biocontrol.

A metagenomics study involving two corn microbiomes following infection with aflatoxigenic and/or non-aflatoxigenic *Aspergillus flavus* strains

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In light of the increasing use of non-aflatoxigenic *A. flavus* strains as pre-harvest biocontrol, a metagenomics study is underway to ascertain the effect of introducing a biocontrol agent on the inherent silk and kernel microbiomes of two corn varieties (B73 = susceptible and CML322 = resistant). Concomitant with the introduction of the biocontrol agent (K49), the impact of exposure of those same microbiomes to a highly toxigenic *A. flavus* strain (TOX4) will be investigated. To more easily distinguish between the introduced *A. flavus* strains, K49 has been tagged with green fluorescent protein (GFP), and TOX4 with a red fluorescent protein (RFP, mCherry). There were four treatments for each corn variety as follows: Control (no spores), K49-GFP alone, TOX4-RFP alone, and mixture of K49-GFP+TOX4-RFP. Epibiota and endobiota were collected from corn silks and kernels throughout a growing season and prepared for metagenomics sequencing. Any changes in microbial community structure (bacterial and fungal) in response to

the introduced *A. flavus* strains will be compared to the inherent microbiomes of ears without spore treatments and documented for evaluation. This analysis may uncover any organism(s) that either help or inhibit the biocontrol strain as it inhibits aflatoxin contamination by the toxigenic strain. Progress of the research study and ongoing metagenomics data will be presented.

Exploring the cell structure and metabolism of *Yarrowia lipolytica*

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Yarrowia lipolytica is an oleaginous yeast that is being developed as a production organism for biofuels and bioproducts. *Yarrowia* produces significant amounts of lipids, including triacylglycerols when nitrogen is a limiting nutrient. We have previously characterized system level metabolic and regulatory changes that occur upon nitrogen limitation using systems and cell biological approaches. Production of biofuels and bioproducts is highly dependent upon monitoring and fostering cellular conditions for their production. Co-factor production, balance and renewal is a key support for oxidation-reduction reactions, such as NAD+/NADH supply during lipid production. We utilized a multi-omics approach to monitor changes to central carbon metabolism enzymes and metabolites. We compare mutant strains for three different NAD-production enzymes grown on media containing with varied carbon sources. Examination of these conditions and mutants provides metabolic and regulatory information that will inform metabolic engineering and biodesign applications in *Y. lipolytica*. Our studies also include the development of a strain library expressing fluorescently tagged proteins that we use to generate a "cell organelle atlas" in *Y. lipolytica*.

Combined meta-omics reveal links among fungal community composition, gene expression, and chemical changes in decomposing leaf litter

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Decomposition of plant litter is essential for nutrient cycling and a key process for ecosystem functioning. Fungi are considered to be the main decomposers of leaf litter in forest ecosystems. They synthesize and secrete enzymes that change the chemical composition of the litter, and thus represent a major effect of the fungal community. However, fungal community composition and their metabolic activity have been rarely analyzed together, and so far never in combination with litter chemistry.

We characterized the chemical composition of autumn leaves of European beech (*Fagus sylvatica*) and the corresponding leaf litter after one year of decomposition by proton nuclear magnetic resonance (1H-NMR) spectroscopy. The composition and transcriptional activity of fungal communities was assessed by high-throughput Illumina sequencing in the same litter samples. These analyses were highly replicated across 14 different forest plots and cover three distant regions in Germany.

We were able to successfully distinguish freshly fallen leaves from one-year-old litter with respect to their chemical composition. Leaves were chemically more distinct among regions than one-year-old litter. Fungal communities were locally structured, however, functionally redundant among regions, i.e. expressing genes coding for similar litter-degrading enzymes. We identified changes in the abundance of putative chemical compounds between freshly fallen autumn leaves and one-year-old that correlated to the transcription level of litter-degrading enzymes. Transcription patterns were also correlated with the abundance of certain fungal species. Overall, we provide strong evidence of a dynamic interaction between substrate chemistry, expression of enzyme coding genes, and fungal community structure in nature.

Concurrent Session 3.4
SYNTHETIC BIOLOGY AND BIOTECHNOLOGY

WEDNESDAY, FEBRUARY 19

Location: **Frentani Convention Center** | Room: **Latini**
CHAIR: ***Cristina Mazzoni*** & ***Saloheimo Markku***

14:00 - 14:15 **Biology by Design**
Yangxiaolu Cao | Gingko Bioworks Design

14:15 - 14:30 **Quantum Mechanics Modeling of Aflatoxin degradation by *Trametes versicolor*'s Laccase**
Marco Zaccaria | Boston College Biology

14:30 - 14:45 **Tailored engineering of fungal nonribosomal peptide synthetases to obtain artificial cyclodepsipeptides**
Charlotte Steiniger | Technische Universität Berlin, Chair of Applied and Molecular Microbiology

14:45 - 15:00 **On the three-dimensional morphology and substrate-diffusion in filamentous fungal pellets**
Stefan Schmideder | Technical University of Munich Chair of Process Systems Engineering

15:00 - 15:15 **Development of a high throughput Enzymes (LDEs).**
Laure Leynoud-Keiffer | BioEnergy Institute, Deconstruction Department, Fungal Biotechnology Division

15:15 - 15:30 **Functional and biological importance of lytic polysaccharide monooxygenases (LPMO) and cellobiose dehydrogenase (CDh) in Aspergillus nidulans**
César Rafael Fanchini Terrasan | University of Campinas

15:30 - 15:45 **Development of the filamentous fungus *Myceliophthora thermophila* C1 into a next-generation therapeutic protein production system**
Anne Huuskonen | Academy of Sciences

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Biology by Design

Yangxiaolu Cao

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Ginkgo's mission is to make biology easier to engineer. Ginkgo makes use of its highly-automated foundry for designing and building new organisms. Today, our foundry is developing over 40 different organisms to deliver technologically advanced solutions across multiple industries. The Design team is made up of synthetic biologists, systems biologists, protein engineers, and computational biologists building the infrastructure for designing DNA, megabases at a time. The presentation will focus on how the Ginkgo Design team contributes to the design of high-throughput experiments that leverage Ginkgo's Foundry, with the aims of solving difficult biological problems and uncovering new biological design principles historically, and in filamentous fungi in the recent years. You can learn how Ginkgo is developing the next-generation synthetic biology tools for bacteria and filamentous fungi—e.g. algorithms, data structures, predictive models, software pipelines, and experimental approaches.

Quantum Mechanics Modeling of Aflatoxin degradation by *Trametes versicolor*'s Laccase

Marco Zaccaria[1], William Dawson[2], Massimo Reverberi[3], Marek Domin[4], Takahito Nakajima[2], Luigi Genovese[5]

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Bioremediation is the process of exploiting natural organisms to address issues of environmental concern. The case-study we present pertains the degradation of food-infesting fungal metabolites of the genus *Aspergillus*, Aflatoxins: the most carcinogenic among natural compounds. To this end, we perform a DFT-based (Density Functional Theory) full Quantum Mechanics Modeling of Aflatoxin B1 (AFB1) and Aflatoxin G2 (AFG2) degradation by the ligninolytic enzyme laccase of *Trametes versicolor*.

An enzymatic assay of laccase efficacy as an aflatoxin degrader is initially used as a reference to highlight reaction bottlenecks through an empirical mathematical reaction model. A full quantum mechanics model, based on a Density Functional Theory approach,

is then performed on the substrate molecules.

The QMM toxin model indicates that: a) degradation is limited by enzyme affinity for the substrate; b) degradation requires environmental stimulation to be completed; c) Laccase shows intrinsic limitations as an aflatoxin degrader, a full QM modelization is required to enable in-depth optimization.

We finally present a series of steps that allow the design of a full-QM enzymatic model of laccase to inform alterations that could be implemented to better approximate the desired enzymatic outcome on aflatoxins. To our knowledge, this work is the first ever full quantum mechanics-based modeling of enzymatic activity as a whole. We move the first steps in the context of mechanistic description to provide theoretical inputs to transition from an empirical (and semi-rational) approach to enzyme engineering to a rational approach *stricto sensu*.

Tailored engineering of fungal nonribosomal peptide synthetases to obtain artificial cyclodepsipeptides

Charlotte Steiniger[1,2], Sylvester Hoffmann[1], Andi Mainz[1], Marcel Kaiser[3], Kerstin Voigt[4], Vera Meyer[2] and Roderich D. Süssmuth[1*]

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[2] Berlin Institute of Technology, Dept. Applied and Molecular Microbiology, Berlin, Germany
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Nonribosomal peptide synthetases (NRPSs) represent promising engineering platforms for the design of structurally complex peptides of pharmacological importance. While previous studies focused on bacterial synthetases, fungal systems assembling drugs like the antibacterial cephalosporins, antifungal echinocandins or immunosuppressive cyclosporine still await in-depth exploitation. In the forefront of our studies, an effective engineering of fungal iterative cyclodepsipeptide (CDP) synthetases in particular has been severely hampered, as various mechanistic features of CDP biosynthesis were only poorly understood.

By combining protein truncation, *in trans* expression and combinatorial biosynthesis, we assigned important functional segments of fungal CDP synthetases. The gained mechanistic knowledge allowed for the design of a variety of *in vivo* functional artificial assembly lines comprising parts from up to three different NRPSs. By active CDP ring size control and the application of the Tet-on expression system in *Aspergillus niger* as a heterologous host, we obtained the new-to-nature CDPs octa-enniatin and octa-

beauvericin, as well as high titers of hexa-bassianolide (g/L scale), which showed superior antiparasitic activity accompanied by cytotoxicity.

As an alternative NRPS engineering approach, we fused up to three CDP synthetases by harnessing their unique terminal C domain as canonical C domain. This induced a switch from an exclusively iterative to a mixed iterative/linear assembly mode, leading to various hybrid CDPs with altered symmetry. Based on a systematic comparison of three fungal NRPS exchange units (C-A-Mt-T, C_{CTD}-A-Mt-T, A-Mt-T), we also demonstrate that fungal NRPSs of different assembly types can be combined using different swapping sites, while respecting the C domain integrity and specificity. Overall, our findings give distinct clues for efficient reprogramming of iterative and linear fungal NRPSs in future protein engineering approaches.

On the three-dimensional morphology and substrate-diffusion in filamentous fungal pellets

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Filamentous fungi are widely used cell factories for the production of biotechnological relevant compounds. When growing in pellet form, their productivity is strongly influenced by morphological features like the distribution and quantity of hyphae, tips, and branches. Depending on the product of interest, saturation or limitation of substrates inside fungal pellets is pursued. To calculate the availability of substrates inside pellets, the so far unexplored three-dimensional (3D) morphology and the diffusive mass transport have to be known.

Based on X-ray microcomputed tomography (µCT) measurements of pellets and the 3D image data thereof, we developed the first method to quantify and locate hyphae, tips, and branches of whole fungal pellets [1]. In a second study, we computed the diffusive mass transport through the 3D hyphal network gained by µCT measurements and subsequent image analysis [2].

In a case study, we applied this newly developed techniques on two morphological different *Aspergillus niger* and one *Penicillium chrysogenum* strains. The investigation showed significant differences with regards to the inner pellet structure. In particular, the diffusivity of *A. niger* pellets was hindered by a dense pellet-shell. Additionally, the number of tips inside pellets, a measure for the production capability of enzymes, differed significantly between the strains.

In future, these new tools can be used to predict the optimal fungal production strain for each desired product from either an existing mutant library, examined in µCT, or even from a set of simulated pellets, whose most favorable morphology can be reconstructed by genetic and process engineering. These possibilities make it a highly promising approach to increase the yield of numerous different biotechnological processes.

1. Schmideder *et al*., Biotechnol. Bioeng., 2019, doi: 10.1002/bit.26956

2. Schmideder *et al*., Biotechnol. Bioeng., 2019, doi: 10.1002/bit.27166

Development of a high throughput biotechnology platform for *Aspergillus niger* to secrete Lignin Degrading Enzymes (LDEs)

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The conversion of the biomass into advanced biofuels faces many challenges, one of which is finding the right organism for the job. The filamentous fungus *Aspergillus niger* has been chosen as a biocatalyst for cellulose, hemicellulose, and lignin degradation because it can secrete numerous hydrolytic enzymes, such as lignin degrading enzymes (LDEs), it is genetically tractable, and its genome sequence is available.

Here we are presentig a novel method to efficiently transform the genome of *Aspergillus niger* that overcomes several of the current challenges encountered when using the CRISPR/Cas9 approach (Laure Leynaud-Kieffer *et al*. 2019). Filamentous fungi, such as *Aspergillus niger*, are very important hosts for multiple scientific and industrial applications, but are notoriously difficult to optimize for a given application using current approaches used in genetic and metabolic engineering. To overcome these challenges and provide a more robust CRISPR/Cas9 toolbox, we have designed a genetic construct that is efficient and precise in terms of knockout efficiency and phenotype control and have demonstrated its utility using several different genetic motifs. This approach will enable the rapid screening and identification of genetic variants that are capable of efficiently producing any targeted compound through the precise manipulation and optimization of the targeted/desired metabolic pathway.

Using the new CRISPR/Cas9 toolbox, we are now developing a high throughput platform to transform *A. niger* using robotics. We are currently testing a library of ~200 different LDEs that could be potentially secreted by *A. niger.* We will then characterize each secreted enzyme by down scaling in micro culture *A. niger* to study their optimum

efficiency e.g. pH, temperature and ionic-liquid tolerance. Furthermore, we will then up scale the fermentation for each strain to obtain a maximum secreted LDEs yield.

Functional and biological importance of lytic polysaccharide monooxygenases (LPMOs) and cellobiose dehydrogenase (CDH) in *Aspergillus nidulans*

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Members from the family AA9 in the Carbohydrate-Active Enzymes Database (CAZy) are fungal copper-dependent lytic polysaccharide monooxygenases (LPMOs) capable of oxidising cellulose and other polysaccharides. A proteomic analysis identified six AA9 LPMOs being secreted by *A. nidulans* during growth with Avicel, sugarcane bagasse and straw. Functional studies of three LPMOs showed they are capable of oxidise a broad range of soluble substrates in addition to cellulose. When applied in enzymatic reactions with cellulases, they significantly contributed to the degradation of crystalline cellulose (Avicel).

Based on the previous analysis, two AA9 LPMOs and one cellobiose dehydrogenase (CDH) were selected as targets for biological importance studies. When cultivated on agar plates with different substrates, no differences in mycelia or conidiation were observed, however, the single KO strains showed faster growth in polygalacturonic acid, Avicel and CMC. After cultivation in liquid medium with Avicel, no differences in dry-weight or protein secretion were observed, but the secretomes of the LPMO KO mutants showed improved activity against some cellulosic substrates. Further MS analysis of the secretomes confirmed differential qualitative and quantitative secretion of enzymes acting on cellulose. Overall, the sum of normalized peptide counts from enzymes with cellulolytic activity were increased by 10 - 20% in the LPMO KO strains.

Development of the filamentous fungus *Myceliophthora thermophila* C1 into a next-generation therapeutic protein production system

Anne Huuskonen[1], Marika Vitikainen[1], Georg Schmidt[1], Marilyn Wiebe[1], Anssi Rantasalo[1], Veera Korja[1], Christopher Landowski[1], Ronen Tchelet[2], Gabor Keresztes[2], Mark Emalfarb[2] and Markku Saloheimo[1]

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Myceliophthora thermophila C1 is a well-known industrial enzyme production host able to reach enzyme titers of over 120 g/l in a 6-7-day process. Enzymes produced from the C1 technology have FDA GRAS status for food applications.

We have further developed the C1 Technology for low cost manufacturing of different types of therapeutic proteins. To this end, we have reduced C1's protease activity and humanized C1's glycans for expressing glycoproteins such as monoclonal antibodies.

We have characterized over fifty C1 proteases with transcriptomics, proteomics and biochemical methods. Deletion of the critical protease genes has led to low protease C1 strains that show up to 50-fold lower total protease activity than wild type C1. With these strains we have produced a number of biologics including full-length mAbs at final titers of up to 22 g/l, Fc-fusion proteins at 12 g/l and Fab fragments at 14.5 g/l. The antigen binding properties of two mAbs produced from C1 were very similar to CHO-produced control mAbs. We believe that there are many additional approaches to generate C1 strains with even greater productivity.

Data has been generated also for difficult-to-express proteins rVaccines, VLPs and bi-specific antibodies. We have managed to correctly express a secreted 60-mer VLP protein at 300 mg/l and another viral antigen at 1.7 g/l. The starting C1 glycan structure consists mostly of high mannose glycans reaching up to Man9 which is a better glycan structure for humanization than that of yeast. We have begun to modify C1's glycan pattern towards complex human glycoforms by deleting native genes and expressing various heterologous genes.

The data generated from our third party and internal research programs supports the belief that the C1 platform has the potential to be further developed into a safe and efficient expression system that may help speed up the development, lower production costs and improve the performance of biologic vaccines and drugs.

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POSTER SESSIONS & FLASH TALKS

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POSTER SESSION 1 & FLASH TALKS

Poster Session 1.1
DEVELOPMENT AND MORPHOGENESIS

TUESDAY, FEBRUARY 18

18:00 - 19:30 | Location: **Frentani Convention Center**

Transcriptional networks controlling asexual development in *Aspergillus nidulans*: An evolutionary perspective

Oier Etxebeste[1], Ainara Otamendi[1], Aitor Garzia[2], Eduardo A. Espeso[3], Marc S. Cortese[1]

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[3] *Department of Cellular and Molecular Biology, Centro de Investigaciones Biológicas (CSIC), Madrid, Spain*

Asexual spores are the predominant vector for the spread of fungi to new substrates. In the order of Eurotiales, within ascomycota, *Aspergillus nidulans* has been traditionally used as a model to study the genetic and molecular mechanisms controlling asexual development, known as conidiation. The C2H2-type TF BrlA is an essential regulator of the development of asexual multicellular structures or conidiophores. BrlA levels are controlled by signal transducers collectively known as UDAs (upstream developmental activators), while it regulates the morphological transformations leading to conidia production through the CDP central developmental pathway. Here we show that BrlA emerged in the fungal order of Eurotiales, late in evolution [1]. Nevertheless, the promoter of *brlA* and the corresponding gene product(s) have developed the ability to recruit and control both upstream and downstream TFs that are more widely conserved in fungi, having emerged sooner. Overall, our analyses suggests that the transcriptional networks controlling conidiophore development in *A. nidulans* were structured as a consequence of BrlA emergence and subsequent rewiring events.

1. Etxebeste, O.; Otamendi, A.; Garzia, A.; Espeso, E. A.; Cortese, M. S. Rewiring of transcriptional networks as a major event leading to the diversity of asexual multicellularity in fungi. Crit. Rev. Microbiol. 2019, 1–16, doi:10.1080/1040841X.2019.1630359.

Sexual hormones from *Pyrenopeziza brassicae* (light leaf spot) for disease control

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Fungal pathogens are responsible for a substantial proportion of crop yield losses and are therefore a major threat to global food security. One such example is *Pyrenopeziza brassicae*, a pathogen of winter oilseed rape (*Brassica napus*) and other *Brassica* plant species, which causes up to £160 million worth of crop loss per annum in the UK alone. Recent evolution of fungicide resistance in this pathogen has rendered current fungicide treatments ineffective, which exemplifies the need for new methods of disease control to be developed. There is preliminary evidence for the production of a hormonal compound by sexual cultures of *P. brassicae* which has the ability to repress asexual sporulation in this fungus. Asexual sporulation is important for the spread of disease throughout Winter, Spring and Summer and so this hormonal compound could be utilised as a novel disease control agent. Mating (*MAT*) types of *P. brassicae* isolates were established and sexual crosses were set up *in vitro*. Cultures were then harvested and any potential hormones extracted via a solvent extraction procedure. Finally, extracts were assayed for their effects on asexual sporulation. Extracts from mated cultures were shown to repress asexual sporulation by over 1000-fold compared to unamended control conditions *in vitro*. Extracts from single isolate cultures did not repress asexual sporulation. Current work is focussed on isolating and identifying the active hormonal compound(s) within the sexual extract, using a combination of High Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometry (LCMS).

Cellular Control of Proteostasis During Infection-Related Development by the Rice Blast Fungus *Magnaporthe oryzae*

Audra Mae Rogers and **Martin Egan**

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Plant pathogenic fungi encounter a barrage of host-derived stressors during the establishment of disease, likely resulting in damage to their cellular proteomes. In eukaryotes, protein homeostasis or 'proteostasis' is achieved through both the coordinated activities of molecular chaperones and disaggregases which rescue misfolded and aggregated proteins and the autophagy and proteasome systems which breakdown irreparably damaged proteins. A growing body of evidence reveals that potentially cytotoxic misfolded proteins can also be triaged into specialized inclusion bodies in a conserved pathway called spatial protein quality control (SPQC). We are

using the rice blast fungus, *Magnaporthe oryzae*, as a model system to understand how stress-damaged proteins are spatially and temporally managed during infection-related morphogenesis and to investigate the consequences of perturbations to these quality control mechanisms. We show that, in the absence of normal molecular disaggregase activity, SPQC operates to sequester and retain stress-damaged proteins within inclusion bodies inside the conidium, thereby promoting the integrity of the differentiating appressorium. In addition, we explore the extent of functional interplay between SPQC and autophagy (aggrephagy) during stress adaptation and infection-related development by *M. oryzae*. We anticipate that improved mechanistic insights into SPQC will inform the development of new strategies to control rice blast disease.

Characterisation of *Fusarium graminearum* chitin synthases

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Fusarium graminearum (Fg) is a devastating, agricultural pathogen which causes significant losses to cereal crops worldwide because it negatively impacts grain yield as well as grain quality by production of carcinogenic mycotoxins. Chemical fungicides are currently used to control this pathogen, but resistance is now common globally which is limiting their sustainable use. Thus, there is an urgent need for new fungicides with different mechanisms of action. Chitin synthases (CHS) are excellent targets for new antifungal drugs, because chitin is essential to the integrity of the fungal cell wall and thus the survival and virulence of fungal pathogens. Furthermore, chitin not made by plants or mammals and consequently, this target is specific for fungi. No CHS inhibitors have been successfully developed for control of human or agricultural fungal pathogens. One reason for this, is the paucity of biochemical information on these enzymes because they are embedded in the plasma membrane and cannot be purified in the quantities needed for biochemical analysis. This is further complicated by the presence of several chitin synthase genes in each fungal species. The aim of this study is to identify the full complement of chitin synthases in Fg and to recombinantly express them in yeast for biochemical characterisation. Fg has at least eight potential CHSs; and using a combination of bioinformatics, functional complementation and microscopy, this study has identified two FgCHSs that are functional homologs of *Saccharomyces cerevisiae* chitin synthases. FgCHS3b and FgCHS4 complemented a chs double knockout in *S. cerevisiae*, were shown to be important in plant infection and were subsequently taken forward for biochemical characterisation. The FgCHS3b and FgCHS4 proteins have been recombinantly expressed as C-terminal GFP-tagged fusions; a challenging prerequisite for enabling purification and biochemical characterisation of these Fg CHS enzymes for the first time.

GUL1 interacts genetically with a subunit of the STRIPAK complex and controls hyphal morphology and development of the fungus *Sordaria macrospora*

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The striatin interacting phosphatase and kinase (STRIPAK) complex is a highly conserved protein complex. In humans, STRIPAK regulates cell division, differentiation and migration. In the model fungus *Sordaria macrospora*, the complex controls multicellular differentiation, hyphal fusion and vegetative growth. The core of STRIPAK consists of striatin, protein phosphatase PP2A, striatin interacting proteins 1 and 2, sarcolemmal membrane associated protein, phocein/Mob3 and two germinal centre kinases. Using phosphoproteom analysis of STRIPAK-deletion mutants, we found GUL1, an RNA-binding protein, among several putative dephosphorylation targets of STRIPAK. Analyzing the genetic interaction of *gul1* and STRIPAK via *pro45* revealed a negative genetic interaction, suggesting that both genes are part of different, functionally redundant pathways. Morphological characteristics of Δgul1 indicates a role of GUL1 in the establishment of cell wall integrity, vegetative growth and in sexual development. A localization study of GUL1 shows cytoplasmic puncta and a colocalization with the nucleus in vegetative hyphae and ascogonial coils. Kymographical analysis revealed that GUL1 shuttles directionally trough the cytoplasm. Since we were able to demonstrate a colocalization of GUL1 with early endosome marker protein Rab5, it is likely that GUL1 shuttling is an active transport by molecular motor proteins. Expanding knowledge of the connection between GUL1 and STRIPAK will provide detailed insight into the fungal development, which might be extrapolated to higher eukaryotes [2].

1. Kurischko C, Broach JR (2017) Phosphorylation and nuclear transit modulate the balance between normal function and terminal aggregation of the yeast RNA-binding protein Ssd1. Mol Biol Cell 28:3057-3069

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Identification of novel proteins for fungal cell-to-cell communication by localization screening from multicellularity-specific uncharacterized genes

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Multicellular organisms have the feature of intercellular molecular exchange for cooperation among the cells or tissues. Multicellular fungi possess a primitive morphological structure for cell-to-cell communication, the septal pore. A specifically evolved fungal organelle Woronin body plugs the septal pore upon wounding, thereby protecting the flanking cell. However, comparative genomic approach between multicellular and unicellular fungal species has not yet been employed to investigate additional components/mechanisms in the regulation of cell-to-cell communication. As Ascomycota, one of fungal divisions, possesses a number of genetically characterized species, we performed a genomic comparison between multicellular and unicellular ascomycetes and subsequent localization screening to find novel proteins regulating the cell-to-cell communication via septal pore.

In this study, we used *Aspergillus oryzae* due to having a unique experiment system of quantitatively analyzing the ability to protect the flanking cell upon hypotonic shock-induced hyphal wounding[1)]. Here, 776 genes were selected as multicellularity-specific uncharacterized by using BLAST-based genomic comparison between multicellular ascomycetes (*A. oryzae*, *Aspergillus fumigatus* and *Aspergillus nidulans*) and unicellular ascomycetes (*Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Candida albicans*) along with gene ontology category "no biological data available". Proteins encoded by the genes were expressed as EGFP fusion in *A. oryzae*, and 8% of the proteins tested were found to localize to the septum. Approximately 40% of deletion strains lacking the septum-localizing proteins exhibited lower abilities to protect the flanking cell upon wounding. In conclusion, the present genomic comparison along with localization screening allowed us to successfully find many novel proteins having a role in fungal cell-to-cell communication.

1. Maruyama and Kitamoto (2019) The Mycota VIII, pp. 3-14

The STRIPAK component Pro22 regulates trap morphogenesis in the nematode- trapping fungus *Duddingtonia flagrans*

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Nematode-trapping fungi (NTF) are carnivorous microorganisms which are able to trap and digest nematodes by forming sophisticated trapping structures. As natural antagonists, NTF offer high potential as economical biocontrol agent. The formation of trapping devices indicates a major switch from a saprotrophic to the predacious lifestyle. Here we studied the trap formation of the NTF *Duddingtonia flagrans* which is able to form three-dimensional trapping networks to capture its prey. In order to understand trap formation at the molecular level, we sequenced and annotated the genome and established a transformation system.

Trap networks usually consist of several hyphal loops. During the formation, a first loop develops from a basal vegetative hypha to form a three-cell structure and curves to fuse with the parent hypha. We discovered that the signalling cascade underlying hyphal fusion in other fungi is crucial for ring closure of the trap (1). Here we were interested in the early stages of trap formation. This stage resembles the spiral growth of ascogonia or the crozier formation of ascomycetes. It has been shown, that the conserved striatin-interacting phosphatase and kinase (STRIPAK) complex is required for ascogonia formation in *Sordaria macrospora*. Therefore, we investigated the role of the STRIPAK complex during trap formation by generating a mutant strain lacking the *D. flagrans pro22* gene, an ortholog of the STRIPAK component STRIP1/2. Hyphal growth of the *D. flagrans* Δ*pro22* mutant strain was decreased. Trap formation was disturbed resulting in incomplete loop formation, often resulting in column-like trap structures. However, the *D. flagrans* Δ*pro22* mutant was still able to capture nematodes, using *Caenorhabditis elegans* as prey.

1. Youssar, L., Wernet, V., Hensel, N., Fischer, R. *et al*. (2019). Intercellular communication is required for trap formation in the nematode-trapping fungus *Duddingtonia flagrans*. PLoS Genet., 15(3):e1008029

The effect of cultivation temperature on the heat resistance of *Aspergillus niger* conidia

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Preventing food spoilage is a major challenge in the food industry. Preservation techniques such as heat inactivation are widely used, albeit with the cost of consequently altering food profiles. In order to know at which temperatures food companies should treat their products to inactivate spores (conidia) from food spoilage fungus *Aspergillus niger*, additional knowledge is required on the intrinsic properties that contribute to heat resistance of these conidia. Our research is focused on the effect of cultivation temperature during conidiation and the resulting heat resistance of conidia from *Aspergillus niger*. We show that different cultivation temperatures have a major impact on the heat resistance of the resulting *Aspergillus niger* conidia. Cultivation of *Aspergillus niger* at 37ºC instead of 28ºC results in conidia with increased heat resistance. Furthermore, this heat resistance increase correlates with an increase in trehalose concentration in the conidia. To determine the role of trehalose accumulation in spores at higher temperature, a trehalose null knock-out mutant was made using CRISPR/Cas9. The trehalose null knock-out mutant produces conidia that lack any trehalose and were indeed more sensitive to heat. However, when cultivating this trehalose null knock-out mutant at 37ºC the conidia were still increased in heat resistance when compared to the conidia of the trehalose mutant grown at 28ºC. This suggests that perhaps other factors such as heat shock proteins play a role in the heat resistance of *Aspergillus niger* conidia.

The role of the Tec1 Transcription Factor (TEAD) during the development and differentiation of the Basidiomycota fungus *Ustilago maydis*

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Ustilago maydis, a member of the Basidomycota is the causative agent of common smut in maize, and its ancestor, teozintle, One aspect of the study of this fungus that has not received adequate attention refers to the transcription factors that orchestrate the gene expression that controls the that among the transcription factors that were particularly over-expressed was TEC-1 (UMAG_02835), a member of the TEA/ATTS family of transcription factors, that have been poorly studied in fungi. Mutants of members of this gene family have been obtained only in the Ascomycota *Candida albicans* and *Saccharomyces cerevisiae*, but not in any Basidiomycota.

Accordingly, we proceeded to carry out the functional characterization of the *U. maydis* transcription factor *TEC1* gene (TEAD1). We obtained mutants of the encoding gene, and examined their phenotype, mainly the development of fruiting bodies and their virulence.

With respect to the fruiting bodies we observed that the mutants showed a significant delay in their formation and a reduction (ca. 50%) either numbers in comparation with de wild type strains. We observe that virulence of the mutants was severely affected. Thus, when sexually compatible mutant (a1b1*tec1*/a2b2*tec1*) were inoculated in maize plants, none of the characteristic symptoms of the *U. maydis* infection: chlorosis, intense anthocyanin production, and tumour formation were induced. Analysis of expression of the mating genes by qRT-PCR revealed that their expression was inhibited. This result is evidence that mating in *U. maydis* is under the control of TEC1.

A transient receptor potential-like calcium ion channel in the filamentous fungus *Aspergillus nidulans*

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The Transient Receptor Potential (TRP) proteins constitute a superfamily which encodes transmembrane ion channels with very diverse permeation and gating properties. In mammals, this ion channel is best known as a sensor for environmental irritants giving rise to somatosensory modalities, such as pain, cold and itch, and other protective responses. According to bioinformatics analysis, filamentous fungi have putative TRP channels-encoded genes but their functions have remained elusive yet. Here, we reported functions of a putative TRP-like calcium ion channel in the filamentous fungus *Aspergillus nidulans*. Hydrophilicity and domain prediction indicated that the AN9146 encodes a protein that contains six TM domains with long N and C termini similar to the topology predicted for some members of the TRP family, so referred as TrpA. Deletion of *trpA* resulted in a sharp reduction in the number of conidial production at 37° , suggesting *trpA* may involve in response to high temperature. However, these defects in mutants can be rescued to the level of wild-type by adding extra-cellular Ca^{2+}. Moreover, the fact that the phenotypic defects are exacerbated by double deletions of TrpA with either of identified high affinity calcium channel CchA, MidA or calcium P-type ATPase PmrA, suggest that TrpA probably plays an important role in high-affinity calcium transportation. Interestingly, we found TrpA's localization was dynamic. When hyphal cells were cultured under the normal condition, majority of them localized in the membrane of vesicles along with the vesicle secretion network. However, when treated with the cell-wall disruption reagent-congo red, TrpA could translocate to plasma membrane. Therefore, together with data for the *trpA* deletion mutant showed more sensitive to congo red and caspofungin than that of wild type, this information implies that TrpA may also works as a plasma-

membrane ion channel which involves the cell-wall integration. Further detail data are ongoing.

Identification and characterization of *Aspergillus nidulans* Δ*flbB* mutants showing an aconidial phenotype under phosphate stress

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Fungi spread to new niches and colonize them by producing thousands of asexual spores. *Aspergillus nidulans* is a primary reference species for the study of the genetic/molecular mechanisms governing fungal asexual development (conidiation). The transcription factor BrlA plays a central role in this process since: 1) its levels are controlled by signal transducers collectively known as UDAs; and 2) it governs the expression of CDP regulators, which control the morphological transitions leading to conidia production. In response to the emergence of fungal cells to the air, the main stimulus triggering conidiation, UDA mutants such as the *flbB* deletant fail to induce *brlA* expression. Nevertheless, the need for FlbB activity can be bypassed by culturing Δ*flbB* colonies in a medium containing high concentrations of $H_2PO_4^-$. We used this phenotypic trait and an UV-mutagenesis procedure to isolate and characterize Δ*flbB* mutants unable to conidiate in these conditions. The characterization of FLIP166 led to the identification of the putative transcription factor SocA as a multicopy suppressor and $PmtC^{(P282L)}$ as the recessive mutant form responsible for the Fluffy (aconidial) In Phosphate phenotype. Taken together, results validate this novel strategy to identify genes and/or mutations related to the control of asexual development.

The mechanism of hyphal aggregation in liquid culture of *Aspergillus oryzae*

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Filamentous fungi generally form hyphal aggregates in liquid culture. Hyphal pellet formation decreases productivity of industrial enzymes in liquid culture, but the mechanism underlying the hyphal aggregates remains unclear. We previously constructed

α-1,3-glucan-deficient mutant in the model filamentous fungus *Aspergillus nidulans*, and the mutant showed fully dispersed hyphae under liquid culture conditions. These results showed that α-1,3-glucan has a role for hyphal aggregation. For the application to the industrial enzyme production by using the α-1,3-glucan mutant, we then constructed the α-1,3-glucan-deficient (AGΔ) mutant in the industrial fungus *Aspergillus oryzae*. Unexpectedly, the hyphae of the AGΔ mutant formed smaller hyphal pellets than the wild-type strain, suggesting that another factor responsible for the pellet formation is present besides α-1,3-glucan in *A. oryzae*. Here we identified the extracellular polysaccharide galactosaminogalactan (GAG) as a such factor. We constructed the disruption strains of GAG biosynthetic genes in an AGΔ mutant. The hyphae of the double mutant (AG-GAGΔ) fully dispersed in liquid medium, suggesting that GAG has a role for hyphal aggregation in *A. oryzae*[1]. We partially purified the fraction containing GAG from *A. oryzae* culture broth, and the fraction was added to the AG-GAGΔ hyphae, resulting in forming aggregated pellets[1]. Aggregation of hyphae mediated by GAG was decreased by the acetylation of amino group in galactosamine of GAG, suggesting that the deacetylation of GAG is necessary for aggregation[1]. We constructed the recombinant protein-producing strain in AG-GAGΔ strain, and the evaluation of the protein productivity of the AG-GAGΔ mutant in flask culture using the several culture media containing some carbon and nitrogen sources used in industrial production.

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Peroxisome and mitochondrial dynamics regulated by Dnm1 and Fis1 are necessary for sexual development in the fungus *Podospora anserina*

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Mitochondria and peroxisomes are organelles whose activity in the cell is closely associated and that play fundamental roles in development. Research on the mycelial ascomycete *Podospora anserina* has shown that peroxisomes and mitochondria play critical roles at different stages of sexual development. In addition, evidence indicates that the activity of these organelles in this process is interrelated, and involves an active crosstalk influencing their dynamics. Peroxisomes and mitochondria share some of the main factors regulating their dynamics, including the proteins driving their division. This process is facilitated by the dynamin-related protein Dnm1, which is recruited to peroxisomes and mitochondria by membrane receptor proteins, like Fis1. Here we show that peroxisome and mitochondrial fission in *P. anserina* depends on DNM1 and its potential receptor FIS1. We demonstrate that DNM1 and FIS1 are both required for peroxisome and mitochondrial dynamics throughout sexual development, and that their

elimination affects the dynamics of both organelles in a developmental stage-dependent manner. Moreover, we discovered that the segregation of peroxisomes, but not of mitochondria, is compromised upon FIS1 or DNM1 elimination both in somatic and sexual development cells –including meiocytes and ascospores. Finally, we found that *FIS1* or *DNM1* deletion results in defective ascospore differentiation. Our findings show that sexual development involves a complex regulation of peroxisome and mitochondrial dynamics, which relies on their common fission machinery. This research was supported by grants PAPIIT IA203317 and IV200519 from DGAPA, UNAM, and CONACYT-DFG 277869 from FONCICYT.

Characterization of the *exp2* gene essential for cap expansion in *Coprinopsis cinerea*

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Cap expansion is critical factor for spore dispersion in mushroom forming fungi. A putative transcriptional regulator gene, *exp1*, has been identified from cap expansion less mutant in *C. cinerea*. The *exp1* gene is predicted to encode an HMG1/2-like protein with two HMG domains and its transcription is strongly induced in the pileus 3h before pileus expansion. A novel cap expansion less mutant, *exp2#13*, in *C. cinerea* is reported in this study. The *exp2#13* mutant has small unmatured cap on top of fruiting body, and is defective in both pileus expansion and autolysis. Linkage analysis revealed that the responsible gene was located on chromosome III. Genome sequencing of the *exp2#13* mutant revealed that a gene located in chromosome III (CC1G_03235) has stop codon insertion. The putative protein of CC1G_03235 has a zinc finger domain in its C-terminus, and the stop codon was inserted into the domain. DNA containing a wild-type CC1G_03235 gene derived from #326 strain was transformed into the *exp2* mutant, and cap expansion was rescued in the transformed strain. The gene CC1G_03235 was deleted by genome editing in #326, and the genome-edited mutant has similar phenotype with the *exp2* mutant. These suggest that CC1G_03235 is the *exp2* gene. Expression pattern of the *exp2* was compared with the *exp1,* the *exp2* was expressed earlier than the *exp1*. The *exp2* was expressed in the *exp1* genome edition mutant, but both *exp1* and *exp2* were not expressed the *exp2* genome edition mutant. This suggests that the *exp2* is in upstream of the *exp1* for cap expansion and autolysis. The *exp2* was not expressed in fruiting body formed under dark and blue light receptor mutant, *dst2*. This suggests that the *exp2* is under regulation of blue light receptor.

Regulation of conidiation by the velvet complex in *Neurospora crassa*

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The *velvet* family of regulatory proteins are defined by the *velvet* domain, a DNA binding domain, presumably for gene regulation and protein-protein interaction between velvet-domain containing proteins. In addition, these proteins can interact with each other forming complexes that regulate different aspects of fungal biology such as sexual and asexual development and the production of secondary metabolites. There are four genes containing a velvet domain in the genome of *Neurospora crassa (ve-1, ve-2, ve-3 and vos-1). N. crassa ve-1* and *ve-2* mutants show defective aerial hyphae growth, increased conidiation and reduced carotenoid accumulation. To study whether the function of the velvet proteins is redundant, we analysed the phenotypes in double and triple knock-out mutants lacking ve-*1, ve-2* and /or *vos*-1genes. Furthermore, we found the presence of VE-1, VE-2 and the methyltransferase LAE-1 in vegetative mycelia, where they formed complexes. Additionally, we characterized the presence of the components of the velvet complex during the early stages of conidiation, and their subcellular localization. We noted that VE-1 was detected in vegetative mycelia and aerial hyphae, but aerial hyphae kept in light accumulated moreVE-1 than aerial hyphae kept in dark, suggesting a regulatory role for light in VE-1 protein accumulation during conidiation. To understand the role of VE-1 as a transcriptional regulator during conidiation, an RNA-seq analysis has been performed. We analysed the transcriptome of the *N. crassa wild* type and *Dve-1* mutant as they progress from a vegetative growth to conidiation in dark and exposed to light. A further screening of most promising genes affected in *ve-1* mutants and the conidiation process was performed. Our results allowed us to identify genes presumably regulated by VE-1 during the transcriptional response to conidiation.

PKC pathway and NOX1 mediate cell wall changes and cytoskeleton alterations caused by chitosan on *Magnaporthe oryzae*

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Chitosan significantly reduces *M. oryzae* pathogenicity to rice. However, chitosan alone does not cause any damage in rice leaves. Chitosan reduces appressorium differentiation in *M. oryzae* conidia. Chitosan disorganizes *M. oryzae* appressorium cytoskeleton blocking CHM1 and TEA1 transmembrane proteins. This leads to plasma membrane permeabilization concomitant with an increase in intracellular reactive oxygen species (ROS). ROS in *M. oryzae* is mainly generated by *NOX* genes, essential for infection. *NOX1* drives sensitivity to chitosan in *M. oryzae* since Δ*Nox1* growth is unaffected by chitosan. The effect of chitosan on *M. oryzae* is reflected in the composition of cell wall of the fungus. Chitosan causes glucan accumulation and chitin content reduction in both Guy 11 and Δ*nox1*. *NOX1* represses cell wall integrity pathway genes with chitosan since in *NOX1, PKC1* and *SWI6* are induced with chitosan after 8h. *MPS1* and *PKC1* are essential for *M. oryzae* sensitivity to chitosan since PKC1as but specially Δ*mps1* can grow in the presence of chitosan. Concluding, we show that chitosan is a useful tool to modify fungal morphogenesis, gene expression and pathogenicity. Our results are, therefore, of paramount importance for developing chitosan as a natural antifungal to control rice blast.

The role of the Endoplasmic Reticulum-Mitochondria Encounter Structure in the sexual development of the fungus *Podospora anserina*

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Cell organelles establish physical interactions through molecular tethers, which perform several functions in the regulation of organelle activity. One of the identified tethers that connects the endoplasmic reticulum (ER) to mitochondria in fungi is ERMES (ER-Mitochondria Encounter Structure). This protein complex is composed of four subunits: Mdm10, Mdm12, Mdm34, and Mmm1, and is involved in lipid and calcium exchange, mitochondrial protein assembly, fission, motility, and mitophagy. In all studied species, the absence of any of the ERMES subunits provokes alterations in mitochondrial morphology and mtDNA distribution. In addition, absence of any member of the complex causes growth defects, which may be moderate as in *N. crassa*, to very severe as in *A. fumigatus*. However, the contribution of ERMES to sexual development has not been addressed. We have shown that the proteins driving peroxisome and mitochondrial fission as well as some proteins regulating ER structure are involved in the sexual development of *P. anserina*, where their elimination affects meiotic development. These observations suggest that the function of these organelles is coordinated during this process. We sought to determine the role of the ERMES complex in this process by analyzing mutants in *MDM10*. We confirmed that a temperature sensitive strain

with a point mutation in *mdm10* has growth defects and alterations in mitochondrial morphology. Besides we found that this mutant is incapable of forming perithecia, and that this defect is suppressed in trikaryon complementation assays with a Δ*mat* strain. Notably, in this context we did not observe defects during meiotic development. These observations suggest that the ERMES-mediated mitochondria-ER interaction is critical for perithecia formation but not for meiotic development.

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The role of the STRIPAK complex in the sexual development of *Sordaria macrospora*

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Striatin-interacting phosphatase and kinase (STRIPAK) complex is conserved in fungi and animals. In the filamentous ascomycete *Sordaria macrospora*, STRIPAK complex has been found to be essential for hyphal fusion and fruiting-body development. The STRIPAK orthologues in *S. macrospora* are PRO11 (striatin), PRO22 (STRIP1/2), PRO45 (SLMAP), the serine-threonine phosphatase scaffolding subunit PP2AA, catalytic subunit SmPP2Ac1, and kinase activator SmMOB3. Unlike mammals, fungi comprise only one striatin gene and are therefore good models to study the cellular function of the STRIPAK complex. Recently, we performed PRO11 and SmMOB3 pull-down experiments coupled to liquid chromatography and mass spectrometry (LCMS) and analyzed the LCMS data for putative interaction partners. An uncharacterized protein was identified as putative PRO11 interaction partner with very high confidence, and was named STRIPAK complex interactor 1 (SCI1). SCI1 is an orthologue of small coiled-coil (CC) domain(s) containing proteins found in STRIPAK complexes in yeasts, fruit fly, and mammals. Deletion of *sci1* revealed its role in cell-cell fusion and sexual development in *S. macrospora,* and SCI1 was found to interact and co-localize with other STRIPAK components at the nuclear envelope *in vivo*. Thus, SCI1 can be considered as a core component of the *S. macrospora* STRIPAK complex and we proved that our proteomics approach is useful to identify STRIPAK components and effectors.

Further detailed analysis of LCMS data from PRO11, SmMOB3, and SCI1 pull-down experiments showed significant enrichment of proteins of the nuclear pore complex, and proteins involved in phospholipid biosynthesis and transport. The nuclear pore complex proteins may act as anchors that link the STRIPAK complex to the nuclear envelope but it may also play a role in phospholipid signaling. We study these new interaction

partners of the STRIPAK complex, to understand the role of STRIPAK complex in sexual development.

The Ca^{2+}-dependent proteins PEF1 and ANX14 are part of two different membrane damage response systems in *Neurospora crassa*

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Germinating spores of *Neurospora crassa* fuse with each other to form a mycelial network. The fusion process involves degradation of the cell wall and subsequent plasma membrane merger, which bears the risk of cell lysis and cell death. Previous analysis of the membrane fusion deficient *ΔPrm1* mutant indicated that a so far unknown Ca^{2+}-dependent membrane repair mechanism exists in *N. crassa*. In this study, we identified the Ca^{2+}-sensing proteins PEF1 and ANX14 as part of different membrane repair systems with some overlapping functions. Subcellular localization studies revealed that both proteins accumulate at the fusion point upon fusion-induced lysis. Since membrane damage can also be induced by membrane-targeting anti-fungal compounds, we also tested the subcellular dynamics of PEF1 and ANX14 in response to membrane damaging compounds, such as the anti-fungal drug nystatin, the plant defence compound tomatine and newly described antifungal salamander alkaloids. Both proteins are recruited to the plasma membrane in response to the treatment with tomatine and salamander alkaloids, while only PEF1 is responding to nystatin.

To functionally characterize PEF1 and ANX14, gene knockout mutants were analysed. The deletion of *anx14* resulted in increased lysis rates of fusing germling pairs compared to the wild type or the Δ*pef1* mutant. Lysis rates further increased by 2-fold in the Δ*anx14* Δ*pef1* double mutant, suggesting that PEF1 and ANX14 function independently. When grown on tomatine, Δ*pef1* is more sensitive to the toxin than Δ*anx14*.

These observations suggest that ANX14 is more important for the repair of mechanically-induced damage, while PEF1 is mainly involved in the repair of drug-induced membrane damage. We hypothesize that membrane repair constitutes the first step of resistance against membrane damaging compounds. Future studies aim at revealing and fully characterizing the PEF1 and ANX14 mediated membrane repair mechanisms in *Neurospora crassa*.

Cell wall remodeling in the mycoparasite *Trichoderma atroviride* as important strategy in biocontrol

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The accelerated rise in global temperature and weather extremes due to the climate crisis with elevated humidity or draught increases the pathogen burden in crop production and mandates new techniques to control and avoid food and crop spoilage. One of the most promising green alternatives to pesticides in agriculture are mycoparasites and plant symbionts such as *Trichoderma spp.*, which have already successfully been applied as biocontrol agents. Conversion of cell wall chitin to chitosan by specialized deacetylases is a potent escape mechanism of human - and phytopathogenic fungi to avoid activation of the host's chitin-triggered immune system. Thus, its importance for virulence is evident. Chitin and chitosan synthesis have so far only been studied in saprotrophic and phytopathogenic fungi, but not in ascomycetes with a mycoparasitic lifestyle. Here, we analyzed the overall cell wall composition of *Trichoderma spp.* and its role as a protective organelle and virulence factor. The elucidation of these mechanisms in mycoparasites to fight pathogens and to identify targets for antifungal drugs is of utmost importance. Microscopic analysis reveals the intricately regulated interplay of eight chitin synthases and more than 15 other enzymes - deacetylases, chitinolytic enzymes, and accessory proteins in the assembly and turnover of chitin and chitosan in the *T. atroviride* cell wall. Confrontation assays with deletion mutants provide the first evidence that chitin and chitosan remodeling is indispensable for host invasion by the mycoparasites. We further highlight the importance of chitosan in serving as disguise towards hostile chitinases and as scavenger for reactive oxygen species during the mycoparasitic attack. These new insights in the cell wall assembly during mycoparasitism, provide valuable, more specific information on targeting key factors for biocontrol applications as well as for therapeutic purposes.

Correlative cellular and organellar changes associated with transcriptional profiles during toxigenesis in *Fusarium graminearum*

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Fusarium graminearum is an ascomycete phytopathogen, causing *Fusarium* Head Blight disease on cereals, such as wheat and barley. This filamentous fungus produces secondary metabolites, including terpenes, which contaminate grains. *Fusarium* mycotoxins, which are frequently detected in food products include deoxynivalenol (DON) and acetylated derivatives of DON (ADON). DON and ADON are sesquiterpenoids called trichothecenes (TRI). While the biosynthetic enzymes necessary for TRI biosynthesis are well-characterized, cellular differentiation and organellar adaptation in response to elevated production of secondary metabolites is still unexplored. Recently, we identified a reorganized endoplasmic reticulum (ER) as the site of TRI biosynthesis in intercalary hyphae in vitro and in planta (Boenisch *et al*., 2017, Scientific Reports 7:44296). We further discovered spatial accumulation of the cytosolic TRI biosynthetic enzyme Tri5 (trichodiene synthase) in the proximity of this reorganized ER (Boenisch *et al*., 2019, Fungal Genet Biol 124:73-7), suggesting enzyme recruitment to specific parts of the ER. In the current study, overall cellular changes (e.g. hyphal morphology) and organellar dynamics in TRI toxigenic cells were elucidated via microscopy. We determined organellar characteristics of cells induced to produce toxin compared to non-induced cells, including significantly different number of nuclei per cell, size of vacuoles, and mitochondrial function using live cell microscopy and organelle specific dyes. Further, we correlated observed cellular and organellar changes of toxigenic cells with changing transcript profiles from RNASeq data of TRI-induced and non-induced cells over time (24h, 48h, and 72h). The combined results provide novel insights about global cell biological changes that occur upon elevated TRI biosynthesis in F. graminearum and potentially other TRI metabolite producing filamentous fungi.

The histone acetyltransferase Elp3 is required for biofilm formation and virulence in *Aspergillus fumigatus*

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In eukaryotes, DNA is wrapped around histones whereas histone acetylation is the process by adding an acetyl group to the histones for which the gene expression can be regulated tightly. There is emerging evidence that histone acetylation plays an important role for the virulence of fungal pathogens either in host plant or animal. However, relationship between the histone acetylation and its virulence in human opportunistic pathogen *Aspergillus fumigatus* remains poorly understood. In this study, according to bioinformatics conserved domain analysis, we have screened thirteen putative *A. fumigatus* histone acetyltransferase (HAT)-encoding gene null mutants for the virulence in *Galleria mellonella* infection model. Strikingly, we found that the deletion of Elp3, a *Saccharomyces cerevisiae* histone acetyltransferase Elp3 homolog in *A. fumigatus*, resulted in significantly attenuated virulence. Further colony phenotypic analysis of

Δelp3 mutant showed a dramatic reduction in conidiation and hyphal growth. In addition, the adherence assay showed that deletion of elp3 led to markedly reduced expressions for biofilm formation-related genes uge3 and agd3 accompanied with less biofilm production while overexpression of them partially rescued these defects. Moreover, western blot assay for acetylation level of histone H3 revealed that Elp3 is essential for the acetylation of H3K14 in vivo and decreased biofilm formation of Δelp3 mutant is phenocopied by unacetylatable H3K14R mutation strain, implying an important role for acetylation of H3K14 in regulation of biofilm formation. Thus, these findings provide insights for the relationship among histone acetylation, biofilm formation and virulence in the opportunistic human pathogen *A. fumigatus*. Future studies are ongoing to elucidate the mechanism of how Elp3 affects virulence-related genes expression transcriptionally.

Exploration of the genetic cause of female sterility in the rice blast fungus

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Ascomycetes are associated with various food, agricultural, and pharmaceutical industries while some species cause animal and plant diseases. Filamentous fungi in this phylum are known to contain many female sterile strains, which are unable to form sexual structures. Among them, rice blast fungus *Pyricularia oryzae* (*Magnaporthe oryzae*) displays especially low fertility. Compatibility of sexual reproduction is determined by mating-type (α or HMG). When a strain possessing α mating-type meets different strain possessing HMG mating-type, perithecia are formed, which contain ascospores covered by asci. However, most of *P. oryzae* isolates do not form perithecia, and the cause of this is unknown. Comparative genomic analysis was performed to investigate the genes responsible for this female sterility. For this analysis, a fertile wild strain (α) was crossed with a female sterile wild strain (HMG). One of the obtained progenies, which is female sterile and possesses HMG mating-type, were further crossed with the fertile wild strain. This was recurred, and 37 F4 progenies were obtained. Genomic DNA of 4 fertile strains and 4 female sterile strains each were mixed and used for next generation sequencing. Genomic comparison revealed 56 genes to be different in amino acid sequences between fertile and female sterile strains, and among them, 11 genes to be located in a 500 kb clistere region on chromosome 5.

Gene knockouts in the fertile wild strain was performed for the 11 genes, and the deletions of 3 genes showed loss of female fertility, while the deletion mutants of 4

genes remained fertile. The other genes are presumably essential, because they could not be deleted.

Introduction of female sterile-type mutations to the fertile wild strain is now carried out. Furthermore, gene deletions in the female sterile wild strain is planned, because a female sterile-type gene may function as a negative regulator of sexual reproduction.

Characterization of extracellular membrane vesicle in liquid culture of *Magnaporthe oryzae* and *Aspergillus oryzae*

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Extracellular membrane vesicles (eMV) are small, membrane-enclosed structures released from a cell into the surrounding environment (Toyofuku *et al*., 2018). eMV contains cargo molecules such as nucleic acids, proteins and chemical compounds, and affect diverse biological processes, including virulence, horizontal gene transfer and cell-to-cell communication. In the case of the fungi, recent studies demonstrated that fungi are able to produce biologically active EVs under culture and during infection (Souza *et al*., 2019). However, most studies have characterized EVs in yeast, while the release and characterization of these structures in filamentous fungi have been poorly explored. In this study, we analyzed the properties of eMV produced by filamentous fungi, *Magnaporthe oryzae* and *Aspergillus oryzae*.

Lipid particles appeared extracellular space during static liquid culture. When these lipid particles were purified and observed by TEM, vesicle structures were observed, indicating that these lipid particles are eMV. Proteome analysis of eMV fraction revealed that it contains plasma membrane-related proteins and secreted proteins. On agar media, production of eMV-like lipid particles on the surface of mycelia was observed by confocal laser microscope. These results suggested that *M. oryzae* and *A. oryzae* produce eMV during cultivation and eMV originate from plasma membrane. eMV is a kind of vector system which enables trans-cell wall transfer of biological components in filamentous fungi, and should be important to understanding the ecology and characteristics of filamentous fungi.

The iron chealtor BPS is a novel inducer of hyphal morphogenesis in *Candida albicans*

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Candida albicans is a commensal fungus that is also an increasingly important cause of systemic infections among immunocompromised individuals, with crude morality rates of 35-60%. The ability of *C. albicans* to undergo a reversible morphological transition from yeast (oval shaped cells) to hyphae (elongated, filamentous cells) in response to a variety of external stimuli has been long associated with the virulence and pathogenesis of *C. albicans*. Well-characterized *stimuli* include elevated temperature (37°C), exposure to serum, to N-acetylglucosamine, alkaline pH, CO2 , and growth in low-nitrogen media, among others. We identified bathophenanthroline disulfonic acid (BPS), a commonly used iron chelator, as another inducer of hyphal growth. The iron chelator ferrozine, in contrast, does not induce hyphal growth. Since BPS inhibits cellular growth due to iron chelation, addition of hemin or hemoglobin as alternative iron sources best enable to follow the BPS-induced hyphal morphogenesis, but these compounds do not seem to be directly involved in the morphogenetic effect of BPS. Addition of iron or of copper, an alternative BPS ligand, can abolish the morphogenetic effect of BPS. We hypothesize that BPS induces hyphal growth either by binding to a receptor or by its ability, not shared with other iron chelators, to penetrate the cells in its free form. Genetic analysis of BPS-induced morphogenesis is currently being carried out.

Role of *Schizophyllum commune* homeodomain transcription factors during mushroom formation

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Development of anatomical structures in multiples organisms such animals, plants and fungi is mainly regulated by homeodomain transcription factors. In mushroom-forming fungi, these genes are essential during sexual development (1), nuclear migration and heterokaryosis (2) and development of fruiting bodies. In *S. commune*, 9 homeodomain genes (hom) have been recognized. Hom1 and hom2 genes have been previously described to be involved in different stages of fruiting body formation. Deletion of hom2 results in symmetrical growth and no mushroom formation after crossing compatible mating-type deletion strains. In contrast, hom1 deletion produce more but smaller mushrooms when compare to wild-type (3). However, the role of each of these genes during developmental processes is still poorly understood. Here, we have deleted 6 homeodomain genes of *S. commune* (Δhom3, Δhom5, Δhom6,

Δhom7, Δhom8 and Δhom9) by using pre-assembled Cas 9 ribonucleoprotein (4). We compared development of fruiting body formation under standard growth conditions against wild-type strain. Additionally, knock out strains were grown in the absence of light and exposed to high CO^2 levels, in order to determine the effect of these conditions during mushroom formation. All knock out strain formed mushrooms when grown under standard conditions. However, impaired mushrooms were observed in Δhom3, Δhom6 and Δhom8 strains, suggesting that these genes play a role at late steps of the fruiting body formation more probably after primordia formation.

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Blue and red light photoreceptors are involved in basidiocarps development by *Ustilago maydis*

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Description of fruiting body (basidiocarps) development in *Ustilago maydis* (DC) Cda., a Basidiomycota fungus, was surprising since the Class Ustilaginomycetes (Subphylum Ustilaginomycotina) has been described as a non-forming basidiocarps group. The observation that basidiocarp formation occurred only under white light illumination, led us to search for the possible photoreceptors involved in the process. Analysis of the genome of the fungus revealed the existence of three homologous white-collar (WC) genes. These were over-expressed during formation of fruiting bodies. A gene encoding a phytochrome was also identified. Hence, we investigated whether the WC homologues and the phytochrome (Phy1) played a role in basidiocarp formation. Accordingly, we obtained mutants in each one of the three *U. maydis WC* homologue genes, *WCO1a, WCO1b*, and *WCO2*, and the phytochrome encoding gene *PHY1*, and analyzed the phenotype of the corresponding mutants. Phenotypic analysis of the mutants under illumination, showed that *Δwco1a* mutants formed similar numbers of basidiocarps than the wild type strain, whereas *Δwco1b* mutants were severely affected in basidiocarp

formation when illuminated with white, blue or red light. More interesting, *Δwco2* and *Δphy1* mutants did not form basidiocarps under any illumination condition. These results indicate that the photosensory system operates as a complex formed by the Wco and the Phy proteins in basidiocarp formation. Wco1a would be the main blue light receptor, and Wco1b would operate as a secondary blue light receptor. Phy1 would be the red-light receptor, and Wco2, the transcription factor that would receive the stimulation of the photoreceptors to activate transcription of genes involved in basidiocarp formation. Efficiency of the light receptors depended on the whole structure of the complex being formed, possibly, because its association is necessary to maintain its active structure.

Poster Session 1.2
CELL REGULATION AND SIGNALING

TUESDAY, FEBRUARY 18

18:00 - 19:30 | Location: **Frentani Convention Center**

Sugars "in-sight" – towards a new view of carbohydrate signaling and perception by 'omics' analyses of *Neurospora crassa*

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Plant biomass is the most abundant carbon source on earth and a major food source for fungi. While the uptake of representative plant cell wall mono- and disaccharides is known to induce specific transcriptional and translational responses, the initial processes related to early signal reception remain largely unknown.

These initial responses are likely dominated by post-translational protein modifications, such as phosphorylations. To capture these quantitatively, we performed phosphoproteomics after a 2 min induction period of the filamentous ascomycete *Neurospora crassa* with representative inducers. The MS/MS-based peptide analysis revealed large-scale substrate-specific protein phosphorylation and de-phosphorylations in 2,563 proteins. We describe the variances in phosphorylation of 78 proteins related to major signaling processes. For example, adenylate cyclase, a key component of the cAMP pathway, was identified as a potential hub for carbon source-specific protein interactions. Further casein-kinases, G-proteins, serine/threonine protein kinases, transcription factors, cAMP-dependent and MAP kinase pathway proteins confirm the importance of phosphorylation for early substrate recognition.

With the goal to visualize the perception of individual polysaccharides within complex plant biomass, we furthermore analyzed a transcriptomics dataset obtained in response to a large array of carbohydrate conditions. Footprints of metabolic states, such as starvation and catabolite repression, could be identified and allow to generate hypotheses about fungal substrate preferences. In addition, we built a gene co-expression network for all *N. crassa* genes, helpful to guide the search for novel pathways components.

Overall, we provide unprecedented insights into the early stages of the fungal response to environmental cues, which contribute to the rational engineering of fungi for

biotechnological applications, but also help to better understand ecological contexts.

Alternative splicing as an element of signal transduction in multi-step phosphorelay systems in fungi

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The increase in protein diversity through alternative splicing is widespread in the Metazoa kingdom, but in fungi, it has not been extensively studied so far. In particular for signal transduction processes, such as in multi-step phosphorelay systems, the role of alternative splicing has not yet been scientifically addressed.

Our project focuses on the high osmolarity glycerol (HOG) signaling pathway. In the HOG signaling pathway of *Saccharomyces cerevisiae*, external stimuli are perceived by one single sensor histidine kinase and transmitted by phosphorylation via one single phosphotransfer protein Ypd1p. In contrast to *S. cerevisiae*, many sensor histidine kinases are present in the higher fungi, which can detect several different external stimuli. However, only one single Ypd1p coding gene is present in their genomes. This leads to the question of how different signals can be received as "input" from several sensor proteins in fungi and how different signals can be transmitted independently from one phosphotransfer protein as different "output" signals to various targets. We address this question using the filamentous phytopathogenic fungus *Magnaporthe oryzae*. In preliminary work in *M. oryzae*, we were able to identify different transcripts resulting from one genome sequence of *MoYPD1*, amplified different cDNA variants thereof and found a previously unknown isoform of MoYpd1p at the protein level. This results in our hypothesis that different, possibly signal-specific, isoforms of the protein MoYpd1p are produced in *M. oryzae* to facilitate a higher variability in signal transduction.

This hypothesis will be comprehensively investigated at the genetic, transcriptional and biochemical level. In addition to complement the "loss of function" mutant ΔMoypd1 with different MoYpd1p isoforms, we intend to use proteome and phosphoproteome analyses to characterize the signaling processes and aim to generate "multi-fluorescence-mutants" of the different isoforms.

Identification of the guanine nucleotide exchange factor for SAR1 in the filamentous fungal model *Aspergillus nidulans*

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Filamentous fungi are widely used as cell factories for biotechnological purposes: the secretory pathway is responsible for the exocytosis of extracellular enzymes of applied interest. Endoplasmic Reticulum (ER) exit of cargo bound for the secretory route is regulated by the GTPase Sar1. We have previously shown the key role of *sarA*, the SAR1 homologue in *Aspergillus nidulans*, in the secretory pathway and hyphal morphogenesis. The Guanine nucleotide Exchange Factor (GEF) for SarA^{SAR1}, named Sec12, has not been yet characterized in any filamentous fungus. Here we demonstrate unequivocally, through a combination of genetic, biochemical and live-cell fluorescence microscopy approaches, that the essential gene AN11127 encodes the single Sec12 homologue in *A. nidulans*. AN11127 protein sequence displays the signature motif GGGGxxxxG φXN found in Sec12 homologues, and the constitutive overexpression of this gene partially supresses the growth defect exhibited by a *sarA^{ts}* mutant at 37ºC. Fluorescently-tagged AN11127 protein localises to the ER. *In vitro* assays with bacterially-expressed proteins demonstrated that the cytosolic domain of AN11127 indeed accelerates nucleotide exchange on SarA^{SAR1} but not on the related GTPase ArfA^{ARF1}. Thus we present undisputable evidence that the AN11127 gene product is the GEF for the ER-exit regulatory GTPase SarA^{SAR1}.

Antifungal Susceptibility is Modulated by pH in *Cryptococcus neoformans*

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Cryptococcus neoformans is an opportunistic fungal pathogen, but its infection is militated by phagocytosis and phagolysosomes in the host immune system. It has been reported that the pH of phagolysosomes containing *C. neoformans* is approximately 5.3. In general, acidic pH condition, in comparison with neutral pH, is known to alter several physiological characteristics of fungi, including susceptibility to azole antifungal drugs. Indeed, antifungal susceptibility of another human fungal pathogen, *Candida albicans*, is modulated by pH, although the underlying mechanism is not clear yet. Therefore, we

investigated whether the environmental pH influences the antifungal susceptibility of *C. neoformans* and how the fungus responds to acidic pH. We found that the minimal inhibitory concentration of *C. neoformans* against fluconazole was increased under an acidic pH condition, and our GC-MS analysis revealed that the ergosterol content in *C. neoformans* that was grown under an acidic condition was increased in comparison to that in cells grown at a neutral pH level. Moreover, a mutant strain lacking *CFO1*, which is the major component in the high-affinity reductive iron uptake system in *C. neoformans*, displayed significantly reduced sensitivity to fluconazole at an acidic pH level. This implies that a different iron uptake pathway governs the transport of iron under such a condition. Considering that a number of the proteins involved in ergosterol biosynthesis require iron as a cofactor, our data implied the involvement of a yet unknown iron uptake pathway, which is independent of the *CFO1* function, in antifungal susceptibility of *C. neoformans* under an acidic pH condition. In addition, we investigated the underlying molecular regulatory mechanism to understand how *C. neoformans* responds to acidic pH condition through transcriptome analysis as well as phenotypic and biochemical analysis of the series mutant strains lacking the genes involved in the major iron uptake pathways.

BRO-1 localize in vesicles and represent a sub- popular special for fusion, The Suppression of *bro-1* expression results in cell-cell fusion deficiencies

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Colony initiation of filamentous fungi commonly involves fusion of germinating vegetative spores. Studies in *Neurospora crassa* revealed an unusual cell-cell communication mechanism mediating this process, in which the fusion partners coordinately alternate between two physiological stages, probably related to signal sending and receiving. This “cell dialog” involves the alternating, oscillatory recruitment of the SO protein and the MAK-2 MAP kinase module to the apical plasma membrane of growing fusion tips.

We identified BRO-1, the homolog of the mammalian ALG-2-interacting protein X (ALIX), as a new factor participating in germling interaction and fusion. Alix is an ESCRT accessory component and mediates the biogenesis of exosomes and the secretion of extracellular vesicles. In *N.crassa,* BRO-1 is essential. Subcellular localization and live cell imaging revealed that BRO-1-GFP is recruited in vesicles in non-interacting germlings and in the mature hyphae. The co-localization of BRO1-GFP and some vesicles markers indicate that BRO-1might be a sub- popular vesicle special for fusing. However, BRO-1-GFP is recruited to the tips of the interacting germlings in a dynamic, oscillating

manner, such that high signal intensity of BRO-1-GFP in one tip correlates with low signal intensity at the tip of the fusion partner. Suppression of *bro-1* gene expression results in a Δ*so*-like phenotype, including the lack of chemotropic interactions and subsequent fusion. In germinating conidia, the knock-down of *bro-1* also results in the formation of multiple germ tubes, while in wild-type cells only one polarity center is established and maintained. Subcellular localization and live cell imaging revealed that the germlings lacking BRO-1 fail to stably maintain the polarity center, which leads to the establishment of additional, new germ tubes.

We hypothesize that BRO-1 plays distinct roles in cell-cell communication and polar growth. Future analysis of its molecular function will greatly contribute to our understanding of the unique "cell dialog" mechanism and the molecular bases of fungal cellular communication.

Dephosphorylation of the stress-activated MAP kinase Hog1 of the maize pathogen *Cochliobolus heterostrophus* in response to a plant phenolic acid

Rina Zuchman, Roni Koren and Benjamin A. Horwitz

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Protein phosphorylation cascades are universal in cell signaling. Kinome diversity allows specific phosphorylation, while relatively few phosphatases dephosphorylate key signaling proteins. Fungal MAP kinases, in contrast to their mammalian counterparts, often show detectable basal phosphorylation levels. Dephosphorylation, therefore, could act as a signal. In *Cochliobolus heterostrophus*, the Dothideomycete causing Southern corn leaf blight, ferulic acid (FA), an abundant phenolic found in plant host cell walls, acts as a signal to rapidly dephosphorylate the stress-activated MAP kinase Hog1 [1]. To identify the protein phosphatases responsible, we are constructing mutants in Hog1 phosphatases predicted from the genome [2] by homology to yeast and other species. Mutants lacking a member of the PP2C family, ChPtcB, show attenuated dephosphorylation in response to FA. We will test whether the sensitivity to FA-induced cell death is altered when FA-induced Hog1 dephosphorylation is compromised. Phosphatase families in pathogens and non-pathogens can be compared. For example, *Cryptococcus neoformans* serotype A H99 shows constitutive phosphorylation of Hog1 [3] and one can ask to what extent phosphatases contribute to the phenotype conferred by the variant Ssk2 MAPKKK gene in this strain [3]. Using PtcB as a test case, searches of some dikarya genomes led to a single candidate ortholog. Mucoromycotina, in contrast, show expansion (4 PtcB candidate orthologs in Phycomyces blakesleeanus and Mucor circinelloides), as found for other signaling-related families [4], not implying, from this one example, any obvious connection between the number of phosphatase genes and

fungal lifestyle.

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Uncovering the essential transcription factors of *Cryptococcus neoformans*

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Cryptococcus neoformans is an opportunistic human fungal pathogen that causes cryptococcosis and fatal meningoencephalitis. Due to its clinical importance and lack of effective, selective and safe antifungal agents to treat cryptococcosis, it is crucial to identify and validate novel antifungal drug targets. Among these, essential proteins can be valuable antifungal targets as they directly affect the growth of a fungal pathogen. The aim of the study is to identify and characterize essential transcription factors in *C. neoformans*. According to our previous report (Jung *et al* 2015 *Nat. Comm.*), 23 transcription factors are suspected to be essential for the growth of *C. neoformans* as they could not be deleted from the genome. For these genes, we constructed *CTR4* promoter-replaced mutant strains to control their expression levels. Among 19 transcription factors tested thus far, 9 of them were found to be required for growth, whereas the remaining 10 were dispensable for the growth of *C. neoformans*. By phenotypic analysis, we confirmed that the growth-required TFs were highly related to DNA damage response and membrane stability. To examine the essentiality of the 9 growth-required transcription factors, we constructed heterozygous mutants in an engineered diploid strain of *C. neoformans*, and are analyzing basidiospores from these strains to explore the role of these genes in viability. That is, if a viable spore with nourseothricin drug resistance is observed, the transcription factor is required, but not essential, for the growth of *C. neoformans* and the spore itself is a knock-out mutant. On the other hand, if there are no viable nourseothricin resistant spores, the target transcription factor gene is truly essential and further analysis will be performed with the promoter-replaced mutants under the BCS condition (overexpression) to various stress responses.

Because lineage matters: Screening *Aspergillus niger* strains for endogenous pectinase activity

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Aligning with the European research and innovation program "Horizon 2020", the use of renewable resources in the chemical industry as well as the development of more sustainable applications should be fostered. Pectin has gained special interest as a bio-based resource in recent years and is abundantly found in agricultural waste streams, such as sugar beet pulp. Due to the variety of achievable fermentation products from its main monomeric component, D-galacturonic acid (D-GalA), pectin is considered a highly promising second generation feedstock for biotechnological fermentations.

While saprophytic fungi are generally important in plant biomass degradation, *Aspergillus niger* (*A. niger*) is specifically known for its strong pectinolytic capabilities, making it a perfect candidate for industrial-scale pectin de-polymerization. However, while specialized strains for the production of citric acid or proteins are openly available, little is known about endogenous pectinolytic capacities of different *A. niger* strains. We therefore systematically compared the pectinolytic capabilities of six *A. niger* strains (ATCC 1015, ATCC 11414, NRRL 3122, CBS 513.88, NRRL 3, and N402) in controlled batch cultivations in stirred-tank bioreactors. Using data on pellet morphology, total protein secretion and endo-polygalacturonase activity, we identified ATCC11414 as a superior strain with suitable morphology and high endogenous pectinolytic activity. Culture supernatants of ATCC 11414 revealed 75% higher D-GalA release from sugar beet pulp as a complex pectinaceous substrate than the standard lab strain *A. niger* N402, aligning with the results of the standardized assays used in strain selection. Our study therefore presents a robust initial strain selection setup and identifies a highly suitable base strain for potential further genetic optimizations to improve D-GalA production from agricultural residues.

A conserved mitogen-activated protein kinase pathway regulates development and secondary metabolism in three *Aspergillus* species

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The fungal genus *Aspergillus* consists of 300-350 species, many of which can exhibit beneficial or detrimental effects with regards to human and plant health. *Aspergillus* species are capable of producing secondary metabolites (SMs) which are bioactive compounds, ranging from beneficial antibiotics to dangerous mycotoxins. In order for *Aspergillus* species to regulate their development and secondary metabolism, an array of protein signalling cascades are utilized, such as Mitogen-Activated Protein Kinase (MAPK) cascades. A MAPK cascade known as the pheromone module has previously been characterised in the model filamentous fungus *Aspergillus nidulans*. This pathway was shown to contain 5 core proteins that function in propagating a signal to the nucleus in response to pheromone detection, resulting in the regulation of asexual and sexual development, as well as secondary metabolism. Homologs of each of these proteins have been identified in two closely related species *A. flavus* and *A. fumigatus*. These species produce dangerous mycotoxins such as aflatoxin and gliotoxin, which can result in crop contamination and infections in immunocompromised individuals, respectively. This work highlights the identification of the pheromone module complex in both species and characterises the roles of this conserved complex in the regulation of fungal development and SM production.

Trichoderma atroviride mycoparasitism and its regulation by the TOR signaling pathway

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Mycoparasitism is an innate property of the fungal genus *Trichoderma* and species like *T. atroviride* are among the best studied mycoparasites due to their ability to antagonize a wide range of fungi including several plant pathogens. *Trichoderma* mycoparasites efficiently overgrow and kill their fungal preys by using lytic enzymes and antifungal metabolites, also in combination with the formation of infection structures.

We used the strong mycoparasite *Trichoderma atroviride* to study how the TOR (Target Of Rapamycin) pathway, one of the central nutrient-sensing pathways in eukaryotes, affects nitrogen signaling, mycoparasitism and secondary metabolite production in this fungus. Similar to other filamentous fungi, the single TOR kinase-encoding gene *tor1* turned out to be essential in *T. atroviride*. Intervening with signaling via the TOR pathway by using the TOR kinase inhibitor rapamycin or by deleting genes encoding pathway components

that act upstream (*rhe2, tsc1, tsc2*) or downstream (*npr1, are1*) of Tor1, however, revealed various roles of this signaling pathway in the regulation of nitrogen source-dependent growth, sporulation, and mycoparasitism-associated processes. Comparative profiling of nitrogen source utilization revealed that *Δare1* mutants produced less biomass than the wild type on most of the 95 tested nitrogen sources, while *Δnpr1* and *Δrhe2* mutants even grew better than the wild type on some compounds. In confrontation assays against fungal preys similar mycoparasitic activities as the wild type were observed for Δ*rhe2* and Δ*npr1,* while virulence of Δ*tsc1* and Δ*tsc2* was reduced. Δ*are1* mutants were completely unable to attack prey fungi on PDA; however, they reached full mycoparasitic activity on glutamine-containing media. Additional experiments further showed an important role of the TOR pathway in the regulation of expression of the mycoparasitism-relevant protease-encoding *prb1* gene and the production of extracellular secondary metabolites.

RGS domain containing G-protein coupled receptors impact chemical communication in *Trichoderma reesei*

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Signaling pathways transmit information on environmental conditions and their changes to the promoters of crucial metabolic genes. The resulting adjustment of gene expression can be used for strain improvement in industrial applications. *Trichoderma reesei* is predominantly known for its efficient production of plant cell wall degrading enzymes. However, the recently shown interconnection of secondary metabolites as well as its chemical communication upon sexual development indicate a broader applicability.

Heterotrimeric G-protein signaling is impacts cellulase gene expression at a posttranscriptional level with distinct regulatory outcomes in light and darkness for the individual subunits. Additionally, this pathway influences secondary metabolism. Regulators of G-protein signaling (RGS) domains can accelerate the GTPase activity of G-alpha subunits and thereby inactivate their signal transmission. Therefore we were interested in the functions of the three RGS domain containing G-protein coupled receptors of *T. reesei*.

Gpr12, *gpr15* and *gpr19* are largely co-regulated, with enhanced transcript levels on inducing carbon sources and positive regulation by the photoreceptor ENV1 in light. Despite their upregulation under cellulase inducing conditions compared to conditions favouring sexual development, only minor effects on cellulase regulation were found.

Deletion of *gpr12*, *gpr15* or *gpr19* causes an altered response to the presence of a mating partner compared to wildtype, hence modulating chemical communication. Accordingly, we saw indications for a positive effect on fruiting body formation for GPR15. GPR12 and GPR15 further impact production of sorbicilline components.

Consequently, RGS domain containing GPCRs regulate transmission of signals relevant for chemical communication, but not enzyme expression in *T. reesei*.

Austrian *Trichoderma* spp. impact mycotoxin production of the plant pathogen *Fusarium graminearum*

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Secondary metabolites (SMs), including mycotoxins produced by various fungal species have their function in the survival in an environment with limited resources. The defense of a colonized food source often leads to the production of toxins protecting the surrounding area against competitors. Many mycotoxins produced on crops represent a serious threat for human health. *Trichoderma* species known for their efficient biocontrol abilities are used for decades as biological supplements to pesticides in agriculture.

A screening for interaction of diverse *Trichoderma* strains with *Fusarium graminearum* suggested a correlation between antagonistic potential and SM production. Here we present that the presence of *Trichoderma* spp. collected from Austrian soils influences the SM composition of *F. graminearum*. An HPTLC (high performance thin layer chromatography) screening of 95 strains representing 20 *Trichoderma* species revealed various interaction types between competing strains with a substantial influence on the production of DON (deoxynivalenol) by *F. graminearum*. The presence of several strains lead to disappearance of DON production whereas others triggered the production up to 70-fold compared to axenic culture. We also could show that DON overproduction correlates with the presence of several other compounds not produced in axenic culture.

The altered SM regulation by *F. graminearum* is likely due to chemical communication. Hence, we studied the reaction of *Trichoderma* strains to the presence of *Fusarium* as well. We found clear indications for chemical communication which causes production of novel metabolites compared to axenic growth.

In summary we show that a strain-specific interaction with *F. graminearum* has considerable influence on mycotoxin production. Moreover, our findings indicate that not

only antagonism impacting biomass formation of pathogens, but also an influence on secondary metabolism is worth considering in screenings for biocontrol agents.

Grx4 influences growth at elevated temperature and cell wall integrity via the calcineurin and Mpk1 signaling pathways in *Cryptococcus neoformans*

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Monothiol glutaredoxins are important regulators of iron homeostasis because of their conserved roles in [2Fe-2S] cluster sensing and trafficking. We previously characterized the role of a monothiol glutaredoxin Grx4 in iron homeostasis and virulence in *C. neoformans*, a basidiomycetous fungal pathogen causing cryptococcal meningoencephalitis in immunocompromised individuals worldwide. In this investigation, we demonstrate that Grx4 is required for fungal growth at elevated temperatures (both 37oC and 39oC) and under additional stress conditions. The grx4 mutants displayed increased sensitivity to FK506 and cyclosporin A, two calcineurin specific inhibitors, indicating that Grx4 likely influences fungal growth at higher temperatures by influencing or interacting with calcineurin signaling in C. neoformans. Loss of Grx4 caused mis-localization of Crz1, a zinc-finger-type transcription factor and a well-known calcineurin substrate in *C. neoformans*. This mislocalization occurred in cells grown either under thermal stress or with calcium treatment, further supporting the hypothesis that Grx4 is involved in fungal adaptation to elevated temperature by interaction with the calcineurin complex. Moreover, the grx4 mutant was hypersensitive to SDS, calcofluor white, or caffeine treatment, suggesting that Grx4 is required for cell wall integrity. Consistent with this idea, the grx4 mutant also showed increased sensitivity to osmotic and oxidative stresses. In this context, our results show that Grx4 regulates the phosphorylation of the Mpk1 mitogen-activated protein kinase (MAPK) in cells grown at either elevated temperature, or upon SDS treatment or oxidative stress. In summary, our current data support the hypothesis that Grx4 regulates cell wall integrity, thermal and oxidative stresses via both calcineurin sensing and the Mpk1 pathway in *C. neoformans*.

Unveiling of Complex Signaling Networks Involved in the Developmental Process of the Fungal Pathogen *Cryptococcus neoformans*

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The fungal pathogen *Cryptococcus neoformans* causes cryptococcosis by the inhalation of infectious spores generated by unisexual or bisexual reproduction. To understand complex signaling networks modulating the developmental process, a complete understanding of genome-scale transcription factors (TFs) and kinases is needed. Previously we reported that 37 TFs and 42 kinase mutants constructed in *C. neoformans* MATα H99 strain background exhibited altered mating efficiency. To further elucidate the mating regulatory mechanism, we constructed knockout mutants of the mating-regulating TFs and kinases in YL99 strain—MAT**a** isogenic strain of H99 strain—to monitor unilateral and bilateral mating, and to perform an analysis of their function in the developmental process. We constructed 22 gene-deletion strains representing eleven TFs and are currently constructing gene-deletion strains for the remaining mating-regulating TFs and kinases. For confirmed mutant strains, we are examining mating phenotypes during bilateral mating: mating pheromone production, cell fusion efficiency, filamentous growth, formation of basidia and basidiospores. Furthermore, we are examining transcript profiles of mating-regulating TFs and kinases at different developmental stages of sexual reproduction. Ultimately, this study will focus on mapping and discovering the functions of the mating-regulating TFs and kinases, and elucidating complex signaling networks in the developmental process of *C. neoformans*.

STRIPAK dependent phosphorylation of target proteins in the filamentous fungus *Sordaria macrospora*

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The ascomycetous filamentous fungus *S. macrospora* is used as a model organism for multicellular development during the fungal sexual cycle and has been scrutinized for signalling pathways and conserved developmental regulators governing sexual fruit-body formation. Involved in sexual development is the highly conserved striatin-interacting phosphatases and kinases (STRIPAK) complex regulating phosphorylation of developmental proteins. In fungi, the complex is involved in cell fusions, multicellular differentiation, symbiotic interactions and pathogenic relations [1]. Similarly, several human diseases such as seizures and strokes are linked to defective subunits of STRIPAK [2]. We performed extensive isobaric tags for relative and absolute quantification (iTRAQ)-based proteomic and phosphoproteomic analyses to identify potential targets

of STRIPAK. In total, we identified 4,193 proteins and 2,489 phosphoproteins, which are represented by 10,635 phosphopeptides. By comparing phosphorylation data from wild type and three STRIPAK mutants, we identified 228 regulated phosphoproteins. Here, we provide an exemplarily functional analysis of proteins, which are putative targets of STRIPAK.

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2. Kück U, Radchenko D, Teichert I (2019) STRIPAK, a highly conserved signaling complex, controls multiple eukaryotic cellular and developmental processes and is linked with human diseases. Biol Chem 400(8): 1005–1022, doi: 10.1515/hsz-2019-0173

Characterization and function of the RNA interference machinery of *Aspergillus fumigatus*

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Aspergillus fumigatus is a ubiquitous fungus of high abundance in natural environments and able to thrive in very diverse environments, ranging from compost piles to even mammalian lungs in some cases. RNA interference (RNAi) is a conserved molecular mechanism in eukaryotes, which uses small RNA (sRNA) molecules to suppress gene expression through sequence-specific messenger RNA (mRNA) degradation. RNAi has been shown to be important for fungal development, physiology, and defense against invading nucleic acids. sRNAs from some pathogens can even be used as effectors to modulate host defense and influence pathogenesis. In this study, we hypothesized that *A. fumigatus* RNAi is important for gene regulation, stress resistance, and survival of the fungus in human hosts. To test this hypothesis, we created single and double knockouts of key components of the RNAi machinery, including orthologs of the dicer, argonaute, and RNA-dependent RNA polymerase proteins in *A. fumigatus*. Only the deletion of both argonaute proteins resulted in a sporulation defect. To gain insight into the active mechanisms of silencing in *A. fumigatus* and characterize the observed phenotypes, we are currently analyzing the contribution of each protein to silencing mediated by an inverted repeat transgene (IRT)-containing plasmid. So far, our results suggest that only one of the *A. fumigatus* argonaute proteins contributes to IRT-dependent silencing of target mRNA. We next performed RT-qPCR to measure transcript levels of RNAi genes,

which revealed high relative expression in conidia and fungal hyphae under starvation conditions. This was followed by sRNA sequencing in these conditions to predict potential sRNAs, which are currently being analyzed. This study has revealed that *A. fumigatus* RNAi regulates several physiological processes, and we hope to fully characterize the *A. fumigatus* RNAi pathway and provide a platform to study RNAi mediated interactions during infection.

Roles of the cytosolic tails and the last two transmembrane domains in NCS1/FUR family of transporters

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FurE, a member of the Nucleobase Cations Symporter 1 family (NCS1), is an Aspergillus nidulans transporter specific for uracil, allantoin and uric acid. Genetic and functional analyses have previously supported that the C- and/or N-terminal domains of FurE interact with each other and with other cytoplasm-facing segments and thus affect transporter turnover, transport activity and substrate specificity. We subsequently identified distinct motifs crucial for endocytosis, transport activity, substrate specificity and folding in both cytosolic termini of FurE, and obtained additional genetic and in silico evidence supporting that the dynamic cross-talk of specific N- and C-terminal regions affect, from a distance and in pH-dependent manner, the gating mechanism responsible for substrate selection. Interestingly, the role of the last two transmembrane domains (TMS11 and TMS12) in the NCS1/FUR family remained unclear, and is generally thought not to be involved directly in transport activity or substrate specificity. Here, we systematically address by genetic and functional studies, and will present our results, on the role of TMSs 11 and 12.

Chitin desacetylase (CDA1) is required for *Ustilago maydis* virulence

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Fungal cell walls are essential for cell shape and integrity, and for their interaction with

the environment. Besides, in phytopathogenic fungi, the cell wall is necessary for host invasion, being the first structure that makes physical contact with the host cells. The most important structural components of the cell walls of fungi are chitin, and β-glucans. Other components that comprise the amorphous core of the wall, are among others, mannans, β-glucans, proteins, and chitosan.

Chitosan is synthesized by deacetylation of chitin, a reaction catalysed by specific deacetylases. Interestingly, despite the wide distribution of chitosan in fungi, its roles remain mainly unknown.

U. maydis is a plant pathogenic fungus responsible for common smut in maize and teozintle. Through our analysis of the transcriptome of the yeast to mycelium dimorphic transition at acid pH, we observed a number of genes encoding chitin deacetylases. The gene encoding one of them (UMAG_11922; *CDA1*) was the one overexpressed at higher values.

Accordingly, we proceeded to carry out its mutation and analysis of the phenotypic characteristics of the mutants. We observed that these mutants contained reduced amounts of chitosan, and more interesting, that they were affected in their mycelial morphology at acid pH, and were severely affected in their virulence to maize. Reinsertion of the wild type *CDA1* gene in the mutants by an autonomous replication plasmid, led to the recovery of their virulence and chitosan levels, indicating that *CDA1* gene is involved in virulence. These data show that chitosan plays a crucial role in the structure and morphogenesis of the cell wall of the fungus, and that in its absence the cell wall becomes altered and unable to support the stress imposed for the mechanism of defense mounted by the plant host during the infection process.

Comprehensive and comparative analysis of transcription start sites suggests diversity in transcriptional regulation of glycolytic genes in *Aspergilli*

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The *Aspergillus* fungi have significant impacts on human society. Because they display the versatility in metabolizing carbon sources, diversity in their primary metabolisms has been discussed mainly based on comparative genomics studies. Meanwhile, our previous study showed that the gene encoding enolase, catalyzing a reversible reaction in glycolytic pathway, has two alternative transcription start sites (TSSs) in *Aspergillus oryzae*, an industrially important fungus. The two TSSs selection is stringently dependent on difference in fermentable and non-fermentable carbon sources such as glucose and

acetate, respectively. In contrast, the two TSSs usage is not conserved in *Aspergillus nidulans*, suggesting the transcriptional diversity in primary metabolic genes in *Aspergilli*. In this study, we compared transcriptional profiles associated with primary metabolisms between *A. oryzae* and *A. nidulans*, by cap analysis of gene expression (CAGE) that allows genome-wide identification of TSSs simultaneously with expression levels . CAGE data were collected from the mycelium grown with glucose or acetate and mapped to 59 orthologous genes of glycolysis/gluconeogenesis, pyruvate catabolism, TCA cycle, and pentose phosphate pathway. Consequently, 59 genes could be divided into 3 groups; group 1 includes 17 genes (17/59, 29%) expressed higher in the presence of glucose in *A. oryzae* than in *A. nidulans*, group 2 includes 10 genes (10/59, 17%) expressed higher in the presence of acetate in *A. oryzae* than in *A. nidulans*, and the rest of genes included in group 3 show similar pattern of expression levels in both species. Notably, glycolytic genes were enriched in group 1 (12/17, 71%) and differential TSSs usage between the two species were observed in glycolytic genes encoding aldolase, phosphofructokinase, and pyruvate kinase, in addition to enolase. These results can provide us novel insights on diversity in transcriptional regulation for primary metabolisms in *Aspergilli*.

Systematic Dissection of Host-derived Cues for the Regulation of Pathogenicity-related Transcription Factors in Human Fungal Pathogen

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Cryptococcus neoformans is a causative agent of global fungal meningoencephalitis. Nevertheless, its treatment option is limited mainly due to a lack of complete understanding of how the pathogen interacts with the host during infection and disease progression. Although a number of signalling components and pathways have been characterized past decades, it remains elusive how complex signalling pathways are coordinated and regulated during the infection process. To analyze this question, NanoString-based in vivo transcription profiling was performed to monitor 180 transcription factors during the whole infection process at diverse infected tissues. Here we focused on 23 transcription factors whose in vivo expression was highly induced during host infection and deletion significantly decreased pathogenicity in STM murine infectivity assay. To further elucidate their regulatory mechanisms during host infection, the expression level of the 23 transcription factors were analyzed under in vitro host mimic condition (HMC). Among these, expression of 12 transcription factors was strongly induced in HMC. To classify which host factor causes the induction of gene during infection, HMC signals were further dissected into five distinct cues: temperature, carbon starvation, nitrogen starvation, serum and high CO_2. Notably, host physiological temperature and carbon starvation made most significant contribution. For example, expression of *PDR802*, *FZC39*, *FZC30*, *BZP4*. *ZNF2* and *HLH1* was markedly induced by temperature

upshift from 30°C to 37°C. Supporting this, double deletion of *PDR802* and *FZC39*, both encoding fungal specific Zn_2Cys_6 domain, but not each single deletion, caused reduced growth rate at 37°C. On the other hand, expression of *FZC30*, *GAT201*, *PDR802*, *BZP4*, *MLN1* and *STB4* was highly induced by glucose starvation. This study provides further insight into complex signalling pathways modulating the host-pathogen interactions of *C. neoformans*.

UspA protein and CandA complex control different stages of protein recycling in filamentous fungi

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The recycling of proteins is essential for fungal development. Proteins directed for degradation are marked with ubiquitin by E3 ligases; the cullin-RING-ligases (CRL) is the largest family and the Skp1/cullin-1/Fbx (SCF) a well studied subgroup, which recognizes specific proteins and adds on them ubiquitin, a mark for degradation via the ubiquitin-proteasome system (UPS). SCF function is controlled by neddylation (Nedd8), a post translational modification, of the cullin protein. The neddylation confers stability to SCF, thus substrates are recognized and ubiquitinated; the deneddylation by signalosome (CSN), destabilizes SCF thus Fbx exchange is possible. Here we elucidate the roles of UspA (ubiquitin-specific protease A) and CandA (cullin-associated-Nedd8-dissociated protein A) complex in distinct stages of protein recycling in filamentous fungi.

UspA is a functional DUB (deubiquitinating enzyme) in *A. nidulans*, interacting with all subunits of CSN. Mutant analysis showed that UspA induces asexual and sexual development but also it represses the expression of secondary metabolite gene clusters. Moreover, UspA is responsible for the reduction at later steps of the sexual and asexual development, of the VeA (velvet domain protein A), a central regulator of the fungal differentiation.

CandA nuclear complex, is consisting by three proteins (CandA-C1, CandA-C and CandA-N) in *A. nidulans* and by two in *A. fumigatus* (CanA and CanA-N). CandA complex is an adaptor-receptor exchange factor for CRLs, required for the re-activation of the ubiquitin labeling apparatus, after its deneddylation by the CSN. Genetic analysis revealed involvement of CandA-C and CandA-N in asexual and sexual differentiation and that CandA-C1 controls the formation and germination of spores, vegetative growth and changes of secondary metabolites.

Our research could contribute to the design of novel molecules able to confer control of the fungal growth.

(Meister *et al*., 2019; Köhler *et al*., 2019)

SIP-1 is essential for germling fusion of *Neurospora crassa*, probably by mediating the initiation of cell-cell communication

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Cell fusion plays a central role in the development and proliferation of eukaryotic organisms. However, the molecular basis for this cellular process remains largely unknown. In the ascomycete fungus *Neurospora crassa*, germinating spores undergo chemotropic interaction and fusion in order to establish an interconnected colony. The proteins SO and MAK-2 are essential during this process. Their coordinated alternating recruitment to the cell tips of interacting germlings suggests a mechanism that enables communication between genetically and developmentally identical cells using only one signal-receptor pair. Cells are thought to alternate between the physiological state of signal sending and signal receiving in the manner of a "cell dialog" to avoid auto-excitation. The MAK-2 MAP kinase module is associated with signal-receiving, while SO is recruited to the cell tip during signal-sending.

By co-immunoprecipitation and mass spectrometry, we identified SIP-1 as a new interaction partner of the SO protein. A deletion of the *sip-1* gene results in a Δ*so*-like phenotype, including the inability to undergo chemotropic interactions and subsequent fusion. Live cell imaging revealed that SIP-1 is also recruited to the cell tip of interacting germlings in an oscillating manner. Translocation to the membrane coincides with the SO recruitment. However, in contrast to all other known factors, SIP-1 is the only protein that is already recruited in individual germlings prior to interaction. Based on this observation, we hypothesize that SIP-1 plays a role in the initiation of interaction or fusion competence. Before interaction is established, fusion competent cells seem to switch between two physiological states with SIP-1 being either recruited to the tip or localized in the cytoplasm of the cell. In further experiments, the positioning of SIP-1 in the hierarchy of the signaling network will be analyzed to expand the existing model of cell-cell-communication.

Functional reconstitution of the fungal UapA transporter in proteoliposomes: role of membrane lipids and stabilizing mutations

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The UapA purine transporter of *Aspergillus nidulans* is among the most extensively studied eukaryotic transporters in respect to structure-function relationships and regulation of subcellular trafficking. Genetic and structural studies have recently shown that UapA functions as a dimer and traffics to the plasma membrane (PM) via a direct route from the ER, bypassing passage from the Golgi. Importantly, functional dimerization and proper sorting to the PM was shown to depend on specific and annular interactions, respectively, with phospholipids. Genetic mutations suppressing the lack of UapA function due to abolished interactions with membrane lipids strengthen hydrophobic interactions within the core of the protein. Additionally, very recent unpublished data showed that mutations in ergosterol biosynthetic genes lead to UapA turnover and lack of transport activity. To further understand how specific membrane lipids affect UapA structure, function and traffic, we aimed at developing a new flexible system to follow the functional expression of yeast-purified wild-type or mutated UapA in proteoliposomes with distinct lipid composition. In ECFG15 we will present the progress of this effort.

Effects of ambient alkaline pH on gene expression: a key regulatory role for the cation-homeostasis transcription factor SltA

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Aspergillus nidulans is able to tolerate ambient alkalinity up to pH 10. The ability to grow at alkaline pH depends on the effective function of, at least, three regulatory pathways mediated by high hierarchy zinc-finger transcription factors: PacC, which mediates the ambient pH regulatory pathway, the calcineurin-dependent CrzA and the cation-homeostasis responsive factor SltA. Using RNA sequencing, we have determined the effect of external pH alkalinisation on gene expression and compared it to saline stress caused by sodium chloride. Transcriptional data demonstrate that the pattern of gene expression is largely modified under alkaline pH and different to that induced by salt stress. The role of SltA has been also studied by sequencing the transcriptomes of the

null mutant under both stress conditions. The transcriptional role of SltA is wider than initially expected and probably implies both inhibitory and positive roles. This includes, for example, the regulation of the PacC-dependent ambient pH regulatory pathway.

SltA is positively involved in the expression of pacC in response to alkalinity. Our data present a new scenario for understanding the transcriptional response to alkalinity and the cross regulation of major regulatory pathways in *Ascomycetes*, specifically in the *Pezizomycotina* subphylum.

Nuclear movements and the cytoskeleton during *Schizophyllum commune* mating interactions in living hyphae

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Nuclei expressing H2B::EGFP and Lifeact-mCherry labeled actin in the haploid (monokaryotic) and dikaryotic living hyphae of agaricomycete *S. commune* indicate that the actin cytoskeleton plays a role in both the placement of the nucleus/nuclei in the middle of the apical cell for nuclear division as well as the subsequent septal formation process (1, 2). The visualization of nuclei in living hyphae also highlighted the reduction of nuclear size during division and the speed at which the small sister nuclei separate after division. These phenomena are also associated with the microtubule cytoskeleton, whose visualization in living hyphae is in progress. The sexual reproduction of heterothallic filamentous agaricomycetes requires hyphal fusions as well as reciprocal nuclear exchange and migration during mating interactions regulated by the A and B mating type genes. It is still unknown whether a mating interaction with compatible B mating type genes, which encode G-protein-coupled-receptors and pheromones, leads to a cell cycle arrest in *S. commune* as it does in the mating of yeasts and the smut *Ustilago maydis* (3). Previous recordings of hyphal fusions between compatible strains of S. commune suggest that nuclear divisions and the migration of nuclei between the fused hyphae occur without the cell cycle arrest (4). Investigation of the microtubule cytoskeleton in living vegetative hyphae and during their mating interactions, together with labeled nuclei and microfilaments, will provide more accurate knowledge about the responses of the nuclei to pheromone receptor interactions at the beginning of mating, and clarify whether the response is different from those in yeasts and smut fungi. 1. Jung, E.M. *et al*. 2018, Fungal Genet. Biol. 111: 85-91, 2. Raudaskoski, M. 2019, Fungal Biology 123, 638-649; 3. García-Muse, T *et al*. 2003, Eukaryotic Cell 2, 494-500; 4. Raudaskoski, M 1998, Fungal Genet. Biol. 24: 207-227.

Crosstalk of Hog1, Mpk1 and Cpk1 MAPK pathways regulate the cell wall and cell membrane integrity in *Cryptococcus neoformans*

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Mitogen-activated protein kinases (MAPK), the pivotal kinases of eukaryotes, play major roles in proliferation, differentiation, metabolism, stress response and survival. The human fungal pathogen *Cryptococcus neoformans* causes meningoencephalitis regardless of immune system disruption and is responsible for approximately 600,000 deaths annually. There are three major MAPK pathways including Hog1, Mpk1 and Cpk1 pathways in *C. neoformans*. Even though the study of individual MAPK pathways have progressed extensively, the research concerning the crosstalk between different MAPK pathways have yet to be elucidated. In this study, we aim to understand the crosstalk among three major MAPKs and the key regulatory subunits to explain the complex signals regulating the virulence of *C. neoformans*. We verified characterizing how Hog1, Mpk1, and Cpk1 MAPK crosstalk regulate the downstream signaling factors, and we constructed the double and triple MAPK deletion mutants (*mpk1*Δ *hog1*Δ, *cpk1*Δ *hog1*Δ, *mpk1*Δ *cpk1*Δ, *mpk1*Δ *cpk1*Δ *hog1*Δ). Through phenotypic analysis, we discovered that all three MAPKs have roles in thermosensitivity, osmotic stress, oxidative stress, and cell wall and membrane stress response. Specifically, Mpk1 is known to play key roles in cell wall stress response, and we discovered that Hog1 and Cpk1 cooperatively contribute to cell wall integrity (CWI). To identify the regulatory mechanism in CWI with the crosstalk of MAPK, we observed the changes of pMpk1, pHog1 and pCpk1 in all double MAPK mutants (*mpk1*Δ *hog1*Δ, *cpk1*Δ *hog1*Δ, *mpk1*Δ *cpk1*Δ) under basal and cell wall disturbing conditions. In addition, we discovered that deletion of all three MAPKs increase cell membrane susceptibility independently. Therefore, we aim to provide insight into the regulation of cell wall and cell membrane integrity by complex MAPK crosstalk.

Subcellular localization of GATA transcription factors AreB and AreA under different carbon and nitrogen regimes in *Aspergillus nidulans*

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Nitrogen metabolite repression modulates the expression of target genes participating in utilization of alternative nitrogen sources, resulting in transcription only when glutamine or ammonium levels are limiting. In Aspergillus nidulans this regulatory mechanism depends on general GATA transcription factors AreA and AreB. Recently, we have shown that the expression of the three areB transcripts is regulated by nitrogen and carbon

source (Chudzicka-Ormaniec *et al*., 2019).

AreB subcellular localisation under different carbon/nitrogen regimes was studied using A.nidulans strains expressing the AreBα, AreBβ and AreBγ fused with the T-Sapphire fluorescent label. As a control we used the strain expressing the AreA::GFP fusion, as the AreA localisation is well studied (Todd *et al*., 2005).The microscopic analysis shows that the three AreB forms differ in their cellular localisation. Under carbon and/or nitrogen repressing conditions and under carbon or nitrogen starvation AreBβ is localised in the nucleus, while under carbon and nitrogen starvation the increased concentration of the protein in the cytoplasm is observed. Under most conditions tested AreBα and AreBγ fusion are observed mainly in the cytoplasm. AreB forms may have different functions in the nucleus and regulate different groups of genes. As expected, under carbon repressing conditions AreA is localised in the nucleus in response to nitrogen starvation.

MAT loci regulate development and virulence of the fungal pathogen *Fusarium oxysporum*

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Mating partner recognition in ascomycetes is mediated by the interaction of specific diffusible peptide pheromones with their cognate receptors. The expression of these proteins regulates sexual identity and is controlled by a single genetic locus named mating type (*MAT*), which exists in two idiomorphs, *MAT1-1* and *MAT1-2*. In the highly destructive asexual tomato pathogen *F. oxysporum* f. sp. *lycopersici* (Fol), pheromones and receptors were recently shown to have mating-independent functions in community behaviour and chemotropic sensing of the plant host (Vitale *et al*., 2019; Turrà *et al*., 2015). However, information on how *MAT* loci regulate these functions beyond sexual development is currently missing. Here we determined the role of *MAT* loci in two Fol isolates, 4287 (*MAT1-1*) and 54003 (*MAT1-2*), by generating strains that either lack the *MAT* locus or contain both *MAT* loci in their genome. Phenotypic assays with these mutants revealed that *MAT1-1* represses conidial germination at high concentration of inoculum, whereas *MAT1-2* promotes germination under these conditions while repressing vegetative hyphal fusion. Interestingly, deletion of *MAT1-1* led to a significant decrease of virulence on tomato plants. Our results indicate that *MAT* loci regulate key

biological and virulence-related processes such as quorum sensing in germinating conidia and vegetative hyphal fusion, thus reinforcing our knowledge on the interplay between sexual development and virulence in fungal plant pathogens.

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Circadian regulation of a mycoparasitic interaction between *Botrytis cinerea* and *Trichoderma atroviride*

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Circadian clocks coordinate organisms' activities with daily environmental rhythms synchronizing their biology with the environment. In the past years, evidence has emerged showing that circadian clocks also impact the outcome of organismal interactions, as in the case of the phytopathogenic fungus *Botrytis cinerea* and the plant *Arabidopsis thaliana*.

In this study, we analyzed the effect of circadian regulation in the mycoparasitic interactions between *Trichoderma atroviride* and *B. cinerea.* While we had described the presence of a functional clock in *B. cinerea,* (which enhances its virulence during night-time), there was no evidence regarding the presence of circadian rhythms in *T. atroviride*, despite the existence in its genome of putative clock components.

We developed a luciferase reporter strain by fusing its open reading frame to the core-clock component FREQUENCY (TaFRQLUC). Thus, we were able to observe daily oscillations in LUC levels. Likewise, we also detected rhythms when using a luc-transcriptional reporter under the control of a *frq* minimal promoter. Taken together, these results show evidence of a functional circadian clock in Trichoderma. We then proceeded to generate FRQ knockout (Δ*tafrq*) and overexpressor strains (OE::*tafrq*), revealing only a minor effect of FRQ in conidiation.

Regarding the circadian regulation of the mycoparasitic interaction, *wt* and clock mutant strains of both fungi were confronted on antagonistic assays at 20°C under constant

light (LL), constant darkness (DD), or light-dark cycles.They were evaluated after 7 days, measuring growth area and necrotic halo produced, observing differences between the abovementioned genotypes. Another variable that we evaluated was time of the day of the initial inoculation (morning or night), revealing distinct and clear interactions phenotypes, suggesting that in this fungal-fungal interaction time-of-the-day may matter.

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Systematic Functional analysis of phosphatase networks in human fungal pathogen *Cryptococcus neoformans*

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Cryptococcus neoformans causes fatal cryptococcal meningoencephalitis mainly in immunocompromised patients, which leads to an average of 223,100 infections and 181,100 deaths annually in worldwide. Despite its clinical importance, comprehensive understanding of its pathobiological signaling networks is far from completion and therapeutic options for treatment of systemic cryptococcosis are highly limited. Here, to further elucidate complex signaling networks regulating the virulence of *C. neoformans*, we aimed to identify and functionally characterize the 139 putative phosphatases, which are major signaling components in the basidiomycete fungal pathogens. We selected putative phosphatases based on annotation in the *C. neoformans* var. *grubii* genome database provided by National center for Biotechnology Information (NCBI) and performed a BLAST search with their protein sequences to identify any corresponding orthologs in *Saccharomyces cerevisiae*, *Aspergillus nidulans*, *Candida albicans*, *Fusarium graminearum* and human. We classified putative phosphatases into 16 groups based on InterPro phosphatase domain annotation. Thus far, we have successfully constructed

230 signature-tagged gene-deletion strains representing 114 putative phosphatases through homologous recombination methods. We are in the middle of examining their phenotypic traits under 30 different in vitro conditions including growth, differentiation, stress response, antifungal resistance, virulence-factor production and in vivo virulence potential in insect and mammalian hosts. Along with our previous functional genetic studies for *C. neoformans* transcription factors and kinases, this study will provide a comprehensive insight into the fungal pathobiological signaling networks.

Rapid adaptation of signaling networks in the fungal pathogen *Magnaporthe oryzae*

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The high osmolarity glycerol (HOG) pathway for osmoregulation in the model organism *Magnaporthe oryzae* is an attractive signaling pathway to study the basics of fungal physiology. The HOG pathway consists of a phosphorelay system and a MAPK cascade enabling to adapt towards extracellular osmotic changes in the environment. The major osmolyte produced as an osmotic stress response is arabitol. Individual "loss of function"(lof)-mutants of the HOG pathway are impaired in osmoregulation. Lof-mutants of the HOG pathway are unable to produce arabitol and thereby being more sensitive towards osmotic changes.

Long-term cultivation at high osmolarity resulted in stable mutants that arose as individuals being restored in osmoregulation from each of the individual lof-mutant. Within a relatively short time period, compared to millions of years of evolution, a rewiring of the signaling pathway in *M. oryzae* could be observed after 4 weeks. Interestingly, all of the "suppressor"-mutants found to be exclusively produce glycerol as a major compatible upon salt stress instead of arabitol. The "suppressors" are reestablished in osmoregulation and are able to memorize osmoregulation-ability even after growing weeks without stress. This phenomenon has been further investigated by DNA and RNA sequencing of *ΔMohog1*(suppressed) resulting in a set of candidate genes may be responsible for the rewiring of the osmoregulation pathway.

We aim to combine theoretical approaches to integrate sequencing data from genomics and transcriptomics with modern quantitative (phospho)-proteomic techniques. Furthermore, reversed molecular genetics will be used to validate the candidate genes or even other factors found to putatively promote or constrain rapid evolutionary adaptation.

We are convinced that this phenomenon is important for the comprehension of rapid evolutionary processes in eukaryotes and can be applied to other organisms apart from *M. oryzae*.

UapA-membrane lipid interactions are crucial for ER-exit, dimerization, function and expression of mammalian transporters in *A. nidulans*

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Transporters are transmembrane proteins that mediate the selective translocation of solutes across biological membranes. Recently, we have shown that specific interactions with plasma membrane phospholipids are essential for formation and/or stability of functional dimers of the purine transporter, UapA, a prototypic eukaryotic member of the ubiquitous NAT family. Here, we show that distinct interactions of UapA with specific or annular lipids are essential for ab initio formation of functional dimers in the ER or ER-exit and further subcellular trafficking. Through genetic screens we identify mutations that restore defects in dimer formation and/or trafficking. Suppressors of defective dimerization restore ab initio formation of UapA dimers in the ER. Most of these suppressors are located in the movable core domain, but also in the core-dimerization interface and in residues of the dimerization domain exposed to lipids. Molecular Dynamics suggest the majority of suppressors stabilize interhelical interactions in the core domain and thus assist the formation of functional UapA dimers. Among suppressors restoring dimerization, a specific mutation, T401P, was also isolated independently as a suppressor restoring trafficking, suggesting that stabilization of the core domain restores function by sustaining structural defects caused by abolishment of essential interactions with specific or annular lipids. Importantly, introduction of mutations topologically equivalent to T401P into a rat homologue of UapA, namely rSNBT1, permitted the functional expression of a mammalian NAT in A. nidulans. Thus, our results provide a potential route for the functional expression and manipulation of mammalian transporters in the model *Aspergillus* system.

Functional studies of the role of the RING-Finger protein CarS in *Fusarium fujikuroi*

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Fusarium fujikuroi produces a large variety of secondary metabolites in response to different environmental factors. Light stimulates the synthesis of neurosporaxanthin (NX) and other carotenoids through the induction of transcription of three genes of the NX pathway: *carRA*, *carB*, and *carT*. Mutants affected in the gene *carS* exhibit deep-pigmented phenotype due to deregulation of the *car* genes. Evidence of the role of CarS as a repressor was obtained by increasing *carS* mRNA using a constitutive *gpdh* promoter or the doxycycline-inducible Tet-on system. In both cases, enhanced *carS* transcription results in albino phenotypes under illumination.

Protein CarS has a LON-protease and two RING-fingers domains, typical of ubiquitin ligases. For identification of effectors of CarS, transformants expressing a CarS protein tagged with a FLAG epitope at either N- or C-termini have been generated. In the dark, transformants with a N-tagged CarS accumulated NX, probably due to altered CarS activity, while those with a C-tagged CarS have a phenotype similar to wild type.

Mutations in *carS* are pleiotropic, since affect not only carotenoid biosynthesis but also conidiation, germination, and sensitivity to antibiotics such as voriconazole and amphotericin B. In order to shed light on the role of CarS in morphogenesis, we checked the effect of antifungals on cell wall components and sterols in a *carS* mutant in comparison to wild type. Although no changes were detected for most of the components, we found that voriconazole increases NX biosynthesis in *F. fujikuroi.* This correlated with enhanced mRNA levels of the structural *car* genes and reduced mRNA levels of *carS*.

A novel lncRNA involved in the regulation of carotenoid biosynthesis in *F. fujikuroi*

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The synthesis of neurosporaxanthin in *Fusarium* is a well-known model in the study of the regulation of carotenoid production in filamentous fungi. In these species the

pathway is stimulated by light, a regulation investigated in detail in *Fusarium fujikuroi* that involves the transcriptional activation of the structural genes *carRA* and *carB*, linked in a coregulated cluster. In addition, the pathway is down-regulated by the RING-finger protein CarS, as indicates the carotenoid-overproducing phenotype of mutants of the *carS* gene. A detailed examination of the region upstream to the gene gene *carS* in RNA-seq analyses identified a putative 1.2-kb non-coding transcript, whose levels increased in *carS* mutants. This nonannotated gene, that we call *carP*, is transcribed in the same direction as the neighbor *carS* gene, and its deletion results in an albino phenotype and a strong decrease in the mRNA levels of the structural *car* genes. In combination with parallel studies in *F. oxysporum*, sequence analysis of *carP* discards a protein-encoding function. Despite the lack of coincident ORFs, their respective *carP* sequences exhibit 75-80% of identity, suggesting a sequence-dependent function. Unexpectedly, the deletion of *carP* does not lead to apparent alterations of carS transcription in *F. fujikuroi*. Our attempts to complement the *carP* deletion by re-introduction of the wild *carP* sequence showed recovery of carotenoid synthesis only in some transformants, suggesting the need for its expression in the native location. Data will be provided on RNA-seq studies in progress on the global effects of *carP* deletion on the *F. fujikuroi* transcriptome. In conclusion, our data point to a key role of *carP* as a regulatory lncRNA needed for the production of carotenoids in *Fusarium*, possibly through the controls of CarS by an unknown mechanism currently under investigation.

CreD ubiquitination required for endocytic degradation of the maltose transporter MalP in *Aspergillus oryzae*

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Aspergillus oryzae produces a large amount of amylolytic enzymes, whereas the expression level of amylolytic genes is strongly repressed by glucose owing to the carbon catabolite repression system. In *A. oryzae,* maltose utilization is required for those production. *malP* encodes a major maltose transporter, and a *malP* disruptant exhibited a significant decrease in maltose consumption and α-amylase activity. In the presence of glucose, MalP also undergoes endocytic degradation. In higher eukaryotes, signal-dependent ubiquitin (Ub) modification of plasma membrane (PM) transporters triggers its selective endocytosis and sorting to the vacuole/lysosome for degradation. The human Nedd4 HECT E3 Ub ligase family, is responsible for ubiquitination of PM transporters, which requires a specific arrestin adaptor protein to be selectively targeted by ligases. In our previous studies showed that HulA, the *A. oryzae* homologue of Nedd4, and the arrestin-like protein CreD involved in glucose-induced MalP internalization. Although CreD could act as a HulA adaptor during the glucose inactivation of MalP, it is unclear

how CreD regulates MalP degradation. The Nedd4 family ligases are known to interact with adaptors harboring the proline-rich (PY) motifs. Here, we identified the proline-rich regions of CreD that act as PY motifs to interact with HulA. Mutational analyses revealed that CreD interaction with HulA was impaired by mutation of multiple PY motifs in CreD, resulting in repressed glucose-induced MalP degradation. Moreover, we found that CreD was also ubiquitinated by HluA and that mutation in the four lysines, conserved among *Aspergillus* species, of CreD remarkably blocked its ubiquitination, leading to marked retardation of glucose-elicited MalP internalization. These results suggested that CreD interacts with HulA through its PY motifs and that the Ub modification state of CreD is important for glucose-induced inactivation of MalP.

Discovering the role of the casein kinase 2 complex in the pathogenicity of the human fungal meningitis pathogen *Cryptococcus neoformans*

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The opportunistic human fungal pathogen *Cryptococcus neoformans* causes fatal meningoencephalitis both in immunocompromised patients and immunocompetent individuals. However, the therapeutic options for treatment of cryptococcosis are currently highly limited. As a potential antifungal drug target, kinases have been considered to be good candidates and play important regulatory roles in cellular mechanisms and virulence of fungal pathogens. In previous studies, we found Cka1, a serine/threonine eukaryotic kinase, is involved in regulation of cell growth, cell cycle, cellular morphology and pathogenicity of *C. neoformans*. In this study, we aim to figure out the regulatory mechanism of Cka1 and its associated protein complex in *C. neoformans*. We found one catalytic subunit Cka1 and two regulatory subunits which are Ckb1 and Ckb2 as a putative complex subunit. To confirm the protein localization, we constructed tagged or co-tagged mutants of Cka1-GFP, Ckb1-mCherry and Ckb2-mCherry and verified their protein localization. And we identified the physical interactions between catalytic subunit and regulatory subunits of casein kinase 2 using yeast two-hybrid system. We also constructed single and double knockout mutants of regulatory subunits to compare the phenotypes with *cka1*Δ. The regulatory subunits were involved in antifungal drugs susceptibility, oxidative stress and DNA damaging responses. Interestingly, when *CKA1* was overexpressed in *ckb2*Δ *ckb1*Δ, it restored the growth defect of *ckb2*Δ *ckb1*Δ. We also constructed *cka1*Δ *ckb1*Δ *ckb2*Δ triple deletion mutant and it showed severe growth defect like *cka1*Δ mutant. As a result, Cka1 plays major roles and Ckb1/Ckb2 have minor roles in casein kinase 2 complex. This study will provide a comprehensive Cka1 cellular mechanism to develop an antifungal drug.

Transcriptomic analysis of the genes involved in the dimorphic transition of *Ustilago maydis* induced by ethanol as a carbon source

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Ustilago maydis is the causative agent of the disease in maize known as common carbon or huitlacoche. The fungus has a sexual cycle that begins when a diploid teliospore germinates producing haploid complementary yeasts which mate. A dikaryon is form that invades the plant, and forms a diploid mycelium. Finally, teliospores are formed, thus completing the fungus life cycle.

The natural dimorphic transition yeast-to hypha of *U. maydis* may be replicated *in vitro* by different means: transition from neutral to acid conditions (1), and use of fatty acids as carbon source (2). More recently it was shown that ethanol induced filamentous growth in solid medium (3). We observed mycelial growth of the fungus occurs in liquid medium.

Taking into consideration that the change of carbon source from glucose to fatty acids brings about a metabolic change from a six- to a two-carbon source (fatty acids are transformed into acetyl units), we considered that both processes might be associated. Accordingly, this change must bring about an alteration in the metabolic routes: glycolysis and TCA cycle, to glyoxylate shunt and gluconeogenesis.

In order to determine if this change also brought about an alteration in the expression of the genes involved in the different form of growth, we proceeded to analyze the transcriptome of the *U. maydis* strain FB2 (*a2b2*) grown in glucose and in ethanol using the Illumina RNA_Seq. Around 18 million of reads were obtained from each treatment. From the 6788 genes reported to exist in the genome of *U. maydis*, 543 (158 upregulated and 385 downregulated) were found differentially expressed. Among the upregulated genes that were over-expressed, with ethanol, we identified the following: those encoding repellent protein 1, aldehyde dehydrogenase, semialdehyde dehydrogenase, acetyl CoA carboxylase, phosphoenol pyruvate carboxykinase, acetylCoA -acetyltransferase and alanine-glyoxylate transaminase, all possibly related to ethanol metabolism.

Dissecting functional domains in the cation stress response transcription factor SltA

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A proper response to elevated extracellular concentrations of mono and divalent cations and alkalinity require the activity of the zinc finger transcription factor SltA in *Aspergillus nidulans*. As the well-known transcription factor PacC mediating ambient pH response, SltA is activated by proteolytic processing. The serine protease SltB cleaves the primary 78 kDa form of SltA to render the functional 32 kDa version. SltA 32 kDa comprises the DNA binding domain plus a very poorly conserved C-terminal region among homologues. In this work we have focused on the identification of proteolytic determinants and the nuclear transport signals in SltA. We found several nuclear localization signals along the SltA32kDa form, the need of SltA proteolysis for its nuclear localization and the cleavage site for SltB protease.

New structural and biochemical insights into gene regulation by MAT1-1-1 transcription factor from *Aspergillus fumigatus*

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The filamentous fungus *Aspergillus fumigatus* is a medically important human pathogen that causes invasive aspergillosis in immunocompromised individuals. *A. fumigatus* is found worldwide in soil and produces vast numbers of spores that are ubiquitous in the atmosphere. Exposure to spores can cause asthma and allergic sinusitis, among other lung diseases [1]. A fully functional sexual reproductive cycle that leads to the formation of ascospore containing fruiting bodies (cleistothecia) was discovered recently [2]. Mating in *A. fumigatus* is, as in many other filamentous ascomycetes, govern by two mating-type (MAT) transcription factors (TFs) MAT1-1-1 and MAT1-2-1, which possess an alpha domain and a high-mobility group (HMG) domain, respectively [3]. A recent study suggests that the pathogenicity of *A. fumigatus* is linked to the mating-type [4]. By now, it became apparent that MAT TFs control a wide variety of target genes also involved in other processes than sexual reproduction. However, it is not clear how MAT TFs selectively find functional genomic regions to regulate the expression of the target genes. Therefore, our main focus here is the biochemical characterization of the DNA-binding properties of the MAT1-1-1. Using the *E. coli* expression system and affinity chromatography, we obtained soluble truncated MAT1-1-1 derivatives. The best version was used for DNA-binding studies. The putative binding motif of MAT1-1-1 was found in *A. fumigatus* sex-related genes, which subsequently was verified by electrophoretic mobility shift assays. Furthermore, *A. fumigatus* MAT1-1-1 bound also the target genes from *P. chrysogenum* MAT1-1-1. These results point towards highly conserved

mechanisms of DNA recognition among the alpha domain TFs present in Ascomycota [5].

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Structure-function analysis of mating-type proteins from the penicillin-producing ascomycete *Penicillium chrysogenum*

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Penicillium chrysogenum is the main industrial source of the β-lactam antibiotic penicillin. Recently, it was shown that *P. chrysogenum* can reproduce sexually under laboratory conditions [1]. The chromosomal regions determining mating compatibility are designated as mating-type loci *MAT1-1* and *MAT1-2*. They encode mating-type transcription factors (TFs) MAT1-1-1 and MAT1-2-1, respectively, which were shown to be involved in sexual reproduction, as well as in the regulation of non-sexually related genes [2]. The presence of a sexual cycle in important industrial fungi provides a valuable genomic tool, which can be used for strain improvement without introducing undesirable mutations, responsible for genetic instability. However, insufficient knowledge about how mating-type TFs find and regulate target DNA is a major limiting factor for industrial strain improvement programs using conventional mating experiments.

The aim of this project is the detailed characterization of the structure and function of the MAT1-1-1 protein because the α-domain of MAT1-1-1 is exclusively present only in filamentous ascomycetes and so far no structural data are available for this domain.

We report about the optimized protein purification method, which was developed to obtain sufficient amounts of functional mating-type proteins for structural analysis. We characterized the DNA binding and dimerization properties of both TFs by performing electrophoretic mobility shift assay. Yeast-two hybrid analysis was used to prove the in vitro formation of a MAT1-1-1/MAT1-1-1 homodimer. In addition, we found the physical interaction between the MAT1-1-1 and MAT1-2-1 proteins, suggesting that the transcription of the correct genomic targets of MAT1-1-1 and MAT1-2-1 during the diploid state can be mediated by a functional MAT1-1-1/MAT1-2-1 heterodimer complex.

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Sensing by GPR16 impacts balanced regulation of enzyme production and chemical communication in *Trichoderma reesei*

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The workhorse *Trichoderma reesei* is one of the most important filamentous fungi applied in biotechnology, especially because of its high secretion of enzymes. However, recently also an interrelationship of enzyme production with secondary metabolism was shown. Transcription and expression of those important enzymes and metabolites is influenced by environmental factors via the light response pathway and the heterotrimeric G-protein pathway.

Our research showed that cellulase gene expression is regulated at a posttranscriptional level, for which the presence of the G-protein coupled receptor (GPCR) CSG1 is essential. CSG1 senses a precise concentration of glucose, hence indicating a sensing mechanism aimed at distinguishing dead litter material from living plants. Based on these findings we analyzed regulatory targets of CSG1 upon growth on cellulose. Interestingly, our results suggest that deletion of csg1 causes alterations in signal sensing as besides other signaling genes, also GPCR encoding genes are concerned. We studied the function of GPR16, transcript levels of which are abolished in the absence of CSG1. GPR16 does not only influence growth in a medium dependent manner, but its deletion causes an almost complete abolishment of cellulase production in constant darkness. Unexpectedly, the deletion also leads to a strong upregulation of the expression of the SOR-cluster genes and subsequently of the production of (various) secondary metabolites. Furthermore GPR16 deficient strains seem to have altered environmental and potentially surface sensing in a light dependent manner.

Consequently, glucose sensing by CSG1 is crucial for adjustment of sensing mechanisms in *T. reesei*. Altered sensing due to the negative effect on GPR16 impacts not only cellulase regulation and secondary metabolism, but also the recognition of the environment itself.

Cell dynamics of the peroxisomal protein Pex13 of *Podospora anserina*

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Peroxisomes are highly dynamic organelles, which perform multiple metabolic functions and that have critical roles in fungal development. Peroxisomes selectively import proteins by two canonical import pathways, which are driven by the receptors Pex5 and Pex7. This process is mediated by a set of peroxisome membrane proteins that are grouped in three complexes: the docking/translocation, the ubiquitination and the dislocation complexes. In particular, the protein Pex13 is associated with the docking complex, where interacts with Pex14 and Pex5, the proteins forming the pore for protein translocation. In *P. anserina* we have shown that Pex13 contributes to the regulation of peroxisome biogenesis to facilitate meiotic development. Here we show that Pex13 activity is subject to a complex regulation that depends on the distinct peroxisome import machinery modules. We found that wild-type cells have low levels of peroxisomal Pex13, which are importantly increased in mutants of Pex14, of the ubiquitination complex, or of the protein linking the docking and ubiquitination complexes (Pex8). Moreover, we found that Pex13 mislocalizes to a network of tubular structures different from peroxisomes in mutants of the dislocation complex, and to small discrete foci in mutants affected in peroxisomal membrane biogenesis. In addition, we found that Pex14 abundance also reciprocally depends on Pex13. Finally, we discovered that Pex13 activity is also regulated during meiotic development progression through changes in its peroxisomal abundance. These results reveal that Pex13 activity is subject to a fine regulation, which probably involves selective ubiquitination and degradation processes and crosstalk with the proteins driving peroxisome biogenesis.

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A novel mechanism of mitochondrial dysfunctions-triggered the calcium signalling-dependent fungal multidrug resistance

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Drug resistance in fungal pathogens have risen steadily over the last decades during long-term azole therapy or triazole usage in agriculture. Modification of the drug target protein to prevent drug binding is a major recognized route to induce drug resistance. However, mechanisms for emergence of new non-drug target-induced resi stance remain only

loosely defined. Here, we explore the molecular mechanisms of multidrug resistance resulted from an efficient adaptation strategy for survival in drug environments in the human pathogen *Aspergillus fumigatus*. We show that mutants conferring multidrug resistance are linked with mitochondrial dysfunction induced by defect in the heme A biosynthesis. Comparison of the gene expression profiles between drug-resistant mutants and their parental wildtype strain shows that multidrug-resistant transporters, chitin synthases and calcium signalling-related genes are significantly upregulated, while the mitochondrially-derived reactive oxygen species (ROS) scavenged-related genes are significantly downregulated. The upregulated-expression genes share consensus calcium-dependent serine threonine phosphatase–dependent response elements (the binding sites of calcium signalling transcription factor CrzA). Accordingly, drug-resistant mutants show enhanced cytosolic Ca2+ transients and persistent nuclear localization of CrzA. In comparison, calcium chelators significantly restore drug susceptibility and increase azole efficacy either in lab-derived or clinic-isolated *A. fumigatus* strains. Thus, the mitochondrial dysfunction as a fitness cost can trigger calcium signalling and therefore globally upregulate a series of embedding calcineurin-dependent-response-element genes, leading to antifungal resistance. These findings illuminate how fitness cost affects drug resistance and suggest that disruption of calcium signalling might be a promising therapeutic strategy to fight against non-drug target-induced drug resistance.

The *Aspergillus fumigatus* transcription factor SomA couples exopolysaccharide galactosaminogalactan synthesis and cell wall integrity

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Aspergillus fumigatus is a common opportunistic fungal pathogen that cause invasive aspergillosis, a lifethreaten disease in immunocompromised patients. The exopolysaccharide galactosaminogalactan (GAG) plays an important role during *A. fumigatus* invasive infection (Gresnigt *et al*., 2014). The GAG biosynthetic gene cluster was composed of five genes on chromosome 3. It has been reported that transcription factors SomA, MedA and StuA were involved in GAG production (Gravelat *et al*., 2013; Lin *et al*., 2015). However, the detail regulatory mechanism of GAG biosynthetic gene cluster remains largely unknown. To further understand the regulatory mechanism of GAG biosynthetic gene cluster, chromatin immunoprecipitation followed by sequencing (ChIP-seq) was used to identify genes under direct SomA transcriptional regulation. These results confirmed the direct regulation of GAG biosynthetic gene cluster by SomA. SomA could directly bind to a conserved motif in the promoters of GAG biosynthesis genes agd3 and ega3. In addition, SomA is also enriched in the promoters of medA and stuA, two developmental regulators of *A. fumigatus*. Moreover, ChIP-seq revealed a

new role for SomA in cell wall integrity. The direct regulation targets by SomA includes genes encoding the cell wall stress sensors MidA and Wsc3, chitin synthases and β-1,3-glucan synthase. Consistent with those findings, loss of somA increases *A. fumigatus* susceptibility to cell wall-perturbing agents. Interestingly, the cell wall stress could induce the overexpression of GAG biosynthetic gene cluster and this induction was depended on SomA. Collectively, this study elaborated the regulatory mechanism of GAG and cell wall integrity, uncovered a relationship between GAG production and cell wall stress.

Survival factor genes of *Mucor circinelloides* and their role in virulence

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M. circinelloides is a filamentous fungus belonging to the order Mucorales. Several members of this fungal group may cause life-threatening infections in immunocompromised patients called as mucormycosis. Survival factor (SVF) protein plays an important role in the protection of cells from oxidative stresses in baker yeast and participates in the sphingolipid biosynthesis of the cell membrane. Sphingolipid signalling plays an important role in the regulation of various cellular processes, including cell growth, survival and differentiation. Transcriptomic studies revealed the upregulation of the encoding genes in several human pathogenic fungi during the host-pathogen interactions. However, the function and regulation of the SVF proteins are still unknown in filamentous fungi.

In the *M. circinelloides* genome, two hypothetical svf genes were identified (svf1 and svf2). We have studied the expression of the genes after culturing the fungus under different conditions (e.g. incubation time, medium or temperature, aerob/anaerob) by real-time quantitative reverse transcription PCR. Using the CRISPR/Cas9 technique, single gene disruption mutants were constructed for each gene and we have started the characterization of the resulting strains. Macro-,micromorphology and sensitivity to different stressor chemicals (e.g. H_2O_2, congo red, calcofluor white) were tested. Mutants showed altered characteristics compared to the original strain suggesting that the cellular integrity may be damaged in the mutants. Pathogenicity of the mutants was also examined in alternative *Drosophila melanogaster* model and a decreased virulence was detected. We also carried out susceptibility test of our strains against various antifungal drugs.

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A ubiquitin-conjugating enzyme regulates *Trichoderma reesei* cellulase gene expression via facilitating Xyr1 binding to promoters

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Cellulase gene expression in *Trichoderma reesei* is exquisitely controlled by an intricate transcriptional network,wherein XYR1 (Xylanase regulator 1) has been identified as the most important transcriptional activator of cellulase/hemicellulase gene expression although its precise transactivating mechanism remains largely unkown. Here we identified a ubiquitin conjugating enzyme belonging to the UBC7 subfamily that participates in regulating cellulase gene expression. Disruption of the E2 gene markedly reduced cellulase biosynthesis.The E2 is mainly localized in the nucleus and co-localizes with XYR1. The TrE2 active site mutation C85A and the E3-binidng double mutant F62A/A96D were all unable to restore the cellulase expression of the Δubc4 strain, indicating that the important function of E2 in *T. reesei* cellulase gene expression requires an active E3/E2~Ub complex. An N77S mutation in TrE2 that specifically eliminates RING E3-mediated isopeptide formation between ubiquitin and substrates but not HECT E3 transthiolation, however, rescued the ΔTrE2 phenotype. Thus the important function of TrE2 in contributing to cellulase gene expression is with a HECT E3. Our results further showed that TrE2 disruption of did not affect xyr1 transcription but led to a significantly lower occupancy of XYR1 on cellulase gene promoters, which could not be restored by overexpressing XYR1. Together, these results suggest that TrE2 participates in regulating cellulase gene expression via ubiquitylation of a specific substrate to contribute tothe efficient XYR1 binding to cellulase gene promoters.

The monothiol glutaredoxin GrxD is essential for sensing iron starvation in *Aspergillus fumigatus*

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Efficient adaptation to iron starvation is an essential virulence determinant of the most common human mold pathogen, *Aspergillus fumigatus*. Here, we demonstrate that the cytosolic monothiol glutaredoxin GrxD plays an essential role in iron sensing in this fungus. Our studies revealed that (i) GrxD is essential for growth; (ii) expression of the encoding gene, grxD, is repressed by the transcription factor SreA in iron replete conditions and upregulated during iron starvation; (iii) during iron starvation but not iron sufficiency, GrxD displays predominant nuclear localization; (iv) downregulation of grxD expression results in de-repression of genes involved in iron-dependent pathways and repression of genes involved in iron acquisition during iron starvation, but did not significantly affect these genes during iron sufficiency; (v) GrxD displays protein-protein interaction with components of the cytosolic iron-sulfur cluster biosynthetic machinery, indicating a role in this process, and with the transcription factors SreA and HapX, which mediate iron regulation of iron acquisition and iron-dependent pathways; (vi) UV-Vis spectra of recombinant HapX or the complex of HapX and GrxD indicate coordination of iron-sulfur clusters; (vii) the cysteine required for iron-sulfur cluster coordination in GrxD is *in vitro* dispensable for interaction with HapX; and (viii) there is a GrxD-independent mechanism for sensing iron sufficiency by HapX; (ix) inactivation of SreA suppresses the lethal effect caused by GrxD inactivation. Taken together, this study demonstrates that GrxD is crucial for iron homeostasis in *A. fumigatus*.

MpkB MAP kinase pathway is required for sexual development, but not for mycotoxin production, in *Aspergillus nidulans* and *Aspergillus flavus*

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In eukaryotic systems, MAP kinase pathways play important roles in regulating stress responses as well as growth and development. MpkB MAP kinase in a filamentous fungus *Aspergillus nidulans* has been known to coordinate sexual development and secondary metabolism, including sterigmatocystin (ST) production. In this study, however, the results of the ST production analysis of wild type and mpkB deletion mutants showed that the mutation did not affect the ST production and ST related gene expression. Furthermore, ST production of ΔmpkB, ΔmkkB, and ΔmpkBΔmkkB mutants in the veA+ background was similar with wild type. Also, MpkB constitutive activation or inactivation mutants showed no significant effect on the ST production. Interestingly, ST production of mpkB and mkkB mutants was remarkably delayed in the veA1 background, suggesting that the ST production is affected primarily by the veA gene. Similarly, in *Aspergillus flavus*,

MpkB ortholog AflmpkB mutant couldn't produce any sclerotia, but it produced aflatoxin B1 normally. Taken together, the mpkB gene alone does not affect mycotoxin production such as ST in *A. nidulans* or aflatoxin B1 in *A. flavus*, indicating that the signaling of MpkB MAP kinase and mycotoxin production were governed by independent pathways.

VosA-dependent ascospore gene expression in *Aspergillus nidulans*

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VosA plays significant roles in asexual sporulation in the model filamentous fungus *Aspergillus nidulans*. In the present study, we characterize the roles of vosA in sexual spores. During ascospore maturation, deletion of vosA causes rapidly decreased spores viability. In addition, absence of vosA results in lack of trehalose and decreased in tolerance to thermal and oxidative stresses. Genome wide analysis demonstrated that loss of vosA induces expression of sterigmatocystin biosynthesis genes and slightly increases sterigmatocystin contents in ascospores. In the vosA deletion mutant ascospores, expression of other secondary metabolite gene clusters including asperthecin, microperfuranone, and monodictyphenone increased, but mRNA expression of genes involved in primary metabolite processes was decreased. Moreover, deletion of vosA results in alters mRNA expression of genes associated with cell wall integrity and trehalose biosynthesis. Overall these results demonstrate that VosA is a key regulator for sporogenesis in both asexual and sexual spores in *A. nidulans*.

Calcium signaling genes play a role in stress tolerance, thermotolarence, cellulose degradation, and circadian clock in *Neurospora crassa*

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We investigated the calcium (Ca2+) signaling process in the model filamentous fungus *Neurospora crassa*. We identified differential expression of various Ca2+ signaling genes under various cellular conditions in *N. crassa*. We determined the expressions of the several Ca2+ signaling genes ncs-1, camk-2, plc-1, cmd, cna-1, cnb-1, and splA2 under various cellular conditions using qRT-PCR. We found that the calcineurin subunits CNA-1 and CNB-1 play a critical role in osmotic stress, thermotolerance and circadian clock in *N. crassa*. The cnb-1 expression was increased about 2.5-fold under the heat-shock condition. In addition, we found that the CRZ-1 transcription factor binds to the promoter of the heat-shock protein (hsp)-80 gene to provide thermotolerance in *N. crassa*. The CRZ-1 protein is dephosphorylated by the Ca2+/calmodulin (CaM) dependent phosphatase calcineurin for its nuclear localization. We predicted transcription factors controlling the calcineurin and the calmodulin (CaM) expressions and validating the candidate transcription factor using several techniques including chromatin immunoprecipitation (ChIP), electrophoretic mobility shift assay (EMSA), co-localization, and co-immunoprecipitation assays. We also found that these Ca2+ signaling genes play a role in the circadian clock by modulating the expression of frq-1 and wc-1 genes. Furthermore, we identified a novel role of sPLA2 in cellulose degradation. We previously showed that the NcZrg-17 gene has a role in tolerance to endoplasmic reticulum stress and cellulose degradation in *N. crassa*. Therefore, Ca2+-signaling plays an important role in tolerance to stress, heat-shock, cellulose degradation, and the circadian clock in *N. crassa*.

Calcium signaling genes play an important role in tolerance to calcium stress and survival under various stress conditions in *Neurospora crassa*

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We studied the calcium (Ca2+) signaling process in the model filamentous fungus *Neurospora crassa*. We found that the Ca2+ signaling plays a critical role in coping with the high extracellular Ca2+ and heat-shock stresses in N. crassa. We previously showed that the crz-1 transcription factor upregulates expression of the Ca2+ sensor for the tolerance to Ca2+ stress in *N. crassa*. Here, we identified that the calcineurin is important for survival under cellular conditions in *N. crassa*. In addition, calmodulin plays a critical role in survival under various stress conditions in *N. crassa*. Furthermore, we also identified a novel role of sPLA2 in cellulose degradation. Therefore, Ca2+-signaling plays an important role in tolerance to Ca2+ and survival under various cellular conditions in *N. crassa*.

Nutrient transporter translocation to the plasma membrane via Golgi bypass in *Aspergillus nidulans*

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Eukaryotic nutrient transporters, being polytopic transmembrane proteins, are thought to traffic from their site of synthesis, the ER, to the plasma membrane, through the Golgi, using the conventional vesicular trafficking pathway. Notably, however, current knowledge on the mechanism of membrane cargo secretion has been obtained by using mostly proteins that are polarly localized, and to our knowledge no report has ever shown formally the involvement of Golgi specifically in the PM localization of de novo made transporters. Here we show that in the *Aspergillus nidulans* several nutrient transporters follow an unconventional trafficking route that initiates at ER-exit sites (ERes) and requires clathrin and actin polymerization, but surprisingly, does not involve passage through the Golgi or other key effectors of conventional secretion (Rab11, AP-1, microtubules or endosomes). Our findings will be discussed relative to other studies concerning unconventional trafficking routes in other systems and within a rationale on why transporter traffic bypasses the Golgi. Last but not least, we will propose that the trafficking mechanism uncovered here in a lower eukaryote might hold true for the sorting of nutrient transporters and other house-keeping non-polar cargoes in higher organisms.

An atypical heat-shock protein interacts with the key ribonuclease of a dicer-independent RNAi mechanism in *Mucor circinelloides*

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The discovery that the RNA-silencing mechanisms of *Mucor circinelloides* regulate the expression of many genes prompted the search for proteins that could regulate these mechanisms in response to environmental signals. We reasoned that some elements of the signal transduction pathway could interact with key components of the RNAi mechanisms. Therefore, we search for proteins that interact with R3B2, an atypical RNase III that is essential in the non-canonical Dicer-independent RNAi mechanism by using a yeast two-hybrid system. Thus, we identified the interaction of R3B2 with itself, and furthermore, with members of the HSPA12 family, an atypical conserved group of heat-shock protein 70 (HSP70) that is suggested to be involved in functions distinct from

classical HSP70. Therefore, we named the *M. circinelloides* protein as HSPA12.

Interestingly, this HSPA12 did not show interaction with any other protein of the RNA-silencing mechanisms descrive in *M. circinelloides*, suggesting that it can only regulate ribonuclease activity of R3B2, and hence, the regulation of the non-canonical Dicer-independent RNAi mechanism. Regulation of R3B2 activity by HSPA12 is supported by the fact that HSPA12 interacts with the R3B2 region that presents the catalytic domain and one of the double stranded RNAbinding domains. Moreover, HSPA12 could regulate the non-canonical RNAi mechanism in response to high temperature because the hspa12 deletion mutants showed a poor growth at high temperature (30º C) in comparison with the wild-type strain. Opportunistic pathogens, like *M. circinelloides*, have to grow at body temperature, hence HSPA12 could be important for virulence.

Genetic regulation of *Ustilago maydis* cellular differentiation processes by polyamines

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Polyamines are low molecular weight molecules, which possess several amino groups and are ubiquitous and indispensable for the normal growth of most of the living beings. Polyamines regulate different cellular process, especially proliferation and cellular differentiation. Nevertheless, their mode of action is not completely known. Previously, we showed that polyamines were required for the dimorphic transition of *Ustilago maydis*. This is a dimorphic phytopathogenic Basidiomycota fungus that produces the maize disease known as common smut. In nature, it grows in the form of haploid yeasts. When two sexually compatible yeasts mate they form a mycelial dikaryon which invades the host plant. In vitro conditions the fungus grows in the yeast form at pH 7 and in the mycelial form in a pH 3 medium. This *in vitro* dimorphic transition requires polyamines. Thus, Δodc mutants are unable to grow in the mycelial form at pH 3, unless supplemented with a high concentration of putrescine.

In the present study we proceeded to analyze if polyamines were also required for the genetic regulation of the natural process of *U. maydis* mating, and the dimorphic transition that occurs therein. We observed that at low polyamine concentrations (0.1 mM putrescine), *U. maydis* sexually compatible Δodc mutants were impaired in the production of conjugation tubes and were unable to form the dikaryotic mycelium. Analysis of the expression of the genes coding for the sexual pheromones (Mfa1 and Mfa2) demonstrated that they are directly regulated by polyamines. With these results, we conclude that polyamines are necessary for mating in *U. maydis* directly regulating the

expression of the essential genes for this process. In adddition, by microarrays analyses, we identified 2959 genes (1531 down-regulated and 1428 up-regulated) differentially regulated by putrescine at pH 7; and 475 genes (146 down-regulated, 329 up-regulated) at pH 3.

Functional characterization of an atypical RNase III involved in a RNAi-related mechanism of RNA degradation in *Mucor circinelloides*

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The filamentous fungus *Mucor circinelloides* shows an intricate RNAi mechanism with at least three pathways that control several physiological and developmental processes. We have identified three RNase III proteins that participate in the RNAi-related pathways. Two canonical Dicer-like proteins produce the small-RNAs that drive the defense against exogenous nucleic acids, the control of some genes at mRNA level, and the production of drugresistant epimutants. Additionally, R3B2, a bacterial-like RNase III with two double-stranded RNA binding domains (dsRBDs), plays a pivotal role in a non-canonical Dicer-independent RNAi mechanism, which is proposed to degrade specific mRNAs with short stretches of dsRNA produced by the RNA-dependent RNA polymerases of the fungus.

In this work, we characterized the RNA binding and cleaving properties of R3B2. We show using Electrophoretic Mobility shift Assays that R3B2 binds both ssRNA and dsRNA through its two dsRBDs, and substitutions of key amino acids or deletion of these domains caused the loss of affinity for RNA. Although it can bind both ssRNA and dsRNA, it only cleaves ssRNA and this substrate preference relies on the RNase III-like domain. The activity and binding features of R3B2 concur with the characteristics of the ssRNAs produced by the non-canonical RNAi mechanism, confirming that this protein is the main ribonuclease of this mechanism. R3B2 is present only in *Mucorales* and shows low sequence identity to bacterial RNase III proteins. However, the crystal structure of the RNase III-like domain of R3B2 revealed that it adopts a homodimeric structure similar to *bona fide* RNase III proteins, which cut dsRNA. The substitution of R3B2 RNase III-like domain with the canonical domain from *Escherichia coli* RNase III restored the substrate specificity against dsRNA, indicating that the substitutions present in the R3B2 catalytic domain determines its unusual substrate specificity.

Intracellular functions of hydrophobins and other surface-active proteins in *Trichoderma*

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The tubular structure of the fungal body requires surface hydrophobicity modulation because absorptive nutrition is efficient if the body is hydrophilic, while reproduction is usually performed by hydrophobic air-dispersed spores. Therefore, the development of a fungal colony is accompanied by the secretion of various surface-active proteins such as hydrophobins (HFBs), cerato-platanins (CPs), or other small secreted cysteine-rich proteins (SSCPs). Located outside the cell, HFBs self-assemble at hydrophobic/hydrophilic interfaces, thereby changing the surface properties and mediating fungal interactions with the environment. Here, we revealed a distinctive HFB secretory pathway in aerial hyphae that includes a massive intracellular accumulation followed by a coordinated release shortly before conidiation. Using the HFB enriched mold *Trichoderma* and the HFB-free yeast *Pichia pastoris*, we proved that despite the presence of the signal peptides that lead HFBs to ER and the conventional vesicle trafficking, only a minor portion of HFBs is secreted through this pathway in both fungi. Instead, HFBs tended to preferentially accumulate in lipid bodies (LBs) and/or vacuoles. The transmission electron microscopy revealed that the internalization of such LBs in vacuoles resulted in the hydrophobic/hydrophilic interface and the subsequent formation of HFB vesicles (multicisternal structures), which putatively contributed to the maintenance of turgor pressure in aerial hyphae and prevented cell aging. The analysis of *Trichoderma* mutants producing fluorescently labeled CPs suggested a similar pattern of intracellular accumulation, albeit associated with different developmental stages. We will present results suggesting that the differential intracellular accumulation of surface-active proteins in functionally specialized hyphae (trophic or spore-producing) plays a pivotal role in *Trichoderma* development, colony architecture, reproduction, and fitness.

Poster Session 1.3
PRIMARY AND SECONDARY METABOLISM

TUESDAY, FEBRUARY 18

18:00 - 19:30 | Location: **Frentani Convention Center**

Rewiring metabolic pathways for organic acid production in the filamentous fungus *Aspergillus niger*

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Itaconic acid (IA), a C5-dicarboxylic acid, has previously been identified as one of the top twelve biochemicals that can be produced by biotechnological means. IA is naturally produced by *Aspergillus terreus,* and in the past we developed heterologous production in the related species *Aspergillus niger*. Remarkably, we observed that during high producing conditions and elevated titers *A. niger* detoxifies the extracellular medium of IA. Transcriptome analysis has led to the identification of two novel and previously unknown IA bioconversion pathways in *A. niger*. One pathway is proposed to convert IA into pyruvate and acetyl-CoA through the action of itaconyl-CoA transferase (IctA), itaconyl-CoA hydratase (IchA) and citramalyl-CoA lyase (CclA), similar to the pathway identified in *A. terreus*. Another pathway putatively converts IA into 1-methyl itaconate through the action of trans-aconitate methyltransferase (TmtA). Upon deleting the key genes *ictA* and *ichA* we have observed increased IA production and titers and cessation of IA bioconversion, whereas surprisingly, deletion of *tmtA* lead to strong reduction of heterologous IA production. Concomitant to the IA biodegradation pathway secretion of an unknown compound was observed. Based on published results on the IA biodegradation pathway, we hypothesized that the final product of IA biodegradation in *A. niger* may be citramalic acid (CM) which could be confirmed by HPLC analysis. Interestingly, further exploration of the effect of metabolic rewiring on organic acid production by transcriptome analysis led to the identification of the genes encoding another unknown biosynthetic cluster that is putatively involved in the biosynthesis of CM. Upon overexpression of the putative citramalate synthase and genomically clustered organic acid transporter, we observe strongly increased CM bioproduction by *A. niger*.

Phylogenetic and binding pocket analysis of fungal adenylation domains towards their substrate specificity predictions

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Non-ribosomal peptide synthetases (NRPSs) are mega enzymes involved in the biosynthesis of non-ribosomal peptides (NRPs). Adenylation (A-) domains are responsible for selective recognition of substrates that are incorporated in NRPs. A-domains activate multitudes of substrates harboring carboxy terminus. 247 NRPS specific A-domain substrates or NRP monomers are listed in Norine database [1].

Prediction of correct substrates is paramount to decoding the final chemical structures of NRPS assembly lines. However, this is challenging for fungal NRPSs because presently available tools, based predominantly on bacterial data, do not give reliable predictions for fungi. In our poster, we will demonstrate the phylogenetic reasons why the tools trained on bacterial sequences fail to give correct predictions for fungal NRPSs.

We have attempted to predict substrates for fungal A-domains by utilising structure-based features in a neural network classifier. In the poster, we will present our novel approach called Neural Network-based A-domain Substrate Specificity prediction Classifier (NNassc). We have combined physicochemical and structural descriptors of nine binding residues for substrate prediction. As opposed to other tools, such as SANDPUMA [2], our approach involves prediction of substrate substructures rather than substrate classes. NNassc was validated on internal and external validation datasets and outperformed SANDPUMA. Our model predicts correct substrates in all cases except a few Phenylalanine binding pockets while SANDPUMA works only with Alanine or Tyrosine binding pockets. In addition, NNassc can predict “unusual” substrates that could not be predicted by other tools.

1. Flissi, *et al*., Nucleic acids research 44.D1 (2015): D1113-D1118

2. Chevrette, *et al*., Bioinformatics 33.20 (2017): 3202-3210

Isolation and identification of an unusual, modified, cyclic hexapeptide from the filamentous fungus *Fusarium graminearum*

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Filamentous fungi produce many secondary metabolites with diverse biological activities interesting for medical applications. Among these are polyketides and non-ribosomal peptides. The fungus *Fusarium graminearum* holds 15 genes coding for polyketide production and 19 genes coding for non-ribosomal peptide production. So far, only 8 polyketides and 4 non-ribosomal peptides have been assigned to the genes responsible for their production. The yet unknown secondary metabolite products hold the potential to provide new candidates for the drug discovery pipeline serving the increasing demand for novel drugs, including antibiotics and anti-cancer agents.

In this poster I present the detection, isolation and identification of a new fungal secondary metabolite applying RP-HPLC-MS-SPE-NMR. It was found to be an unusual cyclic peptide not described in the literature.

Genomic evidence of the involvement of a *cyclase* gene in the biosynthesis of ochratoxin A

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Filamentous fungi produce a multitude of low-molecular-mass compounds known as secondary metabolites (SM). Many of these compounds have known applications in medicine and biotechnology,; but SM are also implicated in food safety and human health as mycotoxins. The widespread use of Next-Generation Sequencing (NGS) for fungal genome sequencing has led to identification of SM clusters for known metabolites as well as a significant number of novel predicted SM gene clusters. However, most of these clusters yet to be examined in the detail needed to completely understand the pathway steps and the regulation of the biosynthesis. This genome sequencing approach led to the identification of the biosynthetic genes cluster of ochratoxin A (OTA) in the *Aspergilli*. Recently, by a gene knock-out approach the role of five genes in the OTA biosynthesis was demonstrated. However, the first step of the OTA biosynthesis polyketide cyclization leading to the formation of 7-methylmellein has not yet been completely clarified. The current accepted hypothesis is that this step is mediated by a C-terminal domain of the PKS protein which has a cyclization activity, namely a cyclase domain.

An alternative hypothesis may involve a cyclase protein encoded by a distinct gene. In this regard, several fungal terpene cyclases genes have been characterized, including the *trichodiene synthase* in *F. sporotichioides*, and the *ggs2* in *G. fujikuroi*, involved in gibberellin production. Recently, detailed analysis of *Aspergilli* genomes has led us to the identification of a gene sequence showing similarity to bacterial polyketide cyclases. This gene is located in the OTA cluster, between the PKS and the NRPS encoding genes, and is present in the genome sequences of all currently sequenced OTA producing fungi. The characterization of the OTA *cyclase* gene, phylogenetic relationships and expression analysis in OTA producing and not producing conditions are reported for the first time in this work.

Cellulolytic activity in *Aspergillus spp*. contaminating livestock feeds and raw materials

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The contamination by *Aspergillus spp*. have become a global concern in food and feedstuffs and can lead to a reduction in yield and quality of agricultural products with significant economic losses. Most species of *Aspergillus* produce cellulose-degrading enzymes and some of them also have mycotoxigenic activity. This study aimed i) to evaluate the *Aspergillus* contamination in feeds (16) and row materials (32) collected in Sicily; ii) to isolate and identify fungi belonging to the genus *Aspergillus* and iii) to analyze their ability to produce cellulolytic enzymes. *Aspergillus* spp. contamination was evaluated on PDA using serial ten-fold dilution and spread plate technique (Mirabile *et al*., 2019) and ranged from 50 to $9x10^6$ CFU/g and from 45 to $3,3x10^7$ in feeds and raw materials, respectively. The most recurrent colonies were identified by morphological features, ITS and β-tubulin sequence analysis as *A. niger, A. tubingensis, A. brasiliensis, A. fumigatus* and *A. flavus*.

Qualitative production of cellulolytic enzymes performed according to Mandels *et al*. (1976) and time course of endo and exo-β-1,4 glucanase activity (UI/ml) determined in solid submerged fermentation (Ghose, 1987), revealed a variability between *Aspergillus* species and was strain-dependent. *A. tubingensis* SAAF14, *A. flavus* MUCL18903 and *A. brasiliensis* MUCL20039 exhibited the highest CMCase and FPase activity of 2.16, 2.37 and 0.99 UI/ml and 0.65, 0.92, and 0.42 UI/ml, respectively. The presence of these *Aspergillus* isolates with high cellulolytic activity could represent a potential risk for the food quality of the contaminated food.

Ghose TK. 1987. Measurement of cellulase activities. Pure & Appl. Chem. 59(2):257-268.

Mandels M, Andreotti R, Roche C. 1976. Measurement of saccharifying cellulase. Biotechnol. Bioengng. Syrup. 6:21-33.

Mirabile G, Bella P, Conigliaro G, Giambra S, Alberto Vazquez M, Davino S, Torta L. 2019. Fungal contaminants in Sicilian livestock feeds and first studies on the enzymatic activity of *Aspergillus* isolates. Cuban J. Agr. Sci. 53(4):1-14.

Genetic engineering of fungi exploiting pyrimidine salvage pathway-based self-encoded selectable markers

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Selectable markers are essential for a wide range of applications in genetic engineering. In this work, we demonstrate the use of pyrimidine salvage-based marker genes for the genomic integration of DNAs of interest (DOIs). The described technology is based on homologous recombination-driven replacement of endogenously encoded, negative selectable markers with DOI giving resistance to 5-fluorocytosine (5FC) or 5-fluorouracil (5FU). Proof-of-principle experiments in the human fungal pathogen *Aspergillus fumigatus* using GFP and LacZ reporter cassettes uncovered three loci suitable for transformation selection: *fcyB* (5FC permease), *fcyA* (5FC deaminase) and *uprt* (5FU phosphoribosyltransferase). Loss of individual activities resulted in differential 5FC/5FU resistance, allowing their consecutive use for the insertion of multiple DOIs. Described applications include simultaneous multicolor localization microscopy and the production of penicillin via genetic insertion of the 17-kb biosynthetic gene cluster. In addition to *A. fumigatus*, we successfully utilized orthologous markers genes in *Penicillium chrysogenum* and *Fusarium oxysporum*. Evolutionary conservation of the pyrimidine salvage pathway and the versatile applicability further highlights the potential of this technology for genetic and metabolic engineering.

Pyrimidine salvage enzymes and their role in the metabolization of fluoropyrimidines in *Aspergillus fumigatus*

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Annually more than 1.5 million people die from fungal diseases. A major proportion is caused by invasive and chronic mold infections, predominantly by the most prevalent airborne mold pathogen *Aspergillus fumigatus*.

Currently only 3 major classes of antifungal acting agents are used to treat *Aspergillus* infections: azoles, echinocandins and polyenes. A fourth class, nucleobase analogs, with its only member 5-flucytosine (5FC) is barely used for the treatment of aspergillosis. 5FC represents a prodrug and requires intracellular, pyrimidine salvage-mediated metabolization into toxic RNA and DNA nucleotides to inhibit fungal growth. Previous work has shown that 5FC is highly efficient against this fungus at pH5 in comparison to neutral pH, where the antifungal activity of 5FC is insignificant.

In this work we functionally characterized pyrimidine salvage enzymes in *A. fumigatus* involved in the metabolization of 5FC as well as its derivatives 5-fluorouracil and 5-fluorouridine and assessed the role of individual genes in resistance to the respective molecules. To evaluate if further environmental triggers interfere with 5FC activity, we tested its antifungal activity during various host-niche related stress conditions. Moreover, we generated fluorescent reporter strains to monitor the expression of genes playing major roles in 5FC metabolization under different stress variables.

Taken together, this work aims to acquire a comprehensive understanding on the genetic and molecular factors contributing to the antifungal activity of 5FC against *A. fumigatus*.

Genus-wide analysis of *Fusarium* polyketide synthases uncovers broad natural product potential

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Collectively, species of *Fusarium* cause economically important diseases on virtually all crop plants, and many species produce secondary metabolites (SMs) that are toxic to animals (i.e., mycotoxins) and can accumulate in crops where they pose health risks to humans, livestock, and pets. Polyketides are the most common group of fungal SMs and

are formed by polymerization of simple carboxylic acids (acyl-CoA) via the activity of large, multidomain polyketide synthases (PKSs). To gain further insight into the biosynthetic potential of *Fusarium*, we examined the content and phylogenetic relationships of PKS genes in genome sequences of 214 species that represent the known breadth of phylogenetic diversity in the genus. Maximum likelihood analysis of the predicted amino acid sequences of 2975 PKS genes retrieved from the genome sequences resolved 130 distinct clades. We propose that most clades correspond to a structurally distinct polyketide product. Comparisons of the genes flanking the *Fusarium* PKS genes to previously characterized SM biosynthetic gene clusters in other Ascomycetes indicate that *Fusarium* has the potential to synthesize multiple SMs, including lovastatin- and cyclosporine-like metabolites, that have not heretofore been ascribed to this genus. This genus-wide study highlights the biosynthetic potential of fusaria and will help identify novel fungal SMs.

Ribosomal peptides derived from KEX2-processed repeat proteins (KEPs) in fungal defense and development

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Fungal peptides are an important source of bioactive natural products and therapeutics. Recently, a novel class of fungal peptides, derived from KEX2-processed repeat proteins (KEPs), was defined. KEPs consist of an N-terminal secretion sequence and repeats of short peptide sequences that are separated by dibasic (KR or RR) residues. The dibasic residues are recognized and cleaved by the Golgi endopeptidase KEX2. Additional exopeptidases can further trim the released peptides before they are exocytosed. A well characterized representative of this class of peptides is the α-pheromone of the yeast *Saccharomyces cerevisiae*. Recent biocomputational analysis of 250 fungal genomes suggested that KEPs are widespread and give rise to a broad variety of secretory peptides of yet unknown function. In this project, we are investigating the biosynthesis and function of predicted KEPs and their derived peptides in the mushroom *Coprinopsis cinerea*. Using CRISPR-Cas9 mediated gene knockouts combined with MS-based peptidomics we aim at the identification and structural characterization of the predicted peptides in the supernatant of *C.cinerea* cultures. Additionally, we express the peptide precursors in the yeast *Pichia pastoris* and synthesize the predicted/confirmed mature peptides using a peptide synthesizer. These peptides will be tested for their function in hyphal growth and development as well as fungal defense against bacteria and nematodes. The gained insight will allow to further define this novel class of precursor proteins and might lead to the discovery of novel peptides with interesting bioactivities.

Bioprospecting a newly identified fungus from the Borneo rain forest regarding its bioactive properties

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Due to the over-use of antibiotics in both, healthcare and agriculture more and more antibiotic-resistant bacteria are emerging. Consequently, novel antibiotics are needed to treat infections caused by antibiotic-resistant pathogens. However, research on fungi as natural sources of antibiotics has declined in the last decade, as the chance of rediscovery of bioactive secondary metabolites (SM) has increased. We decided to take advantage of ant-fungus mutualism as ants and their surrounding fungi can be a promising source of antibiotic substances. The chosen strain derives from the environment of nests of the "exploding ants" *Colobopsis explodens* from the Borneo rain forest. We used different nutritional conditions in liquid and solid media to induce the secondary metabolite production in the fungus. Additionally, we combined the different media with epigenetic modifiers in order to increase the chance of induction of biosynthetic gene clusters which are silent under laboratory conditions. The resulting SM were extracted, and their antimicrobial effect was investigated on different model organisms representing different types of microorganisms (e.g. *Escherichia coli, Bacillus subtilis, Fusarium oxysporum*, *Trichoderma reesei*, *Saccharomyces cerevisiae*). Those extracts resulting in positive bioactivity assays were additionally tested against antibiotic-resistant strains. Further, we sequenced the genome of this fungus to lay the groundwork for metabolite-cluster correlation via transcriptome analyses.

Engineering pentose catabolism of *Aspergillus niger* for the production of metabolites from lignocellulosic biomass

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The filamentous ascomycete *Aspergillus niger* is able to efficiently degrade lignocellulosic biomass to monomeric sugars that it can use as a carbon source. In nature, D-xylose and L-arabinose are the most abundant monosaccharides after D-glucose, being major constituents of the plant cell wall polysaccharides xylan, pectin and xyloglucan. These sugars are converted through the pentose catabolic pathway (PCP), of which the final step links to the pentose phosphate pathway. Up to now, only some of the PCP genes and enzymes of *A. niger* have been characterized in detail. Thus, the aim of this study was to obtain a better understanding of the pentose catabolism of *A. niger* by identifying and characterizing the deletion strains for all the genes related to pentose catabolism, including recently identified back-up genes of this pathway. The influence of the deletion strains on growth on plant biomass and re-routing of sugar catabolism was also studied in order to gain a better insight into the flexibility of this fungus in using plant biomass-derived monomers as substrates. The results of this study will therefore also facilitate strain engineering of *A. niger* as a cell factory for the production of metabolites related to this pathway, such as xylitol.

A portion of the research was performed using EMSL (grid.436923.9), a DOE Office of Science User Facility sponsored by the Office of Biological and Environmental Research.

rmtA-Dependent Transcriptome and its Role in Secondary Metabolism, Environmental Stress, and Virulence in *Aspergillus flavus*

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Aspergillus flavus colonizes numerous oil seed crops such as maize, peanuts, treenuts and cottonseed worldwide, contaminating them with aflatoxins and other harmful toxins. Previously our lab characterized the gene *rmtA*, which encodes an arginine methyltransferase in *A. flavus*, and demonstrated its role governing the expression of regulators in the aflatoxin gene cluster and subsequent synthesis of toxin. Furthermore, our studies revealed that *rmtA* also controls conidial and sclerotial development implicating it as an epigenetic regulator in *A. flavus.* To confirm this, we performed a RNA sequencing analysis to ascertain the extent of *rmtA*'s influence on the transcriptome of *A. flavus*. In this analysis we identified over 2000 genes that were *rmtA*-dependent, including over 200 transcription factor genes, as well as an uncharacterized secondary metabolite gene cluster possibly responsible for the synthesis of an epidithiodiketopiperazine-like compound. Our results also revealed *rmtA*-dependent genes involved in multiple types

of abiotic stress response in *A. flavus*. Importantly, hundreds of genes active during maize infection were also regulated by *rmtA*. In addition, in the animal infection model, *rmtA* was dispensable for virulence, however forced overexpression of *rmtA* increased mortality with respect to the wild type.

Studies on sugar transporter CRT1 reveal new characteristics that are critical for cellulase induction in *Trichoderma reesei*

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Trichoderma reesei is an ascomycete fungus that has a tremendous capability of secreting extracellular proteins. It natively produces a wide range of lignocellulose-degrading enzymes, but it has also been used for the production of heterologous proteins. Although many aspects of the biology of this organism have been unfolded, the roles of the many sugar transporters coded in its genome are still a mystery with a few exceptions. One of the most intriguing discoveries in relation to the sugar transporters of this fungus has been CRT1 (cellulose response transporter 1). Although CRT1 was reported in three different publications in the same year, the conclusions made by the authors were not uniform regarding the biological role and function of this transporter, since there were some indications that it may be a sensor rather than a transporter. In our study, we show that CRT1 is indeed a sugar transporter with high affinity for cellobiose and lactose, and that it does not appear to function as a sensor since the cellulase induction effect could be reconstituted by adding heterologous sugar transporters in place of CRT1. It was also observed that the length of N-terminal sequence, which was found to vary in different sequence annotations, has a strong impact on its ability to transport cellobiose and lactose. In a *crt1* deletion background, which is impaired in cellulase secretion, adding back the longer version of CRT1 or either *N. crassa* CDT-1 or *A. nidulans* LacpB/CltB was enough to restore the wild-type cellulase induction and secretion phenotype. However, when the shorter version of CRT1 was added back it conferred only partial restoration of this function. These results shed light on the metabolism of cellulose-derived oligosaccharides, which are believed to be the inducers of the cellulase and hemicellulase production in many ascomycete fungi, and therefore knowledge about their transport may lead to improvements in strain engineering.

Secondary metabolic response of *Aspergillus nidulans* to intimate interaction with *Aspergillus fumigatus*

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Fungal genome contains a large number of cryptic biosynthetic gene clusters for new compounds which are not expressed under standard laboratory conditions. For expression of cryptic gene clusters, several strategies, such as use of chemical elicitors or heterologous gene expression, have been employed. These days co-cultivation is the method of choice to obtain the biosynthetic products of cryptic genes. Thus, a close study on the molecular mechanisms underlying the activation of biosynthetic gene clusters by co-cultivation is necessary for development of technology to exploit potential of fungal secondary metabolism.

We searched for fungal interactions that stimulate production of secondary metabolites by screening combinations of several *Aspergillus* species. As a result, we discovered that the production of antimicrobial diphenylethers (DPEs) in *Aspergilus nidulans* (*An*) is promoted by combined culture with a pathogenic fungus, *Aspergillus fumigatus* (*Af*). The main products of the combined culture were three DPEs, violaceols I, II, and diorcinol. On the other hand, in co-culture of the two fungi separated by a dialysis membrane (separated co-culture), the main product was diorcinol, which suggested that the contact with *Af* is necessary for the stimulation of violaceols biosynthesis in *An*.

We also found the activation of several biosynthetic gene clusters encoded in the genome of the two fungi for secondary metabolites including the orsellinic acid gene cluster (*ors* cluster) by the combined culture mentioned above by RNA-seq analysis. The *ors* cluster is involved in several bioactive compounds including violaceols and diorcinol in *An*. Furthermore, we revealed that the *ors* cluster excepting for *orsE* is upregulated in the separated co-culture by real-time PCR (fold change of >5). We assume that the DPEs production profiles of the combined culture and the separated co-culture could reflect the *ors* cluster expression profiles specific to the two types of culture.

Sit1 and Sit2 mediate utilization of ferrichrome-type and ferrioxamine-type siderophores in *Aspergillus fumigatus*

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Aspergillus fumigatus employs two high affinity iron uptake systems, the reductive iron assimilation (RIA) and a siderophore-mediated iron acquisition. Siderophores are low-molecular mass ferric iron-specific chelators. *A. fumigatus* secretes two fusarinine-type siderophores for iron uptake, triacetylfusarinine C (TAFC) and fusarinine C (FsC), and employs two ferrichrome-type siderophores for intracellular handling of iron, ferricrocin (FC) and hydroxyferricrocin (HFC). Siderophore biosynthesis has been shown to be essential for virulence of this opportunistic human pathogen and to enable imaging of fungal infections. *A. fumigatus* possesses five putative siderophore transporters, which are transcriptionally repressed by iron indicating a role in iron homeostasis. To characterize siderophore uptake in *A. fumigatus*, mutants lacking the putative siderophore transporters Sit1 and Sit2 were generated in a genetic background avoiding interference with endogenous siderophores and RIA. Lack of either Sit1 or Sit2 did not affect utilization of FC and the fungal xenosiderophore coprogen, while combined lack of Sit1 and Sit2 dramatically decreased their utilization. Lack of Sit1 blocked utilization of ferrioxamines B, G and E – xenosiderophores produced by *Streptomycetes* and decreased utilization of the fungal ferrichrome-type siderophore ferrichrome A. Lack of Sit2 significantly decreased utilization of the ferrichrome-type xenosiderophores ferrirhodin and ferrirubin, which are produced by other *Aspergillus* species. In contrast, Sit1, Sit2 or both did not affect utilization of FsC or TAFC. Notably, ferrichrome A and coprogen were only poorly utilized and ferrioxamines did not support growth and sporulation to the same extent as FC or TAFC. This reveals that *A. fumigatus* utilizes a wide spectrum of xenosiderophores, but with different efficiency. We also identified substrate specificity differences of Sit2 and Sit2 transporters, even within ferrichrome-type siderophores.

Oxygen depletion triggers metabolic and transcriptomic response on wood decay and ethanol production in the white rot fungus *Phlebia radiata*

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Wood decomposing *Basidiomycota Polyporales* are inhabitants of dead wood, and their basidiocarps (fruiting bodies) are found on fallen tree trunks in the forest ecosystems. We hypothesized that fungal hyphae may encounter partial oxygen depletion inside wood upon decay. Therefore, we investigated how white rot fungi simultaneously decompose wood by expressing carbohydrate-active enzymes (CAZymes) and utilize the released sugars in a consolidated bioprocess.

Polyporales species were explored for their capability of simultaneous saccharification and fermentation (SSF) on solid lignocellulose substrates. Under these conditions, the white rot fungus *Phlebia radiata* isolate 79 outperformed in ethanol production[1,2]. We proceeded by genome sequencing the isolate[3] and studying gene regulation *via* RNA-Seq under aerobic and anaerobic conditions.

First, we noticed that expression of CAZy genes was affected by atmospheric changes. Hypoxia (oxygen depletion) evidently is an equally important regulator as composition of the lignocellulose substrate. However, cellulose, hemicellulose, pectin, and lignin acting enzyme-encoding genes have unique response to environmental changes. Interestingly, cellulose-acting genes were induced by the absence of oxygen, which was consistent with enzyme activity measurements. In addition, high pectin depolymerization activity was detected.

Secondly, promoter regions of core metabolism and CAZy genes were studied *in silico*. We noticed that metabolic response of *P. radiata* to hypoxia was similar to observations on the *Basidiomycota* human pathogen *Cryptococcus* rather than cellulose-degrading *Ascomycota* species. In addition, regulation of acetyl-CoA and glycerol metabolism, and especially phosphoketolase pathway are apparent adaptations to hypoxia in *Basidiomycota*.

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Loss of function of the carbon catabolite repressor CreA leads to inducer independent expression of the ferulic acid esterase B gene in *A. niger*

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The *faeB* gene of *A. niger* encodes a feruloyl esterase that catalyzes the hydrolysis of ester bonds between hydroxycinnamic acids (e.g. ferulic acid) and plant-polysaccharides, thereby releasing the hydroxycinnamic acids. With the aim to decipher the mechanisms

involved in the transcriptional regulation of *faeB*, a genetic screen was performed to isolate *A. niger* mutants displaying inducer independent expression of *faeB*. We constructed *PfaeB-amdS* and *PfaeB-lux* reporter strains and isolated trans-acting mutants in which both reporters were induced, based on growth on acetamidase plates and induced luciferase activity respectively. The genetic screen yielded over 120 trans-acting mutants. The genome of one the mutants was sequenced and revealed a point mutation in the *creA* gene. Subsequently, the mutants were also analyzed for defect in carbon repression by determining sensitivity toward allyl alcohol and *creA* sequencing. All isolated mutants were sensitive to allyl alcohol indicating that they all have defects in carbon catabolite repression. The *creA* gene of 27 additional mutants was sequenced and 24 of them contain mutations in the *creA* gene. By targeted deletion of *creA* in the *PfaeB-amdS* and *PfaeB-lux* reporter strain, it was confirmed that loss of function of *creA* results in low but inducer independent expression of *faeB*.

Cinnamic acid and sorbic acid conversion are mediated by the same transcriptional regulator in *Aspergillus niger*

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The aromatic compound cinnamic acid is commonly found in plants and functions as a central intermediate in lignin synthesis. Several filamentous fungi, *e.g. Aspergillus niger*, are able to degrade cinnamic acid through non-oxidative decarboxylation to styrene which is catalyzed by cinnamic acid decarboxylase (CdcA, formerly known as ferulic acid decarboxylase) and the co-enzyme flavin prenyltransferase (PadA). In addition, these enzymes are also essential for the decarboxylation of the food preservative sorbic acid. The corresponding genes are clustered in the genome together with a gene encoding the sorbic acid decarboxylase regulator (SdrA). SdrA is predicted to be involved in the regulation of *cdcA* and *padA*, but this was never functionally analyzed. Here we studied the role of SdrA through whole genome transcriptome analysis using an *sdrA* deletion strain grown on cinnamic acid and sorbic acid. This revealed that additional targets, of which several were clustered with *cdcA*, *padA* and *sdrA* are regulated by SdrA. Synteny analysis using 30 *Aspergillus* genomes demonstrated a conserved cinnamic acid decarboxylation gene cluster in most Aspergilli of the Nigri clade. Aspergilli lacking

certain genes in the cluster were unable to grow on cinnamic acid, but could still grow on related aromatic compounds, confirming the specific role of these three genes for cinnamic acid metabolism.

Solving the polyketide pigmentation puzzle in *Fusarium solani*

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Fusarium pigmentation is dictated by a set of two polyketide synthase (PKS) gene clusters where one is expressed during mycelial growth and the other during perithecial development. In the vast majority of *Fusarium* species, perithecial pigmentation relies on the *PKS3* gene cluster responsible for biosynthesis of fusarubins and bostrycoidins. In these species, mycelial pigmentation is mediated by bikaverin or aurofusarin. However, the situation is different for *F. solani* where mycelial pigmentation is controlled by the *PKS3* gene cluster, while the less common *PKS35* is responsible for the perithecial pigmentation, although no actual compound(s) has ever been associated with the latter.

We set out to characterize the polyketide pigments of *F. solani*. The *PKS3* gene cluster of *F. solani* shares seven genes with the previously characterized clusters in *F. graminearum* and *F. fujikuroi*. However, it differs from the previously described clusters by containing additional genes, some with predicted enzymatic function related to secondary metabolism. When we overexpressed the cluster specific transcription factor in *F. solani*, we observed a massive increase in production of javanicin, bostrycoidin, fusarubin and dihydrofusarubin.

In order to investigate the perithecial pigment chemistry we performed heterologous expression of the *PKS35* in *Saccharomyces cerevisiae*, yielding prephenalenone and dehydroxyprephenalenone as the initial polyketide intermediates. Secondly, we overexpressed the local transcription factor gene in *F. solani*, resulting in a dark green and orange/red phenotype. We identified three new candidate compounds by mass spectrometry. To our surprise, the mutant also produced elevated levels of javanicin and bostrycoidin obscuring the observation and isolation of PKS35-related compounds. Interestingly, this co-expression indicates the regulation of both *PKS3* and *PKS35* gene clusters is somehow connected.

Genome Mining of the Biosynthetic Gene Cluster of Citrinalin A in *Penicillium citrinum* using CRISPR-Cas9

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Based on previous analysis, the production of citrinalin from *Penicillium citrinum* was confirmed. Using a structurally similar compound with a known biosynthetic pathway as a probe, the gene cluster of citrinalin was discovered. After that, *Penicillium citrinum* was turned into a genetic system with CRISPR-Cas9 technology. Using plasmids which were kindly donated by the Mortensen lab, we were able to modify the protospacer of gRNA constructs, and then we reformed the plasmids with DNA assembly. Through the *E. coli* transformation procedure, the amount of plasmids were amplified for future transformation to fungal strains. During the process, plasmids were linearized and were used for transformation of *P. citrinum*. After trying various protoplasting and transformation approaches, a protocol was successfully developed to generate colonies carrying the plasmids with the phleomycin resistance gene and the Cas9 gene. Cas9 will cut in specific regions where the designed protospacers bind to, and therefore generate gene mutations and deletions in that specific regions. Since genes within the citrinalin cluster were disrupted individually, the enzyme function of each gene was analyzed by culturing the fungal mutants under citrinalin-producing conditions, and a further analysis was done via LCMS and NMR. By this approach, the critical enzymes, intermediates, and eventually the biosynthetic pathway of citrinalin will be discovered.

Identification of three transporters involved in di/tri-peptide uptake in *Aspergillus oryzae*

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The filamentous fungus *Aspergillus oryzae* possesses 134 proteolytic genes, and

oligopeptide uptake is thought to be important for nutrient absorption. In eukaryotic cells, uptake of di/tri-peptides is mediated by the proton-dependent oligopeptide transporter (POT) family. In this study, we identified three POT family transporters, designated PotA, PotB, and PotC, in *A. oryzae*. All of these transporters were able to complement the activity of di/tri-peptide uptake in the POT-deficient yeast. Growth comparison of deletion mutants of these transporter genes suggested that PotC and PotB are responsible for di/tri-peptide uptake in *A. oryzae*, whereas PotA, which is the most similar to yeast major POTs, contributed little to di/tri-peptide uptake. Nitrogen starvation induced *potB* and *potC* expression, but not *potA* expression. When three dipeptides were used as nitrogen sources, *potA* expression was induced only by Leu-Gly, and *potC* expression was also induced by Gly-Pro in addition to Leu-Gly. Only *potB* expression was strongly suppressed by Gly-Glu. Disruption and overexpression of the transcription factor PrtR, which regulates secretory proteolytic genes, suggested that PrtR is partially involved in the regulation of *potA* and *potB* expression but not in the *potC* expression. The expression levels of *potC* and alkaline protease *alpA* were dramatically reduced by disruption of *ubrA*, an orthologue of the yeast ubiquitin ligase *UBR1* responsible for the *PTR2* expression, whereas the *potA* and *potB* expression was apparently unaffected by *ubrA* disruption. These results suggest that the expression of each POT gene is controlled by different regulatory mechanisms in *A. oryzae*.

Identification and heterologous expression of putative NRPS-like and PKS coding genes from *Guignardia bidwellii* in *Magnaporthe oryzae*

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Guignardia bidwellii is the causal phytophatogenic fungus of black rot in vines posing a massive threat to organic viticulture. The phytotoxic dioxolanones phenguignardic acid and guignardic acid have been identified as potential virulence factors involved in the infection process of the black rot fungus.

To date a genetic manipulation of *G. bidwellii* has not been successfully established and therefore a heterologous expression system has been established for the study of the secondary metabolites involved in the biotic interaction.

The genome of *G. bidwellii* has been sequenced and two putative NRPS-like coding genes have been identified. Moreover, transcriptome analysis led to identification of

one polyketide-synthase gene which expression correlates with the production of dioxolanones.

In contrast to NRPS, NRPS-likes consist of only one module with adenylation, thiolation and thioesterase domains, but lacking a condensation domain. The catalyzed reaction is the coupling of exactly two deaminated aromatic amino acids as building blocks, which can be modified by other proteins.

These three genes (GbNRPS-like1/2 and GbPKS1) have been introduced into *Magnaporthe oryzae* in order to express these enzymes under the control of a constitutive promoter.

Several new compounds could be isolated from the culture of the heterologue expression strains that are not present in the wild type of *M. oryzae*: phenguignardic acid, when GbNRPS-like 1 was expressed, and guignardianone C, by the co-expression of the GbNRPS-like1 and the GbPKS1 genes.

Parallel phylogenomic roadmapping - disentangling widespread HGTs and recombinations in the evolution of fungal macrolactone clusters

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Fungal benzenediol lactones (BDLs) constitute an ancient subclass of highly bioactive polyketides with many peculiar mechanistic and evolutionary features. The signature tandem polyketide synthase array shared also with azaphilone biosynthetic pathways, earmarks the lactone biosynthetic clusters as a convenient object of study of discrepancies between history of fungal taxa and the individual functional modules (gene families) required for biosynthesis.

Building on the earlier example of phylogenomic roadmap for non-reducing aromatic polyketide synthases, we introduce the concept of phylogenomic atlas where multiple reconciliations are created to investigate the relationships between evolutionary patterns evident in the history of different gene families involved in formation of the biosynthetic cluster, including the core polyketide synthases and crucial accessory enzymes (e.g. flavin-dependent halogenases).

Using over 800, both preexisting and reannotated, as well as newly sequenced fungal genomes we corroborate the hypothesis about widespread horizontal gene transfer between divergent ancestral macrolactone producers. Utilising large scale pairwise

comparisons and sampling the space of cost-optimal reconciliations, we provide a comprehensive look at the history of macrolactone biosynthesis demonstrating the role of recombination and differently fragmented genomes as horizontal gene transfer partners facilitating breakup&formation of new cluster patterns. Taken together, our roadmaps resolve the origins of diversity in both dihydroxyphenolic and resorcylic lactone producers, demonstrating in particular multiple origins of tandem polyketide synthase biosynthetic clusters even in related taxa of ecologically diverse filamentous fungi (*Pochonia* and *Fusarium* genera).

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Screening and annotation of potential benzenediol lactone producers among higher fungi

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Fungal benzenediol lactones are an ancient group of bioactive aromatic polyketides synthesised by combined action of highly reducing (HR-PKS) and non-reducing (NR-PKS) polyketide synthases. In order to better understand the ecological and phylogenetic relationships between taxonomically divergent producer strains, we set out to screen a collection of over 80 strains of filamentous fungi for biosynthetic gene signatures. Utilising a collection of custom degenerate PCR markers designed for HR-PKS (317 primer pairs) and NR-PKS (254 pairs) we identified putative producer strains among members of *Diaporthales* (*Coniella* sp., *Diaporthe* sp., *Leucostoma* sp. and *Valsa* sp. members) and *Hypocreales* orders (*Fusarium* sp., *Ilyonectria* sp., *Pochonia* sp. members), as well as singular representatives of distantly related orders of filamentous fungi (*Acephala*, *Curvularia*, *Phoma* and *Talaromyces* genera). Selected strains were sequenced in Illumina HiSeq technology, assembled and annotated utilising both standard approaches to curation of biosynthetic cluster signatures (AntiSmash/SMURF) as well as a custom in-house 'phylogenomic roadmapping' pipeline.

Thus far, five whole genome assemblies were completed for *Pochonia* sp. J3.5, *Pochonia bulbillosa* CYS17 (a related non-producing strain), *Curvularia inaequalis* SP01, *Diaporthe toxica* MJ01 and *Fusarium equiseti* On2.3. Of particular interest was the finding of atypical dehydrocurvularin-like biosynthetic cluster in a previously uncharacterised representative of the entomopathogenic/nematophagous *Pochonia* genus - *Pochonia* sp. J3.5 isolate. The newly available genomic drafts highlight high diversity of macrolactone

biosynthetic toolkit, as well as support the possibility of multiple, parallel origins of lactone biosynthesis across related fungal taxa.

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Understanding parameters enhancing erythritol consumption, a prerequisite to the development of an efficient erythritol production process in *T. reesei*

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Erythritol is a naturally occurring four carbons polyol mainly used as a sweetener in the food industry. The interest towards erythritol is growing due to its specificities among the polyols family: it has the highest digestive tolerance among polyols, it is a zero-calorie polyol and it is non-cariogenic. Moreover, it is naturally produced by osmophilic yeasts and fungi such as *Yarrowia lipolytica*, *Trichosporonoides* sp, *Moniliella pollinis* or *Trichoderma reesei* making erythritol a good candidate for manufacturing through a biotechnological process.

At the industrial scale, erythritol is currently produced by yeast fermentation of sugars. The use of sugars as substrate along with low yield producing strains contribute to the high manufacturing cost of erythritol and a high environmental impact of the process keeping erythritol from wider use in industry. Strategies to improve erythritol manufacturing are oriented around 3 axes: improve naturally producing strains by genetical engineering, develop the use of cheaper non-sugar substrate and optimize the manufacturing process. As a natural erythritol producer being a saprobe and already industrially cultivated for enzyme manufacturing, *T. reesei* is a good candidate for an approach combining the 3 described axes. Therefore, we chose this organism for the development of a new erythritol production process with reduced environmental and financial cost.

However, the capacity of *T. reesei* to use erythritol as a carbon source is currently still not determined. Yet, if *T. reesei* can use erythritol has a carbon-source it is essential to elucidate the parameters impacting its consumption before starting the manufacturing process optimization. Parameters investigated will be presented and their effect discussed.

The nicotinic acid pathway of *Aspergillus nidulans* includes a reversible conversion to 6-hydroxynicotinic acid

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Nicotinic acid (NA) catabolism in prokaryotes can proceed aerobically or anaerobically. A novel eukaryotic NA catabolic pathway was described by us. Eleven *hxn* genes (encoding eight enzymes (*hxnY/T/S/V/W/X/M/N*), two transporters (*hxnZ/P*) and one transcription factor (*hxnR*)) are regulated by the pathway specific transcription factor HxnR, which is activated by a metabolite of the pathway (Ámon *et al*. 2017 and present authors and M. Flipphi, in preparation). Study of the NA utilization on mutants deleted for enzyme encoding *hxn* genes resulted in the identification of a number of consecutive steps while other steps still remain uncharacterised. The *hxnS* (nicotinate hydroxylase) deletion strain cannot utilize NA as sole N-source, however, *hxnT* (flavin oxido-reductase) deletion strains can utilize NA as efficiently as the *hxnT*$^{+}$ control and shows a reduced utilization of 6-hydroxynicotinic acid (6-NA), a metabolite subsequent to NA. Paradoxically, the *hxnS hxnT* double deletion strain utilizes 6-NA better than the *hxnS*$^{+}$ *hxnT*$^{+}$ control, which could be rationalised if the pathway diverges into alternative routes downstream to 6-NA and simultaneously 6-NA back-converts to NA. Here we provide *in vivo* evidence of the back-conversion of 6-NA to NA through the utilisation of a nicotinic acid auxotroph, *nicB8* (*nicB8 hxnR*$^{+}$, *nicB8 hxnR*^{c}*7* and *nicB8 hxnR*^{c}*7 hxnY/T/S/V/W/X/M/N*) and HPLC/GC-MS detection of the appearance of NA in 6-NA fed *hxnR*^{c}*7* cultures of *hxnX*, *hxnV* and *hxnW* deleted strains.

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In vitro enzyme evolution of Purine Hydroxylase I (HxA) and Purine Hydroxylase II (HxnS)

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We have established that an ancient gene duplication of the xanthine hydroxylase gene (HxA) had led to a novel enzyme of unprecedented substrate specificity (HxnS). HxA accepts xanthine (X) and hypoxanthine (Hx) as a substrate, while its paralogue HxnS

cannot hydroxylate xanthine but can hydroxylate nicotinic acid (NA). A systematic *in silico* comparison of HxA and HxnS orthologues across Pezizomycotina revealed eight conserved HxnS specific amino acid residues, which might be responsible for the HxnS-specific functions (Amon *et al*., 2017, Open Biology, 10.1098/rsob.170199). To verify the functional role of these eight amino acids, 16 different point mutation-carrying HxA and HxnS expressing plasmids were constructed carrying single amino acid changes corresponding to the residue of the paralogue protein at the same position. These mutated genes were transformed into appropriate mutated strains and the NA, Hx and X utilization ability of the transformants was studied. Here we present the properties of the transformants expressing the different mutated enzymes.

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Structural homology function predictions for fungal nicotinate catabolising enzymes

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The genes encoding eight enzymes (Hxn*T/Y/S/V/W/X/*M and *N*) involved in a novel nicotinic utilisation eukaryotic acid catabolic pathway were described by us (Ámon *et al*. 2017 and present authors and M. Flipphi, in preparation). In order to gain a rough perception about the enzymatic steps of the catabolic pathway, 3D protein models were obtained (using I-Tasser and ModRefiner) and compared to their known structural homologs (using Matchmaker in Chimera 1.11.2rc). Here we present the substrate binding active sites of the eight Hxn enzymes superimposed to their cognate known structural homologs and predict their probable substrates and activities. These data together with *in vivo* growth phenotypes of the cognate gene-deleted mutants outlined a catabolic pathway that is split after 6-hydroxynicotinate (6-NA), the hydroxylation product of NA. One of the alternative routes involves the production of 2,5-dihydroxypyridine from 6-NA followed by the probable formation of trihydroxypyridine (the putative metabolite inducer of the pathway specific transcription factor). After saturation of the latter, the pyridine ring is opened and the ring-derived nitrogen is utilized. The alternative route has not been elucidated, since some of the relevant enzymes are versatile and different scenarios can be envisaged.

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Synthetic control devices for gene regulation in *Penicillium chrysogenum*

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Synthetic biology aims at controlled gene regulation that can lead to increased production of chemicals and pharmaceuticals. Here, orthogonal, synthetic control devices were developed for *Penicillium chrysogenum*, a model filamentous fungus and industrially relevant cell factory. In the synthetic transcription factor, the QF DNA-binding domain of the transcription factor of the quinic acid gene cluster of *Neurospora crassa* is fused to the VP16 activation domain.

This synthetic transcription factor controls the expression of genes under a synthetic promoter containing quinic acid upstream activating sequence (QUAS) elements, where it binds. A gene cluster may demand an expression tuned individually for each gene, which is a great advantage provided by this system.

The control devices were characterized with respect to three of their main components: expression of the synthetic transcription factors, upstream activating sequences, and the affinity of the DNA binding domain of the transcription factor to the upstream activating domain. This resulted in synthetic expression devices, with an expression ranging from hardly detectable to a level similar to that of highest expressed native genes. The versatility of the control device was demonstrated with fluorescent reporters and its application was confirmed by synthetically controlling the production of penicillin.

The absence of QUAS elements resulted in transcriptionally silent synthetic promoters which are currently investigated for gene activation via a dCas9-CRISPR based transcriptional regulatory tool.

The characterization of the control devices in microbioreactors, proved to give excellent indications for how the devices function in production strains and conditions. We anticipate that these well-characterized and robustly performing control devices can be widely applied for the production of secondary metabolites and other compounds in filamentous fungi.

Genetic characterization and virulence contribution of the beticolin toxin produced by the sugar beet pathogen *Cercospora beticola*

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Cercospora leaf spot, caused by *Cercospora beticola*, is the most destructive foliar disease of sugar beet worldwide. The *C. beticola* armory includes several secondary metabolites (SMs) that act in concert to make this pathogen a successful colonizer of sugar beet. One such group of SMs is the beticolins, comprised of 20 members (B0 to B19), that demonstrate non-host specific toxic properties. Early research on their biological function indicated that beticolins have antibacterial and phytotoxic properties, which were only observed in the presence of light. Later it was found that due to their ability to form complexes with Mg^{2+}, beticolins can interfere with H^{+}-ATPase activity and are able to incorporate themselves into lipid bilayers to form ion channels. While chemical structures and some biological activities have been identified, the biosynthetic pathway of these toxins is unknown. Therefore, it has not been possible to assess to what extent beticolin production and associated phytotoxic effects contribute to virulence. To help characterize the role of beticolin in virulence, we identified all SM clusters in the *C. beticola* genome and queried expression of these SM clusters under beticolin-inducing conditions using RNAseq. Since beticolin contains a chlorine, we searched among the expressed gene clusters for a halogenase enzyme, which is known to install chlorine atoms on SMs. We identified a single induced SM cluster that contained a halogenase enzyme and developed *C. beticola* knock-out mutants in the PKS gene within this cluster. Using analytical chemistry techniques, we confirmed that this cluster is responsible for beticolin production. Beticolin mutants will be used to assess the role of the beticolin gene cluster in phytotoxicity of sugar beet tissues. Further characterization of genes in the beticolin pathway will be discussed. This research provides an underlying genetic mechanism for the role of beticolin during *C. beticola* infection of sugar beet.

Role of GsfR1 and global regulators on griseofulvin and other secondary metabolites biosynthesis and on growth and virulence of *Penicillium griseofulvum*

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Penicillium griseofulvum is a causal agent of apple blue mold, an important post-harvest disease. This pathogen is able to produce many secondary metabolites both on apples and *in vitro* including mycotoxins (e.g. patulin and cyclopiazonic acid) and the antifungal compound called griseofulvin. In order to investigate the regulation of griseofulvin biosynthesis and to elucidate the role of this compound on the biology of *P. griseofulvum,* deletion mutants for putative genes encoding transcription factors were obtained; furthermore, promoter analysis of biosynthetic genes was performed. The results revealed that the regulation of griseofulvin involves both a pathway-specific transcription factor encoded by *gsfR1* and global regulators of gene clusters. GfR1 regulate negatively or positively griseofulvin biosynthesis depending on external stimuli and seems to be also involved in the regulation of patulin biosynthesis and in the asexual development and virulence of the pathogen. Moreover, many stimuli can trigger activation of griseofulvin biosynthesis, such as nutrient availability, stress response and sporulation; the role of carbon and nitrogen was confirmed *in vitro*. This study allowed to throw light on the complex regulation of griseofulvin biosynthesis in *P. griseofulvum* and revealed a deep interconnection between fungal development and secondary metabolism.

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Diversity of metabolic profiles and evolutionary forces acting in secondary metabolism gene clusters of *Aspergillus nidulans*

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The filamentous fungus *Aspergillus nidulans* has been a primary work horse used to under stand fungal genetics. Much of this work has focused on elucidating the genetics of biosynthetic gene clusters (BGCs) and the secondary metabolites (SMs) they produce. These compounds are both niche-defining in fungi and of great economic importance to humans. Despite this focus in lab, very little is known about the natural diversity in BGCs and SM production in *A. nidulans*. We determined the BGC content and looked for evolutionary patterns in these clusters from whole genome sequence

data of two clinical isolates and the A4 reference genome of *A. nidulans*. Differences in BGC content were used to explain SM profiles determined using liquid chromatography-high resolution mass spectrometry. We found that BGC content was broadly predictive of SM profiles, with both varying substantially between isolates. In addition to SNPs, total loss, and translocations observed in BGCs, we demonstrate that one clinical isolate of *A. nidulans* has received the viridicatumtoxin BGC through horizontal gene transfer, likely from *Penicillium spp*. We identify viridicatumtoxin and several other compounds previously not known to be produced by *A. nidulans*. Lack of sterigmatocystin production by one isolate was not easily explained by sequence data, raising questions about other genes and processes known to regulate this BGC. The diversity in BGC content and SM production observed here offers new avenues to understand the regulation of secondary metabolism in the context of the diversity that exists within this fungal species.

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Comparative performance of *Aspergillus terreus* itaconic acid fermentations on D-xylose and xylitol

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Itaconic acid (IA) is produced by *Aspergillus terreus* mainly from molasses or starch. However, research over the efficient utilization of non-food, lignocellulosic plant biomass is soaring. D-xylose is the most abundant pentose in the lignocellulose complex.

The first enzymatic steps of D-xylose catabolism could lead to cofactor imbalance and low biomass yield. In principle this could be avoided by employing xylitol, the polyol of xylose and a by-product from ethanol manufacturing as carbon source. The aim of this study was to evaluate this option in terms of fermentation performance. To this end, controlled fermentations with D-xylose and xylitol liquid were performed at five different initial concentrations (10, 50, 110, 150 and 200 g L-1), by employing *A. terreus* NRRL 1960, a high IA producer strain.The lowest initial concentration (10 g L-1) resulted in poor molar yield on both carbon sources, particularly on xylitol (0.04 ± 0.01 vs. 0.24 ± 0.01). Differences narrowed with increasing initial carbon concentrations, and eventually, no significant difference was found in the molar yields at the two highest initial carbon concentrations (all in the range of 0.5 ± 0.03). On xylitol, an early lag phase lasting for up to 2 days was observed, and although consumption rate has accelerated afterwards, carbon source utilization rate (g L-1 h-1) remained lower than on D-xylose at any concentration. Importantly, maximal fungal biomass concentrations were not statistically different,

indicating a more efficient biomass formation on xylitol than on D-xylose. We conclude that by facilitating xylitol uptake – particularly at the early stages – this common polyol can be made a superior carbon source over D-xylose for IA fermentations.

Research was supported by the EU and co-financed by the European Regional Development Fund under the project GINOP-2.3.2-15-2016-00008 and by the EFOP-3.6.1-16-2016-00022 project co-financed by the EU and the European Social Fund.

Erythritol in *Trichoderma reesei* - Construction of a multirecombinant production strain

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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.

The industrially used cellulase and hemicellulase production fungus *Trichoderma reesei*, is not a naturally high-level producer for erythritol but the organism is able to degrade lignocellulosic material and can therefore utilize renewable and cheap material as substrate in biotechnological processes. The main goal of this work was the design of a multirecombinant production strain that can use hydrolyzed straw as a substrate for the production of erythritol. Since erythritol is stored within the cell, we introduced a sugar transporter from another microorganism into *T.reesei* to release erythritol into the supernatant, which leads to a simplification in analysis and following downstream processes. Furthermore we deleted or overexpressed several genes, which may be involved directly in the erythritol production pathway or help shifting the process towards the production of erythritol. Right now, a number of recombinant strains were constructed. All of them are carrying the transporter-encoding gene and one additional overexpressed gene directly involved within the erythritol pathway. Also two enzymes leading to byproducts have been deleted to force the flux towards the production of erythritol. The resulted strains will now be cultivated, characterized and compared concerning their erythritol production ability.

A pair of transcription factors regulates the switch between primary and secondary metabolism

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Many fungal secondary metabolites can be used in medicine and biotechnology applications and are potential sources for new therapeutic agents and drug leads. However, under laboratory conditions, the fungal secondary metabolism remains largely silent, hindering the discovery of new secondary metabolites. Previously, we could demonstrate that the transcription factor Xpp1 is involved in the regulation of both, the primary and the secondary metabolism of *Trichoderma reesei*.1 The deletion of xpp1 led on the one hand to a reduced growth rate and on the other hand an enhanced production of low molecular weight compounds in the supernatant, as detected by an untargeted metabolomics approach. Data from an RNA-Seq analysis support the model, that Xpp1 acts as an activator of primary-, and as a repressor of secondary metabolism.

Recently, we identified a putative interaction partner of Xpp1, termed Xpp2. A deletion of Xpp2 resulted in fungal strains exhibiting a strongly reduced growth phenotype on a wide array of unrelated carbon sources compared to the wild-type strain. This growth impairment is carbon source dependent and not a general feature. This suggests that Xpp2 might be involved in the regulation of several different catabolic pathways. Further, we investigated the influence of Xpp2 on the production of secondary metabolites by untargeted, isotope-assisted metabolite profiling, analogously to the studies of Xpp1 and found a strong reduction of low molecular weight compounds (in number and amount). Taken together, our results suggest that Xpp2 is an antagonist of Xpp1. Controlling the transcription factor pair Xpp1 and Xpp2 may offer a novel approach to activate silent gene clusters and facilitate the discovery of new fungal secondary metabolites.

1. Derntl, C. *et al*. Transcription factor Xpp1 is a switch between primary and secondary fungal metabolism. Proceedings of the National Academy of Sciences of the United States of America 114, E560-E569, doi:10.1073

Omphalotin, lentinulin and dendrothelin: Homologous members of a new family of RiPPs

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Backbone N-methylation provides pharmacologically favorable properties to peptides and has long been thought to be exclusively present in non-ribosomal peptides (NRPs) exemplified by the fungal natural product cyclosporine A, a very powerful immunosuppressant. Genome mining revealed, however, that omphalotins A-I, a series of macrocyclic backbone N-methylated peptides from the mushroom *Omphalotus olearius*, represent a class of ribosomally synthesized and post-translationally modified peptides (RiPPs), referred to as borosins (Ramm *et al*., 2017; van der Velden *et al*., 2017). The omphalotin precursor protein OphMA contains a SAM-dependent methyltransferase domain which iteratively methylates the core peptide region located at its C-terminus. A putative prolyl oligopeptidase (OphP), encoded by the same gene cluster, is thought to excise and macrocyclize the methylated core peptide. Both enzymes are currently being characterized for their promiscuity, as they may be applicable for the biotechnological de novo production of therapeutic peptides.

Complementary strategies to unlock secondary metabolite gene clusters in the filamentous fungus *Podospora anserina*

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Filamentous fungi constitute a rich source of bioactive secondary metabolites (SMs). In the case of the model fungus *Podospora anserina*, genome sequencing revealed the presence of more than 40 gene clusters which may be involved in SMs biosynthesis. However, only few clusters and their corresponding SMs are well characterized. In order to fully explore the metabolome of *P. anserina* and reveal the potential of unknown SMs, we previously used a targeted strategy involving the overexpression of PaAflR, the sterigmatocystin cluster-specific transcription factor (Shen *et al*., 2019, https://doi.org/10.1111/1462-2920.14698). In the present study, global approaches have been investigated following two routes: 1) variation of fungal culture conditions and

2) chromatin remodelling by either using the histone deacetylase inhibitor agent SAHA or deletion of KMT6, gene encoding the histone H3K27 methyltransferase. Analyses of the LC-HRMS/MS metabolomic profiles from these experiments evidenced the positive impact of a global deregulation strategy to activate the SM production and its complementarity to targeted cluster overexpression.

Enhancing peptaibols production in the biocontrol fungus *Trichoderma longibrachiatum* SMF2 by elimination of a putative glucose sensor

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Trichoderma spp. are main producers of peptide antibiotics known as peptaibols. While peptaibols have been shown to possess a range of biological activities, molecular understanding of the regulation of their production is largely unclear, which hampers the production improvement through genetic engineering. Here, we demonstrated that the orthologue of glucose sensors in the outstanding biocontrol fungus *Trichoderma longibrachiatum* SMF2, TlSTP1, participates in the regulation of peptaibols production. Deletion of Tlstp1 markedly impaired hyphal growth and conidiation, but significantly increased peptaibols yield by 5-fold for Trichokonins A and 2.6-fold for Trichokonins B. Quantitative real-time polymerase chain reaction analyses showed that the increased peptaibols production occurs at the transcriptional levels of the two nonribosomal peptide synthetase encoding genes, *tlx1* and *tlx2*. Transcriptome analyses of the wild type and the Tlstp1 mutant strains indicated that TlSTP1 exerts a regulatory effect on a set of genes that are involved in a number of metabolic and cellular processes, including synthesis of several other secondary metabolites. These results suggest an important role of TlSTP1 in the regulation of vegetative growth and peptaibols production in *T. longibrachiatum* SMF2 and provide insights into construction of peptaibol-hyperproducing strains through genetic engineering.

Poster Session 1.4
GENOME, CHROMATIN AND EPIGENETICS

THURSDAY, FEBRUARY 20

18:00 - 19:30 | Location: **Frentani Convention Center**

Functional dissection of the meiotic drive of female-inherited accessory chromosomes in *Zymoseptoria tritici*

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Meiosis is a highly conserved cellular process that involves pairing and segregation of homologous chromosomes. How chromosomes lacking a homolog undergo meiosis is poorly understood. Fungal accessory chromosomes that show presence/absence polymorphisms can be unpaired during meiosis. The genome of *Zymoseptoria tritici*, the causal pathogen of septoria blotch, comprises one of the largest known complements of fungal accessory chromosomes. Interestingly, these unpaired accessory chromosomes show a meiotic drive that increases their frequency when solely inherited from the female parental strain. The mechanism of this drive is unknown, but it appears to involve an additional replication of the accessory chromosomes during meiosis. Here, we aim to test whether DNA replication associated with meiosis as well as mitosis differs between core and accessory chromosome in *Z. tritici*. ChIP-seq analyses of ORC2 and MCM2 binding sites, two highly conserved proteins involved in the licensing of the origin of replication, will be used to map this first step in chromosome replication on both core and accessory chromosomes. We are currently establishing synchronized cell cultures of *Z. tritici* in combination with flow cytometry to observe the cell cycle and DNA content as a first step before ChIP-Seq analyses. Our results will allow us to determine the distribution and firing of origins of replication on core and accessory chromosomes and may provide novel insight into the underlying mechanism for the processes involved in the meiotic drive of the accessory chromosomes of *Z. tritici*.

Flow cytometry as the state of the art tool for fungal nuclear DNA quantification: genome size measurement and nuclear cycle analysis

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Genome size information is scarce and patchy across fungi, with information available for less than 2000 species. So far most records were obtained using static, microscope-based cytometry methods or derived from genome sequencing projects. Flow Cytometry is now considered the state of the art method for genome size measurement (D'Hondt *et al*., 2011) and appropriate methods (Sabatinos & Forsburg, 2009; Tavares *et al*., 2014) and DNA standards (Talhinhas *et al*., 2017) are available, enabling the analysis of most genome size ranges and fungal life styles in a rapid, robust and inexpensive way. The average fungal genome size is 60 Mbp, but sizes vary across phylogeny, ranging from 2.2 Mbp (*Encephalitozoon romaleae*) to 3706 Mbp (Pombert *et al*., 2012; Egertová & Sochor, 2017). In several fungal clades genome size expansion seems to go along with evolution either to plant mutualism or to plant parasitism (particularly biotrophy). Nuclear DNA quantification also enables nuclear cycle analysis, which has revealed the presence of 1C, 2C and a low proportion of 4C nuclei in across diverse life cycle stages of several fungi in the Pucciniales. Downstream FISH analyses of nuclei separated by Fluorescence-Activated Cell Sorting have shown the 2C population is composed of mostly diploid nuclei in G1 phase. While Flow Cytometry for nuclear DNA quantification is routinely employed in plant sciences for genome size and ploidy studies, its use in fungal biology is still seldom. Appropriate standards and methods are now available, prompting a more generalised use of Flow Cytometry for genome size and nuclear cycle studies in fungi.

D'Hondt *et al*. (2011) Mol. Plant Pathol. 12: 815-828

Egertová & Sochor (2017) Plant Syst. Evol. 303: 981-986

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Sabatinos & Forsburg (2009) Meth. Mol. Biol. 521: 449-461

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Role of Kmt6, a histone methyl transferase, in plant pathogenicity of the necrotrophic fungus *Alternaria brassicicola*

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Alternaria brassicicola is a fungal necrotroph responsible for the Brassicaceae dark spot disease. This fungus is a seed-borne pathogen that only affects the aerial parts of host plants causing great damages with major incidence on yield and product quality. Its transmission to seed is a major component of pathogen fitness promoting the dispersal and long-term survival of the fungus.

Recently, we showed that several chromatin-modifying protein encoding genes, including the gene encoding the histone lysine methyltransferase Kmt6 (H3K27), were overexpressed in germlings exposed to osmotic and hydric stresses, which are the main constraints encountered by the fungus during the seed colonization process. To understand the potential involvement of Kmt6 in plant pathogenicity, we used a reverse genetic approach by generating and characterizing *kmt6* deficient mutants (*Δabkmt6*). We showed that the leaf and silique colonization processes were impaired in *Δabkmt6* by comparison to the wild-type and that mutant strains exhibited higher sensitivity to various environmental stresses. Moreover, transcriptomic approach showed that genes involved in stress response and secondary metabolite pathway were misregulated in the mutant compared to the wild-type strain.

Key words: pathogen/host interaction, methylation, seeds, fungal necrotroph

Structural dynamics of chromosomes and its role in genome plasticity of *Fusarium oxysporum*

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Fusarium species are among the most pathogenic and toxicogenic fungi described. Intra-specific genetic variability is essential for evolution and adaptation, allowing the pathogen to model and adapt its lifestyle to the host species and to the variable environmental conditions.

Previous studies established that *Fusarium oxysporum* displays a high level of genome instability, reflected by frequent chromosomal rearrangements. These events tend to occur in certain chromosomal regions, after subjecting the fungus to an experimental evolution process under different environmental conditions. These findings are reminiscent of those previously reported in experimentally evolved lined of *Saccharomyces cerevisiae*, which carried numerous chromosomal rearrangements (Dunham *et al.,* 2002) (1).

The main objective of this project is to quantify, on a large scale, the rate of chromosomal reorganizations in *F. oxysporum* and determine their effect on fungal development and pathogenesis.

1. Dunham M, Badrane H, Ferea T, Adams J, Brown P, Rosenzweig F, Botstein D, (2002) Characteristic genome rearrangements in experimental evolution of Saccharomyces cerevisiae. Proceedings of the National Academy of Sciences 99(25): 16144-16149 (DOI: 10.1073/pnas.242624799)

Genomic analysis and intraspecific diversity of a new heat resistant basidiomycetous fungal species

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Heat resistant fungi have the ability to survive exposure to temperatures beyond the typical maximum cardinal temperature. Usually, spores allow the fungus to resist temperature stresses, however mycelia with increased heat resistance have also been described. Heat resistant fungi occur in all terrestrial ecosystems and have their ecological niche in habitats with recurring temperature stress (e.g. forest wildfires, flare pits), but are also regularly isolated from ordinary soil habitats.

Strategies to overcome periodically high temperatures include specially adapted enzymes and the production of protective compounds, making such organisms potentially useful for biotechnological applications. Moreover, frequent contamination of heat processed products, (e.g. in food industry) result in both economic losses and considerable risks for human health.

Although the majority of heat adapted fungi so far detected belonged to the Ascomycota, several studies indicate that a huge number of heat resistant soil fungi, belonging to the Basidiomycota, are still undescribed.

A screening of Canadian forest soils for heat-resistant fungi yielded in four fungal isolates. Morphological characterization and molecular analyses of universal barcodes (SSU, LSU and ITS) let to the assumption that these four heat-resistant isolates represent a new lineage, basal to the Ustilaginomycotina. High quality genomic DNA was isolated using a CTAB based protocol and sequenced with Illumina MiSeq. Preliminary results indicate that the four obtained genomes comprise an average size of 23 Mb, with a high GC content of 59 %. In this study, we aim to characterize elements providing heat resistance and respective regulation, such as synthesis of protective cell compounds (e.g. sugar, alcohols) and heat shock proteins.

We will provide insights into both the intraspecific genetic diversity and structure as well as the composition of the genome of this new heat-resistant Basidiomycete.

Mitochondrial dysfunction in *Pleurotus ostreatus* progeny: a matter of genome conflict

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In basidiomycetes, two monokaryons (mks) with different alleles at two unlinked mating loci can fuse to form a dikaryon. Mks behave as male and female gametes because they fertilize the other by exchanging their nuclei (bidirectional mating/fertilization). Once the dikaryon forms, its cells contain two nuclei and one mitochondrial type because mks compete to transmit it. Moreover, the meiotic spores it produces contain only one mitochondrial type. Genome conflict occurs when an individual bears nuclear and mitochondrial genes of different origins. This situation triggers mitochondrial dysfunction and the Retrograde Signaling Pathway (RSP).

P. ostreatus N001 is a dikaryon whose growth rate has decreased by 20% after 25 years of subculture. Simultaneously, the growth rate of two mks populations (1998 and 2016) derived from it was reduced by half. To find the reason for that reduction, we analyzed 20 transcriptomes of the 2016 population including fast and slow-growing mks. The RNAseq revealed 808 genes positively and 92 negatively correlated with the growth rate.

Further analysis of the differentially expressed genes (DEGs, p-value <0.005, fold change >3) revealed 204 and 327 genes overexpressed in fast and slow-growing mks,

respectively. Focused on DEGs overexpressed in the slow-growing mks, the GO-domain terms ion binding, membrane and component of membranes, oxidoreductase activity, hydrolase activity, and drug binding, were the most abundant. These functions occur upon induction of the RSP in other organisms. Furthermore, the slow-growing mks showed high ROS levels, different sensitivity to benomyl, affection of the membrane and cell wall, overexpression of genes related to mitochondrial dysfunction, and activation of the ERK1/2, FUS3, and HOG-1 signaling pathways. All this data suggest that the performance of the slow-growing mks could reflect the lack of adaptation between their nuclear and maternally inherited mitochondrial genes.

Schizosaccharomyces pombe Mti2 and Mti3 function together in mitochondrial translation initiation

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Mitochondrial DNA (mtDNA)-encoded subunits of the oxidative phosphorylation (OXPHOS) complexes are synthesized in mitochondria. Initiation of translation of mtDNA-encoded subunits requires two general factors mIF2 and mIF3, whose counterparts in bacteria are essential for protein synthesis. In this study, we report the characterization of the fission yeast *Schizosaccharomyces pombe* mIF2 (Mti2) and mIF3 (Mti3). Unlike its *Saccharomyces cerevisiae* counterpart (*IFM1*), which is essential for respiratory growth, *S. pombe cells* lacking *mti2* were able to grow on nonfermentable carbon sources, although they showed a slow growth phenotype as compared with wild-type (WT) cells. The growth defect of the *mti2* deletion mutant could be suppressed by expressing *IFM1*, demonstrating functional conservation between the two proteins. Similarly, unlike its counterpart in *S. cerevisiae* (*AIM23*), deletion of *mti3* did not affect cell growth on respiratory media and mitochondrial translation. However, deletion of *mti3* exacerbated the growth defect of the *Δmti2* mutant, suggesting that the two proteins had distinct, but partially overlapping functions during mitochondrial translational initiation in *S. pombe*. Sucrose gradient sedimentation analyses showed that both Mti2 and Mti3 associated with the small subunit of the mitochondrial ribosome. Our findings support the view that the importance of the mitochondrial translation initiation factors varies among the organisms.

Temperature and histone modifications affect the mutation rate in the wheat pathogen *Zymoseptoria tritici*

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Mutations are the basis for genetic variation along genomes and crucial for adaptive evolution - with mutation rates varying markedly between species. Histone modifications, which influence aspects of chromatin biology, affect mutation rates in mammalian cancer cells but their effect on mutation rates in fungal pathogens is unknown. This is however of particular interest as genomes of many pathogenic fungi comprise supernumerary or accessory regions with markedly different histone modification patterns. Here, we assess the effect of histone modifications on the mutation rate in the wheat pathogen *Zymoseptoria tritici*, which contains up to eight accessory chromosomes and exhibit a high standing genetic variation. A mutation accumulation approach spanning approx. 244800 mitotic cell divisions over the course of 52 weeks allowed us to determine the neutral rate of mutations. We show that the histone modification H3K27me3 is correlated with an increased mutation rate and removal of H3K27me3 by deletion of the methyltransferase *Kmt6* leads to a reduction in the mutation rate. Phenotypic characterisation furthermore showed that mutations are, on average, slightly deleterious, an observation that contrasts with the high standing genetic variation of *Z. tritici*. Interestingly, increasing the temperature increased the mutation rate dramatically and confers chromosome copy number variation. On average, these changes have a highly negative effect on the fitness. We conclude that histone modifications and environmental factors such as temperature affect the rate of spontaneous mutations and thereby the fitness of this important wheat pathogen.

Functional analysis of the tRNA-ome of *Aspergillus fumigatus*

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Aspergillus fumigatus is an opportunistic human fungal pathogen responsible for an

alarming number of life-threatening infections worldwide. There are limited antifungal treatments currently available to clinicians, and rates of resistance of *A. fumigatus* to several key antifungals is increasing. A growing number of transcriptional and post translational factors have defined roles in pathogenicity and drug resistance however little is known about the role of translational factors. Transfer RNAs (tRNAs) are ancient RNA molecules with an integral role in translation. Recently, tRNAs have been implicated in complex stress responses and adaptive translation (Torrent *et al* 2018, Thompson *et al* 2008, Begley *et al* 2007). To investigate the significance of tRNAs in pathogenicity and drug resistance, a genome wide barcoded tRNA knock out library has been generated in *A. fumigatus*. Through library generation we have identified 5 tRNA genes that are essential for viability. We show that under optimal growth conditions, *A. fumigatus* otherwise displays robustness to tRNA gene deletion.

Detection and molecular characterization of novel dsRNA viruses isolated from different Zygomycete fungi

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Presence of mycoviruses can be confirmed by the detection of double stranded RNA (dsRNA) elements in fungal isolates. Mycoviruses are usually asymptomatic in their hosts, but occasionally they may enhance or reduce their virulence. Although mycovirus-harbouring are common among fungi, the different groups of the former Zygomycota are the least explored organisms in this respect.

Our aim was the detection and molecular characterisation of the dsRNA fragments in different fungal strains e.g., *Umbelopsis*, *Mortierella*, *Mucor*, *Rhizopus* and *Lichtheimia* isolates belonging to the phylum Mucoromycota.

We found 31 dsRNS-harbouring strains from the total 229 investigated isolates. The molecular identification of the detected dsRNA elements of five *Umbelopsis*, fourteen *Mortierella*, four *Mucor*, four *Rhizopus* and three *Lichtheimia* strains by whole genome

sequence analyses is in progress. These isolates harbour different dsRNA patterns with 1 to 6 discrete and differently sized (1.7-10.0 kb) dsRNA bands. By now, we have examined *Umbelopsis ramanniana* NRRL 1296 strain in detail, in which four dsRNA fragments (2.8-5.3 kb) could be observed. These fragments correspond four novel dsRNA viruses: Umbelopsis ramanniana virus 1 to 4 are related to viruses of *Totiviridae* family. By transmission electron microscopy, two different isometric virus particle types were detected in this strain.

Attempts to eliminate viruses from virus-harbouring strains are in progress as well as the comparison of macro- and micromorphology, growth and germination rates of virus-harbouring and virus-free isolates. We also plan to examine the possible effect of the virus presence on the virus-harbouring *Rhizopus* and *Lichtheimia* isolates, and the correlation between virus-harbouring and the virulence potential.

This research was supported by „Lendület" LP2016-8/2016 and TUDFO/47138-1/2019-ITM and TK was Supported By The UNKP-19-3-SZTE-130 New National Excellence Program Of The Ministry For Innovation And Technology.

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

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Poly(ADP-ribosyl)ation is a post-translational modification of proteins. This reaction occurs in response to DNA damage. When DNA is damaged, poly(ADP-ribose) polymerase (PARP) recognizes the cutting edge of DNA and forms poly(ADP-ribose) (PAR) on to acceptor proteins. PARP is a highly conserved in all eukaryote except for yeast, and that is encoded by prpA in genome of filamentous fungus *Aspergillus nidulans*. PAR is degraded mainly by poly(ADP-ribose) glycohydrolase (PARG). The physiological significance of PARG is not fully understood. In eukaryote, PARG is encoded at least one gene in the genome, and parg-knockdown results in the lethal sensitivity against DNA-alkylating agents or gamma-irradiation. It is also reported that parg-knockdown occurs the derangement of biological clock in *Arabidopsis thaliana*. PARP homologs are found in mammals, plants, metazoans, protists and filamentous fungi, but not in yeasts, while PARG homologs are identified in all eukaryotes except for yeasts and some fungi including *A. nidulans*.

Here, we searched for the parg gene in the model fungus *A. nidulans*. Seven genes that are putatively catalyzed nucleotide hydrolysis were selected for the candidate of fungal

parg. These proteins were expressed as soluble proteins using the *Escherichia coli* expression system. Among them, one protein hydrolyzed poly(ADP-ribose), and PARG activity was enhanced by the addition of 10 mM MgCl2. These findings are consistent with the conclusion that this enzyme has PARG activity, and thus we designated the novel enzyme as fungal PARG (fPARG). The amino acid sequence of fPARG does not show homology with known PARG. In this study, we discovered novel PARG in *A. nidulans*.

A genetic transformation method for the mycetoma-causing agent *Madurella mycetomatis*

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Mycetoma is a neglected tropical disease that can be caused by both bacteria (actinomycetoma) and fungi (eumycetoma), with the fungus *Madurella mycetomatis* being the most common causative agent [1]. At the moment, treatment options for eumycetoma are limited and life-changing surgery is often required. There is a gap in the knowledge of the pathogenesis of this fungal species on a genetic level. The genome of *M. mycetomatis* has only recently been sequenced and so far it has not been possible to genetically manipulate *M. mycetomatis* [2]. Genetic manipulation of various pathogenic fungal species, including *Aspergillus* and *Candida* species, has increased our knowledge of the function of genes and pathways involved in virulence. Therefore, it is vital that such a tool will be established for *M. mycetomatis* as well.

In this work, a protoplast-mediated transformation protocol was applied to *M. mycetomatis*, as it is one of the most common methods to genetically manipulate fungal species [3]. Protoplast formation was optimized and different selection markers, including hygromycin and terbinafine, were tested. The transformation method was tested with ectopic integration of vectors and targeted gene deletion.

With this method, the genes and pathways involved in the virulence of *M. mycetomatis* can be deciphered. This will lead to more knowledge on the regulation of the pathogenesis, which will lead to new possible targets for antifungal drugs to treat eumycetoma.

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Genome evolution in the new model yeast species *Saccharomycodes ludwigii*: trade-off between meiotic recombination and epigenetic inheritance

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Sexual reproduction evolved early in the evolution of eukaryotes and is a ubiquitous reproductive mode among extant organisms. Despite the remarkable variability in sexual life cycles, resulting from numerous combinations of sexual determination systems and mating preferences, our understanding of the involved cellular machineries and their evolutionary consequences is based on a tiny number of model species. Here we establish the sexual yeast species *Saccharomycodes ludwigii* as an ideal new model system for the study of the impact of meiotic recombination on genome and proteome evolution. We used whole-genome sequencing and comparative genomics of natural isolates, as well as a high-resolution variant segregation analysis in meiosis to dissect the extent of meiotic recombination in the species. Our analyses provided clear evidence that meiotic crossing over occurs extremely rarely, although it is still possible, as also expected by the existence of a nearly complete meiotic gene complement. Non-crossover-mediated genomic interactions are also very limited. Meiotic GC-biased gene conversion has therefore a small contribution to the shaping of genomic GC content in *Sd. ludwigii*. This, together with the mutation bias towards AT that we determined using a mutation accumulation analysis, presumably account for the unusually low genomic GC level in this genome, which is characterized by numerous AT-rich repeats and low-complexity regions. A significant fraction of genomic repeats, many of which were predicted to code for putative prion-forming domains, are found within open reading frames. By using fluorescent tagging, microscopy and time-lapse imaging we determined prion-suggestive protein aggregation and inheritance upon cell budding. Our results altogether showcase *Sd. ludwigii* as an ideal model system for the study of the

roles of recombination in evolution and the dissection of novel evolutionary strategies for adaptation that do not rely on recombination.

Ectopic recombination between solo-long terminal repeats triggered pathogenic changes and genome rearrangement in the rice blast fungus

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The rice blast fungus, *Pyricularia oryzae*, rapidly adapts to newly developed resistant cultivars through frequent loss of the *avirulence* (*Avr*) gene. In addition, chromosomal polymorphisms, including genome-wide multiple translocation of *Avr-Pita* (deletion, duplication, and translocation), are detected in various field isolates (Chuma *et al*., 2011). Considering the DNA repair properties and genome structure of *P. oryzae*, ectopic recombination between solo-long terminal repeats (solo-LTRs) may cause pathogenic and genomic evolution. To confirm this, using engineered nuclease TALEN, we introduced a specific DNA double-strand break (DSB) into the closed-to-solo-LTR, flanking *Avr-Pita,* in the O29-J isolate. Southern blot analysis showed that TALEN-mediated DSB triggered the deletion and translocation of *Avr-Pita*, which enabled it to infect the resistant cultivar Yashiro-mochi. Sequencing analysis revealed that the recombination between the tandem solo-LTRs flanking *Avr-Pita* resulted in the loss of *Avr-Pita*. The loss of *Avr-Pita* was also observed at the subtelomeric region of the supernumerary chromosome in the O23IN isolate. In this case, telomere capping and duplication/translocation of the other subtelomeric region via ectopic solo-LTR recombination led to the loss of *Avr-Pita* and genome rearrangement. Using a marker system to detect ectopic solo-LTR-like recombination showed that various stress conditions dramatically accelerated the recombination in the genome of *P. oryzae*.

Control of virulence by sirtuins in *Ustilago maydis*

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Ustilago maydis is a fungal plant pathogen that produces tumors in several tissues on its host, maize. As most of plant fungal pathogens, *U. maydis* develops many morphological changes during the pathogenic process in order to ensure proper infection. These sequential morphological changes have to be tightly regulated through the control of different transcriptional programs. The control of genetic programs involved in developmental changes has been typically shown to be regulated by chromatin modifying factors. In terms of chromatin biology, *U. maydis* lacks some of the main proteins involved in heterochromatin formation, such as Heterochromatin Protein 1 (HP1), RNA interference machinery and H3K27 methyltranferases. A similar scenario is found in *Saccharomyces cerevisiae*, where heterochromatin-like structures are performed by the complex SIR. The catalytic subunit of this complex is the sirtuin (NAD-dependent histone deacetylase) Sir2. In order to study a possible role of this protein in the pathogenic control of *U. maydis*, we have identified a Sir2 homolog in this fungus and observed that it is involved in the regulation of the infection process, as the overexpression of Sir2 during the pathogenic program reduces infection symptoms in the plant. In addition, we observe that Sir2 is controlling cell to hypha transition and is repressing filamentation-related genes as well as some additional pathogenic related genes during non-pathogenic condition. We are currently studying in depth the role of Sir2 in the transcriptional control of pathogenesis and its possible role in chromatin modification.

Interaction of a fungal lncRNA with a transactivator enhances cellulase production in *Trichoderma reesei*

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The regulatory network for the expression of cellulase-encoding genes in the industrially relevant fungus *Trichoderma reesei* has been studied for decades. It has recently been shown that HAX1, the first long non-coding RNA ever discovered in filamentous fungi, has a positive impact on cellulase expression. Interestingly, three differently sized HAX1 variants could be identified in the investigated *T. reesei* strains. While the shortest version was most abundant in the wild type strain QM6a, the longest variant occurred

most frequently in the hypercellulolytic strain Rut-C30. When the different HAX1 versions were individually overexpressed, we observed increased enzyme production as compared to the wild type strain, depending on the length of the overexpressed HAX1 variant. Furthermore, we demonstrated that HAX1 interacts with Xylanase regulator 1 (Xyr1), which represents the crucial transcriptional activator of cellulase-encoding gene expression in *T. reesei*.

Developmentally-regulated oscillations in the expression of UV repair genes in a soilborne plant pathogen dictate UV repair efficiency and survival

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The ability to withstand UV damage shapes the ecology of microbes. While mechanisms of UV tolerance were extensively investigated in microorganisms regularly exposed to the sun, far less is known about UV repair of soilborne microorganisms. *Fusarium oxysporum* is a soilborne, fungal plant pathogen that is resistant to UV. We hypothesized that its UV repair capacity is induced to meet irregular sun exposure. Unlike the SOS paradigm, our analysis revealed only sporadic increases and even decreases in UV repair gene expression following UVC irradiation or exposure to visible light. Strikingly, a major factor determining the expression of UV repair genes was the developmental status of the fungus. At the early stages of germination, the expression of photolyase increate while the expression of UV endonuclease decreased, then the trend was reversed. These gene expression oscillations were dependent on cell cycle progression. Consequently, the contribution of photoreactivation to UV repair and survival was stronger at the beginning of germination than later when a filament was established. *F. oxysporum* germinates following cues from the host. Early on germination, it is most vulnerable to UV; when the filament is established the pathogen is protected from the sun because it is already within the host tissue.

Genomic Analysis of Ketoconazole Resistance in the Dandruff-associated Pathogenic Fungus *Malassezia restricta*

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Malassezia restricta is an opportunistic fungal pathogen on human skin and is associated with various skin diseases including seborrheic dermatitis, dandruff and atopic dermatitis, which are commonly treated with the azole antifungal drug ketoconazole. In this study, we clinically isolated ketoconazole resistant *M. restricta* strains, designated as KCTC 27529 and KCTC 27550, from dandruff patients. To understand the ketoconazole resistance, the genome and transcriptome of the isolated strains were sequenced and compared with that of the susceptible reference *strain M. restricta* KCTC 27527. With our genomic approaches, we identified multiplications of the genomic locus encoding the homolog of Atm1 in *M. restricta* KCTC 27529, the result of which was supported by our transcriptome analysis showing an increased expression of the ATM1 homolog in the same strain. Atm1 is a mitochondrial iron exporter and is involved in Fe-S cluster transport as well as azole sensitivity in fungi implicating that the protein also contributes azole resistance in *M. restricta*. Furthermore, transcriptome analysis suggested that the homolog encoding the PDR5 homolog is significantly up-regulated in *M. restricta* KCTC 27529 implying that, in addition to the genomic multiplication of the ATM1 homolog, an increased drug efflux influences the ketoconazole resistance of the strain. The mechanism of ketoconazole resistance in the other resistant isolate KCTC 27550 is different from the strain KCTC 27529. The comparative genome and transcriptome analyses revealed that there is a genomic multiplication of the locus encoding homologs of ERG11 in *M. restricta* KCTC 27550 which are highly expressed compared to the reference strain. Overall, our data suggest genomic rearrangement, for example, multiplication of the *locus* encoding genes involved in drug resistance, is a common mechanism of ketoconazole resistance in *M. restricta*. In addition, PDR5 has an important role in the azole resistance of *M. restricta*.

Complete mitochondrial genome sequences of *Aspergillus luchuensis, Aspergillus parasiticus* and *Aspergillus pseudoglaucus*

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Aspergillus luchuensis is a filamentous fungus used for food and alcohol fermentation in many Asian countries. *Aspergillus parasiticus* is a notorious filamentous fungus, which can produce aflatoxin B and G. And *Aspergillus pseudoglaucus* is a xerophilic filamentous fungus which can produce various secondary metabolites. Here, we reported

the complete mitochondrial genome sequences of *A. luchuensis* and *A. pseudoglaucus* isolated from fermented soybean brick, called as Meju, in Korea, and *A. parasiticus* also isolated in Korea. The mitochondrial genomes were successfully assembled from raw reads sequenced using MiSeq by Velvet and GapCloser. Total length of the *A. luchuensis, A. parasiticus* and *A. pseudoglaucus* mitochondrial genomes are 31,228 bp and encoded 44 genes (16 protein-coding genes, two rRNAs, and 26 tRNAs), 29,141 bp and encoded 45 genes (17 protein-coding genes, 2 rRNAs, and 26 tRNAs), and 53,882 bp, which is third longest among known *Aspergillus* mitochondrial genomes and encoded 58 genes (30 proteincoding genes including hypothetical ORFs, two rRNAs, and 26 tRNAs), respectively. The mitochondrial genomes can be used for further analyses of *Aspergillus* mitochondrial comparative genomics to improve understanding of diverse *Aspergillus* species.

The genome of *Geosiphon*: an arbuscular mycorrhizal fungus that forms symbioses with cyanobacteria

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Geosiphon pyriformis is the only known fungus that forms endocyanosis with cyanobacteria (*Nostoc spp*). Ribosomal genes usually place this fungus near the base of phylogenetic tree of arbuscular mycorrhizal fungi (AMF), but no genome is publicly available from this lineage. Here we present the genome sequence of *Geosiphon pyriformis*, which we sequenced using Illumina paired-ends and mate pairs. Using a unique trinucleotide K-mer approach, we obtained a draft genome sequence for this species that includes 931 scaffolds, with a N50 value of 688969 bp (largest scaffold of 2.7 Mb). Genome completeness (BUSCO > 96%) and gene counts (26,000) are comparable to other available AMF genomes. Phylogenetic analyses confirmed the placement of *G. pyriformis* based on ribosomal data, and analyses of Pfam domains revealed enrichment in various transposon and lipase related domains compared to other AMF taxa. Assessment of KOG functions also identified an enrichment for genes involved in lipid transport and metabolism in *G. pyriformis*, but genes implicated in the fatty acid complex are absent from the genome. We also found evidence that *G. pyriformis* carries a one speed-genome. The *G. pyriformis* genome provides new opportunities to understand the evolution of the ancestral AMF toolkit, and we present evidence of horizontal gene transfers and regions involved in sexual reproduction in these ancient plant symbionts.

Trichoderma reesei Rad51 can tolerate mismatch sequences to promote interhomolog recombination and chromosome synapsis during hybrid meiosis

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Homologous recombination directs error-free repair of DNA double-strand DNA breaks (DSBs) during mitosis as well as creates new combinations of genetic materials in gametes during meiosis. Most sexual eukaryotes (budding and fission yeast, higher plants, mammals and some basidiomycete fungi) possess two RecA-like recombinases (ubiquitous Rad51 and meiosis-specific Dmc1), whereas the Rad51-only eukaryotes (e.g., *D. melanogaster, C. elegans* and *Pezizomycotina filamentous* fungi) have lost Dmc1 during evolution. In yeast and mammals, Rad51 and Dmc1 collaborate to mediate interhomolog recombination pathway *via* using a homologous non-sister chromosome as template for DSB repair. Dmc1 is superior to Rad51 in tolerating mismatched sequenze during DNA strand exchange reactions in highly polymorphic hybrid meiosis. In this study, *Trichoderma reesei*, an industrial workhorse filamentous fungus, was used to address whether and how the Rad51-only eukaryotes proceed interhomolog recombination between highly diverse zygotes. We applied multidisciplinary approaches (genetics, genomics, biochemistry and single molecule biophysics) to show that *T. reesei* Rad51, like yeast and mammalian Dmc1 (but not Rad51), can tolerate mismatched sequences during the DNA strand exchange reactions in meiosis.

Poster Session 1.5
OMICS & BIOINFORMATIC

THURSDAY, FEBRUARY 20

18:00 - 19:30 | Location: **Frentani Convention Center**

In-house long read sequencing yields affordable superb fungal genome assemblies

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Filamentous fungi are known to produce a wide range of secondary metabolites that are of food, agrochemical and pharmaceutical interest. The traditional way to discover novel secondary metabolites from fungi is to screen the compounds produced under permissive conditions. However, this is quite tedious and labor intensive since numerous conditions must be screened for each fungus and still no guarantee of achieving permissive conditions exist. An alternative approach is whole genome sequencing followed by genome mining based on predictions of conserved domains, which can be utilized to identify secondary metabolite gene clusters of interest – thus predicting the synthesis potential of filamentous fungi. Promising candidate gene clusters can then be transferred and overexpressed in heterologous production hosts or laborious screening of growth conditions can be undertaken on a limited and qualified set of fungi.

In this poster, we present an in-house and affordable sequencing pipeline utilizing the MinION platform from Oxford Nanopore Technologies for the cost-efficient sequencing of hundreds filamentous fungi. Recent advancements in MinION sequencing technology and bioinformatics data analyses enables quick and straightforward long-read sequencing and highly contiguous assemblies with a low error rate. In relation to genome mining in fungi, an added benefit of long-read sequencing is that single reads spanning entire gene clusters can usually be found, providing experimental validation of the candidate gene clusters. Thus eliminating the possibility of artefact chimeric gene cluster made by assembly of short reads.

Genome sequence of *Stemphylium vesicarium*, the causal agent of Brown Spot disease of Pear

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Stemphylium vesicarium (Wallr.) E. Simmons is the causal agent of several plant diseases as well Brown Spot of Pear (BSP), which is one of the most economically important fungal diseases in European pear-production areas. Moreover, conidia widespread from plant material infected by the pathogen can trigger respiratory allergy. We were aimed to provide the first available genome sequence of *S. vesicarium*. The resource is presented accompanied by genome properties.Genomic DNA was extracted from the monoconidial strain 1731a13FI1M3 isolated from pear, then *de novo* sequenced by shotgun on Illumina Miseq platform v3. 1,127 contigs were assembled and a total assembly length of 38.66 Mb were obtained. The size was similar to those of *Stemphylium lycopersici*, the only other genome currently available for the *Stemphilium* genus. Gene prediction resulted in 12,309 putative genes. A primary functional annotation provided information about Orthologous Groups, Gene Ontology terms, KEGG pathways, and SMART/Pfam domains for each group. Furthermore, combined prediction of transmembrane topology and signal peptide were carried out, and genome sequence was also analyzed for the automatic genomic identification and analysis of biosynthetic gene clusters. Among predicted genes it was also possible to identify several orthologues required in other Ascomycota as related to: pathogenesis or full virulence on plants, signal transduction, cell respiration. Furthermore, the annotation was able to detect the orthologues of alt a 1 major and alt a 7 minor *A. alternata* allergens. The availability of this genome opens a new scenario in the investigation of *S.vesicarium* lifestyle and molecular plant/pathogen interaction, and could be fundamental to design more effective and sustainable fungicides management strategies to control BSP and other plant diseases. The genome sequence has been deposited in the GenBank under the accession number:QXCR00000000 (BioProject: PRJNA470620, BioSample: SAMN09098503).

Comparative genomics of transposable elements and Repeat-Induced Point (RIP) mutation landscapes in *Neurospora* species

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A large portion of nuclear DNA is composed of transposable element (TE) sequences, whose transposition is controlled by diverse host defense strategies in order to maintain genomic integrity. One such host defense strategy, first identified in *Neurospora crassa,* is the fungal-specific repeat-induced point (RIP) mutation that hyper-mutates repetitive DNA sequences and is identified by numerous C-to-T substitutions. RIP in *N. crassa* is extremely effective; all identified TEs show signs of extensive RIP mutation patterns. Whether the TE landscapes and associated RIP patterns observed in *N. crassa* are conserved among closely-related *Neurospora* species is currently unknown. In this study, we compare whole genomes of closely-related *Neurospora* species, which diverged from the last common ancestor about 7 million years ago, to determine genome-wide TE distribution and abundance. The reference *N. crassa* assembly as well as our generated long-read-sequenced genome assemblies, representing 10 lineages and 18 genomes between 37.8 MB and 43.9 MB, show that the TE content varies 8.7-18.9% across genomes. Degraded Long Terminal Repeat- (LTR-) retroelements are most abundant among the identified TEs, which are distributed across all seven chromosomes of *Neurospora* at varying frequencies with highest densities around putative centromeres. In closely-related *Neurospora* species, TE sequences show signs of numerous C-to-T substitutions, suggesting that RIP occurs in these species. RIP signatures in these *Neurospora* species overlap with TE-dense regions, and RIP intensity seems to vary between these species. Leveraging the completeness of the reference assemblies and our generated high-quality genome assemblies, we are now equipped to better understand the evolution of RIP and TEs in *Neurospora*.

Conserved white rot enzymatic mechanism for wood decay in the *Basidiomycota* genus *Pycnoporus* explored by genomics and proteomics

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White-rot fungi (WRF) are pivotal decomposers of dead organic matter in forest ecosystems and play a role in the dynamics of carbon cycling. These fungi typically use a large array of hydrolytic and oxidative enzymes to deconstruct lignocellulose. However, the extent of lignin and cellulose degradation may vary between species and wood type.

Here we combined comparative genomics, transcriptomics and secretomics to identify conserved enzymatic signatures at the onset of wood decaying activity within the Basidiomycota genus *Pycnoporus* including white-rot species widely distributed in temperate, tropical and subtropical land areas.

One originality of our work is the processing of gene expression data which allowed the identification of conserved gene regulations across three species of the genus. Co-regulations were further inspected at the protein level by secretome sequencing.

We observed strong conservation in genome structures and repertoires of protein coding genes within the genus *Pycnoporus*. *P. cinnabarinus, P. sanguineus* and *P. coccineus* mobilized a conserved set of enzymes for breaking down cellulose, hemicellulose and pectin. Cellobiose dehydrogenase genes were invariably co-regulated with at least one AA9 LPMO gene copy, suggesting enzymatic synergy in vivo. The co-occurrence in the secretomes of H2O2 producing enzymes (GMC-oxidoreductases and glyoxal oxidases) with H2O2 consuming enzymes (class II peroxidases and AA9 LPMOs) was a common feature of three *Pycnoporus* species, although each enzymatic partner displayed independent transcriptional regulation.

Finally, the regulation of genes involved in oxidative degradation of lignin and in detoxification was largely species-specific.

Development of single and multitargeted gene deletion methods in the inky-cap fungus *Coprinopsis cinerea*

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The FUNCODE project aims to reconstruct gene regulatory networks (GRNs) in five widely used fungal species to gain deeper insight into gene expression response to environmental changes, lignocellulose degradation and complex multicellular development. To reconstruct GRNs, information obtained from genome-wide mapping of transcription-factor (TF) binding sites will be combined with transcriptome data of TF

knockout strains. To generate TF knockout strains a reliable, homologous recombination-mediated genome editing method is required.

The inky-cap fungus *Coprinopsis cinerea* (Agaricales, Basidiomycota), one of the model organisms of the study, is a litter decomposing fungus, and a model organism of mushroom development. We adapted CRISPR/Cas9-based genome editing via *in vitro* assembled ribonucleoprotein (RNP) complex to induce DNA double-stranded break (DSB) and homology-directed repair (HDR) in *C. cinerea,* in the absence of a deficiency in the non-homologous end joining pathway (NHEJ). The efficiency of gene disruption and its repair via HDR was 0.5 – 3%, which was verified with PCR and sequence analysis. Our approach offers a simpler and HDR-based alternative to a plasmid-based Cas9 and gRNA expression described in *Coprinopsis* [1], similar to genome-editing approaches reported recently for *Schizophyllum* [2]. Using an RNP complex eliminates the need for a time-consuming plasmid construction for Cas9 and gRNA expression and reduces off-target effects. To aid the deletion of large fragments (e.g. complete elimination of DNA binding domain of TFs), we also tested paired gRNAs. DSBs induced with two RNP complexes without HDR were also observed in that case, with which case, the efficiency of gene disruption could be increased more. These results pave the way for routine genetic manipulation of one of the most widely used model system in basidiomycete biology.

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Capturing biochemical information in the CAZy database

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Since 1998, the Carbohydrate-Active enZYmes (CAZy; www.cazy.org) [1] database describes the sequence-based families of enzymes and associated domains that cleave, modify, and build glycans. As of today CAZy lists more than 1.2 million entries arranged in over 320 enzyme families, including data from over 13,300 genomes. This classification provides a system that correlates structural features and molecular mechanism of carbohydrate active enzymes and has become a reference system in our field.

Our expert biocuration of sequence and functional information provides the community a manually curated and up-to-date knowledge. Within the CAZy system, enzyme specificity

is displayed (in the form of an EC number) only based on experimental evidence. The assignment of a function to an entry thus depends on the availability of experimental data, ideally providing substrate (and/or product) specificity. The exponential growth of sequence data requires extensive automation for the identification of CAZymes. After more than 20 years of continuous activity, the classification and modular analysis of these enzymes has reached maturity. However, the management of functional data still relies on limited vocabularies and on heavy biocuration.

In order to pursue this effort and to make functional biocuration data available to the entire community, we have implemented on our website a form to submit the minimum information required to assign a function to a sequence. We reasoned that this process would be less time consuming and less error-prone if the researcher at the origin of a newly characterized sequence provides these details. Thus with the help of our community, we will be able to dedicate our efforts to improving our database and associated tools to cope with the ever-increasing amount of data and contribute a better understanding of the metabolism of complex carbohydrates in general.

Application of comparative promoter analysis for understanding of secondary metabolism regulation in non-model fungi

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Genes involved in secondary metabolite (SM) biosynthesis are often clustered in fungal genomes; this means, they are they are co-localized on a chromosome and co-regulated. About half of known SM gene clusters have an “own” embedded transcription regulator responsible for their induction. But in those cases when there is no cluster-specific TF, it is hard to find out, which TF is involved in the coordination of expression of the cluster genes. The situation becomes even more challenging in non-model fungi, especially in those that are not susceptible to genetic manipulations. In these cases, we have to rely on bioinformatics methods to predict the most probable candidate regulators and therefore reduce the experimental work. One of the fruitful approaches is comparative genomics combined with promoter analysis. In this poster, I will show how it can be applied to getting insights into mechanisms and evolution of SM regulation in fungi. The main assumption is that independently of whether a TF is embedded in the cluster or not, its binding sites must be shared by the promoters of all regulated genes. Conservation of the clusters between species can imply conservation of respective upstream signalling pathways, and so we can assume involvement of the same TF in the regulation in different species. Thus, applying comparative analysis, we come up not only with prediction of common motifs shared between the clusters but also with prediction of

potential regulator, or whether the mode of regulation might differ between the species. In the poster, I will provide examples of how comparative promoter analysis helps us to get new insights into the possible regulatory mechanisms in non-model fungi.

New genomic resources for the brown rot fungal pathogens *Monilinia fructicola*, *Monilinia laxa* and *Monilinia fructigena*

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Monilinia fructicola (MFRC), *Monilinia laxa* (MLAX) and *Monilinia fructigena* (MFRG) are the main causal agents of brown rot on stone and pome fruits. Genomic and transcriptomic data for the three fungal species have so far remained largely unexplored. The complete transcriptomes of MFRC (GenBank accession GGAK00000000), MLAX (GGAL00000000) and MFRG (GGAM00000000) was sequenced (Illumina technology), de-novo assembled and annotated[1]. Comparative analyses among orthologous transcripts led to identify 65 transcripts significantly over-expressed or unique in MFRC, 30 in MLAX and 31 in MFRG. They represent sets of potential key genes associated with fungal morphogenesis and development, secondary metabolism and host-microbiota-pathogen interactions. High-quality draft genomes of *M. fructicola* Mfrc123 strain (VICG00000000)[2], *M. laxa* Mlax316 strain (VIGI00000000)[3] and *M. fructigena* Mfrg269 strain (QKRW00000000)[4] were obtained through a hybrid and hierarchical de-novo assembly strategy using both Illumina and PacBio sequencing technologies. They consisted of 20 (MFRC), 49 (MLAX) and 131 (MFRG) scaffolds, with a total length of 44.05, 42.81 and 43.125 Mb and a scaffold N50 of 2,592, 2,449 and 768 kb, respectively. Genome annotations identified a total of 12,118 (MFRC), 11,163 (MLAX) and 10,502 (MFRG) genes and 13,749, 12,424 and 9,960 proteins, respectively. These newly generated reference genomes improve current available resources in terms of contiguity, gap-free sequences, and read mappability and provide structural and functional annotations of the genomes. They are expected to significantly contribute to comparative analysis of genome biology and evolution within *Monilinia* species.

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Comparative genomics of smut fungi indicate ability of meiotic division and sexual reproduction in the genus *Pseudozyma*

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Ustilaginomycetes are a group of host specific plant parasitic fungi that undergo an anamorphic yeast stage and a teleomorphic parasitic stage during their life cycle. However, species in the genus *Pseudozyma* do not have a described teleomorphic stage and are therefore viewed as asexual species. Using comparative genomics of 28 Ustilaginomycete species, we analyzed the occurrence of 27 core meiosis genes as well as mating receptors and pheromones to assess the question if these species have retained the genetic makeup for mating and meiosis. The presence of pheromone receptor genes and pheromones in the genus *Pseudozyma* leads to the conclusion that these species have the ability to mate, which is further supported by the occurrence of core meiosis genes. Thus, this raises the question whether these species have a parasitic stage or have recently switched to a non-parasitic life style as an evolutionary strategy, maybe after losing access to their host species.

Uncovering long non-coding RNA associated with drug response in *Aspergillus fumigatus*

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Our understanding of the non-coding RNA (ncRNA) repertoire in the pathogenic fungus *Aspergillus fumigatus* is limited. Excluding housekeeping ncRNA, less than 20 ncRNAs have been identified in the sequenced type strain Af293, with the majority of these being small ncRNA. Long non-coding RNAs (lncRNAs) have emerged as important regulatory elements in many organisms and we hypothesised that they could influence the way *A. fumigatus* responds to antifungal drugs. RNAseq data from 6 drug exposure

experiments were used to generate a novel *A. fumigatus* transcriptome assembly and identify lncRNA candidates. Using this assembly, we performed differential expression analysis to discover over 100 candidates which are associated with response to the antifungal, Itraconazole. Gene knockouts of a subset of these transcripts were generated to investigate their influence on stress and drug responses in *A. fumigatus*. This study has revealed novel putative lncRNA in *A. fumigatus* which may contribute to, and inform our understanding of, the mechanisms of drug resistance in this pathogen.

Trichoderma harzianum aluminum tolerance is mediated by a large change in gene expression profile

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Excessive amounts of the metal aluminum (Al) should be considered a concern to the agricultural industry, as it can affect several cellular processes, impairing crops development. The region of Cerrado is the second largest biome in Brazil, being its soils generally acid and poor in nutrients, requiring some treatments before usage for cultivation. Some microorganisms, as the fungus *Trichoderma harzianum*, are commonly applied in seeds to promote plant growth, since it may enhance nutrient uptake, control pathogens and induce stress resistance in plants. In this study, we investigated the effects of aluminum in the global gene expression profile of *T. harzianum*, in order to better understand the applicability of the fungus in this region. Our results showed that of the 13,932 genes predicted by the reference genome, 6,364 were differentially expressed in the presence of the metal, being 3,239 up-regulated and 3,125 down-regulated. Gene Ontology categorization and enrichment indicated that the biological processes related to nucleobase-containing compound biosynthetic process, RNA biosynthetic process and glycosyl compound metabolic process were mainly up-regulated, and carbohydrate metabolic process, organonitrogen compound biosynthetic process, electron transport chain, cellular amide metabolic process and regulation of protein modification process were mainly down-regulated. Molecular functions as transcription regulator, drug binding, DNA binding, ligase and GTPase binding were up-regulated while protein dimerization, hydrolase and electron transfer were down-regulated. Together, these results suggest a great readjustment in the expression of the genes and possibly in the synthesis of proteins of *T. harzianum* in the presence of aluminum in concentration similar to the existent in the Brazilian Cerrado soil. The data obtained in this study provides possible targets for biotechnological improvement of the fungus survival and application in this biome.

Draft genome assemblies of a global collection of *Pyrenophora tritici-repentis*, the causal agent of tan spot disease of wheat

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The fungus *Pyrenophora tritici-repentis* (*Ptr*) causes tan spot, a destructive foliar disease of wheat worldwide. Eight races of this fungus have been identified based on their ability to produce combinations of three necrotrophic effectors: Ptr ToxA, Ptr ToxB and Ptr ToxC. In this study, the complete haploid asexual genomes of 43 *Ptr* isolates representing all known races from various geographical regions were sequenced. In total, twenty-three isolates from Canada, twelve from the Caucasus, and eight from North Africa were sequenced on the Illumina HiSeq X platform. Two isolates out of that pool, representing races 8 and 3, were also sequenced using PacBio RS II to generate hybrid assemblies by assembling the PacBio reads de novo with Flye[1,2] and polished with Illumina reads using Pilon[3], which will be used as references for comparative genomics analyses. Furthermore, de novo assemblies were generated with the Illumina reads only using the following programs and testing different algorithms: Shovill (with Spades)[4,5], Shovill (with Megahit)[6,5], SoapDenovo27, and CLC Genomics Workbench 12 (Qiagen Inc., 2018). Genome annotation was carried out with the FunGAP pipeline[7]. Parameters such as N50, number of contigs (>= 500bp), number of gaps ('N' insertions), and number of genes annotated were used to compare and choose the best de novo assembly algorithm for this dataset. To our knowledge, this is the most comprehensive collection of global *Ptr* genomes assembled, and preliminary results from the pan-genome analysis to investigate genome plasticity will be presented.

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Novel method in genome mining and transcriptome analysis reveal undiscovered RiPPs in *Trichoderma spp*.

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Microorganisms can produce a diverse range of secondary metabolites (SM). With the growing threat of multi resistant pathogens and the high rediscovery rate of known SM by conventional methods, there is a growing need for new bioinformatic approaches targeting SM with diverse modes of action that tackle therapeutic challenges. Ribosomally synthesized and post-translationally modified peptides (RiPPs) are a chemically and structurally diverse group of SM. RiPPs have many different applications such as food preservatives, antibiotics and anti-cancer drugs. RiPPs are commonly produced by bacteria such as *Bacillus spp*. and *Streptomycetes spp*., but only very few fungal RiPPs are known. However, fungal RiPPs are gaining special attention due to their enormous diversity and application potential. We developed a new procedure for mining fungal genomes with the specific focus on the identification of RiPP precursor peptides by using existing tools, such as antiSMASH and RiPPMiner. First, to validate this procedure, we mined the genomes of the known fungal RiPP producers *Aspergillus flavus* and *Amanita phalloides* and could re-identify the biosynthetic gene clusters responsible for the respective RiPPs. Next, we mined the genomes of four different *Trichoderma spp*. representing the whole genus. Our approach found a total of 779 potential RiPP precursor peptides in all mined genomes and recognized 79 known fungal precursor peptides including the ustiloxin B precursor peptide. Furthermore, a phylogenetic analysis revealed conserved putative RiPP precursor peptide genes within the Trichoderma genus. Through transcriptome analysis using previously published RNAseq data and RT-qPCR we showed that the predicted precursor peptides are transcribed in *T. reesei* QM6a. Our study is the first report of the ability of *Trichoderma* to produce RiPPs, the detected putative RiPP clusters encode potential novel uncharacterized RiPPs that may path the way for the discovery of novel antibiotics.

CreA regulation was observed at low free monosaccharide level during *Aspergillus niger* grown on crude plant biomass

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Carbon catabolite repression (CCR) is a universal regulatory system that enables fungi to selectively utilize the energetically most favorable carbon sources and thrive in diverse environments. In most filamentous fungi a conserved zinc finger transcription factor CreA/Cre-1 has been identified as the key regulator to control this process. However, most current knowledge about the CreA regulation is based on fungi grown with high concentrations of monosaccharides, which is clearly different from fungal natural biotopes and growth conditions during their industrial applications that use crude plant biomass as a carbon source.

To assess whether and how the CreA plays a role during growth of fungi on more natural and industrial related carbon sources, we comparatively analyzed the time-course transcriptome of an ascomycete fungus *Aspergillus niger* creA deletion strain (ΔcreA) and reference strain during growth on crude plant biomass (sugar beet pulp and wheat bran). The results revealed that CreA significantly regulates many important genes and pathways involved in lignocellulose degradation and sugar catabolism, but this regulation is strongly carbon source and time dependent. In addition, the integrative analysis of CreA-regulated genes detected in this study and previous data suggest the CreA acts in concert with other important plant biomass related transcription factors for a precise regulation of fungal plant biomass degradation. Our findings support a crucial role of CreA for fungal physiology in natural biotopes and therefore its conservation across the fungi kingdom. In addition, our study provides novel insights into CreA regulation network on lignocellulose degradation, which could contribute to genetic engineering of fungi as cell factories to produce enzyme, biomaterials and biofuel.

A blueprint of the protein secretion machinery in *Neurospora crassa*

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Protein secretion is a fundamental biological process involved in host pathogenesis, immune response, cellular communication and maintaining cellular homeostasis. In eukaryotes, the traffic of proteins destined for extracellular space are processed, packaged and delivered via an intricate network involving several organelles spanning from the cytoplasm to the cell membrane. From an application perspective, elucidating this protein secretion machinery in filamentous fungi is critical for the development

of hypersecretion strains for novel enzymes and/or understanding the host-pathogen interactions in fungal diseases. In this direction, we have built the first genomescale model of the protein secretion system in *Neurospora crassa*. Our computational pipeline to reconstruct the protein secretion system involves a combination of genomics tools and literature mining. Firstly, we have manually compiled and curated from published literature the different components in classical secretion pathway in *N. crassa*. Importantly, our compilation includes evidence, both experimental and/or computational, supporting the annotation of secretion components in *N. crassa*. This effort has led to the function assignment to several proteins with currently unknown function in the published *N. crassa* genome. Secondly, we have captured the protein sorting process in the classical secretion pathway by organizing the components into reactions or mechanisms in the protein secretion system model for *N. crassa*. After reconstructing this model, downstream analysis of next-generation RNA-seq and ChIP-seq data within the network context has shed new insights on the regulation of the protein secretion system in *N. crassa*. Overall, this integrative analysis provides a systems perspective on the protein secretion machinery in *N. crassa* and will have future impact on functional genomics of filamentous fungi.

Prediction and analysis of the secretome of an opportunistic fungal pathogen

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Aspergillus fumigatus and multiple other *Aspergillus* species cause a wide range of lung infections, collectively termed aspergillosis. *Aspergilli* are ubiquitous in environment with healthy immune systems routinely eliminating inhaled conidia, however, *Aspergilli* can become an opportunistic pathogen in immune-compromised patients. The aspergillosis mortality rate and emergence of drug-resistance reveals an urgent need to identify novel targets. Secreted and cell membrane proteins play a critical role in fungal-host interactions and pathogenesis. Using computational pipeline integrating data from high-throughput experiments and bioinformatic predictions, we have identified secreted and cell membrane proteins in ten *Aspergillus* species known to cause aspergillosis [1]. Small secreted and effector-like proteins similar to agents of fungal-plant pathogenesis were also identified within each secretome. A comparison with humans revealed that at least 70% of *Aspergillus secretomes* have no sequence similarity with the human proteome. An analysis of antigenic qualities of Aspergillus proteins revealed that the secretome is significantly more antigenic than cell membrane proteins or the complete proteome.

Finally, overlaying an expression dataset, four *A. fumigatus* proteins upregulated during infection and with available structures, were found to be structurally similar to known drug target proteins in other organisms, and were able to dock in silico with the respective drug [1].

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Comparative genomics between an industrially important species, *Aspergillus sojae*, and harmful one, *Aspergillus parasiticus*

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Aspergillus sojae has been used for soy sauce production for more than 300 years. Taxonomically, *A. sojae* and *Aspergillus parasiticus* are classified into *Aspergillus* section Flavi. This section includes not only industrially important but harmful species. *A. sojae* does not produce aflatoxin, a potent carcinogenic secondary metabolite, while it is almost morphologically-indistinguishable from *A. parasiticus* which infects and damages crops by producing aflatoxin. In 1986, it was reported that the homology between *A. sojae* and *A. parasiticus* was 91% in total DNA hybridization (1). However, the genomic differences between them have not been analyzed in detail (2). In this study, a comparative genomic analysis was performed between *A. sojae* NBRC 4239 and *A. parasiticus* CBS 117618. Using 13,752 annotated open reading frames (ORFs) of *A. parasiticus* CBS 117618 as queries, we searched the *A. sojae* NBRC 4239 genome for homologous genes by Spicio (3). As a result, 11,171 (81.2%) of the 13,752 ORFs were extremely highly conserved (Scipio score >0.95) in *A. sojae*. Scipio score is a value calculated by the following formula: [(the number of amino acid residues that used for actual comparison) – (the number of mismatched amino acid residues in the comparison)] / the number of total amino acid residues of ORF. Similarly, 1,643 (11.9%), 318 (2.3%), 128 (0.9%), and 109 (0.8%) ORFs were conserved in *A. sojae* with Scipio scores of 0.85-0.95, 0.75-0.85, 0.65-0.75, and 0.50-0.65, respectively. Meanwhile, 94 secondary metabolite biosynthesis gene clusters were identified in *A. parasiticus* genome by anti-SMASH (4). In this presentation,

we will discuss the differences between *A. sojae* and *A. parasiticus* from the viewpoint of functions of conserved and non-conserved ORFs.

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Effects of nitrogen deficiency on lipid synthesis and metabolic profiling of xylose assimilating thraustochytrid

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Thraustochytrids are marine heterotrophic unicellular protists. Their lipids rich in fatty acids are expected utilization for biodiesel fuel. However, organic carbon sources such as sugars are required for growth and lipid synthesis of thraustochytrids by contrast with photosynthetic algae. As a cheap carbon source for cultivation of thraustochytrids, lignocellulose containing 30-40% of pentose (mainly xylose) is promising recently. For microbial production of the biofuels from lignocellulosic biomass by thraustochytrids, xylose assimilating thraustochytrids have been isolated. In the present study, metabolome analysis of the cells grown on the medium containing xylose as a sole carbon source were carried out by GC-MS and LC-MS/MS. Moreover, the cells cultivated in the nitrose sufficient medium or nitrogen deficient medium were compared, respectively. As a result, the metabolites of TCA cycle (succinic, fumaric, and malic acids) were decreased and remarkably increased total lipids in the cells cultivated in the medium containing deficient nitrogen. It was suggested that xylose incorporated in the cells were used for lipid synthesis mainly. However, xylose and xylulose were not detected in the cells cultivated in the medium containing sufficient nitrogen. And the cellular lipids decreased compare to the cells cultivated nitrogen deficient medium. Therefore, it was suggested that the nitrogen deficiency affected on the metabolic carbon flow in the isolate. In addition, the contents of cellular coenzymes (NADP, NADPH) showed that nitrogen deficiency accelerated generation of NADPH for active fatty acid synthesis.

Xylose adaptation and metabolic profiling of heterotrophic thraustochytrid for microbial production of biofuels

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Thraustochytrids are marine heterotrophic unicellular protists. They decompose organic matters such as dead leaves of sea grass or mangrove in the ocean, and remarkably accumulate lipids in the cells. Their lipids rich in fatty acids are expected utilization for biodiesel fuel. However, organic carbon sources such as sugars are requie for growth and lipid synthesis of thraustochytrids by contrast with photosynthetic algae. As a cheap carbon source for cultivation of thraustochytrids, saccharified lignocellulose containing 30-40% of pentose (mainly xylose) is promising recently. For microbial production of the biofuels from lignocellulosic biomass by thraustochytrids, xylose assimilating thraustochytrids have been isolated. In the present study, xylose adaptation of xylose assimilating thraustochytrid was carried out for more than three years. The adaptation made the xylose consumption rate of adapted isolate remarkably faster than that of non-adapted isolate. To make clear a mechanism of xylose adaptation, the major metabolites of central metabolic pathway in the cells of adapted isolate were analyzed by using GC-MS and LC-MS/MS, and compared to those of non-adapted isolate. In addition, the contents of cellular coenzymes were also analyzed. These results showed that the optimization of carbon flow and coenzyme balance made the efficiency of the biofuel production from lignocellulosic biomass much more efficient in the future. A part of this research was supported by New Energy and Industrial Technology Development Organization (NEDO) in Japan.

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POSTER SESSION 2 & FLASH TALKS

Poster Session 2.1
ANIMAL – FUNGI INTERACTIONS

WEDNESDAY, FEBRUARY 19

18:00 - 19:30 | Location: **Frentani Convention Center**

Comparative RNAseq analyses of the entomopathogenic fungus *Metarhizium anisopliae* reveal specific signatures of filamentous and yeast-like development

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The fungus *Metarhizium anisopliae* is a facultative insect pathogen used as biological control agent of several agricultural pests worldwide. It is a dimorphic fungus that displays two growth morphologies, a filamentous phase with formation of hyphae and a yeast-like phase with formation of single-celled blastospores. Blastospores play an important role for *M. anisopliae* pathogenicity during disease development. They are solely formed in the hemolymph of infected insects as a fungal strategy to quickly multiply and colonize the insect´s body. Hyphae are are formed as *M. anisopliae* grows in soil, associated with plants or inside killed insect hosts but does not require insects to be formed. *Hyphae and blastospores can be produced in vitro under specific conditions and blastospores have great commercial potential for direct application in biological control. Here, we use comparative transcriptome analyses to characterize physiological changes and metabolic signatures associated with M. anisopliae dimorphism.* Our results show a clear molecular distinction between the blastospore and mycelial phases. In total 6.4% (n=696) out of 10,981 predicted genes in *M. anisopliae* were differentially expressed between the two phases with a fold-change > 4. The main physiological processes associated with up-regulated transcripts in blastospores during liquid fermentation were oxidative stress, amino acid metabolism, respiration processes, transmembrane transport and production of secondary metabolites. In contrast, the up-regulated gene content in hyphae were associated with increased growth metabolism and cell wall re-organization. Our study illustrates important aspects of fungal morphogenesis in *M. anisopliae* and highlight the main metabolic activities of each propagule under *in vitro* growth conditions. Moreover, we found that blastopores produce different secondary metabolites compared to the

hyphal phase, which could be further investigated for potential commercial use.

High efficiency transformation and construction of tools for genetic manipulation of the laurel wilt pathogen, *Raffaelea lauricola*

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The fungal pathogen, *Raffaelea lauricola*, is the causative agent of laurel wilt, a devastating disease of members of the Lauraceae family that includes avocados. The fungus is vectored by ambrosia beetles that carry the fungus in specialized structures (mycangia), with the fungus acting as a food source for beetle larvae growing in tree galleries. Both the beetle and the fungus are considered invasive species and their combined activities have resulted in the death of millions of trees. In order to probe the molecular basis for plant pathogenicity of the laurel wilt fungus, molecular tools including establishment of efficient transformation protocols are required. These data provide a foundation for understanding the molecular factors that make *R. lauricola* such a potent plant pathogen. Resistance marker profiling revealed susceptibility of *R. lauricola* to hygromycin, phosphinothricin, chlorimuron ethyl. *Agrobacterium*-mediated transformation using either the *bar* or the *sur* marker resulted high efficiency transformation. A second protocol using LiCl-PEG treatment of fungal blastopores yielded was also capable of resulting in transformers, although at lower efficiencies. Transformants were mitotically stable (at least 5 generations), and >95% of transformants showed a single integration event. Promoters derived from *R. lauricola* and vectors expressing green and red fluorescent proteins were constructed and successfully transformed into the fungus. A random insertion mutant library of >20,000 clones was generated. Tools for CRISPR-Cas9 mediated genetic manipulation were also constructed. These results establish simple and reliable genetic tools for transformation of *R. lauricola* needed for genetic dissection of the virulence mediated by this plant pathogen.

Expansion of fungi enables high resolution in fluorescence microscopy

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Super-resolved fluorescence microscopy denoted substantial progress in the recent years. In most applications either the optical setup is adapted or certain photophysical properties of the sample are used finally allowing for the record of images below the diffraction limit. In contrast, in expansion microscopy (ExM) the cellular structure is expanded to improve the resolution of fluorescence-based microscopy [1]. Cells are fixed and immunostained before they are embedded in a suitable expandable gel. After enzymatic digestion of all cell components, the gel is expanded thereby uniformly extending the fluorophores.

ExM has not yet been used to visualize fungi. The application of ExM is challenging as fungi are surrounded by a complex cell wall that hampers the uniform expansion of the cell content. Using different model fungi we show that fungi - ascomycetes as well as basidomycetes - are suitable for ExM after treatment with cell wall lytic enzymes. In our approach we expanded about 4-fold *Ustilago maydis* sporidia expressing a fluorescent membrane protein (fungal rhodopsins UmOps1 and UmOps2 [2]) and *Fusarium oxysporum* expressing fluorescent Histone H1 and cytoskeleton-proteins. In addition we also applied the expansion protocol to *Aspergillus fumigatus* hyphae interacting with immune cells. We used confocal laser scanning microscopy (CLSM) and structured illumination microscopy (SIM) to visualise the expanded samples in comparison with the original sample.

Our results indicate that ExM is generally suitable for studying fungal cell biology. Accordingly, ExM offers a simple and extremely versatile method to study fungal cell biology and interaction with immune cells on a confocal microscope close to the resolution of sophisticated super-resolution microscopes.

1. Chen *et al*., Science 347, 543–548, 2015

2. Panzer *et al*., Front. Microbiol., 2019

Comparative genomics of endoparasitic fungi, *Esteya vermicola*

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Esteya vermicola is the first reported endoparasitic nematophagous fungus that attacks

the pinewood nematode, the *Bursaphylenchus xylophilus*. Although the infection activities of *E. vermicola* had been verified as a biocontrol agent of pine wilt disease, their infection mechanism has not been unraveled. To understand their unique pathogenic properties, we compared 6 nematophagous fungi including *E. vermicola* to identify pathogenic genes that are unique and conserved among them. A targeted comparison with nematophagous fungi highlighted nematophagous fungi were equipped with diverse pathogenic genes which hypothetically play role in penetration and digestion processes. Particularly, genes encoding glycoside-degrading enzymes and membrane transporters were highly expanded in *E. vermiocla*. It also permitted the initial characterization of several secondary metabolite gene clusters in *E. vermicola*, which show the great potential of virulence. These results provide a useful genetic information for the understanding of infection mechanism of nematode-destroying fungi and open the way for molecular dissection of *E. vermicola* physiology.

Functional studies of nigerolysins in *Aspergillus niger*

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Proteins of the aegerolysin family have a high abundance in Fungi. Due to their specific binding to membrane lipids, and their membrane-permeabilization potential in concert with protein partners belonging to a membrane-attack-complex/perforin (MACPF) superfamily, they were proposed as useful tools in different biotechnological and biomedical applications (1).

We performed functional studies on expression of the genes encoding aegerolysin and MACPF-like proteins in *A. niger* (2). Following the naming of these proteins in *Pleurotus* sp, these predicted homologues have been named as Nigerolysins (Nig), adding A and a number (1 or 2) for aegerolysins and B and a number (1 or 2) for MACPF-like proteins. Results suggest the sporulation process being crucial for strong induction of the expression of all these genes. However, deletion of either of the nigerolysin A genes did not influence the growth, development, sporulation efficiency and phenotype of the mutants, indicating that nigerolysins A are not key factors in the sporulation process. In all expression studies we noticed a strong correlation in the expression of one of the nigerolysins A and one of the nigerolysins B gene. Nigerolysins A were confirmed to be

secreted from the fungus. We also showed the specific interaction of a recombinant *A. niger* nigerolysin A with an invertebrate-specific membrane sphingolipid. Moreover, using this protein labelled with mCherry we successfully stained insect cells membranes containing this particular sphingolipid.

Our results suggest, that nigerolysins A in this species, and probably also in other aspergilli, could be involved in defence against predators.

1. Novak, Kraševec, Skočaj, Maček, Anderluh, Sepčić. Fungal aegerolysin-like proteins: distribution, activities, and applications. *Appl microbiol biotechnol* 2015

2. Novak, Čepin, Hodnik, Narat, Jamnik, Kraševec, Sepčić, Anderluh. Functional studies of aegerolysin and MACPF-like proteins in *Aspergillus niger*. *Mol microbiol* 2019

Study of the roles of microbial symbionts in the bark beetle holobiont

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Bark beetles (Coleoptera: Curculionidae) are ecologically and economically significant herbivores. Phloeophagous bark beetles primarily feed on plant tissues, but they are also associated with broad range of microorganisms, creating a holobiont more or less specific to beetle. The holobiont, including mutualistic, antagonistic or commensal fungi, bacteria, mites and nematodes, has functional importance to symbiosis. The core symbionts probably improve beetle fitness by aiding with diet, detoxification of plant tissue and suppressing growth of pathogens. Although most bark beetles are attracted by weakened trees, they can became aggressive tree-killing pest during their outbreaks. Among the European species, *Ips typographus* is surely the most important pathogen of spruce. Its fungal associates *Endoconidiophora polonica* and *Ophiostoma bicolor* have been suggested to contribute to tree killing. However, little is known about further functions of associated microorganisms and their effects on *I. typographus* fitness and population dynamics. The aims of our study are to describe the composition and functions of the symbiotic community throughout the life cycle of *I. typographus* and to identify core symbionts. Community composition is assessed by cultivation methods and metabarcoding and metagenomic approaches. Functions of symbionts are derived from metatranscriptomic data. In the next step, we would like to maintain *I. typographus* in axenic cultures to examine influence of core symbionts on beetle fitness. So far, we have obtained 63 bacterial isolates from larvae and 31 bacterial and 30 fungal isolates

from adult beetles. Among them, we have identified bacteria within the genus *Rickettsia* and *Sodalis* which are known endosymbiotic bacteria helping insect with diet. As their importance for *Ips typographus* is not known, we would like to focus in detail on functions of these endosymbionts, including their localization within beetle body by fluorescent *in situ* hybridization.

Analysis of putative virulence factors in the nematode trapping fungus *Duddingtonia flagrans*

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Nematode trapping fungi represent a heterogenic group of microorganisms that is able to capture nematodes with complex trapping structures to utilize them as nutrient source. As natural antagonists of nematodes, they represent an ecological strategy as biological pest control for pathogenic nematodes. In a low-nutrient environment and in contact with nematodes, nematode trapping fungi can switch from saprotrophic growth to a parasitic lifestyle. This switch is indicated by the formation of complex trapping structures, and the interaction is based on a distinct interspecies communication[1]. Therefore, and because many pathogens secrete effector proteins to facilitate the infection, we hypothesize that nematode trapping fungi secrete virulence factors or effector proteins to manipulate their prey[2]. We sequenced and annotated the genome of *Duddingtonia flagrans*, which forms adhesive trapping networks. The secretome was predicted and the putative virulence factor CyrA (**cy**steine **r**ich protein A) was chosen for further studies based on bioinformatic analyses. Secretion of CyrA was confirmed using a laccase assay. A *D. flagrans cyrA*-deletion strain displayed attenuated virulence using *Caenorhabditis elegans* as prey. The *cyrA* gene was only expressed in traps and during the growth inside *C. elegans*, as shown with a promoter fusion with mCherry and qRT-PCR. A CyrA-GFP fusion protein without signal peptide expressed in *C. elegans* localized in the cytoplasm. The CyrA-GFP fusion protein expressed in *D. flagrans* accumulated at the infection site and localized in distinct, dynamic foci in the traps and in hypha inside the captured nematode. These results suggest secretion of CyrA at the infection site and action in the cytoplasm of the host cells.

1. Youssar L., Wernet V., Hensel N., Fischer R., *et al*. Intercellular communication is required for trap formation in the nematode-trapping fungus *Duddingtonia flagrans*. PLoS Genet 15(3): e1008029 (2019).

New prospects for banana weevil (*Cosmopolites sordidus*) biomanagement using volatile organic compounds from entomopathogenic and nematophagous fungi

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We have isolated entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium spp.)* from banana plantations (Canary Islands, Spain). We have tested their pathogenicity on *Galleria mellonella* larvae and banana weevil (*Cosmopolites sordidus*, BW) adults. *M. robertsii* 4TS04 is the most virulent strain on BW (LT_{50} 1.7days) followed by *B. bassiana* 1TS11 (LT_{50} 3.5 days), Gas Chromatography/Mass Spectrometry (GC/MS-SPME) has identified 97 volatile organic compounds (VOCs) from *B. bassiana* (Bb203 and Bb1TS11), *M. robertsii* (Mr4TS04) and the nematophagous fungus *Pochonia clamydosporia* (Pc123). The effect on BW behaviour of seven of those VOCs and two technical repellents of BW (fresh garlic slices and colloidal sulphur) have been tested in a two-way glass olfactometer vs. fresh banana corm. Two fungal VOCs signifficantly repel BW. The other five VOCs and garlic shown a mild BW repellement. Two of the VOCs analyzed still showed activity when tested in banana field plantations naturally infested with BW. We are currently developping appropriate formulations for VOCs field delivery and searching for new volatiles. This could lead to the use of VOCs as a new agrobiotechnological tool for sustainable BW management worldwide.

The lysine deacetylase RpdA is essential for virulence in *Aspergillus fumigatus*

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Current suboptimal treatment options of invasive fungal infections and emerging resistance of the corresponding pathogens urge the need for alternative therapy strategies and require the identification of novel antifungal targets. *Aspergillus fumigatus* is the most common airborne opportunistic mold pathogen causing invasive and often fatal disease. Establishing a novel *in vivo* conditional gene expression system, we demonstrate that downregulation of the class 1 lysine deacetylase (KDAC) RpdA leads to avirulence of *A. fumigatus* in a murine model for pulmonary aspergillosis. The *xylP* promoter used has previously been shown to allow xylose-induced gene expression in different molds. Here, we demonstrate for the first time that this promoter also allows *in vivo* tuning of *A. fumigatus* gene activity by supplying xylose in the drinking water of mice. In the absence of xylose, an *A. fumigatus* strain expressing *rpdA* under control of the *xylP* promoter, $rpdA^{xylP}$, was avirulent and lung histology showed significantly less fungal growth. With xylose, however, $rpdA^{xylP}$ displayed full virulence demonstrating that xylose was taken up by the mouse, transported to the site of fungal infection and caused *rpdA* induction *in vivo*. These results demonstrate that (i) RpdA is a promising target for novel antifungal therapies and (ii) the *xylP* expression system is a powerful new tool for *in vivo* gene silencing in *A. fumigatus*.

Mycobiota of insect herbivores and their host plants: the role of spatial, temporal and ecological variability

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Insect-microbiota-plant interactions have received significant attention recently. Especially an impact of microbiome on trophic relationships can be considered as a hot topic of ecology but also for the application of the knowledge in biological control of pests. Despite the larvae of Lepidoptera (caterpillars) belong to the most important herbivores including also many serious pest species, their microbiota is relatively little known and vast majority of studies is devoted to bacteria. Caterpillar microbiota has been so far regarded as species poor and possibly relictual. Contrary to these conclusions we revealed in preliminary study, that mycobiota could be of importance due to high MOTU richness and incongruence in composition between caterpillar gut and host plant phylloplane. Here we bring first analysis of caterpillarmycobiota-plant tritrophic interactions based on extensive dataset from designed elaborative field sampling. We aimed to determine

the effect of spatial (from micro-to macro-scale) and temporal variability, host plants (trees) and catterpillars feeding strategy. Using DNA metabarcoding (ITS2 on Illumina MiSeq) we obtained mycobiomes from more than 1500 caterpillars and 500 samples of phyllospheres. Our data confirm our expectation that species diversity and structure of microbiomes are influenced by complex of environmental factors including large number interspecific interactions which should be of focus of subsequent experiments.

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Construction of a mutant library to examine the pathogenicity of *Mucor circinelloides* using CRISPR/Cas9 system

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Mucorales species have great biotechnological importance such as producers of extracellular enzymes, carotenoids or organic acids, but several species (e.g., *Rhizopus oryzae, Lichtheimia corymbifera* and *Mucor circinelloides*) can also act as opportunistic human pathogens causing frequently lethal systemic infection in immunocompromised patients, called as mucormycosis. In the recent years, the number of mucormycosis cases has significantly increased. The properties and potential virulence factors that make the *Mucorales* species enable to cause disease in human, are not well known. Potential virulence factor genes may play role in fungal dimorphism, proteolytic enzyme production, iron uptake, and production of cell surface proteins. *M. circinelloides* has been used as model organism in different molecular biological and genetic studies. Recently, we have successfully developed a CRISPR/Cas9 system to modify the genetic background of this fungus. In this study, based on selected, potential virulence factor genes (i.e. cotH, svf, hsbA and ho), a mutant library has been constructed from M. circinelloides. We have started to analyse the role of these genes in the pathogenesis of *Mucor*. Our results suggested that MS12-ΔcotH3 and MS12-Δsvf1 strains are less virulent in the alternative Drosophila melanogaster model, while in DKA mice, the disruption of cotH3 and cotH4 genes resulted decreased virulence. Sensitivity of MS12-Δsvf1 strain to oxidative stress induced by H2O2 also increased.

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The effect of abiotic factors on growth of bark beetle fungal symbionts

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Bark beetles (Scolytinae) belongs between the most diverse and important groups of insects. This group has a global ecological and socio-economical impact. The symbiosis of bark beetles and microorganisms allows them to reach new sources of nutrients, extend their niches, defend themselves against the predators and adapt to fast-changing conditions. My PhD thesis is focused mainly on the abiotic factors like the effect of oxygen, pH or activity of water on the growth of 2 groups of symbiotic fungi (ophisotomatal fungi and genus *Geosmithia*). Above mentioned basic abiotic factors are considered to be responsible for division into 2 ecological groups of bark beetles (so-called "ophiostomatoid fungi associated" and "*Geosmithia* associated" type) according to our hypothesis.

Investigate the relevance of cotH genes in the pathogenicity and other biological mechanisms of *Mucor circinelloides*

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CotH proteins are widely present in *Mucorales* and absent from non-invasive pathogens. Previous studies showed the importance of one of these proteins in the pathogenicity of *Mucorales*. In case of *Rhizopus*, CotH3 mediates fungal invasion of host cells during mucormycosis. Construction of genetically modified strains, in which the cotH genes are

disrupted or overexpressed can provide a good tool to investigate their relevance in the pathogenicity. Five putative spore-coat proteins were disrupted in *Mucor circinelloides* by a recently developed plasmid-free CRISPR/Cas9 method. The mutation was proven for each targeted gene by PCR analysis and the pyrG gene was found to be integrated at the expected positions in all mutants. To identify on- and off-target mutation in an edited fungus, whole genome sequencing was also performed. Growth ability of the mutants, spore germination and the effect of cell wall stressors calcofluor white and Congo red on the fungal growth were tested. Spore surface morphology was imaged with scanning electron microscopy and the inner spore structure was investigated by transmission electron microscopy. Phagocytic assay was performed with the standard macrophage-like cell line J774.16. Pathogenicity of the mutants was examined in the alternative *Drosophila* and a murine model. Stressors affected differently the cotH mutants. The cotH3 and cotH4 mutant strains exhibited reduced virulence in murine model, furthermore the cotH4 mutant showed reduced virulence in D. melanogaster model. Deletion of some of the cotH genes resulted variances in the structure of the inner spore coat, differences in spore size distribution, fungal growth and sporulation.

The study was supported by the "Lendület" Grant of the Hungarian Academy of Sciences (LP2016-8/2016) and the grant TUDFO/47138-1/2019-ITM of the Ministry of Human Capacities. GN is grateful for the support of the Premium Postdoctoral Fellowship Program of the Hungarian Academy of Sciences.

Validation of the *P. teres* f. *teres* effectors VR1 and VR2 conferring virulence on Rika barley identified in the bi-parental mapping population 15A × 6A

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Net form net blotch is an economically important foliar disease of barley (*Hordeum vulgare*) caused by the fungal pathogen *Pyrenophora teres* f. *teres*. Yield losses have been reported in the range of 10-40% and complete yield loss has been observed under environmental conditions highly favorable to the pathogen. Little is known as to the molecular mechanisms involved in the host-pathogen interaction. To investigate the mechanisms of *P. teres* f. *teres* virulence, a mapping population was developed from a cross between two California *P. teres* f. *teres* isolates 15A and 6A that showed a differential response on Rika barley, with 6A being virulent and 15A avirulent on Rika. Two virulence QTL conferred by 6A, VR1 and VR2, were identified. Within the 15A × 6A population, progeny isolate #72 contained VR2 but not VR1 and no isolates were identified containing VR1 but not VR2. Using the full genome sequences of isolates 15A and 6A,

candidate genes were identified for VR1 and VR2. In progeny isolate #72 a CRISPR-Cas9-ribonucleoprotein (CRISPR-Cas9-RNP) mediated gene disruption was performed in the VR2 candidate gene, resulting in a loss of virulence on Rika barley and providing strong preliminary confirmation that the candidate gene was VR2. VR2 encodes a secreted protein with a mature amino acid sequence length of 405 amino acids and contains no predicted protein domains and no homology to any known proteins. The VR2 gene is currently being functionally characterized using heterologous expression in *Pichia pastoris* and gain-of-function transformation into parental isolate 15A. A gain-of-function transformation of VR1 into isolate 15A resulted in isolate 15A becoming virulent on Rika barley and providing strong preliminary confirmation for the candidate being VR1. VR1 encodes a secreted protein with a mature amino acid length of 557 amino acids with no predicted protein domains or homology to any known proteins. VR1 is currently being functionally characterized in the same manner as VR2. Additional characterization is being performed using CRISPR-Cas9-RNP mediated co-editing in isolate 6A to both perform a double gene disruption of VR1 and VR2 as well as *in vitro* pull-down assays using VR1 and VR2 as bait proteins for barley cellular lysates. The characterizations of VR1 and VR2 will contribute significant knowledge to the molecular interaction between barley and *P. teres* f. *teres* during a susceptible interaction.

Staphylococcus aureus in an oral dual-infection model

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The human fungal pathogen *Candida albicans* and the bacterial species *Staphylococcus aureus* are often isolated from the same infection sites. Previously we showed that *C. albicans* hyphae are required for the dissemination of *S. aureus* into the kidneys and to cause death of the animals (Kong *et al*., 2015). We aimed to further investigate this in order to discriminate between different possible underlying mechanisms. We already excluded that the damage caused by an oral *Candida* infection is the reason for *S. aureus* to disseminate as an *als1 als3* mutant causes similar damage but no dissemination. Previously it was shown by atomic force microscopy that the Als3 protein binds to *S. aureus*. This pointed towards a role for Als1 and Als3 in the dissemination process. However, these proteins are only required upon mild immunosuppression of the animals. When the immunosuppression is to strong, it seems that there is no recruitment of macrophages by the *C. albicans* and even for a wild type strain where there are a lot of *S. aureus* cells binding to the hyphae, there is no dissemination. These experiments have been validated using the BioFlux equipment clearly showing the adhesion of *S. aureus* cells to wild type hyphae, but not to those of the *als1 als3* mutant. *In vitro* macrophage

co-culture experiments clearly show that the macrophages are recruited to the *Candida* cells but the hyphae are too big, so only bacteria are taken up. In the animal such macrophages are then going back to the lymph nodes and bacteria seem to be released. We are currently using advanced imaging techniques to visualize the infection process.

Ref: Kong, E. *et al*., Clinical implications of oral Candidiasis: host tissue damage an disseminated bacterial disease. Infect & Immun 83: 604-613, 2015.

Poster Session 2.2
PLANT – FUNGI INTERACTIONS

WEDNESDAY, FEBRUARY 19

18:00 - 19:30 | Location: **Frentani Convention Center**

Comparative transcriptome analysis identified novel genes modulated by *Plasmopara viticola* and resistant/susceptible *Vitis vinifera* during interaction

Giuliana Maddalena[1], Gabriella De Lorenzis[1], Matteo Brilli[2], Mirko Moser[3], Vahid Shariati J.[4], Elahe Tavakole[5], Alessandro Passera[1], Paola Casati[1], Massimo Pindo[3], Alessandro Cestaro[3], David Maghradze[6,7], Osvaldo Failla[1], Piero Attilio Bianco[1], Fabio Quaglino[1], Silvia Laura Toffolatti[1]

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FLASH TALK - *Presenting author' e-mail: silvia.toffolatti@unimi.it*

The oomycete *Plasmopara viticola* (Berk. *et* Curt.) Berlese and De Toni is the causal agent of downy mildew, one of the most important diseases affecting grapevine cultivation worldwide. The application of plant protection products is essential for the control of diseases. However, to preserve human health and the environment, the European regulations strictly points towards a reduction of the fungicide applications. The selection of resistant cultivar represents an additional control method. Unfortunately, *Vitis vinifera* L., the Eurasian grapevine species mainly used in the global wine industry, is extremely susceptible to the pathogen. The authors recently reported the existence of a downy mildew resistant *V. vinifera* cultivar, named Mgaloblishvili, originally from Georgia (Caucasus), the domestication center of the species. Mgaloblishvili exhibits unique resistance traits, not encountered in *vinifera* and non-*vinifera* varieties, against *P. viticola*. Based on comparative transcriptome analysis, the mechanism of resistance and susceptibility to *P. viticola* in *V. vinifera* was dissected analyzing the genes involved

in plant-pathogen interaction. In particular, whole transcriptomes of leaves collected from Mgaloblishvili, Pinot noir (a *V. vinifera* susceptible cultivar), and Bianca (an interspecific resistant hybrid) plants inoculated and not-inoculated with *P. viticola* were used to identify *P. viticola* effector-encoding genes, plant genes involved in the resistance mechanism of Mgaloblishvili, but also to identify a putative susceptibility gene in *V. vinifera.* The unique resistant traits found in Mgaloblishvili highlight a rare defense system in *V. vinifera* against *P. viticola,* which promises fresh opportunities for grapevine genetic improvement. These results open new perspectives for studies aiming at improving breeding for resistance to *P. viticola* inside the *V. vinifera* species through the exploitation of resistance genes or disruption of susceptibility gene.

Exploring the role of plant vesicle trafficking during *Fusarium graminearum* infection

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To protect themselves against microbial attack, plants have to activate and regulate defence responses. In most cases, regulation of immune signals and delivery/discharge of extracellular immune molecules are controlled by vesicle trafficking. Because of this, important trafficking regulators are often the targets of pathogen effectors. Manipulation or disruption of these critical pathways is key for successful pathogen infection. *Arabidopsis thaliana* (*At*), is a model plant and with a range of mutants available, often used to study the importance of trafficking regulators during pathogen infection. In a recent study, we evaluate the infection of the cereal fungal pathogen *Fusarium graminearum* (*Fg*) in 9 *At* knockout mutants targeting genes regulators of vesicle trafficking. We found that AtMIN7, an ARF-GEF protein, also considered immunity-associated in *Arabidopsis–Pseudomonas syringae* interaction, plays a role during *Fg–Arabidopsis* interaction (1). *AtMin7* mutants were highly susceptible to *Fg* infection compared to *At* wild-type (Col-0). We have also identified *AtMin7* homologues in wheat and demonstrated that a similar phenotype was achieved by silencing these genes using BSMV-VIGS. *AtMin7* silenced wheat plants were more susceptible to *Fg* infection. These results suggest that AtMIN7 may also act as an immunity-associated protein during *Fg* infection in Arabidopsis and wheat, or vesicles regulated by AtMIN7 are responsible for delivery of important plant immune molecules. Specific roles of AtMIN7 during *Fg* infection are being explored.

1. Nomura *et al*., 2006. Science 313.5784:220-223

The effect of fruit sugar level of two near isogenic tomato lines, on the pathogenicity mechanism and host response during infection of red tomatoes

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Colletotrichum gloeosporioides and *Penicillium expansum* cause postharvest disease in tropical and deciduous fruits. During host colonization, *C. gloeosporioides* and *P. expansum* differentially modulate environmental pH and gene expression to enhance pathogenicity, as dependent on sugar availability in the host tissue. To uncover the effect of host sugar content on these pathogens' interactions with fruits we performed global transcriptomic and metabolomics study during *C. gloeosporioides* and *P. expansum* inoculation of two tomato lines having similar genetic background but differential level of TSS content: low sugar content (LowSC) and high sugar content (HighSC). *C. gloeosporioides* showed enhanced colonization of the LowSC line containing 7.33% total soluble solids (TSS) with enhanced relative expression of glycosyl hydrolases, glucanase and the major facilitator superfamily transporter genes. Enhanced colonization of *P. expansum* occurred in the HighSC lines with 12.23% TSS, accompanied by an increase in carbohydrate metabolic processes and glycolysis, mainly phosphoenolpyruvate carboxykinase, 2-oxoketoglutarate and the accumulation of gluconic acid. The host gene response to fungal attack differed depending on the sugar level. Reduced colonization of HighSC lines by *C. gloeosporioides* was accompanied by increased induction of glucosyltransferase expression, which regulates the activity of antifungal compounds such as phenylpropanoids, suggesting a new mechanism for modulation of fruit defense against pathogens. While the low sugar content lines downregulate carbon metabolic process and pathogenicity of *P. expansum*.

Overall, host sugar levels in tomato fruits differentially modulate colonization patterns by activating specific fungus-pathogenic and host-response factors. This indicates a pivotal role of the dynamic changes in nutrient availability during fruit development and ripening in determining susceptibility to different fungal pathogens.

National and EU common catalogue of beans varieties Resistance tests to *Colletotrichum lindemuthianum* race 6: methods of examination for listing

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CREA-DC deals with the protection of agricultural, ornamental and forest plants and foodstuffs from biotic and abiotic agents. It is a national reference for protection and certification of pre-multiplication materials. It deals with the control and certification of seed materials, the assessment of the requirements for the registration or release of plant variety rights. The official trials required for registration on the official Italian Catalogue provide for the assessment of the requirements of distinctness, homogenity and stability of the varieties. The tests usually have a two-year duration with two sowing cycles and the distinctive varietal characteristics to be examined concern the morphology and physiology of plants and, for common bean (*Phaseolus vulgaris* L.) species, also resistance to certain pathogens. The technical UPOV guidelines and technical protocol of Plant Community Variety Office (TP/12/4) provide the characteristics to be examined for bean varieties, the examples varieties and the methods of examination. The compulsory physiological characteristics for registration of bean varieties are: resistance to Bean Common Mosaic Necrosis Virus and to antracnose. Antracnose is a resistance to monogenic control, caused by the hemibiotrophic fungus *Colletotrichum lindemuthianum*. This is a parenchymal necrotic disease characterized by the appearance of dehydrated spots and the tissues desiccation is related to the action of toxic metabolites at the level of permeability of cell membranes. The CREA-DC Phyto-pathological Analysis laboratory in Battipaglia evaluates the resistance to the bean antracnose. Resistance validation is calibrated by comparison with the results of susceptible and resistant reference varieties. The test ends when the symptoms are well expressed on the susceptible reference variety. In recent years the selection of new resistant varieties is allowing this disease to be successfully controlled.

Auxin production and impact in *Neurospora crassa*

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Production of the plant phytohormone auxin has been reported in several phytopathogenic

fungi (Jameson, 2000; Tsavkelova *et al*., 2012). In those cases, auxin is considered a regulator of the plant-fungus interaction. Surprisingly, several non-phytopathogenic fungi are also able to produce auxin (Kollath-Leiß *et al*., 2014), however, reportedly only in tryptophan-supplemented media (Gruen, 1959). Our investigations are focused on the auxin biosynthesis in the ascomycete *Neurospora crassa*. We discovered the biosynthetic network with several interdependent pathways (Sardar & Kempken, 2018). Phenotypical analyzes of an auxin-deficient mutant strain led to the conclusion, that auxin does play a physiological role in the fungus and influences its sexual and asexual, development. We investigated the interaction of *N. crassa* with diverse plant species and found, that the plant model organism *Brachypodium distachyon* tends to interact with the fungus. Moreover, we found evidence for a possible influence of the plant on the fungal auxin metabolism. Our data indicate the double-role of auxin as a fungal growth regulatory hormone and as a signal molecule in plant-fungus communication.

Gruen, E. H. (1959). Auxin and fungi. Annual Review of Plant Physiology

Jameson, P. E. (2000). Cytokinins and auxins in plant pathogen interactions-an overview. Plant Growth Regul.

Kollath-Leiß, K., *et al*., (2014). BEM46 shows eisosomal localization and association with tryptophan-derived auxin pathway in Neurospora crassa. Eukaryotic Cell

Sardar, P., & Kempken, F. (2018). Characterization of indole-3-pyruvic acid pathway mediated biosynthesis of auxin in Neurospora crassa. PLOS ONE

Tsavkelova, E. *et al*., (2012). Identification and functional characterization of indole-3-acetamide-mediated IAA biosynthesis in plant-associated Fusarium species. Fungal Genetics and Biology

Isolating Fungal Endophytes to Improve Heat Resistance in *Solanum lycopersicum*

Luisa Liu-Xu, Loredana Scalschi, Emma Fernández-Crespo, Ana González-Hernández, Carlos Albert, Gemma Camañes, Begonya Vicedo, Pilar García-Agustín, Eugenio Llorens

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Phytobiome has been shown to be highly dynamic and capable of conferring abiotic and biotic stress tolerance, as well as health improvement to the host plant (Porras-Alfaro &

Bayman, 2011, Tanaka et *al*., 2012, Zuccaro et *al*., 2011). Since a large number of fungal species and their role as endophyte are still unknown, finding and characterizing them could have big potential for agricultural uses in the current climate change situation.

In this way, our group previously studied endophytes in cereals to improve hydric stress in treated plants (Llorens et *al*., 2019). This time, we focused on endophytes from *Solanum lycopersicum*, because of its great economic value in agriculture. We isolated fungal endophytes from Money Maker variety, considered as heat resistant. Then, inoculated them in traditional varieties named Alcalà de Xivert (ADX) and Thessalonica's Greek Red tomato (TR-TH-0030) that are heat sensitive. Inoculated plants, along with control ones, were subjected to heat stress at 42ºC for a period of 6 hours to test their resistance, and then had a recovery time of 2 hours. Phenotypical and physiological traits were analyzed. Results showed that some of the endophytes from tomato plants help the host plant to overcome the stress, and proved that inoculated plants had different performance from non-inoculated plants. Now, we are currently studying the characteristics of the plant-endophyte interaction to understand the stress tolerance process in order to develop possible field applications.

Llorens, E., et *al.* (2019). https://doi.org/10.1111/1462-2920.14530

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Trichoderma-plant crosstalk is mediated by VOCs emission

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Volatile Organic Compound (VOC) emissions play important ecological and physiological roles for many organisms, mediating interactions at different levels, included those between fungi and plants. Fungal VOCs may act as attractants and deterrents to insects and other invertebrates, or may be beneficial to plants by triggering defense responses and priming them against pathogen attack, as well as by favoring growth promotion of nearby

plants. However, also plants release up to 36% of the assimilated carbon as VOCs, both constitutively and in response to interactions with beneficial or detrimental organisms. Plant VOCs are involved in plant-plant signaling, but they can also play significant roles in shaping phytobiomes.

Fungal strains of the genus *Trichoderma* are well-known producers of VOCs. The scope of this work is the complex volatile-mediated bidirectional crosstalk between different genotypes of *Phaseolus vulgaris* and several *Trichoderma* species. Volatomes emitted during the interaction of *T. harzianum* T22, *T. longibrachiatum* MK1 and *T. atroviride* P1 with four different *P. vulgaris* varieties ('Pinto Villa', 'Flor de Junio Marcela', 'Flor de Mayo Anita') and the landrace 'Negro San Luis' were analyzed using the high resolution proton transfer reaction mass spectrometry (PTR-QiTOF). This innovative tool allows rapid and highly sensitive 'real-time' detection of VOCs.

Through high-throughput *in vivo* analysis, we discovered that distinct and species-dependent VOCs are released by fungi and plants either separately or only in interaction. We also verified that the crosstalk between the two partners is dynamically adjusted and strongly depending on the *Trichoderma* strain, being *Trichoderma*-mediated changes in plant VOCs emission the results of a highly specific response.

Polyketide synthases in the ericoid endomycorrhizal fungus *Oidiodendron maius*

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Fungi produce many biologically active secondary metabolites, a large proportion of them being polyketides, a group of compounds produced by polyketide synthases (PKSs) and characterized by great structural diversity. Several functions have been attributed to fungal polyketides, ranging from ecological and evolutionary adaptation of fungi (Keller, 2019; *Nature Reviews Microbiology* 17, 167–180) to plant-pathogenic fungi interactions.

Potential roles of polyketides have not been investigated so far in mycorrhizal fungi, although a recent comparative genomics and transcriptomics study considering 60 fungi with different taxonomy and ecology (Martino *et al*., 2018, *New Phytologist* 217, 1213–29) has revealed in *Oidiodendron maius*, an ericoid endomycorrhizal fungus, the highest number of PKSs encoding genes, some of them being regulated in symbiosis. We are currently investigating the potential role of *O. maius* PKSs following different approaches that involve: i) i*n silico* prediction of Biosynthetic Gene Clusters in the *O. maius* genome and analysis of the expression in symbiosis; ii) domain prediction, phylogenetic analysis and comparison with other fungal functionally characterized enzymes of *O. maius* PKSs; iii) generation of mutants lacking PKSs genes highly regulated in symbiosis, in order to analyse their mycorrhizal phenotype ; iv) heterologous expression of these *O. maius* PKSs in yeast, in order to identify the polyketide produced ; v) the investigation of the role of *O. maius* polyketides in biocontrol activities and in vi) response to abiotic stress. As *O. maius* is one of the very few genetically tractable mycorrhizal fungi, it represents an interesting model system to investigate the role of polyketides in the mycorrhizal symbiosis. Our investigation should provide a better picture of *O. maius* genetic potential in the polyketide biosynthetic pathway and the characterization of some of these compounds and potential role in symbiosis, biocontrol and stress response.

Terpene synthases in *Trichoderma gamsii* T6085

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Trichoderma is a fungal genus that comprises a large number of species showing a broad spectrum of lifestyles, supported by their Secondary Metabolites (SM) arsenal. The genomes of 21 isolates belonging to 17 *Trichoderma* species were analyzed, and SM backbone genes and clusters were identified. *Trichoderma* contains a striking number of terpenoid synthases (TS) genes ranging from 15 to 26, almost half included in clusters, however most of them have not yet been characterized. The relation between the many terpenes reported in *Trichoderma* and the genes responsible for their synthesis is therefore not known, which defines yet an intriguing area of research. For these reasons, analysis of total core-gene content was focused on TS, whose products have been shown to play important roles in the interactions between *Trichoderma* and its hosts, acting as mycotoxins, antifungal compounds and molecular messengers. In order to investigate the functions and biological roles of TS in *T. gamsii* T6085, able to reduce Fusarium Head

Blight (FHB) symptoms in wheat, we adopted an integrated approach of computational and molecular biology. Characterization based on conserved sequence features and phylogenetic analysis of TS was carried out in order to characterize mono/bi-functional or chimeric Class I and II terpene cyclases, polyprenyl synthases and prenyl transferases. *T. gamsii* T6085 harbours 11 Class I and 5 Class II TS-encoding genes, of which 6 Class I and 1 Class II are embedded in clusters. Expression analyses show that 10 Class I TS are induced in media with different carbon sources and in the stress conditions. Preliminary metabolic profile analysis carried out in 12-day PDB cultures of *T. gamsii* T6085 determined by HPLC-NMR have highlighted the presence of harziandione, a diterpenic compound with known antifungal properties. Aimed at shedding some light on the biological significance of TS in this fungus, expression patterns have also been assessed in *T. gamsii*– wheat and *T. gamsii* – *F. graminearum* interactions, and during the triple interaction *T. gamsii* – wheat – *F. graminearum*.

The functional analysis of a late effector in *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. Previous study showed that 104 effectors are expressed at late infection stages when *U. maydis* undergoes karyogamy and forms hyphal aggregates which are embedded in a mucilaginous polysaccharide matrix. To analyze the function of these late effectors, single gene deletion mutants of the eight candidate late effectors were created and tested for virulence. One mutant named *lep1* (*late effector protein 1*) showed reduced virulence. *lep1* mutants were attenuated in hyphal aggregate formation, failed to undergo massive late in planta proliferation and produced only few spores. *lep1* encodes a small secreted protein which is conserved in all smut fungi. *lep1* gene expression is upregulated most strongly at the time when fungal hyphae forms aggregates. When *lep1* is expressed constitutively by filamentous colonies a mucilaginous polysaccharide matrix is produced whereas wild type colonies do not produce mucilage. During plant colonization, strains constitutively expressing *lep1* show enhanced polysaccharide accumulation in fungal aggregates. These results suggest that Lep1 might either be used as a scaffold or adhesion for hypha-hypha contacts or be used as a signaling molecule to trigger polysaccharide matrix production. We are currently trying to distinguish between these possibilities by treating fungal hyphae with Lep1 peptide.

Defects in iron acquisition result in hypervirulence in *Botrytis cinerea*

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The plant pathogen *Botrytis cinerea*, responsible for the grey-mold disease, infects a wide variety of plant species. The outcome of this host-pathogen interaction, a result of the interplay between plant defense and fungal virulence pathways, can be modulated by various environmental factors. Among them, iron availability and acquisition play a crucial role in diverse biological functions. How *B. cinerea* obtains this essential micronutrient during infection, however, is largely unknown. We set out to explore the role of the reductive iron assimilation (RIA) system in *B. cinerea* infection dynamics. This system is composed of a ferroxidase, that belongs to the multicopper oxidase (MCO) family of proteins, and a membrane-bound iron permease. Gene knockout and complementation studies revealed that compared to the wild-type, a loss-of-function mutant for ferroxidase (*bcfet1*) displayed delayed conidiation, iron-dependent sclerotia production, and significantly reduced whole-cell iron content, as determined on solid culture medium. We observed that while the mutant for iron permease (*bcfth1*) exhibited reduced conidiation but normal virulence and whole-cell iron content, the *bcfet1* mutant displayed a hypervirulence phenotype. Interestingly, though the B05.10 wild-type strain produced slightly reduced necrotic lesions in iron-starved plants, the hypervirulence phenotype of the *bcfet1* mutant led to more prominent necrotic lesions in the presence of iron, but not when infecting iron-deprived plants. This suggests that *B. cinerea bcfet1* knockout mutant requires plant-derived iron to achieve larger lesions. *In planta* analysis of the reactive oxygen species (ROS) generation by both mutant strains shows an iron-dependent increase in ROS in the *bcfet1* mutant, in agreement with larger necrotic lesions, but not in the wild-type or the *bcfth1* mutant strain, suggesting that increased ROS production may at least partly underlie the observed infection phenotype.

Ensembl Fungi: A growing reservoir of fungal interactions

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Ensembl Fungi provides a suite of open-access tools to examine over a thousand fungal genomes, their variants, expression and relationships to other genomes through homology and alignments. The project routinely integrates data from multiple external resources

such as pathogen-host interaction data from PHI-base; a manually curated repository capturing a range of experimentally verified gene functions during interactions with a host as reported in literature. We not only map direct matches to PHI-base data but also make careful extrapolations based on 100% protein similarity to other species within sub-taxonomic groups to elucidate potential gene candidates for experimentation. To date, 6074 fungal genes across 166 genomes in Ensembl Fungi have annotation related to their interactions with 79 different hosts; with the most annotations involving mouse, wheat, rice, tomato and *Zea mays*. Many potential inferences can be drawn from combining this annotation, our gene trees and variation analysis tools.

We present our data capture pipelines and underlying storage of these data which are now capable of integrating other complex pan-species relationships. Fungal interactions with other taxonomic groups are central to many biological questions in agriculture, forestry, environmental science, food production and medicine. The Ensembl Fungi portal remains poised to capture, analyse and disseminate these data to researchers around the world without restriction.

Reactive Oxygen Species (ROS) dosage in *Arabidopsis chloroplasts* improves resistance towards *Colletotrichum higginsianum* in a WRKY33-dependent fashion

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Arabidopsis plants overexpressing peroxisomal glycolate oxidase (GO) in chloroplasts have been established as an elegant system to generate H_2O_2 specifically in a spatiotemporal fashion. In GO plants, glycolate produced during photorespiratory oxygenation of ribulose-1,5-bisphosphate is oxidized to glyoxylate directly in the stroma, generating H_2O_2 as a by-product. The amount of H_2O_2 produced in the GO transgenics can be controlled by adjusting the rate of photorespiration by either increasing light intensity, or by lowering CO_2 concentration. Similarly, photorespiratory mutants deficient in the major peroxisomal catalase CAT2 can be triggered to accumulate H_2O_2 in peroxisomes by the same stimuli. Previous transcriptome analyses of GO plants and cat2 mutants revealed that the transcription factors MYB51, WRKY33 and WRKY40 controlling the biosynthesis of indolic phytoalexins like indole glucosinolates and camalexin are specifically regulated

in response to a burst in stromal H_2O_2 in GO plants. Together with the redox-activated transcription factor ERF6, these three transcription factors represent the overlap to challenge with bacterial and fungal PAMPs. We therefore investigated the influence of H_2O_2 bursts in chloroplasts (in GO plants) and peroxisomes (in *cat2-2* mutants) on the interaction with the fungal hemibiotroph *Colletotrichum higginsianum*. Compared to wild type, GO overexpressors showed improved resistance to *C. higginsianum* after light shift-mediated production of H_2O_2, while *cat2-2* became more susceptible and allowed significantly more pathogen entry. The *cat2-2* mutant suffered from severe oxidative stress after light shifts, as indicated by substantial increase in glutathione pool size and oxidation state. Our data suggest that severe oxidative stress interferes with an effective defense response in *cat2-2*. To resolve the genetic basis of inducible resistance in GO overexpressors, we analyzed GO mediated resistance in mutants deficient in several subsets of indolic phytoalexins. Induced resistance of GO was completely dependent on WRKY33, but only partially dependent on camalexin production, while the absence of indolic glucosinolates did not affect inducible resistance of GO plants to *C. higginsianum*. We conclude that other WRKY33 controlled, yet unidentified indolic phytoalexins contribute to inducible resistance in GO. Furthermore, our data shed new light on the crosstalk between ROS signaling in chloroplasts and the innate immune response in plants.

Comparative -omics analyses to understand Wood-Decay Strategies and Evolution of Pathogenicity in *Armillaria spp.*

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Armillaria spp. (Fungi, Basidiomycota) are a group of devastating pathogens of temperate forests all over the globe. *Armillaria* spp. produce complex multicellular structures called rhizomorphs through aggregation of tip-growing hyphae. They are believed to serve as exploratory organs underneath the soil branching out to various distances to reach carbon sources. Mycelium and rhizomorphs in wood-decay and pathogenic fungi are believed to aid the fungus in making direct contact with the host root followed by colonization. To test this hypothesis, we performed a wood decay assay using *Armillaria ostoyae* and *A. cepistipes*, two conifer-colonizing species. We analyzed their transcriptome- and proteome-level changes from the mycelium and rhizomorphs obtained from colonized dead spruce roots and compared them with mycelium and rhizomorphs growing in the

absence of root. Tissues colonizing on root show similar transcriptomics and proteomics responses and both the omics analyses highlighted a number of gene families that were common as well as species-specific in tissues colonizing the root by the two species. The response however was higher in *A. cepistipes* as compared to *A. ostoyae*, depicted by the higher number of differentially expressed genes (DEGs) and differentially abundant proteins (DAPs) in the former species. We also found the number of DEGs/DAPs to be higher in mycelium as compared to the rhizomorphs. A number of carbohydrate-active enzymes (CAZymes) show upregulation in the tissues colonizing the root, especially in the mycelium, suggesting their role in wood decay. We also found a number of transport proteins upregulated in the rhizomorphs colonizing the root as compared to the invasive mycelium, suggesting their primary role in the transport of the decomposition intermediates. Our data gives the impression that colonization and wood-decay are mediated by mycelium, whereas rhizomorphs are more likely to have role in substrate exploration.

Characterisation of the mycobiome of clonal olive trees cultivated in three distinct environments

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Each time more metagenomic studies have enabled the detection of undescribed microorganisms, many of which occurring endo- or epiphytically on plant hosts, with various roles and lifestyles. In this study we have analysed the mycobiome of three clonal olive plants kept together for five years under green-house conditions and subsequently cultivated for ten years in three distinct conditions in Portugal: in a rural area at Alentejo region with abundant olive tree cultivation; in a pot on a balcony in Lisbon city centre; next to a citrus orchard in São Miguel island, Azores, where olive trees do not occur. As controls, the mycobiomes of neighbouring resident citrus trees in each location were also analysed. Leaves and branches were analysed separately, in each case either subjected to surface disinfection or not. Samples were both used for fungal isolation in semi-selective medium and for DNA extraction, subsequently used for metagenomic ITS sequencing using Illumina 2x250bp PE. Fungi isolated in semi-selective medium were characterized morphologically and, when necessary, also molecularly by ITS, RPB2, TEF1 and TUB2 sequencing. Preliminary results suggest that the mycobiomes of the olive trees studied was mostly acquired in their current environments, with important differences between locations and much less between hosts in each location. While most Fusarium, *Pestalotiopsis*, *Trichoderma* and *Colletotrichum* isolates appear endophytic, most *Alternaria*, *Aureobasidium*, *Epicoccum* and *Dydimella* isolates seem to be epiphytic.

Results of metagenomic analysis (not available yet) will enable a deeper quantification of these occurrences, and may reveal the presence of unculturable fungi. Overall, this study will provide an in-depth analysis of the mycobiome of clonal olive trees cultivated in three distinct environments by comparison to neighbouring citrus plants, revealing novel fungus-host (-location) associations.

Sequence analysis of *Pyrenophora tritici-repentis* effector genes in Tunisia

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Pyrenophora tritici-repentis (*Ptr*) is a fungal pathogen that causes tan spot disease of wheat, and eight races of this fungus (race 1 to 8) have been identified based on the fungus ability to produce combinations of three effectors. The necrosis-causing effector, Ptr ToxA, is a protein encoded by *ToxA* gene and predominate wheat in the Americas and Australia. While Ptr ToxB is a chlorosis-inducing protein, and is encoded by *ToxB* gene. *ToxB* is rarely found in North America and Australia, but is prevalent in North Africa. In this study, a comparative sequence analysis of *ToxA* and *ToxB* genes from a collection of *Ptr* isolates representing various regions in Tunisia and Canada were compared. All *ToxA* sequences were found identical, and belonged to the same haplotype (H15), while *ToxB*-ORF sequences were conserved and matches a previously identified *ToxB* in race 5. A homolog of *ToxB*, known by *toxb* was detected in most Tunisian isolates including races 5 and 7, and its sequence was found identical to the *toxb* sequence present in Canadian race 3 isolate (accession number: AF483833). Characterization of *Ptr* effectors from different populations may help provide a better understanding of the *Ptr* virulence evolution.

Avirulence proteins of *Leptosphaeria maculans*, involved in suppressive interactions, share a common structural pattern and are part of a larger family

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Recognition of a pathogen avirulence (AVR) effector protein by a cognate plant resistance (R) protein triggers a set of immune responses that render the plant resistant. Pathogens can escape this so-called Effector-Triggered Immunity (ETI) by different mechanisms including the deletion of the *AVR* gene, point mutations that allow recognition to be evaded while maintaining virulence function, and the acquisition of new effectors that suppress AVR recognition.

The Dothideomycete *Leptosphaeria maculans*, causal agent of oilseed rape stem canker, is one of the few fungal pathogens where suppression of ETI by an AVR effector has been demonstrated. Indeed, AvrLm4-7 suppresses the recognition of AvrLm3 and AvrLm5-9 by the R proteins Rlm3 and Rlm9, respectively (Plissonneau *et al*., 2016; Ghanbarnia *et al*., 2018). The presence of *AvrLm4-7* does not impede *AvrLm3* and *AvrLm5-9* expression, and the three AVR do not physically interact.

To decipher the antagonistic interaction between *L. maculans* AVR effectors, we determined the crystal structure of AvrLm5-9. Surprisingly, despite a lack of sequence similarity, AvrLm5-9 shares structural analogies with AvrLm4-7 (structure previously characterized by Blondeau *et al*., 2015). Structure-informed searches identified a larger number of putative structural analogues among *L. maculans* effector candidates (including AvrLm3), as well as among effector candidates from other phytopathogenic fungi (including ECP11-1 from *Passalora fulva*; Mesarich *et al*., 2018). We determined the crystal structure of ECP11-1, deduced the 3D structure of AvrLm3, and confirmed that they shared structural analogies with AvrLm4-7 and AvrLm5-9. Remarkably, transformants of *L. maculans* producing ECP11-1 triggered Rlm3-mediated immunity. Furthermore, this recognition could be suppressed by AvrLm4-7. These results suggest that ECP11-1 has the same function as AvrLm3, or that the ECP11-1 structure is sufficiently close to that of AvrLm3 to be recognized by Rlm3.

SIX6: A route to plant cell death

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The soil-borne fungus *Fusarium oxysporum* f. sp. *lycopersici (Fol)* causes fusarium

wilt of tomato. In the *Fol* pathosystem, small secreted fungal proteins, called SIX (Secreted In Xylem) proteins, have been identified in the xylem sap of infected tomato plants. Fourteen *SIX* genes have been identified so far (designated *SIX1–SIX14*). In agroinfiltration experiments, *Fol SIX6* was found to cause cell death when expressed in leaves of *Nicotiana benthamiana* and *N. tabacum*. Purified *Fol* SIX6 protein produced using an *E. coli* expression system was found to cause cell death in leaves of tomato as well as *N. benthamiana* and *N. tabacum*. Infiltration of *Fol* SIX6 protein into cotyledons/leaves of representative species from various plant families, including the Solanaceae, Cucurbitaceae, Brassicaceae and Leguminosae, revealed not only widespread sensitivity to *Fol* SIX6, but also considerable variation in sensitivity, indicating an unexpected degree of specificity. For example, *Fol* SIX6 protein causes a strong cell death response in cotyledons/leaves of bean, calendula, capsicum, eggplant and watermelon; wilting and curling of cotyledons/leaves in cotton, cucumber and flax; but no response in cabbage, pea, radish, spinach, wheat or zucchini. Homologues of *Fol SIX6* have been found in other *formae speciales* of *F. oxysporum* including *F. oxysporum* f. sp. *cubense* TR4 (*Foc SIX6*), which causes panama disease in banana and plantains, *F. oxysporum* f. sp. *vasinfectum* (*Fov SIX6*) which causes fusarium wilt in cotton, *F. oxysporum* f. sp. *melonis* (*Fom SIX6*) which causes fusarium wilt in melons, and many other, but not all, *formae speciales* of *F. oxysporum*, as well as some species of *Colletotrichum*. *Foc* SIX6, *Fom* SIX6 and *Fov* SIX6 proteins have also been found to cause cell death in a wide range of plants, but the patterns of response differed between the *SIX6* variants. An investigation of *Fol* SIX6 function suggests that it may affect plant transpiration.

SnTox2/6 from *Parastagonospora nodorum* uses multiple host targets to induce disease

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Parastagonospora nodorum, causal agent of septoria nodorum blotch of wheat uses multiple proteinaceous necrotrophic effectors that target host genes typically involved in defense to induce programmed cell death (PCD). However, this PCD is to the disadvantage of the host, resulting in proliferation and sporulation of the pathogen. Recently, we used a genome wide association study (GWAS) approach to identify candidate necrotrophic effector genes. We sequenced 198 *P. nodorum* isolates and phenotyped them on host differential lines known to harbor specific necrotrophic effector sensitivity genes including *Snn2* (wheat line BG223) and *Snn6* (wheat line ITMI37), the target genes for SnTox2 and SnTox6, respectively. Using a set of 322,613 markers, we identified a single locus that

was associated with virulence on both BG223 and ITMI37. This locus, which we identified as *SnTox2/6*, harbored a single strong candidate gene encoding a predicted effector. Disruption of *SnTox2/6* resulted in the loss of virulence on both BG223 and ITMI37 and the lack of disease associated with the *Snn2* and *Snn6* loci in segregating mapping populations. Transformation of the avirulent isolate Sn79-1087 with *SnTox2/6* resulted in virulence on lines harboring *Snn2* and *Snn6* and the development of disease associated with the *Snn2* and *Snn6* loci. Preliminary data also indicated that a third sensitivity target, genetically independent of *Snn2* and *Snn6*, was also present in other wheat lines. Collectively, this work shows that SnTox2/6 is targeting multiple wheat sensitivity genes that are important in triggering PCD, resulting in increased pathogen fitness on host lines harboring these target genes.

Using comparative transcriptomic analyses to dissect host specific virulence effectors of wheat and barley pathogen *Bipolaris sorokiniana*

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Bipolaris sorokiniana is a causal agent of spot blotch (SB), common root rot (CRR) and helminthosporium leaf blight (HLB) of both wheat and barley. *B. sorokiniana* is a hemibiotroph which displays a rapid switch in lifecycle from biotrophic to necrotrophic growth during the early stages of infection, ultimately resulting in the death of host tissue. The contribution of fungal virulence factors to this pathosystem is poorly understood. My research is focussed on identifying host specific virulence factors that facilitate pathogenicity in the different cereal hosts and to dissect the underlying mechanisms. A deep RNA sequencing approach was undertaken from wheat and barley leaves infected with *B. sorokiniana*. In this dataset, I found genes encoding small secreted proteins, secondary metabolites, and cell wall-degrading enzymes that showed strongly differentiated transcriptional profiles between the two hosts. Twelve novel small secreted protein (SSP) effector candidates which were significantly differential expressed between wheat and barley infections were identified. Furthermore, a polyketide-derived fumonisin biosynthetic gene and an extracellular iron-chelating siderophore synthetases gene which were both highly upregulated during early stage infection of wheat leaves were also identified. A split marker strategy was employed for targeted gene knockout in *B. sorokiniana* to explore the virulence contribution of those candidates. We generated homologous target gene replacement lines with Hygromycin B in *B. sorokiniana* for each of the candidates. This presentation will outline the progress in characterising these putative pathogenicity genes and their role in facilitating disease.

Effectors from *Alternaria solani* and evidence for a resistance gene in wild potato

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The necrotroph *Alternaria solani* causes early blight on several Solanaceous crops. Potato cultivars show variation in their level of susceptibility to the disease, but no fully resistant cultivars exist. To help understand the infection strategy of *A. solani* and aiming to speed up the identification of resistance genes, we have initiated an effectoromics approach. We have sequenced the genome of *A. solani* using PacBio technology and obtained a gapless genome assembly. Various effector candidates were identified, of which some showed to trigger a cell death response in potato. We are now studying the role of these effectors in pathogenicity of *A. solani* through gene-knockouts and transient expression assays. In parallel, we have explored wild relatives of potato for resistance to early blight. Various genotypes with high levels of resistance were detected. Populations derived from some of these genotypes show a 1:1 segregation of resistance, which suggests that the resistance is caused by a single dominant gene. We have started genetic mapping and found markers that are closely linked to resistance. The effectoromics approach should help us to quickly characterise candidate genes. This research is an important step towards developing a potato cultivar that is resistant to early blight.

The functionally conserved effector Sta1 is a fungal cell wall protein required for virulence in *Ustilago maydis*

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The biotrophic fungus *Ustilago maydis* causes smut disease of maize. The interaction with its host and induction of characteristic tumors are governed largely by secreted effectors. Smut fungi parasitizing different hosts share a small number of core effectors whose function is mostly unknown. Here, we characterize the *sta1* (small tumor associated 1) gene that is upregulated during early stages of infection and encodes a novel core effector. We demonstrate that *sta1* mutants show a dramatic reduction of virulence and the development of tumor cells in infected leaf tissues is abolished. While the *sta1* mutant can colonize the epidermal layer similar to wild type, the *sta1* gene appears necessary for the effective colonization of vascular tissue. Functional orthologs are found in the related

smut pathogens infecting monocot and dicot plants. In budding cells constitutively expressing Sta1, the protein is secreted. However, in filamentous cells Sta1 is specifically bound to the cell wall. Furthermore, the constitutive expression of Sta1 renders the fungal filaments susceptible to Congo red, β-glucanase and chitinase, suggesting that Sta1 alters the structure of the fungal cell wall specifically in filaments. When the *sta1* promoter is exchanged with promoters conferring constitutive or delayed expression during plant colonization, virulence of *U. maydis* is attenuated, indicating that *sta1* expression needs to occur during a specific time window after plant colonization. Our results suggest that Sta1 is a novel kind of effector, which needs to modify the cell wall of fungal hyphae and this is prerequisite for accommodation in vascular tissue and tumor development.

Nuclear calcium spiking as a junction for multiple microbial recognition at the root epidermis

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Nuclear calcium spiking which lies at the core of the so-called Common Symbiotic Signaling Pathway (CSSP), has long been considered a specific response to symbiont recognition at the legume root epidermis. While evidence for an at least partial involvement of the CSSP in other plant-microbe associations has emerged, a clear-cut demonstration of the induction of nuclear calcium spiking by non-symbiotic microbial partners, and more specifically endophytic ones, has not thus far been reported. By using gene expression analysis, phenotypic screening of CSSP mutants, and analysis of nuclear calcium concentration transients via confocal microscopy, we demonstrate the following: an endophytic *Fusarium solani* isolate (strain K; FsK), induces the expression of CSSP- and CSSP-dependent genes, and colonizes legume roots in a CSSP-dependent manner, controlled at a step post the calcium spiking response. We, furthermore, show that the strain's exudates trigger CSSP-dependent nuclear calcium spiking in *M. truncatula* Root Organ Culture epidermis, and this response is also elicited upon recognition of other *Fusaria* exudates, as well as of exudates from mutualistic legume-interacting fungi. The plant cell response upon exogenous *in planta* application of FsK exudates, as well as a preliminary characterization of the triggering molecules, will also be discussed.

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Could small interfering RNA be involved in host specialization in the grey mould fungus *Botrytis cinerea?*

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The grey mould fungus *B. cinerea* infects more than 1400 plant species and thus is considered a broad generalist. However, our recent genomic studies identified lineages that are partially specialized on grapevine or tomato (Mercier *et al*. 2019, *Environ Microbiol* 2019 21:4808) and genes with signatures of divergent selection, representing possible determinants of host specialization (*Unpublished*). Here, we investigated whether small interfering RNAs (siRNAs) could also contribute to this specialization. Indeed, *B. cinerea* produces siRNA that can act as effectors by silencing target genes in the host plant (Weiberg *et al*. 2013, *Science* 342:118). In addition, these siRNAs are derived from transposons and are likely to evolve rapidly in the populations of grey mould.

We compared the repertoires of siRNA in 14 isolates that are either specialized on grape (G) or on tomato (T). While G and T isolate produce a common set of siRNAs, the G isolates produce an additional set of specific siRNAs (3972 unireads). In order to identify the loci responsible for the production of these siRNA, the genomes on one G and one T isolates (G3 and T3) were sequenced with the PacBio technology. The mapping of the siRNA reads on the genomes indicated that the G-specific siRNA are overwhelmingly synthesized from one newly identified retrotransposon that is absent from the genomes of the T isolates.

To identify the grapevine genes potentially targeted and silenced by the G-specific siRNAs, we combine an *in silico* analysis (using the psRNA target software) and a RNAseq analysis in which the transcriptome of grape berries infected by the G3 isolate is compared to the transcriptome of berries infected by the T3 isolate. Further experiments are conducted to validate the role of the identified retrotransposon in the synthesis of G-specific siRNA and to test whether the associated siRNA are able to silence the identified candidate grapevine genes.

Identification of *Trichoderma* species isolated from Algerian soil and evaluation of their antagonist potential against some crops diseases' pathogens

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Screening for antagonistic *Trichoderma* spp. from Algerian soil allowed recovery of 18 isolates from cultivated soil in the central region of northern Algeria and 14 from the southern (desert). Identification based on ITS and tef1 alpha sequencing allowed to identify 5 species *T. atroviride, T. harzianum, T. longibrachiatum, T. viride and T. hamatum*. The species *T. atroviride* and *T. longibrachiatum* were isolated from the northern region; *T. viride* and *T. hamatum* were isolated from the southern region while *T. harzianum* was isolated from both. The antagonistic activity of *Trichoderma* spp. was carried out against *Fusarium oxysporum* f.sp.*ciceris* (Foc), causal agent of Fusarium wilt of chickpea and *Fusarium* spp. causal agent of crown rot (CR) and Fusarium head blight (FHB) of wheat based on *in vitro* and *in vivo* bioassay. Among the *Trichoderma* spp. isolates tested, the Ta.13 (*T. atroviride*) has shown the highest rate (83.92%) of disease reduction against the Foc. Ta.13 also reduced disease index caused by *F. culmorum* (the major pathogen involved on wheat CR and FHB in Algeria) by 70.44%. In the present study, it was also shown that *T. longibrachiatum* isolates induced the highest rates of growth stimulation of chickpea. In parallel, secondary metabolites were obtained by stationary culture of *T. atroviride* (Ta.13) isolate in PDB medium for 30 days. After extraction with ethyl acetate and a rotavapor concentration at 35 ° C under vacuum, the concentrate crude extract was tested on *F.culmorum*, *F. pseudograminearum*, *Microdochium nivale* and *M. Majus*. The growth rate inhibition varied between 27.07 and 48.1%, the highest inhibition rate was recorded with *M. nivale* (48.1%). After crud extract fractionation and purification by column and thin layer chromatography, secondary metabolites with high weight were obtained, 6PP (6 α pentyl pyrone), metabolite A, B and C, and were tested against the same phytopathogenic fungi where 6PP was the most effective.

Role of chitosan and chitin deacetylases during development of *Ustilago maydis*

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The basidiomycete *Ustilago maydis* causes smut disease in maize. This non-obligate pathogen penetrates the plant cell wall using appressoria and then establishes an

extensive biotrophic interaction, where the hyphae are tightly encased by the plant plasma membrane. The fungal cell wall is the first structure to interact with the host and some of its components like chitin are well-known elicitors and trigger plant immune responses. To avoid this from happening in a compatible interaction pathogens have developed strategies to modify or hide chitin. One of these strategies discussed in other pathosystems could be the conversion of chitin to chitosan by chitin deacetylases (CDAs). Chitosan is a poor substrate for plant chitinases and a weak inducer of plant immunity. The aim of this project is to determine whether chitosan is produced by *U. maydis* and which role it plays during colonization. *U. maydis* possesses seven putative *cda* genes which are differentially expressed during development. Using a specific probe for chitosan, a strong signal was detected in appressoria and in biotrophic hyphae. To evaluate the function of chitosan during colonization, we have inactivated all single *cda* genes and have generated strains carrying mutations in multiples *cda* genes. So far, it appears that strong chitosan staining of biotrophic hyphae is not crucial for virulence, although we cannot exclude some form of compensation. Furthermore we show that CDA activity is required for cell viability and in certain *cda* mutants cell morphology is affected. This results indicate that CDAs have discrete but partially overlapping functions during *U. maydis* development.

Genome-wide association studies identify novel candidate genes associated with aggressiveness in the wheat pathogen *Zymoseptoria tritici*

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Plant-pathogenic fungi produce a variety of effectors to overcome plant defense mechanisms, thereby preventing recognition from the host immune system. Identification of these genes in pathogen genomes and natural fungal populations is essential to build durable resistance. In a major crop like wheat, *Zymoseptoria tritici*, the causal agent for Septoria tritici blotch (*Stb*), is the most damaging pathogenic fungi in Europe. Despite this, its genetic basis of virulence is poorly understood. In this study, we performed genome-wide association studies (GWAS) on a panel of 109 re-sequenced field isolates

of *Zymoseptoria tritici*, to identify polymorphisms linked to aggressiveness-related traits on twelve varieties carrying different *Stb* resistance genes. We identified 47 and 32 candidate genes associated with virulence at 10% and 5% False Discovery Rate, respectively, of which six encode small-secreted proteins overexpressed *in planta*. Loci identified by GWAS were manually curated for individual gene models and analyzed for their functional role. The vast majority of detected loci were cultivar-specific suggesting host specialization. Moreover, several candidate genes were found to be located in proximity of transposable elements that may have contributed to local genome plasticity and the evolution of pathogen virulence. The results in our study provide a comprehensive priority list of potential effector genes for functional studies and contribute to the understanding of genetics of virulence in a major wheat pathogen.

Analysis of the effectome of the conifer pathogen (*Heterobasidion parviporum*) and functional roles on interspecific fungal interactions

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Heterobasidion parviporum Niemelä & Korhonen is the most destructive disease agents of Norway spruce (*Picea abies*). During colonization, the pathogen not only infect conifer trees, it also interact with other fungi, such as mycorrhiza symbionts, endophytes or saprotrophs. Our hypothesis is that small secreted proteins (SSPs) from *H. parviporum* (HpSSP) are involved in the interspecific fungal interaction. To identify HpSSP-coding genes potentially involved, we screened the effectome, conducted phylogenetic analysis and identified a subset of 12 SSPs for further characterization. The twelve HpSSPs candidates were selected based on the number of cysteine residues, gene structure, length of amino acids and transcript abundance. Most of the HpSSPs are hypothetical proteins without predicted domain. These sub-set of SSPs were subsequently explored for their roles in the interaction of *H. parviporum* with either ectomycorrhizal *Cortinarius gentilis*, or endophytic *Phialocephala sphaeroides*, or saprotrophs (*Mycena* sp., *Phlebiopsis gigantea*, and *Phanerochaete chrysosporium*). Our results revealed that in co-cultures *Mycena* sp., had antibiosis effect on *H. parviporum*, whereas *P. gigantea* and *P. chrysosporium* had combative competitive or antagonistic interaction with the pathogen. *Phialocephala sphaeroides* and *Cortinarius gentilis* were usually overgrown by *H. parviporum*. Three of the SSPs (HpSSP35.8, HpSSP1.590, HpSSP3.169) with demonstrated in planta expression had similar regulation patterns under different interspecific interactions. Two SSPs (HpSSP2.152 and HpSSP2.330) were barely expressed in paired culture with *Mycena* sp., but were induced in other combinations. The results of HpSSP gene expression patterns provide additional insights on the diverse roles of SSP in pathogenesis and interspecific

fungal interactions.

Functional characterisation of candidate *Fusarium graminearum* effectors

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The effector repertoire of plant pathogens is a key determinant of the success of pathogen-host interactions and could mean the difference between a compromised or a successful crop harvest. One notorious pathogen, the fungus *Fusarium graminearum* is the causal agent of fusarium head blight, one of the most destructive diseases threatening wheat production worldwide.

A main challenge facing *F. graminearum* effector characterisation is pinpointing high quality effector candidates from the predicted proteome. Despite the publication of the refined *F. graminearum* secretome in 2012[1], finding candidates suitable for functional studies from a pool of almost 300 secreted proteins with unknown functions remains problematic.

I have adopted *in silico* bioinformatic pipelines that consider a multifaceted approach to effector discovery such as transcriptional (RNA-seq and microarray), proteomic, taxonomic distribution analysis and the genome location of candidates. This has proven to be successful in finding clusters of candidate effectors in multiple filamentous phytopathogens[2].

For the functional characterisation of candidates, I have taken a two-pronged approach. This includes Agrobacterium-mediated overexpression in the non-host *Nicotiana benthamiana* and the using the BSMV-VOX system to overexpress candidates in the wheat host in combination with Fusarium inoculations. Different types of plant responses to Fusarium effectors will be presented and discussed.

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Uncovering the complex roles of fungal small RNAs in plant pathogenesis

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Small RNAs (sRNAs) are non-coding RNAs with multifaceted roles in gene regulation across all biological kingdoms. They typically silence genes through complementary base pairing with mRNAs in a process known as RNA interference (RNAi). Some plant pathogenic fungi may secrete sRNAs into host cells to mediate RNAi of host resistance genes, but we are only beginning to understand the evolutionary and biological significance of this phenomenon. The parasitic fungus *Sclerotinia sclerotiorum* infects hundreds of diverse plant species. The molecular processes that are key to its success on such a broad range of hosts are elusive. We aimed to uncover the significance of sRNAs in *S. sclerotiorum* pathogenesis and to develop an understanding of their association with disease resistance genes in plants. To this end, we generated fungal sRNA sequencing data during infection and used abundant fungal sRNA sequences to predict candidate target genes in the model plant *Arabidopsis thaliana*. We found that these predicted targets were significantly enriched with several domain annotations related with plant disease resistance. Furthermore, they were significantly more down-regulated during infection with *S. sclerotiorum*. Using data from a genome wide association study (GWAS), we found that the *A. thaliana* target genes were also enriched for SNPs with a significant association with *S. sclerotiorum* resistance. We tested three deletion strains for top candidate genes and found that two were significantly more susceptible to infection (1). Further sequencing data we have generated subsequently have supported a role for RNAi in *S. sclerotiorum* pathogenesis. Combination of different small RNA sequencing methods with GWASs and plant infection bioassays may be a good approach for investigation into the molecular basis of plant pathogenesis in *S. sclerotiorum*.

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Functional characterization of root-associated fungi in *Arabidopsis thaliana*

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Roots of healthy plants are inhabited by a wide diversity of micro-organisms, including bacteria, fungi and oomycetes. While a remarkable conservation of bacterial community composition was observed in roots of natural *Arabidopsis thaliana* across Europe, the composition of filamentous eukaryotic communities displayed greater temporal and spatial variation (Thiergart *et al.*, 2019). A fungal culture collection has been established from *A. thaliana* roots grown in these European soils. Recolonization experiments in germ-free plants using a representative synthetic community revealed a detrimental effect of fungi on both plant growth and survival, which is rescued in presence of bacterial root commensals (Duran *et al.*, 2018).

We sequenced a set of 120 root-associated fungi that are representative of the fungal phylogenetic diversity identified by amplicon sequencing in roots of natural *A. thaliana* populations. They consist in 95% of Ascomycetes with a high representation of Sordariomycetes. Recolonization experiments with individual fungal strains and germ-free plants were carried out in an agar plate system under both phosphate-deficient and phosphate-rich conditions. Phenotyping results revealed a wide diversity of effects on plant development, ranging from destructive pathogens (i.e. *Fusarium* clade) to potentially beneficial symbionts. These fungi promote plant growth exclusively at low phosphate concentration, and evolved independently in five distinct lineages. Using comparative genomics and dual RNAseq, we are currently investigating the conserved and specific strategies employed by these fungi to promote plant growth.

Transcriptomic approach to unveil the interaction between biocontrol yeast and postharvest fungal pathogen on the host fruit: which one is hungrier?

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The attention claimed for ecological concerns affects also postharvest diseases

management. Biocontrol strategies are a promising approach to achieve food safety and food security. The aim of this study is to unveil the molecular interactions involving the biocontrol yeast *Papiliotrema terrestris* strain LS28 (BCA), the postharvest pathogen *Penicillium expansum*, and apple fruits cv. Golden delicious. We performed RNAseq analysis during both their dual and tritrophic interactions to identify the differentially expressed genes of LS28 and/or *P. expansum* applied alone or in combination in apple wounds. For the BCA, gene ontology classification revealed that upregulated genes are involved in transmembrane transport and oxidation-reduction processes, with the glutathione S-transferase gene being the most expressed. For *P. expansum*, transcription and transmembrane transport were the most represented GO categories, with a glycoside hydrolase being the most expressed gene. Analysis of the transcriptomic changes of the host revealed a highly different expression patterns during its interaction with the BCA or *P. expansum*, with the most upregulated genes being involved in cytoskeleton organization and host immunity, respectively. This transcriptomic analysis combined with the recent development of transformation systems for LS28 allows the functional characterization of genes playing a crucial role for biocontrol activity, thus contributing to advance the knowledge on the molecular mechanisms that underlie biocontrol activity.

Compositional and functional analysis of the β-glucan matrix produced by *Serendipita indica* in planta

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Plants continuously survey their environment for the presence of potentially harmful microbes. During microbial invasion, cell-surface receptor proteins perceive microbe-derived or modified-self ligands and initiate appropriate responses. The recognition of fungal chitin and the subsequent activation of plant immunity are well described. In contrast, the mechanisms underlying β-glucan recognition and signaling activation remain largely unexplored.

Recently, we observed that root colonizing ascomycetes and basidiomycetes surround themselves with a β-glucan matrix during plant colonization using a lectin from the root endophyte *Serendipita indica* (Wawra *et al*. 2019, New Phytologist). Since β-glucans can act as potent microbe associated molecular patterns (MAMPs) that activate the plant immune system, information about matrix composition and effects on the plant immune

system are crucial to understand how fungi establish themselves in this ecological niche. Proteomics analytics of the matrix obtained from the root endophyte *S. indica* showed, a strong significant enrichment for proteins carrying the β-1,3-glucan binding WSC domain including members that have the potential to act as β-glucan matrix markers. Furthermore, glycan analytics of the matrix revealed that the β-glucans present in the matrix are distinct from the ones found in the cell wall and can be specifically targeted by plant glucanases to release bioactive MAMPs. In summary, our current data indicate that comparable to animal pathogenic fungi the polysaccharide matrix of plant colonizing fungi has to be treated as a distinct 'compartment' rather than just as an extension of the fungal cell wall. Here we will present our latest data on the topic.

Regulation of *Glycine max* and *Colletotrichum truncatum* gene expression during colonization

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Soybean (*Glycine max* L.) is one of the major crops worldwide as a source of protein-rich foods and animal feeds. Soybean anthracnose, mainly caused by the hemibiotrophic fungus *Colletotrichum truncatum*, is one of the major limiting factors to soybean production. Losses of up to 90 kg/ha are estimated for each 1% incidence of the disease. While anthracnose was historically considered a late-cycle disease, researchers and producers have recently reported its appearance since the early stages of crop production indicating that the program of chemical control has not been effective. Losses due to anthracnose in soybeans have been neglected, but their impact may threaten grain production in up to 53% of the Brazilian production. In order to gain a better understanding of the genetic basis of the *C. truncatum*/soybean interaction, we generated a deep RNA sequencing dataset by examining the transcriptional pattern of isolate LFN150, that had the genome recently sequenced and annotated, in three stages of soybean colonization: the biotrophic, biotrophic-necrotrophic transition and necrotrophic phases. The identification of differentially expressed genes in different *C. truncatum* genotypes may indicate which biochemical pathways are recruited at each stage of host development. A clear understanding of the factors responsible for C. *truncatum* infection and colonization in soybeans has direct implications for the development of long-term control measures for the management of anthracnose.

Investigating the role of the circadian clock in the wheat fungal pathogen, *Zymoseptoria tritici*

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The fungus *Zymoseptoria tritici* causes Septoria Tritici Blotch (STB), one of the most devastating diseases of wheat in Europe which accounts for up to 40 % yield loss. There are currently no fully durable methods of control against *Z. tritici*, so novel strategies are urgently required.

The purpose of this research is to investigate whether the circadian clock is important for pathogenicity and development in *Z. tritici.* Circadian clocks are molecular machineries which are entrained by environmental signals such as light and temperature. Previous research has shown that the host circadian clock can regulate defence against pathogen attack. However, research from the pathogen perspective is limited.

In order to identify the core circadian clock components in *Z. tritici*, known fungal clock genes were BLAST searched against the genome database of this pathogen. The expression patterns of the potential *Z. tritici* clock components identified are currently being investigated using a combination of RNAseq and RT-qPCR studies. These candidate genes are being knocked-out in *Z. tritici via Agrobacterium*-mediated transformation, and the phenotypes assessed *in vitro* and *in planta*. To date, two of the mutant lines tested have displayed developmental defects with possible implications in pathogenicity.

This study is one of the first documented investigations into the role of the circadian clock in an economically important cereal pathogen using a crop plant host. Findings from this research will impact future control strategies against *Z. tritici* such as timing of fungicide application, disease severity measurements, and genes for future fungicide target screens.

Genome rearrangements drive evolution of virulence-related genes in the genomes of *Colletotrichum gloeosporioides* species complex

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Members of the *Colletotrichum gloeosporioides* species complex are causal agents of anthracnose in a wide range of commercially important plants. We sequenced the genomes of fungi from this species complex, *C. fructicola* and *C. siamense*, as well as representatives of three previously unsequenced species, *C. aenigma, C. tropicale* and *C. viniferum*, providing an in-depth overview of its diversity. Comparisons between multiple *C. fructicola* and *C. siamense* isolates led to the identification of large-scale, strain-specific genomic rearrangements and segmental duplications/loss in these genomes. Accessory regions present in *C. fructicola, C. siamense* and *C. aenigma* were found to be associated with secondary metabolite and effector candidate genes, which may contribute to host virulence. Analysis of near chromosomal-level assemblies of four isolates from these species reveal the presence of such accessory regions in sub-telomeric repeat-rich regions and in putative repeat-rich chromosomes, with exchange of genetic sequences occurring between such regions independently in different strains. Together, our results contribute to the understanding of genome evolution in the *Colletotrichum gloeosporioides* species complex.

Identification, characterization, and *in vitro* biocontrol of pathogenic fungi associated with blister blight lesions of Tea (*Camellia sinensis*)

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Blister blight is a devastating fungal disease that affects tea foliages in moist and foggy environments particularly in the tea growing areas of Assam, Darjeeling, and Meghalaya in India. Taking effective control measures of tea blister blight disease has been a challenging task for phytopathologists. For its control chemicals are routinely applied in tea fields which subsequently cause health hazards, environmental pollution and resistance acquisition amongst causal pathogens. In this study, we have surveyed blister blight disease symptoms and collected samples from seven different tea estates of Darjeeling and Meghalaya during the monsoon months (June-November) of 2018. Affected tea plants showed yellow translucent spots in the initial stage, sporulating blisters at the matured stage, and necrotic lesions at the later stage. *Pestalotiopsis* and

Nigrospora were consistently isolated from the leaf lesions. Morphological studies and molecular characterization by ITS-RFLP further confirmed colonization of the leaf lesions by these fungal species. Further, an endospore-forming xylan degrading bacterium *Paenibacillus xylanisolvens* was isolated from tea rhizosphere soil sample from the Bateli tea estate of Assam in India. The identification of the isolate was done by morphological and molecular characterization. *P. xylanisolvens* showed promising *in vitro* antifungal potency which was confirmed by various techniques like the determination of MIC against blister blight associated fungi, rate kill assay, and interaction with the isolated fungal pathogens by SEM analysis. GC-MS was performed to identify the chemical constituents present in *P. xylanisolvens* strain. Thus, the objectives of this present study were to gain a better understanding of the etiology of blister blight disease, to identify fungi that colonize blister blight leaf lesions, and to evaluate the efficacy of a xylan degrading bacterium as potent antifungal agent against blister blight disease of tea.

A bZIP transcription factor of *Colletotrichum higginsianum* is associate with osmotic stress and appressorium formation

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Species of *Colletotrichum* caused a tremendous number of diseases on crops, especially on agricultural production of tropics and subtropics. The economic loss was hard to estimate. *Colletotrichum higginsianum*, the pathogen of cruciferous anthracnose, can infect many cruciferous vegetables and the model plant, Arabidopsis thaliana. This study was aimed to identify virulence-related transcription factors with a primary screening of uncharacterized transcription factors based on gene expression in early stage patterns in stress conditions. A homolog of *C. higginsianum YAP1*, named *ChYAP2*, was identified by phylogenetic tree and sequence alignment analysis. Both *ChYAP1* and *ChYAP2* genes contained a bZIP domain and a PAP1 domain. *ChYAP2* were relevantly expressed during infection based on a published transcriptome database. To investigate gene functions, the deletion strain (Δ*ChYAP2*) confirmed by southern blot and PCR was further examined in phenotyping. The deletion strains Δ*ChYAP2* showed slightly slower growth on colony and much lesser disease incidence to both Chinese cabbage and A. thaliana in pathogenicity assays. The development of its invasive hyphae was restricted in primary invasive hypha stage in planta. Interestingly, the number of appressorium formed was much fewer in Δ*ChYAP2* than wild-type in vitro and in vivo. Notably, *ChYAP2* was down-regulated in response to osmatic stresses. Under different osmotic stress (NaCl, MgCl2 and sorbitol) conditions, the number of appressorium formation in wild-type was reduced, while the Δ*ChYAP2* was still defeated in appressorium formed and the hyphae became shorter. It would be interesting to figure out *ChYAP2* its gene function and the correlation

in appressorium formation during the invasion process. From these results, we found that a bzip transcription factor was required for full virulence. Additional investigation on their interacting proteins may reveal new molecular pathways associated with fungal virulence to plants.

Vegetative compatibility groups and biocontrol of *Pyricularia oryzae* in Taiwan

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Rice blast disease caused by *Pyricularia oryzae* is a serious disease that globally reduces rice production. Vegetative compatibility has been thought as an important mechanism which contributes genetic exchange in ascomycetous fungi. In order to assess the vegetative compatibility groups (VCG) of *P. oryzae* populations in Taiwan, non- utilizing (*nit*) mutants of 87 isolates were generated from minimal medium amended with potassium chlorate. Sulfate non-utilizing (*sul*) mutants were also recovered from 53 isolates which showed no complementation between their nit mutants. A peculiar high percentage of heterokaryon self-incompatible (HSI) isolates was determined by no complementation in pairings of their *nit* / *nit* mutants and *nit* / *sul* mutants. To study the population structure of the 87 isolates, isolate 63 (a heterokaryon self-compatible isolate) was chosen to be a tester in order to pair with other 86 isolates with all possible combinations, resulting in four types of heterokaryon formations: negative type, weak type, medium type and strong type. Remarkably, among those isolates showing strong type of heterokaryon formation with isolate 63, heterokaryon formation may not always occur between any pair of two isolates. This study suggested that *P. oryzae* isolates may derive from a predominant VCG group, indicating that genetic exchange from the parasexual mechanism could commonly occur. This explains the rapidly increasing number of races in field. Biocontrol was considered as a prospective strategy for disease control. Several microorganisms against *P. oryzae* were screened from collections of rice endophytic bacteria and antagonistic bacteria. These strains can inhibit mycelial growth, conidial germination and appressorial formation of *P. oryzae*. Rice treated with these strains obviously reduced the disease severity of rice blast in seedling trays. The detailed mechanisms of disease control through these strains will be demonstrated.

Unravelling the molecular basis for chilling tolerance of the gray mold phytopathogenic fungus *Botrytis cinerea*

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Fungal pathogens are responsible for a significant loss of fresh fruits and vegetables after harvest, estimated at around 30% of the total crop yield worldwide. Low temperature (LT) storage is an effective method to prolong postharvest crop performance with a minimal negative impact on human health and the environment. LT reduces cell respiration and metabolism, which delays fruit senescence / ripening as well as slows down the development of pathogenic microorganisms. *B. cinerea* is a necrotrophic fungal pathogen, causing grey mold disease during pre- and post-harvest on many economically important crops. To uncover the molecular basis of *B. cinerea* cold tolerance, we have characterized the morphology and physiology of *B. cinerea* under several chilling conditions. It showed marked variability in their hyphal, growth pattern, conidiation, sclerotia formation and pathogenicity under LT. Cold-stress tolerance should generate active intra- and extracellular enzymes for development and infection at low temperatures and also essential to cause the system to retain elevated metabolic activity at low temperatures, although at LT all the biochemical and physiological processes needed for growth function. Several pathways, including detoxification of ROS, remodeling of membrane lipids and biosynthesis of osmo-regulators, were evaluated to determine its involvement in the cold-stress response. By correlating the morphology to transcriptomic and metabolic changes during growth at LT advance our understanding of the molecular basis of *Botrytis* infectivity at cold conditions. This knowledge may pave the way to reduce food loss by developing environmentally sustainable control treatments against cold tolerant fungal pathogens in cold storage.

Preparing for battle: characterisation of fungal cellular processes during early infection of wheat by *Zymoseptoria tritici*

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The fungal wheat pathogen *Zymoseptoria tritici* is responsible for major crop losses through causing the disease Septoria leaf blotch (STB). Mitigating this disease is of major economic importance, with an estimated annual fungicide input worth €1 billion targeted to controlling STB in Europe. The infection cycle of *Z. tritici* displays two distinct phases, beginning with an extended symptomless phase (> 7 days) as the spore germinates and

extends hyphae across the leaf surface and through stomata into the intercellular space. The fungus then induces host cell death and tissue collapse in the leaf. Recent evidence from transcriptome analysis suggests that the fungus uses little host-derived nutrition during the early stages of infection, instead relying on macromolecules within the fungal spore. Our research aims to elucidate how *Z. tritici* remobilises stored nutrients to support growth during colonisation of the wheat leaf.

We are currently investigating whether *Z. tritici* uses the self-degradative process of autophagy to recycle cellular resources for germination and growth during the initial symptomless phase. The key autophagy genes ATG1, encoding a protein kinase involved in autophagy initiation, and ATG8, encoding a ubiquitin-like protein required for autophagosome formation, have been characterised by gene deletion and GFP-tagging to assess the importance of autophagy to *Z. tritici* cellular differentiation and virulence. Furthermore, we are investigating the importance of lipid metabolism during *Z. tritici* infection, through disruption of fatty acid β-oxidation. Our results suggest that autophagy is not required for growth of *Z. tritici* during early infection, while mitochondrial metabolism of fatty acids plays a key role in supporting hyphal growth and wheat infection. These findings will provide fundamental knowledge of the molecular mechanisms of *Z. tritici* infection, as well as identifying potential new targets for control of this devastating disease.

PHI-base, a multispecies phenotype database for pathogens, hosts and their interactions to enhance global food security and human health

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PHI-base, www.phi-base.org, is a gold-standard database storing phenotypes on genes implicated in virulence for 268 pathogens tested on 210 hosts. Information is also given on the target sites of anti-infective chemistries. PHI-base's mission is to be a primary information source for researchers studying plant, animal and human pathogens. Manually curated information from more than 3,400 peer reviewed articles are made accessible and searchable to provide relevant molecular and biological facts on pathogenicity, wild-type/mutant genes and fungicide target sites. High-level phenotypes are used to describe the overall experimental interaction outcomes enabling comparative analysis across different pathosystems.

Here we describe our new PHI-base Version 4.8 release (September 2019) and its

increased data content[1]. Since 2015 we observed an increase in the curation of pathogen gene modifications that result in hypervirulence. Hypervirulence phenotype interactions now account for 4.6% of all database entries and are particularly prevalent amongst bacterial pathogen entries. PHI-base is part of the ELIXIR life science infrastructure and phenotype data are already disseminated to Ensembl Genomes and FungiDB to link phenotypes to genomes. This allows further computational analysis including variant analysis, RNAseq and mapping to biochemical pathways. Network analysis approaches to investigate virulence in *Fusarium graminearum* to explore novel candidate virulence genes using PHI-base data are discussed. A newly developed web-based community annotation tool, called PHI-Canto, captures mutant phenotype data during manuscript submission by authors. The aim is to enable phenotype data deposition for the plant-pathogen interaction community, reduce time spend on searching for information and enabling computational approaches for further exploration of pathogen-host interactions datasets.

[1] Urban *et al*., (2020) Nucleic Acids Research database issue, doi: 10.1093/nar/gkz904

Metabolomics of non-host switchgrass plants expressing a poplar lectin receptor-like kinase in response to the mycorrhizal fungus, *Laccaria bicolor*

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A black cottonwood poplar (*Populus trichocarpa*) lectin receptor-like kinase (PtLecRLK1) was recently identified that mediates the symbiosis between *P. trichocarpa* and *Laccaria bicolor* (Labbé *et al*. 2019). When PtLecRLK1 was heterologously expressed in *Arabidopsis thaliana*, a non-host species for *L. bicolor*, the transgene induced the ability of *Arabidopsis* to display interstitial hyphal growth and Hartig net-like extracellular structures created by *L. bicolor*, and suppressed the host's defense responses upon exposure to *L. bicolor*, a key mechanism initiating colonization. Given that Arabidopsis is not known to harbor ectomycorrhizal relationships, a new study was initiated to determine if heterologously expressing PtLecRLK1 in a grass species that is a known host of ectomycorrhiza can result in it establishing a symbiotic relationship that, otherwise, would not occur. Four transgenic switchgrass (*Panicum virgatum*) lines expressing PtLecRLK1 were thus generated and gas chromatography-mass spectrometry-based metabolomics were conducted on roots of transgenic plants in contrast with wild-type plants growing in the presence of *L. bicolor*. The largest metabolomic responses of transgenesis were associated with accumulation of numerous nitrogenous metabolites, which are likely associated with the observed

decline in plant growth. Given that there were declines in fatty acids and organic acids, which have been observed previously with symbiosis, the metabolomic results suggest that many of the early steps in successful colonization occurred, but that later stage events were lacking.

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Chitosan and *Pochonia chlamydosporia* both induce plant hormones and defences in tomato root exudates

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Root exudates include a wide variety of molecules that plants and root microbiota use to communicate in the rhizosphere. Tomato plants were irrigated with chitosan, inoculated with the endophytic nematophagous fungus *Pochonia chlamydosporia* (Pc) or both. Root exudates from those plants were analysed at 10, 20 and 30 days after planting (dap). We found using HPLC and EEM fluorescence both chitosan and Pc to induce plant hormones and defence compounds in tomato roots exudates. High doses of chitosan increase the secretion of plant hormones such as salicylic acid, jasmonic acid or abscisic acid in roots. ^{1}H-NMR detected the largest number of signals in 20 dap root exudates. Among them, phenolics such as trigonelline, cinnamic acid and p-aminobenzoic acid. We also found organic acids, aminoacids and monosaccharides. Root exudates from plants irrigated with chitosan inhibit ca. 2-fold growth kinetics of the tomato root parasitic fungus *Fusarium oxysporum* f sp. radicis-lycopersici. Root exudates from plants colonized by Pc and irrigated with chitosan reduced ca. 2-fold egg hatching of the root knot nematode *Meloidogyne javanica*.

Proteomic and metabolomic approach to understand the molecular interaction between wheat plants and *Trichoderma spp.*

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Trichoderma species used as biocontrol agents have been shown to have direct effects on plants, increasing their growth potential and nutrient uptake, fertilizer use efficiency, percentage and rate of seed germination, and stimulation of plant defences. However, the molecular basis of these changes in wheat introduced by *Trichoderma* spp. is almost totally unknown.

This study focused on the identification of changes in the metabolome, proteome and selected gene expression in two wheat cultivars induced by root colonization with *Trichoderma atroviride* and *Trichoderma cremeum*.

To determine the profiles of metabolites and proteins present in leaves and roots of wheat and to identify the differences in obtained profiles between samples under control treatment, *T. atroviride* treatment and *T. cremeum* treatment, under laboratory and field conditions several complementary methods and mass spectrometry systems were used.

Metabolic analysis resulted in the identification of metabolites in the terpenoids, lipids, plant hormones, starch and sucrose metabolic pathways, that were significantly up- or down-regulated after *Trichoderma* inoculation. Proteomic results demonstrated that many of the proteins were common in both control and treated with *Trichoderma* wheat proteomes; while, from 9 to 223 (depending on the *Trichoderma* strain and plant cultivar, organ, developmental stage, growth conditions) proteins were detected as differentially expressed. Several of them were selected and further analyzed by quantitative real-time PCR at the transcriptome level to compare with the proteomic data.

Our results provide new insights into the response of wheat plants to *Trichoderma* spp. root colonization and indicate that the effect of the association of plant with *Trichoderma* depends on fungal species, wheat genotype, maturity state, type of plant organ and environmental factors.

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Fungal attack: chemical communication and protection strategies based on the secondary metabolism associated with tree canker

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Neonectria ditissima, the causal agent of tree canker, affects a wide range of hardwood plant hosts like *Fagus*, *Malus* and *Pyrus*. To date, there is no effective method to eradicate the disease and little is known about virulence factors.

In this study we are interested in secondary metabolites as determinants of interaction between the pathogen *N. ditissima* and fungal endophytes within the plant environment. The chemical communication between host and pathogen may give hints for plant protection strategies. Our research is based on the antagonistic and mutualistic balance of the pathogen *N. ditissima* and the endophytic consortia in different host trees. A broad variety of organisms were isolated from healthy plants and these organisms were tested for antagonistic activity against the pathogen. Several compounds were identified from submerged cultures of various endophytic organisms with a protective effect against *N. ditissima.*

During our studies, tree canker inducing metabolites as well as phytotoxins produced by *N. ditissima* were examined. Extracts of the pathogen cultivated in different conditions were tested in an apple assay. Citrinin and p-hydroxybenzoic acid among other substances, were detected. In addition, components with fungicide activity such as Ilicicolins and ascochlorin were identified.

The genome of *N. ditissima* has been sequenced and potential gene clusters responsible for the secondary metabolite biosynthesis were determined. Moreover, based on transcriptome analysis, corresponding genes related to phytotoxicity are the target of further studies. In order to assess the function of proteins involved in the biosynthesis of secondary metabolites loss of function mutants are being generated. Another approach is the heterologous expression of the corresponding genes in other fungal hosts.

Determination of the interaction type between fungi isolated from the wheat endosphere

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The interactions between endophytic fungi and their host are complex and the outcomes diverse. Based on several investigations, growing evidence exists that the functions of fungal endophytes, and accordingly the types of their interactions with plants, are affected by various abiotic and biotic factors, including environmental conditions, plant genotypes, plant tissue type, the fungal taxon and strain type, as well as the dynamic network of interactions within the plant microbiome, including mycobiome.

The aim of this study was to investigate the interaction between endophytic fungi isolated from the endosphere of ten polish wheat cultivars. Fungal isolates used in present study were identified at the species level by a combination of morphological and molecular analyses. In order to evaluate antagonistic activities of all endophytic fungi, dual cultures on potato dextrose agar were assessed. The radial growth of each fungus was measured daily with a ruler until contact and inhibition growth coefficients and their standard errors was calculated. After co-incubation, a qualitative evaluation of fungal interactions based on four-point scale and microscopic observation of the mycelium of confronting fungi in the interaction zone was carried out.

Based on the knowledge of antagonistic behavior, fungal isolates were divided into various functional groups and will be used in further studies on the determination of the effects of endogenous fungal isolates on the morphology, anatomy, physiological parameters metabolom and transcryptom of wheat.

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Impact of *Trichoderma* fungi on wheat (*Triticum aestivum* L.) seedlings in *in vitro* culture

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Fungi of the genus *Trichoderma* (teleomorph *Hypocrea*) are widespread microorganisms in environment. The main habitat of this fungi are decaying wood, soil and rhisosphere.

Species of this genus can lead a saprophytic as well as a parasitic lifestyle. By antybiosis, hyperparasitism, and competition can affect on plants pathogens e.g. *Fusarium spp*. *Trichoderma* can cause induced systemic resistance and promote growth and development of plants. *Trichoderma* are classified as biological control agents (BCA) and are used commercially for the production of pesticides such as biopesticides and biofertilizers. Nevertheless interactions of *Trichoderma* with plants are not well recognized, especially with wheat plants.

In this study we used system where wheat seedlings grew on semi solid agar and were inoculated by two species of *Trichoderma* differentiated in terms of lifestyle and production of metabolites: *Trichoderma atroviride* strain AN35 - an efficient producer of glucanases and volatile metabolites, including 6-n-pentyl-2H-pyran-2-one, with the highest antagonistic potential towards mycotoxigenic *Fusarium spp*., and *Trichoderma cremeum* strain AN392 – representing saprotrophic lifestyle an efficient producer of cellulolytic and xylanolytic enzymes. Both species of *Trichoderma* originated from Poland, with that *T. cremeum* AN392 was obtained from sample of decaying wood and *T. atroviride* AN35 was isolated from maize kernels.

Research focused on the quantitative evaluation of transcription profiles of selected genes [pathogenesis-related defence gene PR2 and SOD (superoxide dismutase) gene, known to be related to the antioxidative defence responses in plant cells] and on the determination of the selected plant hormones profiles present in roots of wheat seedlings treated and not treated with Trichoderma strains.

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LC-MS-based Peptidomics, Mass Spectrometry Imaging and Bioinformatics Approaches Used to Identify Peptides in the Wheat-*Puccinia triticina* Interaction

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Puccinia triticina (*Pt*) is an obligate fungal parasite that causes leaf rust on wheat. This

disease occurs annually and potentially results in large yield losses. Upon germination *Pt* enters wheat leaves through open stomata and colonizes the apoplastic space with hyphae, and these ultimately penetrate cells to form haustoria. Eventually uredinia are formed and spores are released. Host-pathogen communication at the protein level has been well-studied in this and similar pathosystems, but the potential roles of secretory peptides (smaller than 5 kDa) has not been examined. This poster describes a new project aimed at investigating the role(s) of peptides in this plant-pathogen interaction using top-down LC-MS analyses and MALDI mass spectrometry imaging to detect novel, endogenous peptides which are missed by conventional bottom-up proteomics analyses. For the LC-MS approach, peptides were obtained from rusted wheat leaves by precipitation from acetone, followed by separation by reversed-phase HPLC using a C_4 column at high pH. Intact fractionated peptides were analyzed by LC-MS using a C_8 column for separation and a high-resolution Orbitrap (ThermoFisher QExactive) mass spectrometer for detection. Correct identification through homology matching required us to construct new *Pt* databases containing hypothetical peptides down to 10 amino acids in length, as such small peptides have been observed and shown to be translated from small open reading frames in other fungi. MALDI imaging was performed on formaldehyde-fixed, paraffin-embedded tissue sections of rust foci on susceptible leaves, using a high-resolution TOF/TOF instrument (Bruker Ultraflextreme), and the experimental conditions are currently being optimized for *Pt*. The poster will highlight our most recent findings and discuss potential roles of peptides in this important plant-pathogen interaction.

A 20 kb region absent in a non-symptomatic isolate reduces virulence in a symptom-inducing isolate of *Verticillium longisporum*

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Verticillium longisporum is an amphidiploid hybrid, which evolved from at least three separate hybridization events involving different haploid parental lineages. Lineage specific (LS) regions of plant pathogenic Verticillium species were suggested to be enriched in genes coding for proteins, which are required for virulence and host adaptation. Here, two *V. longisporum* strains isolated from rapeseed fields in the same area in Germany were investigated. The A1xD1 isolate Vl43 induces strong disease symptoms in rapeseed, whereas plants treated with the A1xD3 isolate Vl32 are symptomless, although both fungi are able to colonize plant roots to a similar extend. A 20 kb LS region originating from the parental D1 lineage was identified to be present in the pathogenic isolate Vl43, but absent

in the asymptomatic strain Vl32. Deletion of this LS region in Vl43 led to an increase of induced disease symptoms in rapeseed plants. This suggests a role of the LS region encoded proteins in reduction of disease severity.

Genomic perspectives on the evolution of the mating-type locus in *Phyllosticta*, with emphasis on *Citrus*-associated species

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Sexual reproduction influences fungal evolution by increasing genetic variation and purging deleterious mutations, enabling species to deal with environmental challenges and genome instability. However, sexual reproduction is also costly, as it can break apart well-adapted genomic configurations. In this sense, the balance between inbreeding and outcrossing may differ between plant pathogens and endophytes and may require different reproduction strategies. The elucidation of reproduction mechanisms is particularly important in pathogens, not only to understand how recombination may affect the structural and genetic integrity, but also because sexual structures may play a role in the interaction with the host. For example, *Phyllosticta citricarpa* causes Citrus Black Spot (CBS) disease and is heterothallic: its ascospores can infect fruits and leaves and play an important role in the cycle of CBS disease in populations where both *MAT1* idiomorphs are present. Seven additional species of *Phyllosticta* are known to be associated with citrus, presenting endophytic and pathogenic lifestyles, being host-specific or cosmopolitan species, with different reproduction strategies: *P. citriasiana*, *P. paracitricarpa* (pathogenic species) and *P. citribraziliensis* (endophyte) are heterothallic and host-specific, but *P. capitalensis* (endophytic in citrus and a cosmopolitan species), *P. paracapitalensis* (endophyte in citrus) and *P. citrichinaensis* (pathogen in citrus) are homothallic. Since sexual reproduction is involved in the *P. citricarpa*-citrus interaction, it may be related to the differences in the lifestyles of *Phyllosticta*. To assess this hypothesis, our approach includes: (a) a detailed characterization of the mating-type locus and flanking genes in *Phyllosticta* species; and (b) a reconstruction of the ancestral character state to determine the ancestral thallism state for the genus *Phyllosticta*. We expect the organization and content of the mating-type region to be highly variable, in agreement to the very diverse lifestyles observed in *Phyllosticta* species.

β-Glucosidase enzyme associated with pathogenicity in *Colletotrichum abscissum*

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The Post bloom Fruit Drop (PFD) disease, in Brazil, is mainly caused by *Colletotrichum abscissum*. The disease is characterized by the presence of necrotic lesions on the petals, which lead to premature fruit drop, and its control depends of fungicide application, and outbreaks are observed when rainy days occurs during the flowering periods. The identification of genes associated with pathogen-host interaction in *C. abscissum* is crucial not only for understanding the disease but also for the identification of new control targets. In this way, the research group Genetics of Microorganisms of UFPR has carried out studies of functional genetics by deletion of candidate genes, obtaining and analyzing a collection of transformants of the fungus *C. abscissum*. During these studies, an ectopic insertion transformant (IAC142EC) was non-pathogenic in citrus flowers, and the location of the cassette insertion was unknown. Thus, the present study aimed to identify the region where the deletion cassette was inserted into the genome of the IAC142EC using the *Genome Walking* methodology. The Genome Walking methodology and the genomic analysis revealed that the insertion of the cassette into the IAC142EC mutant occurred in the *BGL1* gene, which encodes an enzyme of Glycoside Hydrolase Family 3 (EC 3.2.1), probable the β-Glucosidase enzyme (EC 3.2.1.21). This enzyme acts on the degradation of cellobiose, a disaccharide resulting from the degradation of cellulose and has cellulase inhibitory activity. The cellobiose also acts as an inducer of the Pathogen-Associated Molecular Pattern Triggered Immunity (PTI) against cell wall-degrading pathogens. Thus, the deletion of this gene may lead to loss of pathogenicity due to the action of PTI, and in view of the inability of the pathogen to degrade the plant cell wall. This hypothesis is going to be confirmed by the deletion of *BGL1* gene from the *C. abscissum* wildtype strain and phenotypic evaluation of the deleted strain in citrus flowers.

Expression profiling of candidate genes under positive selection among different pathotypes of the coffee obligate pathogen, *Hemileia vastatrix*

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Hemileia vastatrix (Hv) is a complex biotrophic fungal pathogen that causes coffee leaf rust (CLR), a disease that has been a permanent threat to Arabica coffee production. Under the constant threat of new pathotypes emerging under a strong selective pressure and becoming epidemically spread on a continental scale, a better understanding of the adaptive genetic variation of Hv populations is an immediate priority. Since genes involved in coffee-rust interaction are expected to evolve under strong selection, the analysis of genetic and expression differences in putative candidate genes could provide insights on the pathogen virulence evolution. In this study, we identified 34 Hv candidate genes with a signature of positive selection by applying a genome-wide scan integrated into a phylogenomics framework previously developed (Silva *et al*., 2015). Functional annotation assigned the higher proportion of these genes to the categories "Posttranslational modification, protein turnover, chaperones" (21%) and "Energy production and conversion" (15%). Given that the signal of positive selection was captured at the phylogenetic branch leading specifically to *H. vastatrix*, these genes may have a role in adaptive changes at the species level. To assess their potential association with Hv virulence profiles, the analysis of gene expression profiles of 4 selected candidate genes was initiated by qPCR for 5 isolates with contrasting pathotypes during compatible interactions, at 3 key stages of the infection process. First results show differences in expression among isolates, either regarding up or down-regulation at different infection stages, or the level of expression. Putative causal relations and possible adaptive significance is being assessed. This study provides a first insight on the molecular variation underlying virulence divergence in coffee rust.

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M. acuminata root colonization and growth promotion by *P. chlamydosporia*

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The biocontrol fungus *P. chlamydosporia*, colonizes endophytically banana roots.

Root hairs and root surface were found colonized using a using Pc123 GFP modified strain. Hyphal penetration of root cells was observed. Spores of *P. chlamydosporia* 123, significantly increase root and leaf length and weight in banana plantlets (*Musa acuminata* cv. Little drawft) in growth chamber experiments at 30 days post inoculation (dpi). In greenhouse 8L pot experiments, *P. chlamydosporia* 123 spore inoculation significantly increases leaves root and pseudostem length and leaf weight in banana plants. Spore inoculation of worldwide *P. chlamydosporia* strains (Pc 399, Pc cat and Pc 21 in banana plantlets, significantly increases leaf and corm length and weight, and root weight. Root colonization by *P. chlamydosporia* also was detected using cultural techniques and qPCR.

Characterization of the candidate effectors repertoire of *Colletotrichum spp*. pathogenic to soybean

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Colletotrichum is considered to be one of the most important genera of fungi by plant pathologists due to its great potential for destruction. *Colletotrichum spp*. have a hemibiotrophic lifestyle and can infect a wide range of hosts, causing losses in crops of major importance worldwide. Soybean (Glycine max) is one of the most important agricultural commodities worldwide, and in 2018 was valued at nearly 32 billion dollars. The large soybean cultivation, mostly under monoculture and non-tillage systems, affects the intensity of diseases, amongst them, anthracnose. Soybean anthracnose is mainly associated with *C. truncatum*, but during the last few years, other species have emerged from commercial fields becoming one of the biggest limiting factors for soybean production in several regions. In order to identify candidate effectors putatively involved in soybean anthracnose we sequenced the genome of 4 isolates representative of *Colletotrichum* species pathogenic to soybean: *C. truncatum, C. plurivorum, C. sojae* and *C. musicola*. The genomes were assembled and annotated to identify and characterize protein encoding genes. The proteomes and secretomes of the newly sequenced genomes along with those of an additional 10 species covering the diversity of the genus and not pathogenic to soybean were classified into protein families using a variety of bioinformatic approaches. Comparative analysis highlighted families of small secreted proteins specific to soybean pathogens. These specific extracellular proteins may be effectors, proteins that have important roles in modulating the plant's immune system and in host specialization. In this study, we present the draft genome sequences of four *Colletotrichum* species identified as destructive pathogens of cultivated soybean. The

sequences and results represent a new resource that will be useful for further research into the biology, ecology, and evolution of these key pathogens and in the management of soybean anthracnose.

Comparative and functional genomics of *Plectosphaerella* isolates with different life styles

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Fungi can establish different types of interactions with plants that range from the negative interactions that result in plant disease to positive interactions that result in different kinds of benefits for the plant. The outcome of the fungus-plant interaction depends on complex mechanisms that include the pathogenic ability of the fungus and the ability of the plant to respond to fungal attack. Fungi of the genus *Plectosphaerella* are frequently found at the phyllosphere and rhizosphere and are adapted to colonize different plant hosts. We have characterized three *Plectosphaerella* isolates that display distinct types of interactions with the model plant *Arabidopsis thaliana*: *Pc*BMM, a pathogenic isolate; *Pc*2127, a non-pathogenic isolate in wild-type plants; and *P*0831, an epiphytic isolate identified in wild populations of *A. thaliana* in central Spain that colonizes wild-type plants without causing disease symptoms.

Although these isolates have similar gene arsenals and share more than 90% of gene families, comparative transcriptomic analyses identified gene families differentially regulated in planta vs in vitro, notably, a repertoire of *Pc*BMM secreted carbohydrate active enzymes (CAZYmes) that may be involved in the pathogenicity of this isolate. The transcriptomic analysis of the plant response to the different isolates reveals dramatic differences in gene expression, both qualitative and quantitative. Strikingly, the plant barely responds to the epiphytic isolate, which does not elicit a canonical defensive response. In turn, the epiphytic isolate only expresses a few hundreds of genes in planta, most of which have an unknown function. These data indicate a possible strategy of slow growing and low aggressiveness for the epiphytic isolate to avoid plant perception, in a kind of non-aggression pact between fungus and plant.

The fungal endophyte PRB110 improves yield in corn, tomato and pepper crops

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The fungal endophyte PRB110 establishes a mutualistic interaction with *Arabidopsis thaliana*, promoting plant growth and silique production under low phosphate (Pi) growing conditions (Hiruma *et al*., Cell. 2016)(1). Until now, this interaction had only been observed in nature in Pi poor soils of Central Spain. In this study, we show that PRB110 is able to improve yield in different crops, such as tomato, pepper and corn. The effect in tomato and pepper was shown in plants cultivated in greenhouse that where treated with PRB 110 by irrigation at seedling stage. The treated plants displayed a higher root weight, higher number of buds, flowers and fruits per plant, and a higher weight per fruit, what overall resulted in a significant higher yield than mock inoculated plants. The effect in corn was evidenced by both seed treatment and foliar application in open-field conditions. Results showed a yield increase of more than 20%, that rose to 30% in plants subject to a water-restricted regime. Either tomato, pepper and corn where grown under the normal nutritional conditions for commercial crops. These results indicate that the beneficial effect of PRB110 is not restricted to the Brassicaceae *A. thaliana*, but it is applicable to other plant species, such as the Solanaceae (tomato and pepper) and the monocots (corn), which are taxonomically very distant from Arabidopsis, and opens the avenue for PRB110 application in a broad range of agricultural crops. The fact that these plants where not suffering low Pi conditions and that the effect in corn was incremented under drought conditions, points to different modes of action that are currently being investigated in our group.

1. Hiruma, K; Gerlach, N; Sacristán, S; Nakano, Ryohei T; Hacquard, S; Kracher, B; Neumann, U; Ramírez, D; Bucher, M; O'Connell, Richard J; Schulze-Lefert, P. 2016. "Root endophyte *Colletotrichum tofeldiae* confers plant benefits that are phosphate status dependent". Cell 165: 464-474

Understanding the bakanae disease: looking for disease-related genes through the study of avirulent strains of *Fusarium fujikuroi*

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Fusarium fujikuroi is an hemibiotrophic fungus, responsible for the bakanae disease on rice. The pathogen causes crop losses whose magnitude is largely dependent on climate and rice cultivars, varying from 3% to 75%, with the most common symptoms being abnormal height, thin leaves and empty grains, although some strains have been known to induce stunting and withering instead. The induction of elongation is linked the production of gibberellins, but the mechanisms by which *F. fujikuroi* can induce the disease are not completely clear. Even if a high number of *F. fujikuroi* strains has been deposited on online databases, no genome of avirulent strains is available, and no comparative genomic study between virulent and avirulent isolates was ever conducted. In the present work, we confirmed the low virulence of *F. fujikuroi* strain C2S and the avirulence of strain SG4, we sequenced their genomes by Illumina MiSeq and we compared them to the virulent strain I1.3. Two methods were used to compare the genomes: gene prediction and SNP calling. Gene prediction was conducted with MAKER, finding 13,527, 14,795 and 14,534 genes for I1.3, SG4 and C2S, respectively. SNP calling was performed using the genome of *F. fujikuroi* strain IMI 58289 as reference. Genes present in I1.3 and not in SG4, as well as genes with putatively impactful polymorphisms in SG4 and not in I1.3, were identified and analysed, producing a set of 13 genes putatively related to pathogenicity in *F. fujikuroi*.

A single *Verticillium dahliae* effector induces defoliation of cotton plants

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Verticillium dahliae causes wilt disease on a wide range of dicotyledons. Although stunting and withering are symptoms typically induced by pathogenic strains, differential aggressiveness towards hosts is observed among isolates. On cotton, *V. dahliae* strains either exhibit an aggressive phenotype, including defoliation, or milder symptoms that don't include defoliation. In order to understand the molecular basis of this phenomenon, we used a comparative genomics approach, which permitted the identification of two,

identical, genes that are defoliator-specific and highly induced *in planta*. Thus, we hypothesized that the effector encoded by these genes is responsible for defoliation. Indeed, lines of a non-defoliating strain expressing the effector gained the ability to defoliate cotton, while double deletion lines of a defoliating strain lost pathogenicity on cotton. Furthermore, cotton seedlings treated with heterologously-produced effector protein exhibited defoliation symptoms. Taken together, we identified a defoliation-inducing effector that is a pathogenicity factor of *V. dahliae* on cotton.

Cloning of *AvrStb9*, a gene of *Zymoseptoria tritici* conferring avirulence on wheat cultivars carrying the *Stb9* resistance gene

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The septoria leaf blotch disease is caused by the ascomycete fungus *Zymoseptoria tritici*. Nowadays, it is one of the most damaging diseases to wheats and triticale cultures in many regions of the world. The interaction between wheat and *Z. trititi* is complex because of its highly polygenic and mostly quantitative nature. Nevertheless, several wheat resistance genes, called *Stb* genes, correspond to the description of major resistance genes, i.e. qualitative phenotypic effect and involved in a gene-for-gene interaction with the fungus. So far, twenty-one qualitative *Stb* genes have been mapped in bread wheat. *AvrStb6* was the first gene identified that confers avirulence to wheat cultivars carrying a known *Stb* gene, *Stb6*. Here, we report the cloning of *AvrStb9* conferring avirulence to wheat cultivars carrying *Stb9*. *AvrStb9* was identified by GWAS with *Z. tritici* isolates pathotyped on cultivar Soissons. The isolate IPO-09593 is avirulent while the reference isolate IPO-323 is virulent on Soissons. Replacement of the virulent allele with the *AvrStb9* allele from IPO-09593 conferred an avirulence phenotype to transformed IPO-323 strains. Moreover, different recombination events in the *AvrStb9* gene among the IPO-323 transformants allowed us to identify the protein domains involved in virulence. *AvrStb9* encodes a large potentially secreted protein of unknown function. It is expressed *in planta* during the transition from the asymptomic to the necrotrophic phases of infection. The resistance *Stb9* was first reported in cultivars Courtot and Tonic. We identified *Stb9* following a genome-wide association study (GWAS) on elite bread wheat cultivars, and confirmed its presence in Soissons by QTL mapping in a population Beaver/Soissons. The gene-for-gene relationship between *AvrStb9* and *Stb9* was shown by phenotyping *Z. tritici*

transformants with either the virulent or the avirulent allele of *AvrStb9* on near-isogenic lines carrying the resistant or susceptible allele at *Stb9* in Courtot.

Mycotoxins as host tissue colonization factors

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The biological role of mycotoxins remains unclear. It is believed that producing fungi might be better protected against organisms sharing the same trophic niche. However, some mycotoxins seemed to enhance fungal aggressiveness during host exploitation. For example, *Alternaria alternata* produces many toxic secondary metabolites, of which the most relevant is alternariol (AOH) and its derivative monomethyl ether (AME). Recently, the central role of the polyketide synthase gene *pksI* for the biosynthesis of AOH and AME has been established (Wenderoth *et al*., 2019, Mol. Microbiol. 112, 131-146). Moreover, the *pksI*-deleted *Alternaria* strain displayed reduced virulence on tomato, citrus and apple, suggesting AOH as virulence and colonization factor. A polyketide synthase is also the first step of the biosynthesis of the mycotoxin patulin, mainly associated to *Penicillium expansum*. The disruption of the 6-methyl-salicylic acid synthase allowed to obtain mutants that produced less patulin than their wild-type (WT) strain and showed a significantly reduced virulence on apples (Sanzani *et al*., 2012, Int. J. Food Microbiol. 153, 323-331). Moreover, when patulin was exogenously restored, mutants recovered their virulence as compared to that of the WT. Finally, mutants were susceptible to the antioxidant quercetin at 1/100 of the concentration needed for the WT. Finally, the quinone menadione was used as stressing agent for uncovering the molecular determinants driving *Aspergillus flavus* in challenging oxidative stress conditions by the host (Zaccaria *et al*., 2015, Toxins 7, 4315-4329). Metabolic and transcriptional analyses were conducted. Under oxidative stress conditions, *A. flavus* proved to activate several metabolic processes for limiting the ROS-associated detrimental effects, as well as for triggering adaptive and escape strategies, including aflatoxin B1 production. The results reported herein encourage investigation of mycotoxins from a plant pathologist perspective.

Computational and functional analyses of three paralogous effectors proteins in *Colletotrichum graminicola*: the causal agents of maize anthracnose

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The fungal genus *Colletotrichum* is recognized by the scientific community as one of the 10 most important plant pathogens causing disease in almost all crops worldwide. *C. graminicola* is highly specialized and is the causal agent of maize anthracnose. In phytopathogens, effectors modulate the plant defense to allow the establishment of infection and a successful colonization. Previously, we used a computational approach to identify candidate effector proteins in the *Colletotrichum graminicola* genome. We identified 27 putative effector proteins targeting the host nucleus as they are characterized by secretion and nuclear localization signal sequences. One of these, CgEP1, has been shown to be essential for infection and that it is translocated to the host nucleus. Depending of the allele, its structure has from four to six tandem repeats. Interestingly, we found other two secreted proteins encoding genes CgEP4 and CgEP5 in the *C. graminicola* genome that show high similarity with the N-terminal region of CgEP1. Transcriptomic analyses showed that CgEP1 and CgEP4 are upregulated at early stages of infection, while CgEP5 is upregulated during the necrotrophic phase. The CgEP1 and CgEP4 mature proteins are predicted to target the nucleus. Neither has homology to proteins of known function, outside the genus *Colletotrichum* nor do they contain predicted functional domains. We studied the evolution of these three genes in 42 species of *Colletotrichum* covering the diversity of the genus. Homologs of the three genes were found in distant *Colletotrichum* species. We selected CgEP4 for further functional characterization studies. Like CgEP1, CgEP4 is overexpressed in the early stage of infection and is predicted to be translocated to the host nucleus. CgEP4 is composed of a set of five α-helix chains and five β-sheet chains. Preliminary studies show that the CgEP4 deletion mutant has reduced virulence compared to the wild type strain confirming its role as an effector.

The necrotrophic effector SnTox1 of *Parastagonospora nodorum* harbours a promoter variant associated with gene repression

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The fungus *Parastagonospora nodorum* uses proteinaceous necrotrophic effectors (NEs) to cause tissue necrosis on wheat to cause the disease septoria nodorum blotch. The

virulence functions for three NEs, SnToxA, SnTox1 and SnTox3 have been well established. It has been observed that *SnTox1* can epistatically suppress *SnToxA* and *SnTox3*, reducing their contribution to the disease. Little was known about the mechanisms controlling NE expression until the recent discovery that the Zn_2Cys_6 transcription factor PnPf2 was an essential regulator of *SnToxA* and *SnTox3,* but had no obvious role for *SnTox1*. *SnTox1* is expression is significantly higher in the Australian reference strain SN15 compared to the American reference SN4. Closer inspection of the generally well conserved promoter region revealed a 401 bp sequence in SN4 positioned 267 bp upstream of the *SnTox1* start codon that is absent in SN15. Analysis of the world-wide *P. nodorum* population revealed that the indel is present in 89% of isolates outside of Australia. However, only 14% of all Australian isolates possess the indel. To test for any direct regulatory role, mutants with the same indel were produced in both the SN15 and SN15 *pnpf2* knockout mutant. In the presence of the indel, *SnTox1* expression was reduced in SN15 and abolished in SN15 *pnpf2*. There was also no *SnTox1* expression in SN4 *pnpf2*. This indicates that repressor elements associate with the indel and inhibit *SnTox1* transcription but whose activity is reduced in the presence of *PnPf2*. Promoter replacements in isolates carrying the indel and substitution with an unrelated 401 bp sequence are underway to better understand this mechanism. It remains to be seen whether SnTox1 contributes more towards virulence and effector epistasis in isolates without the indel based on modulation of gene expression.

A specific fungal transcription factor controls effector gene expression and orchestrates the establishment of the necrotrophic pathogen lifestyle

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Parastagonospora nodorum causes septoria nodorum blotch (SNB) of wheat. SNB is largely dictated by interactions between necrotrophic effectors (NEs) and host dominant susceptibility genes. SnToxA-*Tsn1*, SnTox1-*Snn1* and SnTox3-*Snn3* are three well characterised NE–sensitivity gene interactions. *SnToxA* and *SnTox3* are positively regulated by a Zn_2Cys_6 transcription factor (TF) PnPf2. Mutants deleted in *PnPf2* lost the ability to infect wheat lines carrying the matching sensitivity genes *Tsn1* and *Snn3* but remained infectious on *Snn1* wheats. RNAseq was used to compare the transcriptomes of the *P. nodorum* wildtype and a *PnPf2*-deficient strain (*pnpf2*) to further identify other

aspects of PnPf2 regulation. Gene ontology and CAZyme analyses of the differentially expressed (DE) genes revealed that genes associated with plant cell wall degradation and proteolysis were enriched in the down-regulated gene set in *pnPf2*. In contrasts, genes associated with nutrient and ion transport were up-regulated in *pnpf2*. Further analysis of the DE gene set revealed that PnPf2 positively regulates effector-like genes encoding proteins. Sequence analysis of the promoter region of DE genes revealed 3 motifs that were over-represented. This includes a putative Zn_2Cys_6 binding site that was enriched in the promoter region of *pnpf2* down-regulated gene set. This motif was present in *SnToxA* and *SnTox3* promoters. Functional characterisation indicates that PnPf2 does not interact with this motif. Instead, PnPf2 may exert its regulatory function on *SnToxA* and *SnTox3* through other downstream TFs. Functional analysis of two PnPf2-regulated bZIP and Zn_2Cys_6 TFs via gene deletion revealed critical roles in vegetative development, virulence and asexual reproduction. We conclude that PnPf2 play a broader role in establishment of a necrotrophic lifestyle by orchestrating the expression of genes associated with nutrient acquisition in tandem with modulating effector expression to disable the host.

Diversity and pathogenicity of the *Alternaria* species complex involved in apple leaf blotch and fruit spots in France

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Apple leaf blotch and fruits spots caused by *Alternaria* species are common diseases in apple production regions. Several *Alternaria* species or complex species including *A. arborescens, A. longipes, A. gaisen* and *A. alternata* seem responsible of fruit losses and decrease tree vigor. Serious defoliations linked to *A. alternata* f. sp *mali* have been observed in the past in Japan and US. This fungus classified as quarantine pathogen (A1 list of EPPO, 2000/29/CE directive) carries a gene cluster involved in the biosynthesis of a host-specific toxin: AM-toxin. However, several cases of *Alternaria* leaf blotch have been reported recently which suggests a possible emergence of this pathogen in Europe. The goal of this study is to achieve the taxonomic assignation of >200 *Alternaria* isolates responsible for symptoms observed in France, by a multilocus sequence typing approach. A specific detection of *Alternaria* f. sp *mali* was performed by targeting three genes involved in AM-toxin biosynthesis. Pathogenicity assays of 30 strains collected

worldwide was conducted on detached leaves to compare their virulence on Golden and Gala varieties. Preliminary prospections of orchards showed that the fruit spots disease seems to represent a minor problem in France. On the other hand, leaf spots have been annually observed, with different levels of severity, on 10 different apple varieties. Collected isolates corresponded mostly to *A. arborescens* and to *the A. alternata* complex. Neither *Alternaria* f. sp *mali* nor *Alternaria gaisen* were isolated from French orchards. Finally, pathogenicity assays suggest that all strains may produce symptoms with a great variability within strains, regardless if they carry or not the gene cluster involved in the production of the AM-toxin.

Development of a conditional gene expression system using a copper responsive promoter in the plant pathogenic fungus *Fusarium graminearum*

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Fusarium graminearum is an important plant pathogenic fungus that causes Fusarium head blight on wheat and barley and ear rot on maize worldwide. *F. graminearum* infections result in reduced grain yield and contamination of mycotoxins, such as trichothecenes and zearalenone, which are harmful to humans and animals. Control of gene expression, such as targeted gene deletion or overexpression/constitutive expression, is necessary to investigate the molecular mechanisms underlying fungal development, virulence, and mycotoxin production. Until now, the zearalenone-inducible promoter (PZEAR) is the only available conditional gene expression system in *F. graminearum*. In this study, we propose a new conditional gene expression system using a promoter of copper responsive FgCTR4 gene (PFgCTR4). FgCTR4 encodes a putative high-affinity copper transporter and its expression is dependent upon copper ion concentrations. In response to excess copper, FgCTR4 was highly repressed and copper deprivation by using copper chelator led to overexpression of FgCTR4. Consistently, GFP fluorescence was markedly reduced in copper-enriched condition and increased in copper-deprived condition in the transgenic *F. graminearum* strain carrying the PFgCTR4-GFP. To validate its general applicability in *F. graminearum* functional genetics, we replaced the promoters of METE, methionine biosynthetic gene, and FgENA5, cation stress-related gene, with PFgCTR4. We are expecting the growth to be reduced in the medium supplemented with copper and stress agent, and growth to be recovered in the presence of the copper chelator. This study reported the first conditional gene expression system in *F. graminearum* where both

repression and induction is available. This system would be a convenient way to precisely control gene expression, and might be used to figure out the biological function of various genes including essential genes.

Identification and functional characterization of novel transcription factors involved in ion homeostasis in the plant pathogenic fungus *Fusarium graminearum*

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Ionic homeostasis plays important roles in the regulation of diverse processes in living cells. In particular, Ca2+-mediated signal transduction pathway is closely involved in fungal pathogenesis as well as various developmental processes. Until now, the study for transcription factors (TFs) related to the Ca2+ signaling pathway in plant pathogenic fungi are scarce. The plant pathogenic fungus *Fusarium graminearum* causes a devastating disease called *Fusarium* head blight (FHB) on cereal crops. In addition to grain yield losses, infection of this pathogen causes severe contamination of mycotoxins, which are harmful to human and animal health. In the previous study, we constructed a mutant library of 709 putative TFs of *F. graminearum*. To understand the underlying mechanisms of TFs involved in Ca2+ signaling of plant pathogenic fungi, we screened a TF mutant library with three chemical chelators known as Ca2+- and Mg2-specific chelating agents. Among them, 16 TF mutants displayed increased sensitivities against not only Ca2+ but also Mg2+-deprived conditions. To confirm the amount of chelated ions and ion contents by chemical chelators, ion concentration was measured by Inductively coupled-MS (ICP-MS). Chelating agents decreased not only Ca2+ but also other ions suggesting that those TFs are important for ion homeostasis and/or ion-mediated signaling pathways. Ongoing study will reveal novel TFs which regulate ion signaling pathways as well as their molecular mechanisms that would be novel targets for controlling plant diseases.

To have or not to have: A dispensable chromosome enables host colonization in the pathosystem *Colletotrichum higginsianum – Arabidopsis thaliana*

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Colletotrichum higginsianum is a hemibiotrophic plant pathogen whose hosts are different members of the Brassicaceae family. Together with *Arabidopsis thaliana*, it represents a prominent model system to investigate various ecologically important fungal pathogens and their infection strategies. The infection cycle starts with the mechanical penetration of the plant cell enabled by specialized cells called appressoria. Subsequently, *C. higginsianum* establishes large biotrophic primary hyphae in the first infected cell. Shortly thereafter a switch to necrotrophic growth occurs, leading to the invasion of neighboring cells by secondary hyphae. We characterized a dispensable mini chromosome (chr11) enriched with effector genes that is essential for virulence *on A. thaliana. C. higginsianum* strains lacking chromosome 11 (chr11Δ) do not show any obvious vegetative defects but are not able to switch from biotrophy to necrotrophy during infection. Analysis of plant defense mutants showed that genes encoded on chromosome 11 are required to suppress PAMP triggered immunity especially the production of tryptophan derived secondary metabolites. By comparative genomics and karyotype analysis of different fungal isolates, we identified genetic variations between mini chromosomes. This enabled us to identify the region on chromosome 11 whose presence correlates with successful *A. thaliana* infection. We further present genetic analysis of this region which allowed us to identify important virulence factors necessary for necrotrophic colonization of the host plant.

The asymptomatic infection of sweet orange by *Alternaria alternata* citrus pathogen

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Alternaria alternata is an ascomycete widely known as pathogenic for a large range of hosts, most of *Alternaria* species are necrotrophic and produce a host specific toxin that induce the plant hypersensitivity response, the tangerine pathotype also produces a host-selective toxin, the *Alternaria* citrus toxin (ACT-toxin), which affects resistant to ABS, once its symptoms have never been reported in the field neither in controlled conditions. *In vitro* Pathogenicity tests are well established to characterize pathogenic strains, performing detached leaves inoculation. We evaluated the tangerine and sweet orange symptoms after an *Alternaria alternata* strain inoculation, and also the ACT-toxin gene expression under different conditions. Therewith, we demonstrate that the gene expression of ACTtoxin is constitutive, host independent. Which indicates that sweet orange has no the

correspondent ACT-toxin binding site, thus the host do not respond to *A. alternata* infection. Furthermore, we confirmed the infection and colonization of citrus sweet orange leaves by *A. alternata*, behaving as endophyte, which is the first report of this kind of interaction and has a significant importance to disease control and management techniques, once sweet orange plants are a pathogen repository.

A fungal family of lytic polysaccharide monooxygenase-like copper proteins

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Lytic polysaccharide monooxygenases (LPMOs) are copper-containing enzymes that play a key role in the oxidative degradation of various biopolymers such as cellulose and chitin. While hunting for new LPMOs, we identified in various fungal lineages a new family of proteins, defined herein as X325. The X325 three-dimensional structure revealed an overall LPMO fold and a histidine-brace with an additional aspartate ligand to Cu(II). Although LPMO-type activity of X325 members was initially expected, we demonstrated that X325 members do not perform oxidative cleavage of polysaccharides, establishing that X325s are not LPMOs. Investigations of the biological role of X325 in the ectomycorrhizal fungus *Laccaria bicolor* revealed exposure of the X325 protein at the interface between fungal hyphae and tree rootlet cells. Our results provide insights into a family of copper-containing proteins widespread in the fungal kingdom, which is evolutionarily related to LPMOs but has diverged to biological functions other than polysaccharide degradation.

Rapid and efficient transformation of the plant pathogen *Microbotryum*: A milestone to understand the evolution of host-specific parasitism

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Biotrophic fungi are characterized by intimate interactions with highly specific hosts and often cause devastating plant diseases. Growth and proliferation during biotrophic phases necessitate an adapted set of genes to manipulate the host plant and complete the fungal life cycle. The genus *Microbotryum* includes species of closely related fungal parasites

infecting specific host plants. Microbotryum has a diphasic life cycle consisting of a haploid, saprotrophic sporidial stage and a dikaryotic biotrophic hyphal stage in planta. The sporidial stage of *Microbotryum* was transformed via *Agrobacterium tumefaciens*-mediated transformation (ATMT) before, but ATMT protocols are time consuming. We established an efficient genetic transformation protocol using electroporation with linear and circular DNA fragments resulting in stable *Microbotryum* transformants. The results show initial incubation at low temperatures is crucial for cell regeneration and formation of resistant colonies. Our results indicate that false positive rate of transformants is minimized by electroporation at 0,5 kV yielding up to 2,5 x 102 transformants. Evaluation of bleomycin resistance marker genes indicates higher yield. Lacking efficient genetic tools to assess the role of putative virulence genes, we further aim to establish a modular molecular toolbox utilizing the Golden Gate cloning strategy. Using BsaI we designed specific restriction and re-ligation sites for each module and each library in our toolbox. This library includes different classes of donor plasmids, which corresponds to multiple research issues like heterologous gene expression and fluorophore tagging. In addition, it enables us to express RNAi and CRISPR/Cas9 constructs in *Microbotryum* to assess gene function. In summary, we present two versatile and time-saving tools, which allow access not only to a single parasitic fungus, but to an entire parasitic genus to understand the evolution of host specificity.

An association genomics approach to identify candidate virulence genes in *Cercospora sojina*

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Frogeye leaf spot, caused by the fungal pathogen *Cercospora sojina*, is a persistent threat to soybean production in the southern United States. Approximately 9.27 million and 6.02 million bushels were lost to this disease in 2017 and 2018, respectively. Host genetic resistance is a viable disease management strategy and several dominant resistance genes have been previously identified. However, distinct pathogen races have evolved to overcome specific host resistances, likely through the diversification or modification of effector genes. Presently, little is known on the genetic diversity of *C. sojina* at the whole-genome scale and the molecular mechanisms of pathogen virulence are currently unexplored. To address these knowledge gaps, we have sequenced the whole genomes

of 53 *C. sojina* isolates (collected from Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, South Carolina, Tennessee, Brazil, and China), which had previously been phenotyped on a soybean differential set. Sequencing resulted in the identification of 29,429 single nucleotide polymorphisms (SNPs) or insertions/deletions (InDels). Analysis of genetic population structure failed to yield any evidence of stratification. Association mapping identified significant marker-trait associations for virulence on soybean lines Lee and Kent (Rcs2). A gene encoding a predicted effector was identified underlying the significant association for virulence on Lee. This candidate gene exhibited hallmark signatures of an effector, including a small size of 75 amino acids, presence of a predicted secretion signal, and having approximately 10.7% cysteine content. The marker significantly associated with virulence on Kent was located within the coding region of a gene encoding a kinase. These effector/virulence gene candidates are currently being validated through the generation of gene-disrupted mutants, as well as stable expression in virulent and avirulent isolates.

Pseudopyrenochaeta lycopersici, agent of Corky Root Rot of tomato: a case history of a less studied soilborne pathogen

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The ascomycete *Pseudopyrenochaeta lycopersici*, formerly *Pyrenochaeta lycopersici*, is the causal agent of Corky Root Rot of tomato. This soil-borne pathogen is classified among the imperfect fungi which lack the sexual form. Population structure analysis based on AFLP technique confirmed the existence of two groups, previously identified by ITS analiysis. AMOVA showed a moderate genetic divergence between the two groups, suggesting the hypothesis of two different evolutive entities, named type 1 and 2 (Infantino *et al*., Plant Pathol 2015; 64:941–50). The genomes of both types have been recently sequenced by Illumina and PacBio technologies (Aragona *et al*., BMC Genomics 2014, 15:313; Dal Molin *et al*., PLoS ONE 2018, 13(7): e0200217). The gene content of the two *P. lycopersici* isolates was very similar, however, while they showed to align for more than 97% of their length, they exhibit low sequence identity. The gapless genome assembly of 62.7 Mb, obtained by PacBio technology, showed a large fraction (30% of the total bases) of repetitive sequences, including transposable elements. Their abundance in *P. lycopersici* next to heterokaryon incompatibility genes, a family largely expanded in both sequenced isolates, suggests the presence of possible mechanisms alternative to gene

reassorting mediated by sexual recombination. An expansion of glycoside hydrolase (GH) family has been also observed, underlying the importance of this component in *P. lycopersici* pathogenicity and virulence (Aragona & Valente, Curr Genet 2015, 61:211–220). The most effective tool to control the Corky Root Rot, is given by the breeding for resistance. A comparative RNA-Seqbased transcriptional profiling suggested that susceptibility and resistance may share overlapping signalling pathways and responses (Milc *et al*., Funct Integr Genomics 2019, 19:811–826). The overall results could be useful for better understanding of other less studied soil-borne fungal pathogens.

A rice/ *Arabidopsis thaliana* glycosyl hydrolase gene displays ambivalent immunity with diverse types of phytopathogens

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Plants encounter diverse pathogens that employ different infection strategies. We have isolated the MOR1 (*M. oryzae* Resistance1) gene of *Arabidopsis thaliana* which required for resistance against *M. oryzae* 70-15 infection, based on a T-DNA insertion allele. The MOR1 genes is also required to restrict the spread of necrotrophic fungal pathogen, *Alternaria brassicicola*, suggesting a common host response strategy against these pathogens; salicylic acid (SA)-related PR genes and other phytohormone-associated genes expression. However, *mor1* mutation had no effect on growth of the virulent *Pseudomonas syringae* pv. tomato DC3000. OsMOR1a was identified by sequence similarity as an orthologue of *Arabidopsis* MOR1 gene in rice. A novel *osmor1a* mutant generated by CRISPR/Cas9 shows increased resistance against *M. oryzae* and *Xanthomonas oryzae* pv. oryzae (Xoo), while significantly enhanced susceptibility to *Cochliobolus miyabeanus* infection. Current findings expand and deepen our understanding of ambivalent immunity and concurrently provides new evidence on plant immunity using genome editing technology for improving the crop breeding.

Diversified modulation of transcriptome complexity by alternative splicing during rice-*Magnaporthe oryzae* interactions

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In post-genome era, one gene to one protein paradigm has shifted to one gene to multiple proteins or functions in whole genome. Among mechanisms, alternative splicing (AS) have potential roles in modulating genomic systems. Although 80.4% of the genome contains introns, only less than 8% of genome was revealed as AS containing genes in *Magnaporthe oryzae*. Similar to transcriptome alterations, AS genes would be reprogrammed during rice-*M. oryzae* interaction. To decipher ASome networks, we compared genome-wide transcriptional profiles of AS isoforms of fungal pathogen and host plant. We collected infected rice sheaths under microscope to enrich fungal samples as a sequential manner. These included vegetative condition and five different infection stages covering pre-penetration, biotrophic, and nectrotrophic stages. In this study, at least one AS isoform of 5,987 genes were found from total conditions, including 5,569 genes from in planta conditions. Two-thirds of isoform patterns showed intron retention, and an increase of this pattern led to the upsizing of ASome infection stages. We also identified AS isoforms assigned differential functional domain compared with stages by ab initio proteome. We validated conserveness of ASome in other *Magnaporthe* strain including 98-06, BR32. This profiling of ASome provides expanded AS repertoire of *M. oryzae* and shows neofunctionalization possibility of proteins by alternative splicing transcription during rice-*M. oryzae* interactions.

Valorization of by-products from oleaginous crops production using *Trichoderma* spp

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Olive, rapeseed and sunflower are three of the world's major oleaginous crops, widely used in Mediterranean countries for food and non-food applications, in green chemistry and for energy production. Olive pomace (OP), sunflower and rapeseed meals (SFM and RSM) are the by-products of oil extraction. Because of their wide production, their recovery and successful utilization as useful products is a relevant opportunity to increase the efficiency of the whole industrial process. These residues, rich in carbohydrates and fibres, can be used as an economical substrate for microbial cultures and for the generation of high value-added products. Since they may still contain substantial amounts of organic carbon and nutritive compound, such as nitrogen and phosphorous, they may be suitable as organic fertilizers and amendments. The aims of this project were to determine: 1) the ability of selected *Trichoderma* spp. to grow on by-products; 2) the enzymatic activity of these *Trichoderma*; 3) the effect of *Trichoderma* and by-products combination in plant growth promotion and soil amendment; and 4) potential application of *Trichoderma* in bioremediation. Several assays with *Trichoderma* spp. (strain T22, T25, E45, T34) were carried out using solid and liquid media supplemented with different quantities of by-products. Results indicate that all strains grew rapidly on the residues when used in low %. When tested in solid state fermentation on 100% of RSM and SFM the strains T22, T25 and E45 produced 1 X 10^{*9} CFU in 48 hours. Preliminary results suggested that the cultural filtrates of *Trichoderma* grown in OP an SFM could have a positive effect on the germination and growth of tomato plants in vitro when used in low quantities. Assays using tomato plants are in progress to determine if *Trichoderma* could also reduce the phytotoxicity of OP filtrates. *Trichoderma* spp. have potential in the valorization of by-products for sustainable application in agriculture.

Study of the beneficial interaction between *Trichoderma* and *Brachypodium distachyon* by RNA-sequencing

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Brachypodium distachyon is a small, temperate grass considered a suitable model plant to study cereal and potential biofuel crops, such as *Miscanthus giganteus* or switchgrass (*Panicum virgatum* L.). Some *Trichoderma* spp. are recognized as successful fungal Biological Control Agents (BCAs), as well as beneficial microbes able to promote plant growth. The objective of this work was to identify genes and signaling pathways activated during the *Trichoderma-Brachypodium* interaction to support scientific and industrial interests for developing renewable and sustainable energy resources. Two *Trichoderma harzianum* isolates (T22 and M10) were selected that demonstrated beneficial effects on *B. distachyon* in the early stages by increasing seed germination rate. The growth promotion effect was continuous in subsequent development stages, exhibiting an increase in biomass yield up to four-fold greater than the control. RNA-seq analysis of the plants after the *Trichoderma* seed coating treatment revealed that both BCAs stimulated plant growth, positively modulated primary metabolism and the expression of genes associated with phytohormone production (auxins, gibberellins and cytokinins). Furthermore, the ability of *Trichoderma* to increase uptake of nutrients was confirmed by the over-expression of genes encoding different transporters (sugars, amino acids and minerals). Both *Trichoderma* strains were able to stimulate the plant defense response, mainly by activating the ethylene-JA signaling, that may be associated with priming/alerting to biotic stress. Furthermore, the two fungal strains demonstrated a gene expression modulation that was specific to the vegetative plant parts whereby the effect of T22 was consistent on plant shoots, while the effect of M10 was mainly associated to a root-response. Results demonstrated that *Trichoderma* is capable of inducing important transcriptomic adjustments that have implications in *B. distachyon* development and biomass accumulation.

Towards the identification of virulence factors of the broad host range plant pathogen fungus, *Sclerotinia sclerotiorum*

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The Ascomycete fungus *Sclerotinia sclerotiorum* (Ss) is a generalist plant pathogen, responsible for white mold diseases on many cultivated species such as oilseed rape, sunflower, tomato, melon and soybean. It also infects the model plant *Arabidopsis thaliana*. An improved understanding of the molecular basis of Ss-induced susceptibility in plants will lead to conceptual advances in plant pathology and renewed opportunities

for engineering durable disease resistance in crops. Soybean is a valuable crop used for oil and protein production as well as nitrogen fertilization, which is severely impacted by *Sclerotinia* stem rot disease. We recently established the Ss-soybean pathosystem at LIPM as a basis to decipher the determinants of disease susceptibility in this crop. As with other necrotrophic fungi, damages caused by Ss are due to the secretion of molecules (including small proteins) referred to as effectors that alter host cells and promote infection. To understand how Ss triggers susceptibility in *A. thaliana* and soybean, we will address the following specific questions: (1) Which Ss genes encode putative effectors induced during the infection of *A. thaliana* and soybean? (2) Which Ss effector candidates contribute to fungal virulence on *A. thaliana* and soybean? (3) Which plant genes respond to Ss effectors? We will present our new home –made *Sclerotinia sclerotiorum* genome browser, and current work on candidate virulent fungal effectors.

Protease and chitinase activities as modes of antagonism of the yeasts *Aureobasidium pullulans* and *Candida subhashii* against *Fusarium oxysporum*

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Fungi of the genus *Fusarium* are among the most important soilborne plant pathogens, they threaten crop production worldwide, and their management by traditional methods has inconsistent results. Some filamentous fungi (e.g., *Trichoderma*) and bacteria have been described to inhibit *Fusarium*, but the biological control of this pathogen is still ineffective. Yeasts are ubiquitous in all environments, show remarkable stress tolerance and harbor great potential as plant protection agents. However, they are underexplored for the application against plant pathogens: a mechanistic understanding of how yeasts suppress *Fusarium* is missing. We have quantified the antagonistic activity of 40 naturally occurring yeasts against a broad range of saprophytic, beneficial and pathogenic fungi *in vitro*. Our preliminary results demonstrate strong plant protection activity for six isolates and indicate different metabolic capabilities and modes of antagonism. The production of enzymes that degrade fungal cell walls has been identified as an important factor contributing to yeast antagonistic activity. Therefore, we used biochemical assays to measure enzymatic activity in culture supernatants. These have revealed our isolates of *Aureobasidium pullulans* and *Candida subhashii* to secrete protease and chitinase activities. Proteolytic enzymes were detected in media containing peptone, while chitinase activity was induced in the presence of N-acetyl-D-glucosamine. Co-culture experiments with *Fusarium* showed an increased protease activity. To identify the proteases and chitinases and to characterize their contribution to the antagonistic activity, we performed differential gene expression analysis and mass spectrometry. Overexpression and gene

deletion experiments are planned to further study the role of the identified enzymes in biocontrol activity. The characterization of these proteases and chitinases will lay the foundation for developing promising isolates as plant protection agents.

Chitosan biosynthesis and degradation: a way to modulate plant defenses in endophytic biocontrol agents?

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Nematophagous fungi have been employed in biological control to protect crops of interest because of their ability to manage nematodes sustainably. *Pochonia chlamydosporia*, a fungal parasite of nematode eggs and females is present worldwide responsible for natural suppression of soils to plant parasitic nematodes. The nematode egg-shell mainly is composed of a protein matrix embedding chitin microfibrils. *P. chlamydosporia* is a true endophyte of both mono and dicot crop plants which modulates their local and systemic defenses. Extracellular depolymerases of nematophagous fungi reflect their parasitic, endophytic and saprophytic traits. *P. chlamydosporia* genome show genes adapted to infect hosts with external barriers based on chitin/protein. For instance, *P. chlamydosporia* shows a highly expanded family of hydrolases and other enzymes related with chitin modification. Chitosan is a highly deacetylated form of chitin with antimicrobial activity. Entomopathogenic and nematophagous fungi are resistant to chitosan. Genomes of most isolates of *P. chlamydosporia* from worldwide origin show genes similar to those encoding putative chitin deacetylases and chitosanases. Most of these isolates display high parasitism to nematode eggs and degrade chitosan. RNA seq indicates that chitosan and nematode eggs induce polysaccharide catabolism, transmembrane transport and oxidation/reduction processes. We also found no correlation between egg-parasitism and chitosanolytic activity. Chitin perception is a key component of the Plant Immune System. Chitin shielding/deacetylation in fungi is a way to circumvent plant defenses. Plant chitinases show less affinity for chitosan than chitin. Therefore, chitosan is a less efficient plant defense elicitor than chitin. We propose that chitosan production and degradation could protect endophytic biocontrol fungi such as *P. chlamydosporia* from plant defenses in the rhizosphere.

Genome-wide association mapping identifies SnTox5 in *Parastagonospora nodorum*

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The *Parastagonospora nodorum*-wheat interaction is comprised of *P. nodorum* necrotrophic effectors (NEs) and their corresponding host sensitivity genes. To date, nine such interactions have been identified in this pathosystem and three necrotrophic effectors, SnToxA, SnTox1, and SnTox3 have been cloned and functionally validated. In our research, we collected 198 isolates of *P. nodorum* representing different wheat growing regions of the United States. Whole genome resequencing was done for all the isolates. Isolates were inoculated on the differential line for Snn5 (LP29) that confers sensitivity to SnTox5, and the resulting phenotypic data was used to carry out genome-wide association analysis with the use of 402,612 single nucleotide polymorphisms (SNPs). A single locus was associated with virulence on LP29. A gene that was predicted to encode a small secreted protein was identified as the strongest candidate for SnTox5. The transfer of a functional copy of the candidate SnTox5 into the avirulent isolate Sn79-1087 resulted in virulence on wheat lines harboring Snn5. In addition, mutants of the SnTox5 candidate were developed by deleting the gene in the isolate Sn2000. Inoculation of gene-disruption mutant strains on the LP749 population and subsequent QTL analysis failed to detect the QTL on chromosome 4B that is associated with the SnTox5 -Snn5 interaction. During the infection process SnTox5 was upregulated in planta, reaching its highest expression at 24 hours post inoculation prior to the on-set of symptoms. The evidence presented here validates the identification of SnTox5 that encodes for the NE, SnTox5.

The effects of light on *Trichoderma atroviride* conidiation and mycoparasitic activity are partially dependent on the strain and the Tmk3 MAP kinase

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The genus *Trichoderma* is a large group of filamentous fungi, which are soil-borne, green-spored ascomycetes and some of its members are well known for their mycoparasitic properties. For this reason, they are considered as biocontrol agents for plant protection against phytopathogenic fungi. While single studies on the influence of light on fungal conidiation and secondary metabolite production are available, nearly nothing is known on how light affects *Trichoderma* mycoparasitism. In this study, we investigated the

influence of light on conidiation and the mycoparasitic activity of *Trichoderma atroviride* using two wild-type strains, IMI 206040 (ATCC 20476) and P1 (ATCC 74058). We found different conidiation patterns in the two strains after exposure to several light and dark regimes, although the expression of the genes encoding the two main photoreceptors regulating photoconidiation, Blr1 and Blr2, was similar in both strains.

In dual confrontation assays employing various host fungi such as *Botrytis cinerea*, *Rhizoctonia solani, Fusarium oxysporum*, and *Rhizopus microsporum*, differences in the mycoparasitic activity of *T. atroviride* P1 and IMI206040 upon cultivation under light and dark conditions were evident. An inhibition test based on diffusible metabolites further showed that light influences the production of secondary metabolites by *T. atroviride* and by the fungal hosts tested. In contrast, the sensitivity of *T. atroviride* spores to cell wall and osmotic stress agents was lightindependent but depended on the Hog1-like MAPK Tmk3, with strain P1 being generally more resistant than IMI206040. Tmk3 as well affected conidiation in both P1 and IMI206040 in a light-dependent way.

Mycoparasitism-related chemotropic sensing in *Trichoderma atroviride* germlings and hyphae

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Trichoderma atroviride is a mycoparasitic fungus used as biological control agent against fungal plantpathogens. Because sensing and recognition of signals derived from the fungal host and plants are essential for the successful mycoparasitic interaction, we applied three different chemotropic assays to study the sensing capacity of germlings, micro-colonies and mature hyphae of *T. atroviride* towards selected compounds that could play an essential role in the mycoparasite-plant-host interaction. Culture supernatants of different fungal preys such as *Rhizoctonia solani, Botrytis cinerea, Sclerotinia sclerotiorum*, and *Fusarium oxysporum* were used to evaluate the response of the mycoparasite to fungal prey-derived signals. *T. atroviride* culture supernatants and 6-pentyl-α-pyrone (6PP) were used to study *Trichoderma* self-interaction, and the use of the oxylipin 13-(s)-HODE allowed us to evaluate the response of the mycoparasite to plant-derived compounds. Glucose and copper were used as controls for positive and negative chemotropism, respectively. 6PP and 13-(s)-HODE emerged as positive chemotropic compounds which triggered germ tube growth towards substance inoculation point. Micro-colonies and mature hyphae visibly reacted with a modification of the colony periphery, where growth speed and hyphal density increased. Culture supernatants of different fungal preys only

elicited a low chemotropic response; however, size fractionation of the supernatant of R. solani cultures resulted in the identification of a single fraction containing the best chemotropic substances. Evaluation of polarized tip growth modifications using a CRIB reporter was used to observe the responses of *T. atroviride* during prey recognition and chemoattraction, revealing a prey-induced cell polarity stress in the mycoparasite in all pairings tested and with certain supernatants, suggesting that mycoparasitism in *Trichoderma* is strongly determined by its ability to sense and overcome prey defences.

Understand the exchangeable chemical signals that influence fungal interactions

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The Populus root microbiome harbors a diverse community of fungi that significantly increase nutrient uptake and acquisition by the plant host while also providing protection against antagonistic parasites. Similar to the bacterial microbiome, the function and organization of the fungal microbiome is also influenced by the chemical environment surrounding the host plant, but relatively little is known about the chemical signals and fungal mechanisms that shape fungal interactions and their organization in community. Here, we characterized diverse signaling molecules and metabolites (e.g., lipooligosaccharides) produced by various fungal species of the Populus rhizosphere and investigated their effect in other fungi. Moreover, we tested various lipo-oligosaccahrides from *Laccaria bicolor* and other fungi and investigated the transcriptome response of L. bicolor and Populus to these molecules. This study provides insights into the role of such molecules in shaping interactions between fungi and organizing the fungal community and their function in the Populus rhizosphere.

The putative dual function of a secreted pathogenesis-related 1 (PR-1) family protein in *Ustilago maydis*

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Plant PR-1 proteins play an important role in plant defense and highly induced on pathogen attack. The CAP-derived peptide of tomato PR-1 (CAPE) can enhance the resistance to

microbial pathogens. In contrast to plant PR-1, the PR-1 proteins in fungi act as virulence factors but the detail mechanism of PR-1 proteins in fungal virulence is not well studied. Here, we functional characterize the two PR-1 family proteins in *U. maydis* UmPR1a and UmPR1b, which are highly and specific regulated during the biotrophic stage. The single deletion of umpr1a results in virulence reduction while the deletion of umpr1b has no impact on virulence. The amino acid sequence alignment reveals the presence of the conserved CAPE cleavage site at the C-terminus of UmPR1 proteins, implying the putative UmCAPE peptides might be generated. Furthermore, the strain constitutive expressing UmPR1a showed better survival in the medium containing antifungal lipid. This indicates that the UmPR1a might function in lipid detoxification. The presence of UmCAPE and detoxification function of UmPR1a suggest that UmPR1a could be a dual function secreted effector required for *U. maydis* virulence.

Effector gene turnover in blast disease fungi

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Several fungal species of the genus *Pyricularia* cause blast disease to distinct plants, including staple crops such as rice and wheat. Effector genes play a key role on the plant-pathogen interaction. We explored the evolutionary dynamics of blast disease effectors previously-reported and effectors predicted on four blast fungal species. Most of the avirulence effectors and about half of predicted effectors were specific to *Pyricularia*; however, many other effectors shared homologs with distant fungal taxa. There was a large variation in effector genes gains and losses among blast fungal species suggesting gene turnover as a significant source of evolution in these genomes. We reported a potential effector family present in all *Pyricularia* genomes that varied from 1 to 8 copies across isolates of the same species. This family was a metalloprotease different to the well-known AVR-Pita. The events of effector gene turnover in blast pathogens might be linked to the arm race between plant receptors and effectors, and probably impact pathogenicity and host specificity.

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Seed trade risks forest biosecurity: An overview with a focus on *Colletotrichum fructicola* and *C. kahawae* associated with *Eucalyptus spp.*

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The global and regional transport of seed risks an inadvertent introduction and distribution of pests and pathogens to unaffected regions. The international trade of forest seed is less regulated than trade in live plants or wood products as it is generally considered to be resilient and free from harmful organisms. Despite concerted action by various governments to implement phytosanitary measures against introduction of quarantine pathogens, serious new or previously unreported and emerging pathogens continue to be reported. Infected and/or infested seeds may distribute these pathogens further through seed trade. In this study, the cryptic nature of *Colletotrichum* species associated with commercial *Eucalyptus* seeds produced in South Africa was investigated. Identity of *Colletotrichum* species was investigated by a polyphasic approach that used both morphological characters and multilocus phylogenetic analyses of the concatenated sequences of ITS regions, β-tubulin, actin, and glyceraldehyde-3-phosphate dehydrogenase genes. Due to previous limitations of selected genes at delineating the *Colletotrichum kahawae* up to subspecies level, additional biochemical tests were performed based on a known fact that the quarantine coffee bean pathogen (*C. kahawae* subsp. *kahawae*) is capable of utilising ammonium tartrate or citric acid as a sole carbon source. Both multigene analyses and biochemical tests confirmed identity to be *Colletotrichum fructicola and C. kahawae* subsp. *ciggaro*. Screening of commercial seed samples showed detected the two *Colletotrichum* species on *Eucalyptus dunnii* (3.5 and 0.5%, respectively), *E. nitens* (2.6 and 1.2%, respectively), and *E. macarthurii* (0.8 and 0.1%, respectively). Greenhouse trials showed that both *C. fructicola* and *C. kahawae* subsp. *ciggaro* to be seed-transmitted and pathogenic on *E. camaldulensis*, *E. dunnii*, *E. nitens* and *E. viminalis* seedlings. Disease symptoms included anthracnose leaf spots that appeared as irregular dark-brown leaf spots on seedlings six days after inoculation. This study reveals the complexity of cryptic species in safeguarding forest biosecurity. Apart from the possibility of misidentifications of commonly occurring anthracnose leaf spots in nurseries, detection of these fungi for the first time on *Eucalyptus* seed is more evidence against the dogma of biosecurity. There is a need for plant protection authorities to be alert to the potential risk associated with seed trade and consider precautionary phytosanitary measures to minimize the risk of introducing seed-borne and seed-transmitted pathogens.

Poster Session 2.3
ANTIFUNGAL AND FUNGICIDES

THURSDAY, FEBRUARY 20

18:00 - 19:30 | Location: **Frentani Convention Center**

The mechanism behind the intrinsic resistance of *Madurella mycetomatis* to the echinocandins

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The fungal species *Madurella mycetomatis* is the main cause of mycetoma, a granulomatous infectious disease that can be caused by both bacteria (actinomycetoma) and fungi (eumycetoma). Actinomycetoma is generally easier to treat than eumycetoma, for which treatment options are limited (1). Azoles, such as itraconazole, are often the drug of choice for treating eumycetoma. Other options include amphotericin B and terbinafine. *M. mycetomatis* is not susceptible to the echinocandins, which target β-1,3-glucan synthase (2). In this work, the mechanism behind this intrinsic resistance to the echinocandins was investigated.

A blast search against the protein sequence of β-1,3-glucan synthase from other fungal species, encoded by the FKS gene, was conducted to find the homologue in *M. mycetomatis*. A single gene, MMYC01_201347, was found to be homologous. The protein sequence of the FKS homologue in *M. mycetomatis* was further analyzed *in silico*. This analysis revealed that the *M. mycetomatis* FKS protein sequence contains a substitution of the first amino acid in the Hot Spot 1 region, which is shared with other fungal species resistant to the echinocandins. This particular substitution could be the cause for the intrinsic resistance of *M. mycetomatis* to the echinocandins. Other molecules targeting the FKS protein are investigated for their binding properties to the *M. mycetomatis* FKS gene and *in vitro* susceptibilities are performed with them to predict if the *M. mycetomatis* FKS protein could still be a valuable antifungal target to treat mycetoma.

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mycetomatis is not susceptible to the echinocandin class of antifungal agents. Antimicrob Agents Chemother. 2010;54(6):2738-40.

Deciphering the antifungal mechanism of HSAF using the model filamentous fungus *Neurospora crassa*

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Heat-stable antifungal factor (HSAF) is an antimycotic compound isolated from a biological control strain Lyaobacter enzymogenes C3. It belongs to polycyclic tetramate macrolactams (PTM) and has shown a broad-spectrum of antifungal activities. However, little is known about its mode of action due to the lack of molecular, genetic and biochemical techniques developed in pathogenic fungi. In this study, we used the model filamentous fungus *Neurospora crassa* to investigate the antifungal mechanism of HSAF. We first mutagenized a *N. crassa* strain using atmospheric and room-temperature plasma (ARTP) and screened for mutants able to grow on medium containing HSAF. Three mutants with significant HSAF resistance were screened after two rounds of mutagenesis. To identify the causative mutations in these mutants that led to HSAF resistance, bulk segregation analysis (BSA) was applied following the next generation sequencing. The results showed that all of these mutants contained point mutations in several genes involved in chitin metabolic process, indicating chitin might be a part of HSAF targets. Considering that chitin is an important component of the fungal cell wall, we subsequently removed the cell wall to obtain protoplasts to test the effect of HSAF on the regeneration of protoplasts. Our data showed that HSAF did not affect the regeneration of protoplasts, but could inhibit the germination after protoplasts regeneration, suggesting that HSAF might inhibit fungal growth via interacting with the fungal cell walls.

Sensitivity of Algerian *Pyrenophora teres* population to QoI and SDHI fungicides as reveled by Pyrosequencing

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Net blotch of barley caused by *Pyrenophora teres* (Died.) Drecshler is currently one of the most destructive diseases on Barley (*Hordeum vulgae* L.) crops in Algeria, fungicides treatments are mainly used to its control. A total of 212 mono-conidial isolates of this fungus were sampled in 2015/16 and 2016/17 seasons, from 17 Algerian provinces, in 58 distinct geographical localities where barley is cultivated. In order to identify fungicides resistant individuals, and prevent their extension in the pathogen population, isolates were assessed for resistance to Quinone outside inhibitors (QoI) and to Succinate dehydrogenase inhibitors (SDHI) fungicides. Resistance was screened using Pyrosequencing technology. F129L and G137R mitochondrial cytochrome b substitution associated with QoI resistance and SDHI related substitutions (B-H277, C-N75S, C-G79R, C-H134R and C-S135R) occurring in the three subunits B-C and D (of the SDH complex) were looked for. Our results showed that all tested isolates were identified as QoI and SDHI sensitive, since all possessed the wild-types alleles. According to these, *P. teres* Algerian population is considered sensitive toward QoIs and SDHIs, which is reassuring for farmers, and fungicides market in Algeria. This study is the first investigation related to mutations associated to QoI fungicides resistance in Algerian population of *P. teres*.

Sensitivity monitoring of *Plasmopara viticola* to oxathiapiprolin, a new member of piperidinyl thiazole isoxazoline fungicides

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Oxathiapiprolin is the first member of a new class of piperidinyl thiazole isoxazoline fungicides and it is classified under the FRAC code 49. This fungicide inhibits an oxysterol binding protein (OSBP) homologue. OSBP are lipid-binding proteins implicated in many cellular processes including signaling, vesicular trafficking, lipid metabolism, and non-vesicular sterol transport [1]. OSBP represents a novel target for oomycete disease control.

The aim of this work was to evaluate the sensitivity of oxathiapiprolin (Zorvec™ – Corteva) on *Plasmopara viticola* samples coming from different area of Northern Italy in 2018 (first year of authorization) and 2019 seasons. Bioassays on leaf discs were carried out, and the results showed the complete sensitivity of *P. viticola* populations to the active ingredient.

The availability of oxathiapiprolin may now improve significantly the downy mildew

disease control in field. Unfortunately, the fungicide is prone to resistance phenomenon then it has to be monitored and follow the guidelines provided each year by Fungicide Resistance Action Committee (FRAC) is essential.

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Antifungal potential of the Temporin B-derived synthetic peptide TB_KKG6K

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Temporins are antimicrobial peptides secreted by the granular glands of the European red frog (Rana temporaria). They are amphipathic -helical polypeptides of 8-14 amino acids (aa) in length, have a low positive net charge at neutral pH, and are active against Gram-positive bacteria (1, 2).

The rational design of Temporin B derived synthetic peptides indicated that hydrophobicity and positive net charge were critical regulators of its antimicrobial activity (2). The most active variant was TB_KKG6K (TB peptide), which contained three extra lysine residues, raising its net charge from +0.9 to +3.9 and expanding its spectrum of activity to include gram-negative bacteria as well (3).

However, the antifungal potential of the TB peptide has not been extensively investigated for its efficacy against two opportunistic human fungal pathogens, *Candida albicans* and *Aspergillus fumigatus*. The TB peptide inhibited the growth of both fungi at low micromolar concentrations and acted in a fungicidal way.

The killing mechanism linked to the rapid uptake of the TB peptide into the cytoplasm.

To evaluate its commercial feasibility, the peptide stability and its harmlessness to human cells is currently evaluated. The TB peptide was found to be stable against proteolytic digestion as well as high temperature and extreme pH conditions, and showed no hemolytic activity. The tolerance of this peptide by primary human skin and lung cells awaits further investigation.

Thus, the TB peptide has the potential to be developed as an effective alternative

therapeutic option for hard-to-treat fungal infections.

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Avitabile C. *et al*. 2013, Biochimica et Biophysica Acta

Avitabile C. *et al*. 2018, ChemMedChem

Alternative control system of *Pleurotus ostreatus* against green mold disease

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Green mold, caused by *Trichoderma pleuroti* and *T. pleuroticola*, represents a very important disease for *Pleurotus ostreatus* (oyster mushroom), commonly controlled by the use of fungicides. *Aureobasidium pullulans* is a biocontrol agent naturally found in a wide range of habitats.

The effect of *A. pullulans* L1 and L8 strains on *P. ostreatus, T. pleuroti* and *T. pleuroticola* was evaluated in the present study by in vitro assays. Both *A. pullulans* strains showed to be compatible with *P. ostreatus* growth and effective as antagonists in reducing the *T. pleuroticola* and *T. pleuroti* colony growth. The inhibitory effect of the two strains was similar in the majority of the in vitro assays. Both strains were more efficient than *Trichoderma* spp. in substrate colonization, and produced volatile (VOCs) and non-volatile compounds (NVOCs) which reduced *Trichoderma* growth. The antagonistic activity of L1 and L8 strains was tested by in vivo assay under controlled conditions similar to those of a mushroom farm. Only L8 was effective in controlling the disease showing an effect similar to prochloraz fungicide against *T. pleuroticola*, the less aggressive *Trichodema* sp., but a lower effect with respect to the fungicide against *T. pleuroti,* the most aggressive tested *Trichodema* sp. *Aureobasidium pullulans* L1 and L8 strains showed the ability to act against green mold by antibiosis and competition for space and nutrients. In addition, both strains showed the ability to promote *P. ostreatus* growth, also probably improving mushroom fruit-body nutraceutical properties.

Cellular response to farnesol and the role of nitric oxide production in *Aspergillus fumigatus*

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Plants produce aromatic essential oils including thymol, farnesol, nerol, and citral to protect themselves from pests and pathogens. Although in general they show inhibitory effect on fungal growth, the molecular mechanism of the antifungal action remains to be elucidated. Recently, thymol has been reported to control fungal growth by inducing generation of reactive oxygen (ROS) and nitric oxide (NO) and apoptosis in the spores of ubiquitous saprophytic *Aspergillus* (1). Here, we investigated the response of *Aspergillus fumigatus* to farnesol, which is sesquiterpene alcohol and one of the components of plant essential oil. Farnesol showed antifungal activity against *A. fumigatus* at concentration above 0.12 mM. In the hyphal state, intracellular NO and ROS levels that were detected by fluorescent probes were increased upon farnesol at the concentrations of the growth inhibition. Interestingly, NO scavenger, 2-(4-carboxy-2-phenyl)-4,4,5,5,-tetramethylinidazoline-1-oxyl-3-oxide (cPTIO), suppressed ROS level as well as NO level in the farnesol-treated hyphae. While the germination rate of *A. fumigatus* was inhibited by farnesol, cPTIO diminished the growth inhibition by farnesol. These results suggested that farnesol-derived NO affects ROS production in the hyphae and germination in *A. fumigatus*. Furthermore, *A. fumigatus* produced secondary metabolites that were dependent on farnesol treatment. In the natural habitat, farnesol is secreted by not only plants but also some bacteria and fungi. Therefore, farnesol possibly plays an important role in interactions between *Aspergillus* fungi and other organisms.

Exploring the variety of interactions between Fungi and Bacteria

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Fungi and bacteria comprise a large fraction of biomass in the soil and since they interact with each other, bacterial-fungal interactions are crucial for understanding the microbial ecosystem which is closely related to agriculture, medicine and the environment. Although, majority of the studies based on the dynamics of microbiota

have used monocultures. Microbial interactions are known to promote the activation of cryptic biosynthetic pathways leading to the production of secondary metabolites which possess not only defense functions but also steer cell to cell communication and other interactive dynamics. Coculturing have been proven to be an effective method to mimic the conditions existing among the microbial interactions in the natural environment, hence may be of potential to facilitate the production of novel antimicrobials as well as facilitator molecules.

This study investigated different combinations of fungi and bacteria in coculture to observe the interactive dynamics of bacteria and fungi. Cocultures were incubated for 1 day spanning to 4 days prior to microscope imaging. The amount and the rate of growth, the affinity of the bacterial cells to the fungal hyphae, velocity of the movement of the bacterial cells (using kymograph analysis) and the distance travelled along the hyphae were examined. According to the degree of these interactions and dynamics the combinations were classified into positive, negative and neutral genres. A selected array of combinations was subjected to HPLC and LC-MS analysis and difference of the chemical profiles of pure and co cultures were analyzed to determine and contrast the levels of bioactive compounds production.

This approach would gain more perspective on an ecological context on the interactions of the environmental microbiota, since there is high potential to increase the metabolic capacity of chemically prolific microorganisms.

Role of the MAPK signaling pathways and chitin synthases of the phytopathogenic fungus *Penicillium digitatum* in sensitivity to antifungal proteins

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Penicillium digitatum is the main postharvest pathogen of citrus fruit and is responsible for important economic losses. Our group has proposed antifungal proteins (AFPs) and peptides as new alternatives to the use of fungicides against phytopathogenic fungi. The involvement of fungal cell wall (CW) and MAP kinase (MAPK) signaling pathways in the interaction of AFPs has been reported. Specific CW components such as chitin, and MAPK routes were induced by AFPs. Chitin is synthesized by a complex family of chitin synthases (Chs), some of which contain a myosin motor-like domain (MMD). Furthermore, three MAPK routes are described in fungi: Kss1/Fus3 involved in the invasive growth and virulence of pathogens, Hog1 in response to osmotic stress, and

Slt2/Mpk1 in response to CW stress. We generated null deletion mutants of *P. digitatum* in MMD-Chs and in the three MAPK from each pathway to study their role in the mode of action of the antifungal protein AfpB obtained from *P. digitatum*. Treatment of *P. digitatum* with its self-AfpB resulted in an overall reduction of the *chs* gene expression and produced a gradual increase of Hog1 and Slt2 phosphorylation. MMD-Chs mutants showed different sensitivity to AfpB, however, none of the three MAPK mutants showed increased sensitivity to this protein, contrary to previous reports of other AFPs. Taking into account all the above, we conclude that MMD-Chs affect sensitivity to AFPs in *P. digitatum* and that the activation of Hog1 and Slt2 by AfpB would not have a defensive role [1][2].

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Comparison of the production pattern and antifungal activity of three antifungal proteins from the phytopathogenic fungus *Penicillium expansum*

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Antifungal proteins (AFPs) are small and cationic defensin-like proteins that are produced and secreted to the culture medium by filamentous ascomycetes. Filamentous fungi encode a wide repertoire of AFPs belonging to different phylogenetic classes (A, B and C) [1], which offer a great potential to develop new antifungals for the control of pathogenic fungi in medicine and agriculture. The phytopathogenic fungus *Penicillium expansum* is one of the few reported to encode three AFPs namely PeAfpA, PeAfpB and PeAfpC, each belonging to a different phylogenetic class [2]. *P. expansum* naturally produces PeAfpA and PeAfpC; however, PeAfpB was not detected in culture supernatants, in agreement with the expression pattern of each *afp* gene, and despite the wide variety of culture conditions tested. AFP production in *P. expansum* is strain-dependent. None of the three AFPs was detected during *P. expansum* infection in apples, suggesting that AFPs are not related to pathogenesis. Production of PeAfpB, and PeAfpC was achieved in *Penicillium chrysogenum* with the *P. chrysogenum*-based expression cassette [3].

Regarding antifungal activity, PeAfpA was the most active protein against plant and human pathogens. PeAfpB showed moderate antifungal activity against filamentous fungi, whereas no activity could be attributed to PeAfpC under the conditions tested. *P. expansum* was also sensitive to its self-AFPs PeAfpA and PeAfpB. Furthermore, both proteins showed positive synergistic interaction when combined against *P. expansum*. Finally, PeAfpA was demonstrated to efficiently protect against fungal infections caused by *Botrytis cinerea* and *Penicillium digitatum* in tomato plants and orange fruits, respectively, although PeAfpA did not confer protection against *P. expansum* infection in apples.

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Signals in pathogen and host sensing: free fatty acid and oxylipins

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Lipids play important roles at various stages of host–pathogen interactions and are crucial in determining the virulence of pathogens and modulating plant defences (1). Free Fatty Acids (FFA) may act as substrates for oxidizing enzymes [e.g., lipoxygenases (LOXs) and dioxygenases (DOXs)] forming oxylipins that have been extensively studied in plant–pathogen interaction. Free fatty acids (FFA) and oxylipins might also function as modulators of several pathways in cell-to-cell communication (2). The structural similarity of plant, fungal and bacterial oxylipins prompts the hypothesis that they are important in cross-kingdom communication. We present here, two case studies: the first regards an eukaryotic pathogen (*Fusarium verticillioides*) infecting maize (*Zea mays*); the second case study deal with a prokaryotic pathogen, *Xylella fastidiosa* subsp. *pauca* infecting *Olea europaea* and artificially *Nicotiana tabacum* (2). These two studies pin point out that the FFA and the oxylipins derived by the oleic, linoleic and linolenic

acid are crucial to modulate the pathogen lifestyle and the interaction with the host. In particular, in *F. verticillioides* the oxylipins cross-talk modulate the fumonisin synthesis. The increase of linoleic acid-derived oxylipins favour the fumonisins production, driving the plant towards the PCD (plant cell death). In *X. fastidiosa*, the oxylipins modulate the planktonic and biofilm formation and within the host seem to pave the way for the Olive Quick Decline Syndrome symptoms. Thanks to similar and overlapping results in different pathosystems, we can assume that lipids, at the interface of interacting organisms, may act as signals able reshaping the lifestyle of the contenders and sometimes determining the fate of the challenge.

Improvement of a lux-system that detect new antifungals

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Fusarium species are plant pathogens and toxin producers causing food contamination and diseases in animals and humans. They cause infections in humans such as keratitis, onychomycosis, and disseminated infections, the later mainly found in immunocompromised patients. Among the human pathogenic *Fusarium* species, *Fusarium oxysporum* has been reported to be one of most abundant.

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 *F. oxysporum*. The method consists in expression of the luciferase gene (*mluc*) under control of a promoter that responds signaling via the CWI pathway. A number of promoters were selected from upregulated genes detected by analysis of RNA-seq data derived from wild-type *A. fumigatus* treated with calcofluor white (CFW) (unpublished) or detected in microarray data of WT treated with Congo Red (CR) versus non-treated controls. To identify an appropriate CWI-inducible *F. oxysporum* gene the following criteria were taken into account, i) the sequence of the *F. oxysporum* promoter should contain canonical Rlm1 boxes, ii) the gene should be induced by CFW and iii) the *A.niger* orthologue should be repressed in *Aspergillus rlmA* mutants. Three putative CWI-inducible *F. oxysporum* genes were selected and their expression studied by qPCR after treatment with CFW. Promoter of gene *chs3*, coding for a chitin synthase 3, contains 3 putative Rlm1-recognition sites (boxes). In order to optimize the detection system, fragments of different length containing 1, 2, or 3 putative boxes, were used to construct three versions of *Pchs3::mluc.* After introduction of the three different constructs in *F. oxysporum,* the transformants were checked for luminescence emission after growth in the presence of

different concentration of different commercially available antifungals. Our current data about the detection system and its optimization will be presented.

Molecular identification and disease management of stem canker of royal poinciana caused by *Neoscytalidium dimidiatum* in the United Arab Emirates

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In the United Arab Emirates (UAE), royal Poinciana suffers from stem canker disease. Symptoms of stem canker can be characterized by branch and leaf dryness, bark lesions, discoloration of xylem tissues, longitudinal wood necrosis, and extensive gumming. A general dieback signs were also observed leading to complete defoliation and ultimately the death of the tree in advanced stages. The fungus, *Neoscytalidium dimidiatum*, was consistently recovered from diseased royal Poinciana tissues, and this was confirmed by the molecular, structural and morphological studies. Phylogenetic analyses of the internal transcribed spacer and translation elongation factor 1-a (ITS/TEF1–α) of *N. dimidiatum* from the UAE with reference specimens of Botryosphaeriaceae family validated the identity of the pathogen. To manage the disease, the chemical fungicides such as Protifert®, Cidely® Top and Amistrar® Top, significantly inhibited mycelial growth and reduced conidial numbers of *N. dimidiatum* in laboratory and greenhouse experiments. The described approach of the "apple bioassay" can be very useful when performing fungicide treatment studies. Under field conditions, Protifert® proved to be the most effective fungicide against *N. dimidiatum* among all tested treatments. Our data suggest that the causal agent of stem canker disease on royal Poinciana in the UAE is *N. dimidiatum*.

A rapid CRISPR-mediated Tet-Off system reveals the phosphoinositide kinases Stt4 and Mss4 are essential for viability of *Aspergillus fumigatus*

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Invasive fungal infections kill over 1.5 million every year. Currently, only four classes of

antifungals are used in clinical practice for the treatment of invasive fungal infections. The first line therapy for treatment of all forms of aspergillosis is voriconazole, a member of the triazole class of antifungals. The extensive use of azoles has led to the emergence of resistance. For those patients who are infected with a resistant isolate, mortality rates are almost 90%. To treat these infections, there is a critical need for novel antifungal agents. This study explores the potential of phosphoinositide kinases (PIKs) and phosphoinositide phosphatases (PIPs) to be novel targets for the development of the next generation of antifungal drugs.

Phosphoinositides (PIs) have been found to have many crucial roles, including regulating intracellular membrane trafficking, membrane recycling, signal transduction and directing the localization and activity of effector proteins. PIs metabolism is governed by a series of highly specific PIKs and PIPs, which are able to sequentially phosphorylate and dephosphorylate at the D-3, D-4 and D-5 positions of the inositol ring. In *Saccharomyces* cerevisiae, several PIKs and PIPs are critical for cellular viability. We therefore hypothesised that one or more PIKs and PIPs could be critical for viability of the human pathogen *Aspergillus fumigatus* (*A. fumigatus*).

Here we used comparative genomics to elucidate the PIs metabolic pathway in *A. fumigatus* and reveal the PIKs and PIPs encoding genes. Using a directed mutagenesis approach, we were only able to isolate heterokaryotic null mutants for these genes, indicating they might be essential for viability. To assess two of the PIKs in more detail, we employed a rapid and simple CRISPR-mediated Tet-Off strategy to replace the native promoters of Stt4 and Mss4. Down-regulation of Stt4 and Mss4 using doxycycline confirmed both genes are non-redundant and required for viability of *A. fumigatus*.

Azaphilones biosynthesis in *Trichoderma harzianum* benefits fungal survival to oxidative stress

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Azaphilones, a large class of fungal secondary metabolites, mainly pigments, characterized by a pyrone-quinone structure, have antimicrobial, antiviral, antioxidant and anti-inflammatory activities. In this study, we present a functional, genetic and biochemical characterization of a group of antioxidant azaphilones produced by mycotrophic *Trichoderma guizhouense* (Hypocreales, Ascomycota) during antagonistic interactions with *Fusarium oxysporum* f. sp. *cubense 4* (Foc4) (Hypocreales, Ascomycota) and abiotic oxidative stress.

Generally, Foc4 is highly resistant against mycoparasitic attacks of the majority of Trichoderma spp. However, one species - *T. guizhouense* (*Harzianum* Clade), can antagonize it by producing an excessive amount of reactive oxygen species (ROS), mainly hydrogen peroxide (H_2O_2) in addition to the array of secreted proteolytic and chitinolytic enzymes. The transcriptomic analysis of these interactions pointed to the specific activity of the PKS cluster (OPB37942-OPB37951) [3]. The deletion of the pks gene (OPB37945) and overexpression of the respective transcription factors (OPB37944 and OPB37950) from the same SM cluster demonstrated that indeed these genes were responsible for the production of the dark yellowish pigmentation noticed during the interaction between *T. guizhouense* and Foc4, but also other fungi, and abiotic oxidative stress. The purified compounds revealed the pyrone-quinone structure and antioxidant activity and were attributed to azaphilones. In this presentation, we will demonstrate the putative biosynthetic pathway of the group of novel antioxidative secondary metabolites of filamentous Ascomycota and show that the production of azaphilones is most likely evolutionary conserved in these organisms.

Amplicon-deep sequencing using Oxford-Nanopore® technology to quantify multi-drug-resistant strains in *Zymoseptoria tritici* populations

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Disease control of septoria leaf blotch (*Zymoseptoria tritici*) on wheat relies mainly on resistant wheat cultivars and fungicide applications. The fungus, however, displays a high potential to circumvent both methods. Resistance due to target site modifications affects all available uni-site fungicides. A different type of resistance has evolved among *Z. tritici* populations impacting multiple chemical families. Strains considered as multi-drug resistant (MDR) have been isolated since 2008. MDR is a common trait developed by many organisms to counteract chemicals and relies on overexpressed drug efflux transporters that expulse the drug outside the cell. In *Z. tritici* the major-facilitator gene, *MFS1,* as principal player of this emerging resistance mechanism is overexpressed in MDR field strains (Omrane *et al*. 2015). We identified three different types of *MFS1* promoter inserts as responsible mutations for MDR in *Z. tritici* field strains (Omrane *et al*. 2017).

This study aimed to develop a molecular tool allowing to detect and to quantify the frequency of MDR strains in *Z. tritici* populations. We decided to apply an amplicon-deep-sequencing based assay in order to correlate the number of sequencing reads to the frequency of the strains. We chose the Oxford-Nanopore sequencing technology for the

long sequencing read length and its low cost. The principle of the method on complex DNA mixtures is to add unique sequence tags to each molecule in the mixture, prior to PCR amplification to minimize amplification bias. Each population is labeled with a barcode, allowing mixing up to 96 populations per sequencing run. Using this methodology, we successfully detected the four *MFS1* promoter alleles in our sequence data of home-made DNA mixtures. Further adjustments of the protocol are needed for quantification in complex DNA mixtures and especially in DNA from infected wheat leaves.

Omrane *et al* 2015, Env. Microb.

Omrane, *et al* 2017, mSphere

Selection, genetic characterization and aflatoxin production of *Aspergillus flavus* strains resistant to SDHI boscalid

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Aspergillus flavus is an ubiquitous fungal species able to colonize several agricultural commodities, in both pre- and post-harvest conditions. This species represents a harmful plant pathogen for its ability to synthesize the human carcinogenic aflatoxin B1. Several approaches are proposed to control *A. flavus* development and related aflatoxin production in field and storage conditions, including the use of the Succinate Dehydrogenase Inhibitor (SDHI) fungicide boscalid. However, this compound is classified as medium-high risk fungicide for trigging fungal resistance in field strains isolated from crops treated with boscalid.

We selected *A. flavus* strains resistant to boscalid, grown on agar medium containing 50 mg/L of the compound. We investigated the molecular mechanism responsible for the resistant phenotype, by designing specific primer pairs to amplify the whole *SdhB*, *SdhC* and *SdhD* genes. By sequencing *Sdh* genes and comparing the amino acid sequences of sensitive and resistant strains, two point mutations were identified in resistant strains: at codon 249 of *SdhB*, where Tyrosine replaced Histidine; at codon 91 of *SdhC*, where Arginine replaced Glycine. The resistant strains response to SDHI boscalid and isopyrazam, for mycelial growth and conidial germination inhibition, was evaluated. Both genotypes showed a high resistance level to boscalid. A positive cross-resistance was found between boscalid and the other SDHI fungicide isopyrazam. Both fungicides interfered with the mechanisms associated to pigmentation of colonies, that appeared

depigmented, lacking the typical *A. flavus* green colour shown on un-amended fungicide medium. A strict correlation between lack of pigmentation and increasing aflatoxin production was also observed.

Comparative genomics of *Aspergillus fumigatus* and the influence of agriculture on ecology and azole resistance

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Aspergillus fumigatus is a ubiquitous saprophyte capable of causing life-threatening invasive infections. Unfortunately, over the last decade there has been a global emergence in azole resistance in *A. fumigatus* and the dominant resistance mechanism is of environmental origin, suggesting that resistance is emerging through a selective pressure applied by the widespread usage of azoles in agriculture. To examine the link between azoles in agriculture and the emergence of clinical resistance, systematic soil sampling was performed over a three-year period before and after the vegetative period and azole application. We observed a reduction in the abundance of *A. fumigatus* on fields following azole treatment – a finding that was not repeated on an organic agriculture control field – suggesting that the application of azoles imposes a bottleneck on *A. fumigatus*. The overall resistance frequency among agricultural isolates was low, with only 1-3% of isolates from 2016-2018 showing resistance. Importantly, isolates from after the growing season and azole exposure showed a subtle, but consistent reduction in susceptibility to medical and agricultural azoles. To examine the population genomics of *A. fumigatus*, we performed WGS on 215 environmental and 50 clinical isolates. Among the environmental samples, isolates from different regions, types of agriculture, and time periods did not cluster separately, indicating a lack of population structure. Comparison of environmental isolates with clinical isolates revealed several subgroups present in the environment that were not represented among clinical samples. Resistant environmental isolates were exclusively either wild type at the cyp51a loci or carried the TR34/L98H allele, while clinical isolates showed a much wider range of cyp51a mutations. Ongoing work is focused on defining fungal determinants enriched in human infection, as well as genetic changes associated with azole resistance.

Discovery of the biosynthetic pathway for the antifungal hymeglusin in *Scopulariopsis candida*

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Filamentous fungi are prolific producers of bioactive secondary metabolites (SMs), which we exploit for human health. Fungi from extreme environments are a great resource of interesting SMs, some of which help them thrive in harsh conditions. Among their many bioactivities, SMs can be powerful antifungal agents. Recent collective efforts have focused on developing antifungals that target invasive yeasts such as *Candida*, as these species have become significant threats to susceptible patients. This work details our efforts to screen a library of irradiated, Chernobyl nuclear accident-associated, fungal strains for their activity against *Candida albicans*. One of the most potent strains demonstrating anti-*Candida* activity was *Scopulariopsis candida* IMV00968, which was found to produce significant levels of the HMG-CoA synthase inhibitor, hymeglusin. The antimicrobial activity of hymeglusin has previously been reported, yet no group has identified its biosynthesis genes. Whole-genome sequencing data for IMV00968 were collected, assembled, and annotated. SM prediction then revealed 21 putative biosynthetic gene clusters within the genome. CRISPR/Cas9 technology facilitated the deletion of the gene encoding a polyketide synthase (hnmA), that was necessary for hymeglusin production.

Characterization of azole resistant *Aspergillus fumigatus* strains isolated from imported tulip bulbs that were purchased in Japan

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Aspergillus fumigatus is a major causative pathogen for invasive pulmonary aspergillosis. Although antifungal azole is a first-line for the treatment, azole resistant *A. fumigatus* (ARAF) strains have been widely isolated worldwide in the last decade. One possibility is that agricultural products such as plant bulbs transport the ARAF between countries via import-export trade. So far, ARAF strains isolated from plant bulbs that were imported from the Netherlands had been reported in Ireland. In the present study, we tried to isolate the ARAF strains from tulip bulbs that were imported from the Netherlands and were purchased in Japan. Total of 22 *A. fumigatus* strains were isolated from 11 bulbs of 4 tulip species. MICs for itraconazole and voriconazole were determined in these isolates by microdilution method and E-test, respectively. Eleven out of 22 isolates

showed resistance to any of medical azoles, and all of the isolates possess a 34 bp or 46 bp of tandem repeat (TR) in the promoter region of *cyp51A* gene. Interestingly, some of the TR strains also possess G448S substitution in Cyp51A that had been identified to be involved in resistance to voriconazole. In addition to the medical azoles, these strains with TR34 or TR46 showed high resistance to fungicides, prochloraz and triflumizole, that are DMI used for disinfection of tulip bulbs. Phylogenetic analysis using microsatellite method estimated that some ARAF strains in our collection were genetically close each other. These results warranted further surveillance of ARAF in agricultural product of international trade in order to reduce unintended spreading of ARAF strains.

In vitro study of *Agaricus bisporus* proteomic response to *Trichoderma aggressivum* f. *europaeum* supernatant

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Agaricus bisporus is the most commonly cultivated mushroom in North America and Europe. Green mold disease results in severe crop losses and is caused by some species of the genus *Trichoderma* but in the early 1980s a new species was identified in Ireland as *T. aggressivum*. This filamentous micro-fungus is a rapidly growing, aggressive competitor that has evolved and adapted to mushroom growing casing and substrate. In this work wet mass was measured, and shotgun proteomics was conducted on *A. bisporus* grown for 10 days and supplemented with *T. aggressivum* supernatants (25% v/v) for additional 2, 4 and 8 days. *T. aggressivum* supernatant statistically significantly inhibited growth by 48% after 4 days, and by 27.9% and 13.16% after 2 and 8 days, respectively. Label free proteomic analysis of changes in the abundance of *A. bisporus* proteins following exposure of cells to *T. aggressivum* supernatant indicated the greatest change after 4 days of coincubation. Exposure lead to an oxidative stress response (proteins that increased in relative abundance: zinc ion binding (6.6-fold); peroxidase activity (5.3-fold); carboxylic ester hydrolase (2.4-fold); biosynthetic process (3.1-fold); 60S, ribosomal protein L36 (3.3-fold); dipeptidase (3.2-fold); [2Fe-2S] cluster assembly (3.3-fold)). Proteins decreased in relative abundance in *A. bisporus* exposed to *T. aggressivum* supernatant were associated with growth such as: structural constituent of ribosome, translation (12-fold), deadenylation-dependent decapping of nuclear-transcribed mRNA (3.4-fold), superoxide dismutase (3-fold), metal ion binding (2.7-fold), small GTPase mediated signal transduction (2.6-fold), deoxyribonucleotide catabolic process (2.6-fold), GTP binding (2.7-fold), glycine cleavage system P protein (2.3-fold), and proteasome subunit beta (2.3-fold). The results indicate that exposure of *A. bisporus* to *T. aggressivum* culture filtrate induces an oxidative stress response and a severe

reduction in growth.

Activity of oligosaccharides derived from Tramesan on aflatoxin inhibition in *Aspergillus flavus*

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Asperigllus flavus is a well-known ubiquitous fungus, which in certain conditions can produce secondary metabolites, namely, mycotoxins, that are hazardous for animal and human health. Mycotoxins are able to contaminate different feed and food commodities and due to their direct influence on human health, this issue is raising the attention of the European Community. The awareness that different chemicals, largely used in agriculture to control pests, could be hazardous for both the environment and human health, led to large limitation of their use. In particular, since 2014, the EC has banned about 50% of chemicals used in agriculture. Furthermore, presently the EC is encouraging the researchers to investigate more environmental friendly "green" approaches and eco-compatible tools a in control of plant fungal diseases, for preventive and/or detoxification strategies. Thus, the necessity of environmentally friendly alternatives, able to control aflatoxin synthesis has increased. One of the most promising biocontrol agents is *Trametes versicolor*, an edible and non-toxic Basidiomycete, considered "healing mushroom" for its bioactivity towards some pathologies. The exo-polysaccharide was extracted, purified, its structure was characterized and the product was named Tramesan. Our work demonstrates that oligosaccharides derived from polysaccharide Tramesan®, have the ability to counteract the mycotoxin synthesis in *Aspergillus flavus*. The inhibiting effect on aflatoxin synthesis of these compounds was evaluated. The aim of the study was to determine the smallest part of the polysaccharide of the Tramesan, active in the inhibition of aflatoxin B1 synthesis. The results have shown that the heptasaccharides have the most inhibiting effect on aflatoxin synthesis. In conclusion, a biocontrol agent from *T. versicolor* could be considered new eco-compatible tools for mycotoxins control, in line with EU directives.

Increase of reactive oxygen species contributes to growth inhibition by fluconazole in *Cryptococcus neoformans*

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Cryptococcus neoformans is an encapsulated yeast causing deadly meningitis in severely immunocompromised hosts. The antifungal drug fluconazole (FLC) causes cell growth defects in *C. neoformans* by preventing ergosterol production. Previous studies suggest that FLC leads to a moderate increase in reactive oxygen species (ROS) in fungal cells. We determined that FLC leads to an increase in ROS in *C. neoformans*. Treatment with antioxidants resulted in reduced ROS in the presence of FLC and led to rescue of *C. neoformans* from FLC induced inhibition of growth. Further, we found that mutants lacking Cu-detoxifying metallothionein (CMT) proteins are more sensitive to FLC and do not rescue cell death and ROS induced by FLC. Metallothionein genes regulate copper homeostasis, scavenge free radicals, and are upregulated during oxidative stress in fungi. Our study indicates that FLC causes a moderate increase in ROS in *C. neoformans* and identifies the metallothionein proteins to be involved in FLC resistance in *C. neoformans*.

The *Aspergillus fumigatus* transcription factor RglT is important for gliotoxin biosynthesis and self-protection, immunomodulation and virulence

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Aspergillus fumigatus is an opportunistic fungal pathogen that secretes an array

of immune-modulatory molecules, including secondary metabolites (SMs), which contribute to enhancing fungal fitness and growth within the mammalian host. Gliotoxin (GT) is a SM that interferes with the function and recruitment of innate immune cells, which are essential for eliminating A. fumigatus during invasive infections. We identified a C6 Zn cluster-type transcription factor (TF), subsequently named RglT, important for A. fumigatus oxidative stress resistance, GT biosynthesis and self-protection. RglT regulates the expression of several gli genes of the GT biosynthetic gene cluster, including the oxidoreductase-encoding gene gliT, by directly binding to their respective promoter regions. Subsequently, RglT was shown to be important for immunomodulation and virulence in an immunocompetent murine model of invasive pulmonary aspergillosis. Homologues of RglT and GliT are present in eurotiomycete and sordariomycete fungi, including the non-GT-producing fungus A. nidulans, where a conservation of function was described. Phylogenetically informed model testing led to an evolutionary scenario in which the GliT-based resistance mechanism is ancestral and RglT-mediated regulation of GliT occurred subsequently. In conclusion, this work describes the function of a previously uncharacterised TF in GT biosynthesis and self-protection in both GT-producing and non-producing Aspergillus species.

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POSTER SESSION 3 & FLASH TALKS

Poster Session 3.1
EVOLUTION

WEDNESDAY, FEBRUARY 19

14:00 - 15:30 | Location: **Frentani Convention Center**

Genomic and phenotypic divergence among heavy-metal tolerant and sensitive isolates of the ericoid fungus *Oidiodendron maius*

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Oidiodendron maius is a symbiotic ascomycete forming ericoid mycorrhiza (ERM) with plants in the family Ericaceae. ERM increases metal tolerance of the host plant in soils enriched in heavy metals, a property ascribed to the fungal partner. Previous experiments on heavy-metal tolerant and sensitive isolates of *O. maius* suggested, for the Cu/Zn superoxide dismutase gene, a higher rate of DNA polymorphism in the tolerant fungal isolates. The aim of our study was to associate phenotypic and genomic characterization of 18 isolates of *O. maius* to identify genomic regions involved in metal tolerance. When tested *in vitro* for their growth with different metals, ten isolates were sensitive and eight were tolerant to at least one of the metals. The genome of these isolates was sequenced and the reads were aligned onto the reference genome of *O. maius* Zn, a metal tolerant isolate. Alignment with stringent parameters led to the identification of a total of 1,874,886 Single Nucleotide Polymorphism (SNPs). A neighbor-joining tree built using these SNPs showed that the isolates grouped according to their metal tolerance suggesting a genomic evolution of this phenotype. Furthermore, two genetic differentiation indices were calculated. The Tajima's D identified 25 genes putative candidates under positive selection (D>2), with an enrichment of the genes without functional (KOG) classification. By comparing the tolerant with the sensitive groups, 4815 genes were found with Fst>0.5, suggesting an important differentiation. These genes were distributed into many KOG categories, mainly in the primary metabolism (i.e: RNA and DNA metabolism and structure, cell cycle control) and included the transporter *OmZnT1*, already known to be involved in the response of *O. maius* Zn to metals. Our study identified several candidate genes involved in metal tolerance in *O. maius*, and the functional characterization of these genes is ongoing.

Exploring the genomic diversity related to wood degradation within the order Polyporales, *Basidiomycota*

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In order to reduce our dependence on fossil carbon for the production of bio-energy and commodity chemicals, plant biomass is being a keen renewable feedstock as an alternative resource, not competing with food production. However capturing the potential of agricultural wastes and woody resources remains a big challenge due to the highly recalcitrant lignocellulose. Only, some microorganisms such as wood decay fungi, have the ability to decompose all lignocellulose components into simpler carbon polymers and carbohydrates by producing a large range of lignocellulosic enzymes. Among those fungi, basidiomycetes from the order Polyporales are very efficient wood decayers. They are thought to have emerged some 125-143 million years ago and have adapted to survive on the very recalcitrant wood.

The objective of this study is to investigate whether specific adaptations related to wood decay have emerged within Polyporales as compared to other fungi that live on wood. For that, we compared the genomes of 50 selected Polyporales, including 30 newly sequenced genomes, to 57 Agaricomycetes species with contrasted lifestyles. We analyzed genome macrosynteny and observed poor conservation in genome structure across Polyporales, indicative of rapid evolution despite low contents in Transposable Elements. The comparison of specific traits between fungi of different lifestyles strengthened the idea of a continuum from saprotroph to biotroph lifestyles. Finally, we identified gene repertoires for plant cell wall degrading enzymes and Cytochrome P450 were the most discriminant traits between lifestyles.

Our results highlight the huge diversity which can be explored inside Polyporales species and makes them a very attractive source of biocatalysts to promote *a* sustainable, primary or post-waste production bioeconomy.

Evolutionary histories of type III polyketide synthases in fungi

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Type III polyketide synthases (PKSs) produce secondary metabolites with diverse biological activities. While they have been extensively studied in plants and bacteria, only a handful of type III PKSs from fungi has been randomly characterized in the last 15 years. The exploitation of fungal type III PKSs to produce novel bioactive compounds requires understanding the diversity of these enzymes, as well as of their biosynthetic pathways. Here, phylogenetic and reconciliation analyses of 522 type III PKSs from 1,193 fungal genomes revealed complex evolutionary histories with massive gene duplications and losses, explaining their discontinuous distribution in the fungal tree of life. In addition, horizontal gene transfer events from bacteria to fungi and between fungi could be inferred. Ancestral gene duplication events have resulted in the divergence of eight phylogenetic clades. Especially, two clades show ancestral linkage and functional co-evolution between a type III PKS and a reducing PKS genes. Investigation of the occurrence of conserved domains in fungal type III PKS predicted gene clusters highlighted the diversity of biosynthetic pathways, likely reflecting a large chemical landscape. Yet, type III PKS genes are most often located next to genes encoding cytochrome P450s, MFS transporters and transcription factors, defining ancestral core gene clusters. This analysis also allowed predicting gene clusters for the characterized fungal type III PKSs and provides working hypotheses for the elucidation of the full biosynthetic pathways. Altogether, our analyses provide the fundamental knowledge to incent further characterization and exploitation of fungal type III PKS biosynthetic pathways.

Evolutionary odyssey of effector-like proteins in the mycoparasitic fungus *Trichoderma*

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Cerato-platanin proteins (CPs) are the small secreted cysteine-rich proteins that play central roles in interactions between the fungi and other organisms performing as signals, toxins, and effectors. Most of the so-far reported CPs are from plant pathogenic fungi and act as virulence factors. However, they are also present in mycoparasitic fungi that are not able to parasitize plants. For example, in *Trichoderma* (Hypocreales, Ascomycota), three homologous copies of CP-encoding genes (*epl1*, *epl2*, and *epl3*) were found in all genome-sequenced species except *T. parareesei,* which lacks *epl3*. Although

the role of CPs in the root colonization by some *Trichoderma spp. was demonstrated*, the genus-wide function of these proteins remained unknown. Here we will present the evolutionary analysis of CPs in 34 genomes of hypocrealean fungi, including three saprotrophs, nine herbivore, 12 carnivore, and ten fungivore species. The preliminary results suggest that *Trichoderma* EPL1 and EPL2 evolved from a protein that was putatively laterally transferred to hypocrealean fungi from white wood rot Basidiomycota and undergone a subsequent gene duplication event. Interestingly, the *Trichoderma* orphan *epl3* could be putatively transferred from *Colletotrichum* sp. (Glomerellales) and duplicated in the *Trichoderma* Section *Trichoderma*. The analysis of *epl1* deletion mutants in *T. guizhouense* and *T. harzianum* suggested no active involvement in interactions with fungi and bacteria, while the deletion of *epl1* in *T. guizhouense* resulted in increased colonization of *Solanum lycopersicum* (tomato) roots. Heterologous EPL1 produced in *Pichia pastoris* triggered the jasmonic acid-mediated signaling pathway in tomato what could interfere with root colonization by the fungus. In this presentation, the details of the comprehensive molecular evolutionary analysis and functional genetic investigation of all CP genes in *Trichoderma* will be presented.

Large diversity of species-specific fungi inhabit fungal sporocarps

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Fungal interactions with human, animal and plant hosts are getting more and more attention in mycology, probably because of health and economic reasons. But, fungi are themselves suitable hosts for many invertebrates, bacteria, virus and other fungi. In this study, we explored the host-specificity, diversity and taxonomic composition of fungicolous fungi residing inside large fungal sporocarps in the forest ecosystem. We inferred the influence of various reproductive traits, such as sporocarp morphology and lifespan on the fungicolous fungi. Using DNA metabarcoding, we revealed an astounding diversity of fungicolous fungi residing in sporocarps. The level of colonisation of fungicolous fungi, measured as the proportion of non-host ITS2 reads, varied from 2.8-39.8% across 11 species and consisted mainly of ascomycetes, basidiomycetes and mucoromycetes. The host fungal species were the main determinant of the community composition and diversity of fungicolous fungi, indicating that the diversity of fungicolous fungi is to a large extent species-specific. In addition, alpha-diversity indexes were higher in short-lived and resupinate sporocarps compared to long-lived and pileate sporocarps, suggesting that moisture is an important factor driving species richness. Finally, the putative ecological roles of fungicolous fungi in different types of interaction with their fungal hosts will be discussed in the light of large fungal genome sequencing projects.

Evolution of the stress-adapated black Antarctic cryptoendolithic fungus *Friedmanniomyces endolithicus*

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Black fungi are among the most resistant extremo-tolerant microorganisms and represent a constant presence in the Antarctic endolithic communities of the ice-free areas of Victoria Land (Continental Antarctica). There, epilithic life is too challenging even for black fungi, and endolithic niche, where more buffered conditions occur, offers an ultimate chance of survival [1]. The genus *Friedmanniomyces* is exclusively associated with Antarctic endolithic ecosystems and, to date, described with two species; *F. endolithicus* and *F. simplex*. Among the black meristematic fungi inhabiting these communities, *F. endolithicus* is undoubtedly the most widespread and frequently isolated [2], suggesting a high degree of adaptation. Recently it was also proven to withstand up to 400 Gy gamma radiation and also to increase metabolism after ionizing radiation exposure [3]. Despite these advances, our understanding on the evolution and adaptation strategies of this peculiar fungus remains still scant. In this study, an isolate of *F. endolithicus* was sequenced and compared to other fungi, including those occurring in different extreme environments; we also looked into the genome evolution to understand important genetic factors of this hyper-adapted fungus.

The genomes here compared differ for major aspects, including the size and number of predicted genes, which were much higher in *F. endolithicus* (46.7 Mbp and 18,070, respectively), showing an ancient Whole Genome Duplication. We have also found that *F. endolithicus* evolved in late Neogene (~ 3MYA), when Antarctica reached the present geographic and climatic conditions.

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Comparative performance of *Aspergillus terreus* itaconic acid fermentations on D-xylose and xylitol

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Itaconic acid (IA) is produced by *Aspergillus terreus* mainly from molasses or starch. However, research over the efficient utilization of non-food, lignocellulosic plant biomass is soaring. D-xylose is the most abundant pentose in the lignocellulose complex.

The first enzymatic steps of D-xylose catabolism could lead to cofactor imbalance and low biomass yield. In principle this could be avoided by employing xylitol, the polyol of xylose and a by-product from ethanol manufacturing as carbon source. The aim of this study was to evaluate this option in terms of fermentation performance. To this end, controlled fermentations with D-xylose and xylitol liquid were performed at five different initial concentrations (10, 50, 110, 150 and 200 g L-1), by employing *A. terreus* NRRL 1960, a high IA producer strain. The lowest initial concentration (10 g L-1) resulted in poor molar yield on both carbon sources, particularly on xylitol (0.04 ± 0.01 vs. 0.24 ± 0.01). Differences narrowed with increasing initial carbon concentrations, and eventually, no significant difference was found in the molar yields at the two highest initial carbon concentrations (all in the range of 0.5 ± 0.03). On xylitol, an early lag phase lasting for up to 2 days was observed, and although consumption rate has accelerated afterwards, carbon source utilization rate (g L-1 h-1) remained lower than on D-xylose at any concentration. Importantly, maximal fungal biomass concentrations were not statistically different, indicating a more efficient biomass formation on xylitol than on D-xylose. We conclude that by facilitating xylitol uptake – particularly at the early stages – this common polyol can be made a superior carbon source over D-xylose for IA fermentations.

Research was supported by the EU and co-financed by the European Regional Development Fund under the project GINOP-2.3.2-15-2016-00008 and by the EFOP-3.6.1-16-2016-00022 project co-financed by the EU and the European Social Fund.

Occurrence, distribution, multiplicity and origins of the divalent metal/proton symporter (NRAMP/DMT) in the *Ascomycota*

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In fungi, trace amounts of Mn(II) are necessary to sustain vegetative growth, to progress through sexual and asexual development, in oxidative stress response and secondary metabolite production. Numerous metalloenzymes require the redox-active transition metal Mn as a co-factor, while Mn(II) is also an antioxidant in its own right. However, at higher concentrations Mn becomes deleterious. Multipass membrane proteins of the NRAMP family are crucial in maintaining the balance between essentiality and toxicity. In Saccharomyces cerevisiae, two paralog NRAMPs (Smf1 & Smf2) are implicated in Mn(II) homeostasis, while a third (Smf3) transports Fe(II). By contrast, *Schizosaccharomyces pombe* only has one NRAMP (Pdt1). We have studied NRAMP evolution by molecular phylogeny. 384 proteins from *Saccharomycotina* and *Pezizomycotina* were obtained from JGI Mycocosm. The analysis could be substantially improved further with the addition of NRAMPs from: (i) *Taphrinomycotina*; (ii) four early divergent Saccharomycotina families; (iii) other underrepresented taxa. We added/changed 265 proteins, deduced manually from NCBI-sourced DNA sequences. Our NRAMP phylogeny suggests early separation in almost subphylum-specific branches. Duplications that gave rise to paralogs in *Pezizomycotina* are thus independent from the two major duplication events in the *Saccharomycotina* lineage that resulted in the Smf1-, Smf2- and Smf3-like NRAMPs found in most ascomycete yeast taxa. Smf2 and Smf3 are paralogs but not functional homologs. Conversely, certain taxa (a.o., *Magnaporthe*) lost their NRAMP, suggesting that (a) structurally unrelated system(s) exist(s) to capture adequate Mn(II).

Research was supported by the EU and co-financed by the European Regional Development Fund under the project [GINOP-2.3.2-15-2016-00008] & the [EFOP-3.6.1-16-2016-00022] project co-financed by the EU and the European Social Fund.

Involvement of spliceosomal twin introns in instances of alternative splicing in *Aspergillus*

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In the primary transcript of nuclear genes, coding "exons" usually alternate with non-coding "introns". The latter are precisely excised by the U2 spliceosome to create the ORF that translates into the correct peptide. Spliceosomal twin introns ("stwintrons") are complex intervening sequences where an "internal" U2 intron interrupts one of the canonical

splicing motifs of an "external" U2 intron (viz. 5'-donor; 3'-acceptor; motif around branch point A) and consequently, are removed by consecutive splicing reactions. Originally, alternative splicing was presented as a means to increase protein diversity but more often it yields "dysfunctional" RNAs (not encoding the correct peptide), which are rapidly degradated by nonsense-mediated mRNA decay. We investigated functional relations between bona fide stwintrons, and extant exon skipping and intron retention events. A donor-disrupted stwintron in a ubiquitous gene occurs broadly in the *Pezizomycotina* subphylum. The stwintron is crucially involved in "skipping" the exon behind it in certain species, like *A. niger* and *Neurospora crassa*, by using alternative 3'-splice sites for its internal intron. A branch-point motif-interrupted stwintron was found in *A. nidulans*. Orthologue genes in related species specify a standard intron at the very same position as the internal intron of the *A. nidulans* stwintron. Excision of the new external intron removes the AUG, implying that it must be retained to deliver a protein.

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Formation of a new intron within an extant intron: how can stwintronisation happen?

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In the *Pezizomycotina* subphylum, a [D5,6] stwintron exists in the reticulon-like rtnA gene, where the internal U2 intron is nested in the donor of the external U2 intron after nucleotide (nt) 5 (5' GUAAG|U)(1). Bona fide *rtnA* orthologs are present in *Lipomyces* (*Saccharomycotina*). In 7 species, the genus-specific first intron position is occupied by different complex intervening sequences (CIS) consistent of nested U2 introns. In *L. lipofer*, it is a [D4,5] stwintron, where the internal intron is nested in the donor of the external intron after nt 4 (5' GUGA|GU). In *L. suomiensis* and *L. japonicus*, the donors of the internal and external introns are separated by one nt (*L. japonicus*: 5' GUAAGUGGUAAGU) and a pair of canonical 3' splice sites (acceptor & lariat branch point motif) is available halfway the CIS: a [D7,8] configuration. Finally, in four other species (including *L. starkeyi* and *L. kononenkoae*), the donors of the CIS-constituent introns are abutting (5' GUACGUGUAAGU), leading to a [D6,7] configuration. Interestingly, the CIS in

these four species can be removed alternatively: it can be excised in one reaction using the proximal donor (5'-GUAAGU) and the distal canonical 3' splice site, or by consecutive reactions as a [D6,7] stwintron, using an imperfect 3' splice site halfway the CIS to define the internal intron, and the distal donor (5'-GUACGU) at the 5' splice site of the external intron. Our work provides clues to how stwintrons evolve endogenously, involving small duplications of (part of the) intron donor element at 5'.

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Transposable elements (TEs) in *Citrus*-associated *Phyllosticta* species

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Transposable elements (TEs) are important for fungal genome evolution, leading to changes in genomic structure and gene expression. TEs represent an additional source of genetic variability other than recombination, being related to accelerated evolution of genes involved in pathogenicity and host range. However, depending on the insertion site, TE proliferation can lead to deleterious effects. Thus, TE expansion may be limited by defense mechanisms, such as Repeat-Induced Point Mutation (RIP). RIP detects DNA duplications and permanently inactivates them with C:G to T:A mutations. TE dynamics differ between fungal species, and similarities in TE content tend to be related to lifestyle instead of phylogenetic relationships. In this sense, our goal is to investigate how TE dynamics influence the genome evolution of *Citrus*-associated *Phyllosticta* species. These species present endophytic and pathogenic lifestyles, are host specific or cosmopolitan and employ different reproduction strategies. They represent an interesting model for evolutionary and comparative genomics studies, and we aim to investigate and understand the biological processes involved with their associations with citrus plants. Our results show that the TE content in *Phyllosticta* genomes is low, ranging from 0.5 – 1%

in *P. capitalensis*, 2% in *P. citribraziliensis*, 2.5% in *P. paracitricarpa*, 3% in *P. citrichinaensis*, 8% in *P. citriasiana* and from 1.3 to 8% in *P. citricarpa*. From these TEs, more than 80% are Class I elements, mainly LTRs, but LINEs, SINEs and DIRS were also observed. Moreover, many of these TE locations show a strong RIP signature, suggesting that this defense mechanism may be involved in the low proliferation of TEs at *Phyllosticta* genomes. Further steps of our study on TE dynamics in *Phyllosticta* include an evaluation of inter and intra-specific variabilities of TE copies and a characterization of the insertion sites, to detect possible effects in gene evolution and gene expression.

A forest *Saccharomyces* population is robust to environmental changes

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Microorganisms, including yeasts, evolve remarkably quickly in laboratory environments. But do observations of rapid evolution in the laboratory apply to wild microbial populations? We investigated the response of a wild Saccharomyces paradoxus population to environmental changes in its native forest environment. *S. paradoxus* is the wild sister species of the model laboratory organism *S. cerevisiae*, and is an emerging model organism for ecology and evolution. The wild *S. paradoxus* population was robust to seasonal abiotic environmental changes and intraspecific competition: over the course of four seasons, the population's structure did not change and secreted intraspecific toxins did not select for resistant strains. The forest *S. paradoxus* population was also inbred, and we speculate that *S. paradoxus* individuals are well adapted to their changing environments such that monthly environmental changes are not effective selective pressures for the wild *S. paradoxus* population.

A macro-evolutionary perspective on long-distance mass transport in fungi

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The evolution of complex multicellular life implies the effective mass transport between spatially distant cells, leading to the evolution of specialized tissues and cells in animals and plants. Fungi can produce rhizomorphs, hyphal strands and cords (here Fungal Long-distance Transport Structures, FLTSs) that enable the transport of nutrients and water, have fundamental role in plant pathogenicity (e.g. *Armillaria* spp.), in the functioning of common mycorrhizal networks and in exploring and degrading wood material (e.g. white rot fungi). Despite of the essential biological functions and the immense importance of FLTSs, we have limited knowledge of its taxonomic distribution and macro-evolution.

Therefore, we reviewed more than 500 literature sources to discover the morphological and taxonomical diversity of FLTSs. We found that species in the phyla Ascomycota and Basidiomycota can develop FLTSs, but the most complex morphologies can be found in the Agaricomycetes. FLTSs are typical in most orders of Agaricomycetes, but they are most abundant in the Agaricales and Boletales. We performed macro-evolutionary analyses using a published mega-phylogeny (Varga *et al*. 2019, Nat. Ecol. Evol.) containing 5,284 species of mushroom-forming fungi (Agaricomycetes). We coded the presence or absences of FLTSs in species across the phylogeny and inferred character state transition rates using both maximum likelihood and Bayesian analysis in BayesTraits. We also estimated trait dependent diversification rates by using the BiSSE model in diversitree R package. We found, that despite the higher transition rate towards non-FLTS state than the reverse direction (i.e. loss of FTLSs), the diversification rate of species with FLTS is higher than species without it. Our work points out the importance and prevalence of long-distance nutrient transport among fungi and that the complex multicellular life affected similarly the body plan of fungi than that of plants and animals.

Population genomics of *Trichaptum abietinum* – a window into fungal speciation

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In spite of the immense species diversity of fungi, the genetic mechanisms underlying speciation remain less studied here than in animals and plants. Genetic studies have revealed that cryptic species are common in fungi, and suggests that reproductive barriers may arise rapidly. In the saprotrophic fungus *Trichaptum abietinum* (Basidiomycota, Hymenochaetales), reproductive barriers between two cryptic lineages in North America was found by Dr Ruth Macrae in the 1960s. Interestingly, both of these lineages were

partially interfertile with European samples. We aim to investigate the genomic basis of the reproductive barriers observed in *T.* abietinum to understand the speciation, and possibly reinforcement mechanisms, in this species complex. Furthermore, we explore the phylogeography of the species complex worldwide to compare and understand recurrent origins of reproductive barriers. We have obtained more than 800 samples from 90 populations, covering North America, Europe, and Asia. Monokaryotic cultures have been isolated from a selection of these samples for crossing experiments and whole genome Illumina sequencing. In our initial ITS barcoding of cultures, we have identified a European lineage, a Circumboreal lineage, and a North American lineage. Through crossing experiments of pairing monokaryons we have confirmed previous findings of the reproductive relationships among these lineages. Moreover, the European and Circumboreal lineages appear to be completely interfertile in Europe with frequent admixture. Here, we will present the first results from phylogeographic analyses on the whole genome sequence data and genomic scans for regions connected to the reproductive incompatibility.

Pathogen adaptations to host and climate in a wild plant-pathosystem

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In 2019, we still face major dilemmas concerning plant diseases and epidemics in both our agricultural fields and precious wilderness. Especially in large, uniform monocultures, proliferation is bolstered which in turn facilitates rapid adaptation of pathogen populations. On top of continuing arms races between hosts and pathogens, shifting environments due to climate change necessitate better strategies to combat and predict the risk of disease.

By evaluating agriculturally relevant pathogens in a natural system and identifying highly influential environmental factors, we have the potential to improve our ability to model and therefore predict disease dynamics. Wild tomato species, provide one such natural system. They occur in a variety of geographically distinct habitats from Ecuador to northern Chile. Many of these natural populations show a range of *Alternaria* blight symptoms, with some populations being more and others being less resistant. We want to understand how pathogen populations vary between host populations, how pathogen populations are structured, and whether we can find particular correlations between pathogen and host evolution. We have sampled over 250 Alternaria strains and

reconstructed their relationships using multi-gene phylogenetic analyses with ITS, *gadph* and RPB2.

Here we will present the latest results on their infectivity. We also perform genomic diversity analyses using whole genome resequencing data for the strains. In addition we share the first insight on chromosomal variation using long read sequencing on a subset of dominant strains. Given that wild tomao species occurs in a large range of unique environments, exhibit local adaptations and resistance variation, this system has the potential to provide a unique opportunity to study how genetic diversity naturally arises and how pathogens adapt to environment and host.

Transposable element diversity drives genome dynamics in the plant pathogenic fungus *VerGcillium dahliae*

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Transposable elements (TEs) are a major source of variaGon and play important roles in evoluGon. Many fungal pathogens have a so-called "two-speed" genome, with gene-dense and TE-poor regions harboring housekeeping genes, and gene-sparse and TE-rich regions containing fast evolving genes. *VerGcillium dahliae* is a fungal plant pathogen that causes disease on hundreds of plant hosts. The *V. dahliae* genome of strain JR2 contains four large lineage-specific (LS) regions. These are enriched for TEs and for in planta induced effector genes with important roles during host infecGon. Here we aim to determine the impact of TEs on the *V. dahliae* genome. To idenGfy dynamic TEs we exploited next-generaGon sequencing analysis of 42 *V. dahliae* strains. We idenGfied frequent presence/absence polymorphisms, collecGvely affecGng 54% of the annotated TEs in *V. dahliae* strain JR2. Three TE superfamilies (Mite, Copia, and Gypsy) are highly dynamic and account for half of the presence/absence polymorphisms. Transcriptome sequencing revealed that TEs belonging to these three TE superfamilies are also acGve (as determined by their expression), irrespecGve whether they localize in core or LS regions. While 35% and 15% of also acGve (as determined by their expression), irrespecGve whether they localize in core or LS regions. While 35% and 15% of all TE polymorphisms concern TEs located in LS and in centromeric regions, respecGvely, about 50% concern TEs located in the core genome. Moreover, we observed that acGve TEs in proximity of genes encoding different gene repertoires correlate with expression under both *in vitro* and in planta condiGons. As expected, within these catalogs, genes located in LS regions are significantly upregulated when compared to expressed genes in the core genome. InteresGngly, we observed an inverse relaGonship between TE acGvity based on expression and the frequency of TE polymorphisms. In this manner,

our analysis indicates that dynamic TEs contribute to genome plasGcity and gene expression in *V. dahliae*.

Poster Session 3.2
MOLECULAR TAXONOMY & PHYLOGENOMICS

WEDNESDAY, FEBRUARY 19

14:00 - 15:30 | Location: **Frentani Convention Center**

Characterisation of *Aspergillus* and *Penicillium* populations of pomegranate fruit by high resolution melting (HRM)

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Because of its nutraceutical and cosmeceutical properties, the consumption of pomegranate fruit and its derivatives and consequently the cultivation of this crop in Europe are increasing. Postharvest fruit losses caused by fungal pathogens are one of the main issues, even because of the risk of contamination by mycotoxins of both fresh and processed products. Indeed, for example, within *Penicillium* and *Aspergillus* genera there are species producing mycotoxins as ochratoxin A (OTA) and fumonisins, hazardous to human health, so that the European Commission regulates the relative thresholds for food and feed. In this investigation, two collections, one of *Penicillium* and one of *Aspergillus spp.*, from symptomatic pomegranate fruit of various cultivars were characterized. However, since their morphological identification at species level was not easy, particularly for *Aspergillus* species belonging to sect. *nigri*, and to avoid the misidentification between *Talaromyces* and *Penicillium* genera, a Talaromyces-specific PCR assay and two genus-specific and species-discriminating HRM assays were set up; moreover, the presence of OTA and fumonisin genes was evaluated. The 10% of the collection of *Penicillium* sensu lato proved to be made up of *Penicillium* sensu stricto strains. Furthermore, *P. glabrum* proved to be the most represented species. None of them seemed to produce OTA. Whereas within sect. nigri, *A. tubingensis* and *A. welwitschiae* were the most represented species, and several isolates of this last species seemed able to produce fumonisins.

Drivers of the biogeographical patterns of the endophytic fungal community in the roots of the Greek olive tree variety Koroneiki

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Olive trees are spread across the arid or semi-arid ecosystems of the Mediterranean basin. They are adapted to drought and low soil fertility and their cultivation has had historically an important role in the economy of olive oil producing countries, like Greece. In the present study we aim to shed light on the factors that determine the endophytic fungal diversity in the roots of the widespread Greek olive tree variety “Koroneiki” across different locations in Greece.

To explore the role of soil conditions and geographic location, we sampled roots from different trees derived from the same propagation origin, cultivated at seven distinct locations across Greece and during two seasons spring and autumn. The endophytic fungal diversity was determined by amplicon sequencing of the ITS2 region via Illumina Hiseq 2x250 bp.

Root endophytic fungal communities were found to differ significantly between the sampled locations (observed richness & Shannon). PERMANOVA showed that location explained 33.7% (p<0.001) of the community dissimilarity (Bray-Curtis), while season explained only 2.2% (p<0.001). Distance-decay analysis revealed a strong increase between the communities dissimilarity and geographical distance (r_{mantel}:0.44,p<0.001), underlying an important role of deterministic processes in the assembly of these communities, potentially environmental gradients or dispersal limitations. From the monitored soil parameters electrical conductivity (r_{mantel}:0.1,p<0.05), organic matter (r_{mantel}:0.13,p<0.01) and exchangeable Fe (r_{mantel}:0.28,p<0.001) correlate with root endophytic fungal communities. Beside the biogeographical variation, we identified a core root endophytic fungal community, represented by 67 OTUs.

Our study constitutes the first attempt to elucidate the factors determining endophytic fungal community assemblage in olive tree roots.

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Limited DNA repair gene repertoire in Ascomycete yeast revealed by comparative genomics

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Ascomycota is the largest phylogenetic group of fungi that includes species important to human health and wellbeing. DNA repair is important for fungal survival and genome evolution. Here, we describe a detailed comparative genomic analysis of DNA repair genes in Ascomycota. We determined the DNA repair gene repertoire in Taphrinomycotina, Saccharomycotina, Leotiomycetes, Sordariomycetes, Dothideomycetes and Eurotiomycetes. The classes of yeasts, Saccharomycotina and Taphrinomycotina, have a smaller DNA repair gene repertoire comparing to Pezizomycotina. Some genes were absent from most, if not all, yeast species. To study the conservation of these genes in Pezizomycotina, we used the GLOOME algorithm that provides the expectations of gain or loss of genes given the tree topology. Genes that were absent from most of the species of Taphrinomycotina or Saccharomycotina showed lower conservation in Pezizomycotina. This suggests that the absence of some DNA repair in yeasts is not random; genes with a tendency to be lost in other classes are missing. We ranked the conservation of DNA repair genes in Ascomycota. We found that Rad51 and its paralogs were less conserved than other recombinational proteins. This suggests that there is a redundancy between Rad51 and its paralogs, at least in some species. Finally, based on the repertoire of UV repair genes, we found conditions that differentially kill the wine pathogen *Brettanomyces bruxellensis* and not *Saccharomyces cerevisiae*. In summary, our analysis provides testable hypotheses to the role of DNA repair proteins in the genome evolution of Ascomycota.

CAZymes associated with adaptation of basal fungi to their lifestyle

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Carbohydrate-active enzymes (CAZymes) are enzymes involved in the synthesis, metabolism, and transport of carbohydrates. Fungal CAZymes play a great role in the decomposition of biomass and fungal cell wall re-arrangement. In consequence, these enzymes are involved in the host-fungus interactions and they play a pivotal role in nutrient acquisition.

Analysis of the genome sequences of ECM fungi showed a lack of many classes of secreted enzymes involved in plant cell wall degradation. Not only plant symbionts have developed such adaptations. Genomes of saprotrophic fungi contain many homologs of laccases and peroxidases necessary for wood degradation. Animal parasites evade host's immune system hiding behind polysaccharide walls.

Body of knowledge of Dikarya CAZymes is well established, but still little is known about their distribution among evolutionary older lineages. This work focuses on the recognition of key CAZymes families associated with adaptation of basal fungi to their lifestyle and to trace their evolution within the kingdom Fungi.

To achieve this goal we identified the CAZymes with significant differences in family size among fungi with diverse ecological properties. Gene count differences are a strong but not sole determinant of the genomic basis of ecological capabilities. In order to strengthen our line of evidence, transcriptomic information was used (if available). We used all genomic assemblies of basal fungi available at GeneBank in 2018. Sequence alignments were manually inspected to discard inactive homologs. Phylogenetic inference showed the individual expansions of paralogs in particular organisms. Most significant results were obtained for acetylesterases, xylanases, galactosamidases, and chitinases.

Our results show that expansions of particular families of proteins possibly contribute to the ecology basal fungi. We also broaden current knowledge about distribution, potential role, and evolution of carbohydrate-active enzymes in basal fungi.

Exploring *Ciborinia camelliae* diversity

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Ciborinia camelliae Kohn is the causal agent of camellia flower blight on plants of the genus *Camellia*. The fungus belongs to the Ascomycota family of Sclerotiniaceae. It has been reported in Japan, North America, Europe, Asia and Oceania. It is an interesting fungus from the phytopathological perspective as it is able to specifically colonize camellia producing molecules with potential insecticidal properties.

Fungal diversity of a collection of *Ciborinia camelliae* (n= 68), including Australasian and European strains was assessed. Multilocus sequence typing using rDNA internal transcribed spacer (ITS1,5.8S, ITS2), beta-tubulin (TUB), elongation factor (EF1), and

Glyceraldehyde 3-phosphate dehydrogenase (GPDH) sequences was carried out to correctly define phylogenetic position of the isolates. Morpho-phenotypic analysis on four culture media at six different temperatures and UP-PCR polymorphism (primer combination n=6) allowed to assess the level of diversity within the species.

MLST showed high uniformity among Italian and European isolates, but significant differences with respect to isolates from China and New Zealand. UP-PCR allowed to discriminate isolates from the same collection site without strong correlation with morphotypes. Further studies aimed at characterising worldwide population are warranted.

Genetic diversity within *Colletotrichum lupini,* the causal agent of lupin anthracnose, and its virulence on white lupin (*Lupinus albus)*

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White lupin (*Lupinus albus*) is a grain legume known for its high protein content and quality, efficient nutrient acquisition and health benefits (Lambers *et al*., 2013, Arnoldi *et al*., 2015). Its high yield potential could make it a sustainable alternative to soybean in cooler regions (Lucas *et al*., 2015). However, since the 1980s anthracnose disease, caused by the air- and soil-borne fungal disease *Colletotrichum lupini*, threatens lupin cultivations worldwide (Nirenberg *et al*., 2002, Damm *et al*., 2012, Talhinhas *et al*., 2016). Even low levels of seed infestation can lead up to total yield loss (Thomas 2004, Diggle 2002). To assist white lupin breeding programs, we analyzed the genetic diversity of globally collected lupin-infecting *Colletotrichum* isolates by multi-locus sequencing (Pecchia *et al*., 2019, Dubrulle *et al*., 2019). First analyses indicate that all isolates belong to the species *C. lupini* and that the genetic diversity of isolates collected from Europe and Australia is lower compared with isolates collected from the South American Andes, showing different genetic groups. An indoor screening assay was developed and validated by field performance, allowing to determine differences in virulence between *C. lupini* strains under controlled conditions. Currently, virulence tests of selected *C. lupini* isolates are being performed on two white lupin cultivars, the susceptible variety Feodora and the tolerant breeding line Blu-25 from Erik von Baer. Our study will shed light on the genetic makeup of the species *C. lupini* and its relation to virulence on white lupin, thereby providing valuable information to improve white lupin breeding programs.

Taxonomy and diversity of Diaporthe endophytic species from Pantanal and Cerrado biomes in Brazil

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Endophytic fungi from several genera have been explored as a source of metabolites with biotechnological potential. Among them is the *Diaporthe* genus, with many species found around the world as saprobic, pathogenic and endophytes on a wild range of hosts. A previous study performed by our research group identified endophytes from the medicinal plants *Vochysia divergens* located in Pantanal and Stryphnodendron adstringes in Cerrado – Brazil. The isolates were grouped by morphological analysis and *Diaporthe* was the predominant genus colonizing the medicinal plants. . Here we intend to study the phylogenetic diversity of these isolates to determine the relationship among them and others species already described. We identified 150 strains from *Diaporthe* genus through Bayesian inference and maximum likelihood (ML) phylogenetic analyses, using a partial fragment of the translation elongation factor 1-alpha (TEF1). The isolates are distributed in 20 different clades in *Diaporthe* genus, nine known species, *D. infertilis, D. batatas, D. racemosae, D. oxe, D. inconspicua, D. pterocarpi, D.* cf *heveae* 1 e 2 and 42 of them clustered close to *Diaporthe* sp. 1. We intend to further explore the genetic diversity of these isolates, to determine which isolates are most closely related. Moreover, we will also perform multi-locus phylogeny and morphological analysis for evaluating the forty possible new species in our sample.

Genome sequence of *Ganoderma lucidum* (Curtis) P. Karst. from Finland

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Ganoderma lucidum (Curtis) P. Karst. is a saprophytic species recognized for its bioactive properties. These properties have been associated with the synthesis of primary and secondary metabolites. So far, more than 400 bioactive compounds have been identified from this species. The current taxonomy of *G. lucidum* from Europe and Asia, as well as other Ganoderma species are not well resolved, leading to confusion

with species identification. The recent advances in molecular technologies that includes whole genome sequencing have allowed more accurate taxonomic identification. It also provides opportunities to explore the bioactivity potential of many fungi based on analyzing a larger range of genes identified directly from the genomes. Our aim was generate a draft genome sequence for *G. lucidum* to aid with the accurate taxonomic identification and to explore its bioactive potential. This was achieved by a combination of Nanopore long-read and Illumina sequencing technologies. Genome sequencing and assembly resulted in an assembled genome consisted of 18 scaffolds, with scaffolds N50 of 3.46 Mb, and an estimated genome size of 43.15 Mb. The genome sequence will be use to clarify the taxonomy of *G. lucidum* from Asia and Europe, and to identify secondary metabolite gene clusters present in this species.

Activation of Silent Gene Clusters in *Aspergillus nidulans* Using Hybrid Transcription Factors

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Fungal secondary metabolites (SMs) represent a vastly underexploited source of clinically and industrially relevant molecules. Unfortunately, the majority of putative SM gene clusters are silent under standard laboratory conditions, underscoring the need for the development of techniques that can lead to their activation. This study aimed to activate three silent gene clusters in *Aspergillus nidulans* through the engineering of hybrid transcription factors (TFs). In this approach, the DNA-binding domains (BDs) targeting three gene clusters were maintained, while the native activation domains (ADs) were replaced with the highly active AD of the asperfuranone gene cluster. This approach successfully resulted in the increased production of several SMs.

Identification of toxigenic fungal species associated with maize ear rot: calmodulin as single informative gene

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Accurate identification of fungi occurring on agrofood products is the key aspect of any prevention and pest management program, offering valuable information in leading crop health and food safety.

Fungal species misidentification can dramatically impact biodiversity assessment, ecological studies, management decisions, and, concerning toxigenic fungi, health risk assessment, since they can produce a wide range of toxic secondary metabolites, referred to as mycotoxins. This can be especially important with maize, since it is a valuable food resource globally. Since each toxigenic fungal species can have its own mycotoxin profile, a correct species identification, hereby attempted with universal DNA barcoding approach, could have a key role in mycotoxins prevention strategies. Currently, identification of single marker for fungi has not been achieved and the analysis of multiple genes is used, with the advantage of an accurate species identification and disadvantage of difficult setting up of PCR-based diagnostic assays.

In the present paper, we describe our strategy to set up DNA-based species identification of fungal species associated with maize ear rot, combining DNA barcoding approach and species-specific primers design for PCR based assays. We have (i) investigated the appropriate molecular marker for species identification, limited to mycobiota possibly occurring on maize, identifying calmodulin gene as single gene taxonomically informative; (ii) designed 17 set of primers for rapid identification of 14 *Fusarium*, 10 *Aspergillus*, 2 *Penicillium*, and 2 *Talaromyces* species or species groups, and finally (iii) tested specificity of the 17 set of primers, in combination with 3 additional set previously developed.

Poster Session 3.3
APPLIED AND ENVIRONMENTAL MICROBIOLOGY

THURSDAY, FEBRUARY 20

14:00 - 15:30 | Location: **Frentani Convention Center**

Exoproteome and transcriptome of a potential new fungal cell factory, *Penicillium subrubescens*: Target specific biomass degrading enzyme production

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Over a century, fungal cells have been used for the production of enzymes and metabolites in many industries, e.g. food & feed, paper & pulp, textiles, and more recently biofuels & biochemicals. For the latter application, they play an important role as producers of plant cell wall degrading enzymes to deconstruct plant biomass into its monomeric units. However, only limited fungal species have been developed as fungal cell factories, namely *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei* and *Myceliophthora thermophila*. This is partly due to the narrow substrate range, low enzyme production level, low secretion capacity, toxin producing, and poor behavior under fermentation conditions of other species preventing them to be attractive for industrial-scale development.

Recently, *Penicillium subrubescens*, isolated from soil of a Jerusalem artichoke field in Helsinki, Finland, was evaluated for its ability to produce plant cell wall degrading enzymes. *P. subrubescens* showed significant potential as its enzyme production levels and saccharification abilities were similar to that of the industrial species *A. niger*[1]. The genome sequence of *P. subrubescens* revealed expansions in specific CAZy families related to hemicellulose, pectin and inulin degradation[2]. Exoproteomic and transcriptomic analyses on two common feedstocks revealed that *P. subrubescens* produced highly specific plant biomass degrading enzyme sets matching the composition of the two

substrates. These results make *P. subrubescens* a highly promising candidate novel potential fungal enzyme factory.

This work was supported by the European Union, Grant agreement no: 613868 (OPTIBIOCAT)

1. Mäkelä, *et al*., 2016. *N. Biotechnol.* 33:834-841

2. Peng, *et al*., 2017. *J. Biotechnol*. 246: 1-3

GalR, GalX and AraR co-regulate D-galactose and L-arabinose utilization in *Aspergillus nidulans*

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Filamentous fungi produce a wide variety of enzymes in order to efficiently degrade plant cell wall polysaccharides. The production of these enzymes is controlled by transcriptional regulators, which can also control the catabolic pathways of the released monosaccharides. Two transcriptional regulators, GalX and GalR, control D-galactose utilization in the model filamentous fungus *Aspergillus nidulans*, while the arabinanolytic regulator AraR regulates L-arabinose catabolism. D-galactose and L-arabinose are commonly found together in polysaccharides, such as arabinogalactan, xylan and rhamnogalacturan-I. Therefore, the catabolic pathways that convert D-galactose and L-arabinose likely often be active simultaneously.

In this study, we investigated the possible interaction between GalX, GalR and AraR in D-galactose and/or L-arabinose catabolism. For this, we generated single, double and triple mutants of the three regulators using CRISPR/Cas9 technology, and analyzed their growth, enzyme and gene expression profiles. These results clearly demonstrated that GalX, GalR and AraR co-regulate D-galactose catabolism in *A. nidulans*.

Combinatorial control of transcription factors involved in sugar beet pulp utilization in the industrially relevant fungus *Aspergillus niger*

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Degradation of plant biomass polysaccharides into simpler and fermentable sugars is crucial for many industrial and biotechnological applications. Filamentous fungi produce a wide variety of extracellular enzymes that target the main cell wall polysaccharides, cellulose, hemicellulose, and pectin. The complex structure of the cell wall requires the synergistic interaction of multiple hydrolytic and oxidative enzymes for its degradation. Fungi regulate the production of these enzymes at transcriptional level to ensure a space-time balanced and optimized enzyme production. So far, several transcription factors (TFs) involved in the regulation of plant biomass utilization have been characterized in fungi, however, only a few are conserved among ascomycetes. Previous studies revealed that the TFs GaaR, AraR and RhaR all contributed to the regulation of pectin degradation in *Aspergillus niger*, with GaaR playing the most dominant role. The present study aims to expand on this topic by studying the contribution of a more complex set of TFs in sugar beet pulp utilization, a by-product of the sugar-refining industry from which sugar beet pectin is obtained. For this purpose, we used the CRISPR/Cas9 genome editing technology in order to generate a combination of single and multiple deletant strains in six different regulators involved in plant biomass degradation: XlnR and ClrB, which are mainly involved in (hemi-) cellulose degradation, RhaR, GaaR and GalX, mainly involved in pectin degradation, and AraR that is involved in both pectin and hemicellulose degradation. The growth phenotype and protein production profiles of the mutant strains were analyzed, and several enzyme activity and saccharification assays were performed in order to determine the relative importance of each regulator in the process of sugar beet pulp utilization in *A. niger*.

Multi-omics analysis of wood-dependent induction of lignocellulolytic enzyme secretion in cultures of the white-rot basidiomycete *Pleurotus ostreatus*

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Fungi interact with their environment by secreting proteins to obtain nutrients, elicit responses and modify their surroundings. The white-rot basidiomycete *Pleurotus ostreatus* degrades lignocellulose attacking the recalcitrant lignin using a set of oxidative secreted enzymes. To produce a comprehensive picture of its strategy to degrade lignocellulose, we bioinformatically identified 538 and 554 secretable proteins in the genome of the monokaryotic strains mkPC9 and mkPC15 and classified them as proteins with unknown functions (37.2%), glycosyl hydrolases (26.5%) and redox enzymes (11.5%). Then we combined these results with RNA-seq analyses, which showed that the relative importance of each group varied in different strains and culture conditions. Only a few genes were actively expressed in a given culture condition in expanded multigene families, suggesting that family expansion could increase adaptive opportunities rather than activity under a specific culture condition. Finally, we studied the experimental secretome complexity and lignocellulose degrading capacity of mkPC9 and mkPC15 and their mated dikaryon dkN001 in cultures containing wood, glucose, and wood plus glucose as carbon sources. The study revealed that P. ostreatus secretes a variety of glycoside hydrolases, carbohydrate esterases, and polysaccharide lyases, especially when wood was the unique carbon source. The presence of wood increased the secretome complexity whereas glucose diminished the secretion of enzymes involved in cellulose, hemicellulose and pectin degradation. On the contrary, the presence of glucose did not influence the secretion of Red-Ox enzymes or proteases, which shows the specificity of glucose on the secretion of lignocellulolytic enzymes. The comparison of the secretomes of monokaryons and dikaryons reveals that secretome complexity is unrelated to the nuclear composition of the strain.

Development of an improved menopausal symptom-alleviating licorice (*Glycyrrhiza uralensis*) by biotransformation using *Monascus albidulus*

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Licorice (*Glycyrrhiza uralensis*) contains several compounds that have been reported to alleviate menopausal symptoms *via* interacting with estrogen receptors (ERs). The compounds exist mainly in the form of glycosides, which exhibit low bioavailability and function. To bioconvert liquiritin and isoliquiritin, the major estrogenic compounds, to the corresponding deglycosylated liquiritigenin and isoliquiritigenin, respectively, licorice was fermented with *Monascus,* which has been demonstrated to deglycosylate other

substances. The contents of liquiritigenin and isoliquiritigenin in *Monascus*-fermented licorice increased by 10.46-fold (from 38.03 μM to 379.75 μM) and 12.50-fold (from 5.53 μM to 69.14 μM), respectively, compared with their contents in non-fermented licorice. *Monascus*-fermented licorice exhibited 82.5% of the ERβ binding activity of the activity observed in the positive control (17 β-estradiol), whereas the non-fermented licorice exhibited the 54.1% of the binding activity in an *in vivo* ER binding assay. The increase in the ERβ binding activity was associated with increases in liquiritigenin and isoliquiritigenin contents. Liquiritigenin acts as a selective ligand for ERβ, which alleviates menopausal symptoms with fewer side effects, such as heart disease and hypertension, compared with a ligand for ERα. In addition, *Monascus*-fermented licorice contained 731 mg/kg of monacolin K, one of the metabolites produced by *Monascus* that reduces serum cholesterol. Therefore, *Monascus*-fermented licorice is a promising material for the prevention and treatment of menopausal syndrome with fewer side effects.

Fungal melanin-based electrospun membranes for heavy metal detoxification of water

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In recent years, heavy metal pollution in water resources has become a severe environmental and public health problem worldwide. Thereby, enhanced treatments are urgently needed with respect to eco-friendliness, filtration efficiencies and low operational costs. In this study, fungal melanin extracted from the fungus *Armillaria cepistipes* (Empa 655)[1] was applied as a promising biosorbent for heavy metals removal from water systems. For this aim, an electrospinning technique to incorporate fungal melanin particles based on the rheological behavior and electrical conductivity was developed. Produced membranes were characterized according to their morphological and surface properties. Metal adsorption assays were then performed on both melanised membranes and raw melanin for comparison. At the physiotoxic concentrations of Pb^{2+}, Cd^{2+}, Ni^{2+} and Cr^{2+}, fungal melanin was able to remove more than 90% of heavy metals in single-component solutions. In multi-component solutions (containing Ca^{2+} and Zn^{2+}), fungal melanin showed a different affinity to the studied metals with the following order: Pb^{2+}>Cr^{3+}>Ni^{2+}>Cd^{2+}>Zn^{2+}>Ca^{2+}. An extreme preference for Pb^{2+} (80% removal) over the essential metals (0% and 12% removal for Ca^{2+} and Zn^{2+}, respectively) was observed. The metal adsorption performance of melanised membranes did not demonstrate significant differences compared to the adsorption capacity of the raw melanin. Thus, it was

concluded that these novel melanised membranes can be efficiently used as filtration method for removal of heavy metals from water system.[2]

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2. Tran-Ly A.N., Ribera J., Schwarze F.W.M.R., Brunelli M., Fortunato G. (2019) Fungal Melanin-Based Electrospun Membranes for Heavy Metal Detoxification of Water. SM&T. *Accepted.*

Stress tolerance in microcolonial black fungi can be studied with new techniques: presenting a genetic toolbox for *Knufia petricola*

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After their discovery on rock surfaces in cold and hot deserts, a polyphyletic group of ascomycetous black fungi was found to dominate a range of hostile environments – natural and man-made, from salterns to dishwashers, roofs and solar panels. Together with bacteria and algae they may establish subaerial biofilms and cause weathering of the surfaces they grow on. Their impressive survival abilities as well as their constitutive protective pigmentation and cluster-like microcolony organisation are similar in environmental isolates as well as in heat-tolerant opportunistic pathogens of animals and humans. The exact genetic properties that ensure their survival in extreme environments can be studied if some black fungi were amenable to genetic manipulations. We selected the rock-inhabiting fungus *Knufia petricola* (class Eurotiomycetes, order Chaetothyriales) that grows moderately in axenic culture and exhibits all the characteristics of microcolonial black fungi such as yeast-like cell growth, absence of reproductive structures and constitutive dihydroxynaphthalene (DHN) melanogenesis (Nai *et al*. 2013, *Fungal Genet Biol*). We developed protocols to efficiently generate and transform protoplasts resulting in stable homokaryotic transformants by targeting genes involved in pigment synthesis and expressing fluorescent reporter genes. Hence, endogenous and foreign genes can be expressed from episomal AMA1-containing plasmids and genome-integrated DNA constructs. Moderate rates of homologous recombination allow for both ectopic and

targeted integrations. CRISPR-Cas9 was further validated as a strategy for obtaining selection marker-free mutants and silencing via RNA interference as an approach to study essential genes. Availability of this genetic toolbox and an annotated genome sequence of the strain A95 is paving the way for studying interactions of *K. petricola* with environmental stressors, material surfaces, soil matrices and phototrophic symbionts.

Comparative genetic and transcriptome analyses of *pex1* and *gat1* single-gene mutants between *Ceriporiopsis subvermispora* and *Pleurotus ostreatus*

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Wood biomass is primarily composed of cellulose, hemicellulose and lignin. In nature, white-rot fungi degrade wood lignin almost exclusively. Recently, several genes in which mutations caused significant defects in wood lignin degradation by *Pleurotus ostreatus* were identified. For examples, *pex1* encoding a peroxisome biogenesis factor and *gat1* a putative Agaricomycetes-specific transcription factor. In this study, we examined the effects of single-gene mutations in *pex1* and *gat1* on wood lignin degradation by another white-rot fungus, *Ceriporiopsis subvermispora*, with the aim of investigating conserved and diverse mechanisms underlying wood lignin degradation among white-rot fungi. *C. subvermispora pex1* and *gat1* single-gene mutant strains were generated from monokaryotic wild-type strain by CRISPR/Cas9. We designated these strains as *Cspex1*m#1 and *Csgat1*m#1, respectively. To examine their abilities to degrade wood lignin, each strain was grown on beech wood sawdust medium (BWS) for 10 days and 20 days. It was shown that their abilities to degrade lignin present in BWS were lower than that of their parental strain. We then performed RNA-seq analysis to examine the effects of each gene mutation on transcriptional expression of wood-degrading enzyme genes when grown on BWS for 10 days. Three putative ligninolytic enzyme genes, *mnp4*, *mnp6* and *mnp7*, of which transcripts accumulate predominantly in parental strain grown on BWS, were significantly downregulated in *Csgat1*m#1. This result is similar to that in *P. ostreatus gat1* mutant strains. However, unlike the case of *P. ostreatus*, aforementioned three *mnp* genes were not downregulated in *Cspex1*m#1. A significant upregulation of cellulolytic and xylanolytic enzyme genes was not found in *Cspex1*m#1 and *Csgat1*m#1, which is also not similar to the results in *P. ostreatus*. RNA-seq analysis using total RNA obtained from each strain grown on BWS for 20 days are now being performed.

Draft genome sequences and annotation of *Trichoderma lixii* and *Trichoderma capillare* isolated from PAH-contaminated soil and industrial wastewaters

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Several *Trichoderma* species (Hypocreaceae) can synthesize molecules of high biotechnological value, including antifungal compounds and Carbohydrate-Active enzymes (CAZymes). Moreover, some *Trichoderma* species have the ability to tolerate toxic compounds and could be used in mycoremediation. *Trichoderma lixii* MUT3171 and *Trichoderma capillare* MUT5453, both deposited at the Mycotheca Universitatis Taurinensis (MUT) of the Department of Life Sciences and Systems Biology, University of Turin (Italy), were isolated from a soil contaminated with polycyclic aromatic hydrocarbons (PAHs) and from a industrial wastewater, respectively.

To evaluate their mycoremediation potential, we sequenced the genomes of both fungi, and performed preliminary explorations of their gene reservoirs. *T. lixii* (40.94 Mbp) and *T. capillare* (39.24 Mbp) encode for 11,920 and 11,491 genes, respectively. We predicted a high number of secreted proteins (858 and 849), with a considerable amount of small-secreted effectors. The other portion of the secretome consisted in a rich arsenal composed of ferroxidases, monoxygenases, CAZymes, lipases and proteases. In addition, both fungi produce biosurfactants such as hydrophobins. While evaluating the potential drawbacks of employing these fungi in mycoremediation, we found that they may synthesize the mycotoxins citrinin, alternariol and leucinostatin, but further confirmation is required. As an added value to their biotechnological potential, both fungi may be able to produce compounds of pharmaceutical interest such as antibiotics and antitumor molecules. Finally, through a genome-scale phylogeny, we show that *T. lixii* and *T. capillare* diverged from their closest sequenced relative, *T. harzianum*, around 4 MYA, and investigate the trajectory of their gene family evolution, which probably resulted in their adaptation to polluted environments.

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Functional diversification of cellobiose dehydrogenases uncovers their involvement in multiple nutritional strategies of the mycoparasite *Clonostachys rosea*

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Clonostachys rosea (Hypocreales, Bionectriaceae) is an ubiquitous fungus that colonizes living plants, digests organic material in soil (saprotroph) and parasitizes on or kills other fungi (necrotrophic mycoparasite) or nematodes, and is thus of interest as a biological pest control agent. We recently found a high number of genes coding for cellobiose dehydrogenases (CDHs, EC 1.1.99.18; AA3_1) in *C. rosea* genome compared to significantly reduced or even missing gene content in saprotrophs such as *Neurospora crassa* and *Trichoderma reesei* and in other *Trichoderma* mycoparasites. Structural analysis of CDH enzymes in *C. rosea* revealed members in AA3 and AA3/AA8 CAZy families, from which two enzymes also possess fungal cellulose binding domains CBM1.

CDHs are extracellular enzymes produced by various wood-degrading fungi, as they are proposed to be involved in cellulose, hemicellulose and lignin biodegradation. However, the functional transcript analysis in the mycoparasite *C. rosea* revealed activation of almost all *cdh* genes during self-recognition, sensing and contact with the host fungus as well as during the growth on cellulolytic substrates. The differential expression analysis indicated specific involvement of CDHs in mycoparasitism, and in degradation of simple and complex cellulolytic compounds. Furthermore, two CDH enzymes, both possessing a carbohydrate binding domain, were detected on microcrystalline cellulose in a time-dependent proteome of *C. rosea*.

Expression profiles of amylolytic genes in the black *koji*-mold *Aspergillus luchuensis*

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The black *koji*-mold *Aspergillus luchuensis* that belongs to *Aspergillus* section *Nigri* has been employed in the production of traditional Japanese spirits (*awamori* and *shochu*) manufactured mainly in southern districts of Japan, and produces a large amount of several amylolytic enzymes. In contrast to the yellow *koji*-mold *Aspergillus oryzae*, *A. luchuensis* produces two types of α-amylases, i.e. acid-unstable (AmyA) and acid-stable (AsaA) α-amylases. In this study, we examined the expression profiles of these α-amylase genes to elucidate the regulatory mechanisms for amylolytic gene expression in *A. luchuensis*.

We first constructed the disruptants for the amylolytic transcriptional activator gene amyR and carbon catabolite repressor gene creA in *A. luchuensis*. The amyR disruptant showed almost no growth but interestingly could form a clear zone around the inoculated area on the starch medium, suggesting that AmyR is essential for starch assimilation but not for α-amylase production. The expression of asaA was abolished but that of amyA was unaffected by amyR disruption in the presence of starch. Furthermore, amyR disruption resulted in loss of the expression of the glucoamylase gene glaA and α-glucosidase gene agdA, which can lead to the growth phenotype of ΔamyR on the starch medium. The creA disruptant showed the upregulation of asaA, glaA, and agdA, but not of amyA. These results indicated that three amylolytic genes, asaA, glaA, and agdA, are regulated by AmyR and CreA, but amyA is not regulated by these transcription factors and constitutively expressed regardless of the carbon source species.

Boosting plant growth: fungal metabolites as biostimulants for growth promotion of *Hypericum perforatum* (L.)

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Fungal culture filtrates have been reported in literature [1] as effective biostimulants for the plant growth. Studies on fungal strains metabolism showed that the metabolites produced in cocultures were clearly different from single cultures [2]. Therefore, *Chaetomium globosum* and *Minimedusa polyspora* showing characteristics with a high potential for plant growth promotion were incubated in single cultures and cocultures in two different culture media (Malt extract agar and Murashige & Skoog) for 15 days. The culture filtrates were applied to in vitro root cultures of *Hypericum perforatum* to test the effect in terms of biomass production. The culture filtrates were analysed by NMR-based metabolomics to identify the metabolites released by the fungi. Through the analyses were detected, identified and quantified about 30 low weight molecules belonging to the classes of: aminoacids (e.g. valine, GABA, phenylalanine), organic acid (e.g. acetic acid,

fumaric acid), alcohols (e.g. ethanol, 2,3-butanediol), carbohydrates and other molecules. Several of the identified metabolites may be of interest for the plant growth stimulation.

1. Bagde *et al*., 'Influence of culture filtrate of *Piriformospora indica* on growth and yield of seed oil in *Helianthus annus*', Symbiosis, vol. 53, no. 2, pp. 83–88, May 2011

2. Bertrand *et al*., 'De Novo Production of Metabolites by Fungal Co-culture of *Trichophyton rubrum* and *Bionectria ochroleuca*', Journal of Natural Products, vol. 76, no. 6, pp. 1157–1165, Jun. 2013

Ecophysiology and applied biodiversity of phillosphere fungi in tropical and subtropical forests

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The phyllosphere microbiomes are exceptionally taxonomically diverse in canopies of tropical and subtropical forests where trees do not shed leaves annually. The adaptation of epiphytic and endophytic microorganisms to their habitat requires the development of such specialized functions for an efficient attachment to the leaf surface, resistance to oxidative stress and survival in the oligotrophic environment. To solve these ecological challenges, phyllosphere microorganisms produce a diversity of secondary metabolites, secrete enzymes that hydrolyze lignocellulose and cutin and adapt to stress. We therefore hypothesize that phyllosphere fungi may have a number of potentially beneficial properties suitable for applications in biotechnology and pharma.

In this study, we focused on the diversity and ecophysiological properties of over 450 filamentous and unicellular fungi isolated from the two evergreen forests: the tropical rain forest on Borneo and the moist subtropical forest of Huangshan mountains (China). The resulting collection of over 350 tropical and 120 subtropical fungi was subjected to the molecular identification and screened for the ability to produce antimicrobial compounds and biodegradable polyesters (PCL). DNA barcoding resulted in the identification of only 70% of tropical and 90% of subtropical isolates. Up to 10% of isolates were not attributed

to genera and some represented putatively novel families. Although many generalist fungi such as *Trichoderma spp., Xenoacremomiun spp., Penicillium spp., Mucoromycotina* and members of *Saccharomycetales* were isolated, many fungi belonged to plant pathogens or had unique physiology. In particular, we detected several novel dimorphic melanised 'black yeast' fungi with a remarkable antifungal potential. The majority of the isolated fungi were able to degrade cellulose and cutin, while the ability to degrade PCL was higher in fungi from the tropical rain forest (10 – 15%) compared to subtropical strains (5%).

Antifungal potential of endophytes from brazilian medicinal plants against the *Colletotrichum abscissum* and *Phyllosticta citricarpa*

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Endophytic fungi associated with medicinal plants have been shown to possess a high potential to produce new bioactive metabolites, to be used in the pharmaceutical and agronomical industries. In the present study we investigated the antifungal potential of fungal endophytes isolated from the medicinal plants, *Vochysia divergens* and *Stryphnodendron adstringens*, located in two hotspot biome in Brazil, Pantanal and Cerrado. The biotechnological potential was evaluated against two important citrus pathogens, *Colletotrichum abscissum* and *Phyllosticta citricarpa*. The endophytes were isolated from 19 plants of *V. divergens* (Pantanal biome) and 20 plants of *S. adstringens* (Cerrado biome), from which five mature, green, asymptomatic leaves and petioles were randomly collected in January of 2018. The isolates were grouped in morphotype based on the macroscopic and microscopic characteristic, such as shape, size, color, texture, and growth pattern. One strain was selected as a representative of each morphotype and studied concerning to activity against the plant pathogens. A total of 1304 fungi were isolated and assigned to 159 distinct morphotypes. Among the 159 representative strains, twelve endophytic fungi produced metabolites that inhibited the mycelial growth (IG) of *Colletotrichum abscissum* (≥40%), the greats result was observed for the extract from strain *Pseudofusicoccum stromaticum* CMRP4328 (IG: 83%). In the analysis of the minimal inhibitory concentration (MIC) the extract from strain *Diaporthe* sp. 2 CMRP4322 showed the lowest MIC, 0.001 mg/mL that inhibited the germination of *C. abscissum* conidia. Against the phytopathogen *Phyllosticta citricarpa*, seven extracts inhibited more than 70% the mycelium growth, and the highest inhibition was performed by metabolites produced by *Diaporthe* sp.4 CMRP4330 (IG: 92%). This data reinforce the ability of medicinal plants found in Brazil to host a diverse group of endophytic fungi with

biotechnological potential.

Bioremediation of polluted soils - the role of fungi

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Oil hydrocarbons, including n-alkanes, cycloalkanes and polycyclicaromatic hydrocarbons (PAHs) are the most persistent and widespread environmental pollutants, thus representing a serious ecological threat. The EU is becoming more and more aware of the need to find effective and environmental-friendly technologies for the treatment of contaminated sites. Bioremediation is a biological approach that relies on the metabolic potential of microorganisms to degrade contaminants. So far most of the attention has been dedicated to bacteria, even though their combination with fungi and plants may enhance the total organic pollutants removal. This approach has been here validated in a heavily polluted site in Italy, recognized as a Site of National Interest. The microbial community of the soil was studied by applying both culture-dependent and culture-independent (Next Generation Sequencing) methods: fungi belonged mainly to Ascomycetes such as *Cladosporium, Aspergillus, Penicillium, Fusarium, Scedosporium, Trichoderma* and *Epicoccum*, while bacteria were ascribable to the genera *Pseudomonas, Sohingobacterium, Pseudoxanthomonas, Rhizobium* and *Acinetobacter*. Several fungal and bacterial strains here isolated were capable of using pyrene, phenanthrene and alkanes as carbon source, indicating a high level of adaptation of these organisms. Microcosms and mesocosms trials were set up: bacteria and fungi were both inoculated in batches of polluted soil and the bioremediation yields were evaluated from a chemical and ecotoxicological point of view. Pollutants concentration was significantly reduced together with the toxicity of the samples. Finally, the ability of plants to germinate, grow and reduce the content of organic pollutants was considered. *Sorghum bicolor, Trifolium repens, Zea mays* and *Trifolium pretense* were the best performing. The final validation of this approach on-field is currently ongoing.

Simultaneous gene mutations in both nuclei of dikaryotic strain of *Pleurotus ostreatus* using CRISPR/Cas9

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We recently reported an efficient genome editing to introduce gene mutations in a monokaryotic strain of *Pleurotus ostreatus* using CRISPR/Cas9. However, it would be required to obtain dikaryotic strains with mutations in the targeted genes in both nuclei for molecular breeding of cultivated mushrooms. In this study, we attempted to introduce gene mutations into both nuclei of a dikaryotic strain of *P. ostreatus* by CRISPR/Cas9 at one transformation experiment with the aim of developing an efficient methodology for molecular breeding of *P. ostreatus*. As a target gene to be disrupted We selected *msh4* and *mer3*. We previously reported that single-gene disruption of these genes by gene targeting impairs basidiospores production/formation in *P. ostreatus*. Molecular breeding of sporeless strains contributes to solving health problems among workers engaged in mushroom cultivation as well as to preventing genetic contamination due to dispersal of basidiospores. Firstly, Plasmids containing the hygromycin B-resistant gene and expression cassettes for Cas9 and gRNA were introduced into the dikaryotic strain, PC9×#64. Among the obtained hygromycin B-resistant transformants, strains with clamp cells were selected, followed by performing genomic PCR to check if mutations were introduced in the target genes. Basidiospores produced by fruiting bodies from the selected strains were then counted and calculated using a hemocytometer. Genomic PCR suggested that mutations in *msh4* or *mer3* were introduced into both nuclei in some transformants. Fruiting bodies from these transformants produced only few basidiospores. These results suggest that gene mutations can be introduced simultaneously in both nuclei of cultivated mushroom strains by our CRISPR/Cas9 system.

In silico identification of the oosporein gene cluster in the genome of *Victoriomyces antarcticus*

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The peculiar features of *Victoriomyces antarcticus* (*Cephalothecaceae*), including the production of red pigments, make this polar microfungus a promising candidate in biotechnology. However, the molecular basis of the majority of its biological aspects has to be yet understood. Here, we report the mitochondrial genome (31.08 Kb, GC % 27.04) and the nuclear genome (24 Mb, GC % 57.89) with annotation of *V. antarcticus* FBL 165^{T}. Considering the genomes of *V. antarcticus* and the sequenced species

within *Sordariomycetes*, the close evolutionary relationships of *Cephalothecaceae* with the *Coniochaetaceae* is confirmed in a phylogenomic framework. Sequence analyses indicated that *V. antarcticus* could act as saprobe, processing soil nutrients. In particular, among the CAZymes, the most abundant GHs were cellulases (GH5, GH6, GH45), endo-1,4-ß-glucanases (GH5, GH7), xylanases (GH10), and amylases (GH13_1, GH31, GH133), which can be related to the decomposition of plant biomass. Moreover, a relatively low number (10) of chitinases (GH18) indicated limited lytic activities on the chitin. Among the SM biosynthesis gene clusters identified, a pks cluster had sequence homology as well as synteny conservation with a *pks* gene cluster present in *Beauveria bassiana* (*Hypocreales*) encoding the pathway for the production of the red pigment oosporin, a symmetrical 1-4 dibenzoquinone derivative with different biological activities. The putative oosporein biosynthesis in *V. antarcticus* begins with a *pks*, utilizing acetyl-CoA and malonyl-CoA to synthesize orsellinic acid, which is enzymatically hydroxylated and then oxidated to benzenetetrol. The dimerization of the benzenetetrol produces oosporein, which is transported across the cell membrane by a protein encoded in the gene cluster. While the regulation of gene expression remains to be elucidated, our data contribute to the understanding on the evolution of the oosporein gene cluster in *Sordariomycetes*.

Functional and evolutionary genetics of zinc tolerance in *Suillus luteus*

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Suillus luteus is a cosmopolitan fungal species, symbiotically associated with pine trees. In primary successions of pines this species is abundant and involved in seedling establishment. On severely metal-contaminated soils, metal-tolerant *S. luteus* populations evolved by natural selection. Tolerant individuals effectively protect their host tree from metal toxicity on these soils. However, the molecular and genetic mechanisms underlying adaptive metal tolerance in *S. luteus* are unknown. We hypothesize that tolerance phenotypes are due to an adaptation in the common metal homeostasis network. By comparative and functional genetics, we identified several *S. luteus* genes encoding transporters involved in metal homeostasis. One of these transporters, SlZnT2, a CDF family transporter exhibits a differential gene expression among Zn-tolerant and Zn-sensitive phenotypes. The difference in expression level seems to be due to an extensive gene multiplication and differences in cis-regulation. Comparative genomics of different isolates representing distinct metal tolerance phenotypes is ongoing to identify the genetic loci associated with adaptive Cd and Cu tolerance. Altogether results of this study will be valuable to select ectomycorrhizal genotypes to support restoration

of metal polluted soils.

Diversity of *Penicillium roqueforti* isolates from Turkish mold-ripened cheeses

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Penicillium roqueforti is the principle filamentous fungal species isolated from blue cheeses worldwide. Turkey has a variety of traditional blue cheeses. The production of these cheeses involve ripening in the cool and humid atmosphere of cellars or caves allowing the growth of filamentous fungi on the cheese surface. During mold-ripening, no specific starters cultures are used; therefore, the resulting mycobiota is spontaneous, but frequently involves *P. roqueforti*. In this study, we have isolated 54 *P. roqueforti* isolates from 26 Konya kuflu tulum cheese, 1 ripened golot cheese from Rize and 4 Erzurum kuflu civil cheese and identified by ITS sequencing. The *P. roqueforti* isolates were determined to be phenotypically diverse using four different media, potato dextrose agar (PDA), yeast extract sucrose agar (YES), malt extract agar (MEA) and oatmeal agar (OA). Rep-PCR using (GTG)5 primer resulted in slight differences in banding patterns. The isolates will also be screened for their mating types and for the presence of the *CheesyTer* and *Wallaby* loci, which are recently detected horizontally transferred regions among cheese fungi. The results will be important in determining the diversity of *P. roqueforti* isolates originating from Turkish cheeses and their relationships to their European counterparts.

The white-rot fungus *Obba rivulosa* shows expression of a constitutive set of plant cell wall degradation targeted genes during growth on spruce wood

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The basidiomycete white-rot fungus *Obba rivulosa* is an efficient degrader of softwood. The dikaryotic *O. rivulosa* strain T241i (FBCC939) has been shown to selectively remove

lignin from spruce wood prior to depolymerization of plant cell wall polysaccharides, thus possessing potential in biotechnological applications such as pretreatment of wood in pulp and paper industry. The genome-sequenced monokaryotic *O. rivulosa* strain 3A-2 derived from the dikaryon T241i, expressed a constitutive set of genes encoding putative plant cell wall degrading enzymes during 8-weeks cultivation on spruce wood. High level of expression of the genes targeted towards all plant cell wall polymers was detected at 2-week time point, after which majority of the genes showed reduced expression. This implicated non-selective degradation of lignin by the *O. rivulosa* monokaryon and suggests high variation between mono- and dikaryotic strains of the white-rot fungi with respect to their abilities to convert plant cell wall polymers.

Integrating field surveys and molecular data to assess the phytosanitary status of cashew in Guinea-Bissau (West Africa)

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Cashew is a major agriculture commodity in Guinea-Bissau, providing significant export earnings and households' income and food security at the smallholder level. Thus, evaluation of the cashew orchard diseases is of chief importance for sustainable production of this crop. For three years, a series of field surveys was conducted throughout Guinea-Bissau. Data collected included ecology of orchards, social organization of households, evaluation of the incidence and severity of relevant cashew-related diseases, and understory vegetation. A total of 25 cashew orchards were evaluated, covering main administrative cashew-producing regions (i.e. Biombo, Bafatá, Cacheu, Oio, Quinara, Gabú and Bolama). Our results show that cashew orchards are a monoculture cropping system with irregularly spaced trees, in most cases with more than 20 years old. Several diseases' symptoms related to gummosis, anthracnose, and dieback were positively identified, which seem to be uneven distributed along dry/rainy seasons. From infected tissues (trunk, bark, leaf, flowers, apple and nut) close to 200 fungi were isolated and morphologically identified followed by ITS sequencing for confirmation. Reported causal agents of gummosis and dieback were identified in all surveyed regions, namely genera from *Botryosphaeriaceae* (*Lasiodiplodia* sp., *Neofusiccocum* sp., *Cophinforma* sp.); while

the anthracnose agent *Colletotrichum* spp. seems to be restricted to Bolama region. Pathogenicity tests are under progress to relate possible causal agent to the diseases identified. These preliminary results suggest that gummosis is the major cashew disease, and further studies using ecological, biological and environmental data are currently underway to determine main factors associated to the expansion of fungal diseases. This work represents the first assessment of the cashew diseases in Guinea-Bissau, a main step towards sustainable production.

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Fungal highway and bacterial toll

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Microbes ubiquitously live in nearly every ecological niche. Different species coexist in certain habitats and share available metabolites. A novel mutualistic growth mechanism is discovered between models of filamentous fungus and bacteria, *Aspergillus nidulans* and *Bacillus subtilis*. The bacterial cells move faster along fungal highway and disperse farther on fugal growth, while bacterial cells deliver thiamine to tips of fungal hyphae and support the fungal growth. The simultaneous spatial and metabolic interactions indicate a mutualism that facilitates the bacterial-fungal species to compete for environmental niche and nutrient respectively. The bacterial cells move along fungal highway and pay thiamine as a toll to extend fungal highway. An example of co-isolated bacterial- fungal species from nature supports the ecological relevance of the mutualistic interaction.

Functional validation of Carbohydrate Esterase family 1 subfamily 1 and 2 by characterization of fungal esterases from uncharacterized branches

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The fungal members of Carbohydrate Esterase family 1 (CE1) from the CAZy database include both acetyl xylan esterases (AXEs) and feruloyl esterases (FAEs). AXEs and FAEs play significant roles as accessory enzymes in biomass saccharification for the

production of biofuels and biochemicals, but are also used in many other biotechnology applications. AXEs release acetyl group from xylan, while FAEs release ferulic and other hydroxycinnamic acids from xylan and pectin. AXEs and FAEs from CE1 share the same catalytic triad and a G-X-S-X- G signature motif. Both AXEs and FAEs are predicted to have evolved from the same ancestor, however they target substrates with a different molecular structure.

Previously we reported a phylogenetic analysis for the fungal members of CE1, establishing five subfamilies (CE1_SF1-SF5). The largest subfamily CE1_SF1 contains mainly characterized AXEs, whereas CE1_SF2 contains mainly characterized FAEs. These two subfamilies are closely related and one may have evolved from the other. Six candidates from ascomycetes belonging to SF1 and SF2 were heterologously produced in *Pichia pastoris* and characterized with respect to their biochemical properties and substrate preference towards model and plant biomass substrates. We confirmed that CE1_SF1 and CE1_SF2 exhibited AXE and FAE activity, respectively. In addition, we also identified a novel dual AXE/FAE activity enzyme, which can support the evolution within these two subfamilies. The newly characterized fungal AXEs and FAEs from CE1 also show promising biochemical properties and have potential for bio-industrial applications.

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Release and recovery: Twenty years' evolution of a fungal population after releasing exotic strains to control insect pests

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Entomopathogenic fungi are the key regulators of insect populations in nature. Some species such as *Beauveria bassiana* have been developed as promising biocontrol agents against insect pests. The effect of biocontrol release of mycoinsecticides on local fungal and non-target host populations remains elusive, but it is a considerable concern with respect to environmental safety. We report the temporal features of the *Beauveria* population evolution over 20 years after the releases of exotic strains to control pine caterpillar pests. We found that the isolates within the biocontrol site were substantially clonal to each other, and the population was largely replaced by genetically divergent isolates once a decade through adaptive selection, non-random outcrossing, and isolate migration. However, fungal population evolved preferentially towards a balancing selection with marginal differentiations over time. The released strains could be present in the environment for a long time with low recovery rates and

without obvious perturbation on population structure. The non-strict control of isolate host preference is genetically evident. Thus, similar to the reoccurrence of host jumping by local isolates, the infection of non-target hosts by the released strains endemically occurred in association with host seasonality. This study not only unveils the unique real-time features of entomopathogenic fungal population evolution but also provides added values to alleviate the concerns of environmental safety regarding the biocontrol application of mycoinsecticides.

Regulation of wheat bran utilization in the industrially relevant filamentous fungus *Aspergillus niger*

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The filamentous fungus *Aspergillus niger* has long been applied in the field of industrial biotechnology for the production of several metabolites, such as citric acid, or different enzymes involved in plant biomass degradation. Fungal enzymes are widely used for the saccharification and conversion of plant biomass waste materials into valuable biochemicals or biofuel. One such waste material is wheat bran, a byproduct of milling in the production of refined grains. Some *A. niger* transcription factors were previously reported to be involved in the control of (hemi-)cellulose or pectin degradation. In our project, we used the CRISPR/Cas9 genome editing system in *A. niger* to delete the genes encoding the (hemi-)cellulolytic transcription factors XlnR, AraR, ClrA and ClrB alone and in all possible combinations in order to determine the hierarchy of these transcription factors in the process of wheat bran utilization. We constructed single, double, triple and quadruple deletion mutants of these transcription factors and compared their phenotypes to that of the wild type strain during growth on wheat bran. Moreover, strains carrying further deletions of the amylolytic and inulinolytic transcription factor genes amyR and inuR, respectively, or the D-galacturonic acid-responsive transcription factor gene gaaR were also considered in order to determine the growth abilities on starch or pectin contained in wheat bran. Protein profiles, enzyme activities and gene expression patterns were analyzed to determine the relative importance of each transcription factor involved in the process of wheat bran degradation and utilization in *A. niger*.

Thriving after host extinction: intraspecific variation and isolate-specific metabolic capacities of *Batrachochytrium salamandrivorans*

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Pathogens rarely drive their hosts to extinction. The chytrid fungus *Batrachochytrium dendrobatidis* (Bd) is, however, frequently associated with population extinctions in amphibians, resulting in the most biodiversitydevastating epidemic in history (Scheele *et al*., 2019). In 2013, a closely related chytrid, *Batrachochytrium salamandrivorans* (Bsal), was discovered to be the cause of the collapse of salamander populations in northern Europe. Analyses of host-pathogen dynamics suggest Bsal poses a similar extinction threat as its sister fungus Bd (Martel *et al*., 2014; Stegen *et al*., 2017). However, all studies of Bsal to date have focussed on a single isolate, assuming the European epidemic to be homogenous. Using genomic and phenotypic analyses from seven Bsal isolates from five outbreaks we found the European epidemic to comprise multiple introductions of isolates with highly divergent genomic landscapes and exceptional evolutionary rates. We identified genomic hallmarks of cripti metabolic functions, which appear to be selected for following host population collapse. These could allow certain isolates to persist indefinitely even once the host population has declined to low densities, supporting pathogendriven host extinction and suggesting environmental elimination in certain outbreaks may be impossible.

Martel, A. *et al*. Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. Science 346, 630–631 (2014).

Scheele, B. C. *et al*. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. Science 363, 1459–1463 (2019).

Stegen, G. *et al*. Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*. Nature 544, 353–356 (2017).

A Genome Wide Association Study reveals genomic insights in conidial heat resistance of *Paecilomyces variotii*

Tom Van Den Brule[1], Maarten Punt[2], Jos Houbraken[1], Robin Ohm[2], Han Wösten[2], Jan Dijksterhuis[1]

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Fungal food spoilage often begins with contamination by spores. In general, fungal spores are more resistant against environmental stress than vegetative cells. The commonly occurring fungus *Paecilomyces variotii* is a known spoilage organism of various food products. Recently, it was shown that distinct *P. variotii* strains produce more heat resistant conidia (asexual spores) than other strains. Here, we quantified conidial heat resistance of 20 *P. variotii* strains. The genomes of all strains were sequenced and annotated. A genome-based phylogenetic tree revealed a clade consisting of only heat resistant strains. This result indicated that conidial heat resistance has a background in genomic differences. A further Genome Wide Association Study (GWAS) revealed a list of 22 genes of interest that are possibly involved in conidial heat resistance. Five of these genes were located on a 60 kb gene cluster that was present in all heat resistant strains, but absent in all sensitive strains. Further research is necessary to see how this cluster is involved in conidial heat resistance. The GWAS presented in this poster provided an elegant way to identify possible loci involved in phenotypical differences.

Deletion of the target extracellular protease genes identified by secretomics for high-level production of cellulolytic enzymes in *Trichoderma reesei*

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Trichoderma reesei is a famous filamentous fungus for production of cellulolytic enzymes in industry. However, undesired degradation of cellulases often happens in culture filtrates and commercial enzyme preparations. Even studies have been reported about describing proteolytic degradation of heterologous proteins in *T. reesei*, there are few systematic explorations concerning the extracellular proteases responsible for degradation of cellulases. In this study, the cellulase activity was observed to rapidly decrease at late cultivation stages in *T. reesei*. It was discovered that this decrease may be caused by proteases. To identify the proteases, comparative secretomics was performed to analyze the concomitant proteases during the cellulase production. 12 candidate proteases from the secretome of *T. reesei* were identified and their encoding genes were individually deleted via homologous recombination. Furthermore, three target proteases (tre81070, tre120998 and tre123244) were simultaneously deleted by one-step genetic transformation. The triple deletion strain ΔP70 showed a 78% decrease in protease activity and a 6-fold increase in cellulase activity at the late stage of fermentation. These results demonstrated the feasibility of improvement of cellulase production by genetically disrupting the potential protease genes to construct the *T.*

reesei strains with low extracellular protease secretion. This dataset also provides an efficient approach for strain improvement by precise genetic engineering combined with "omics" strategy for high-production of industrial enzymes to reduce the cost of lignocellulose bioconversion.

CRISPR/Cas9 in mushrooms without integration of ectopic DNA

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Genome editing is a powerful tool for molecular breeding of various organisms, since a targeted mutation can be introduced in a gene of interest. However, integration of the vector plasmids to the host chromosome may cause problems to avoid generation of a genetically modified organism (GMO). In mushrooms, including edible and medicinal ones, CRISPR/Cas9 is considered as a promising protocol for molecular breeding but has been introduced in a limited number of species. We successfully introduced this technique to oyster mushroom, *Pleurouts ostreatus*, and a selective lignin degrader, *Ceriporiopsis subvermispora*. Using a transient DNA transformation system, we set out for developing a new technology of CRISPR/Cas9 without integration of ectopic DNA in the host chromosome. Here we show examples of efficient and non-GMO type introduction of mutations in the target gene.

Directed evolution of melanized fungi to investigate mechanisms of adaptation and resistance to ionizing radiation

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Lonizing radiation is highly damaging to biological systems and therefore represents a significant health threat. Despite efforts to prevent nuclear proliferation and increase safety at nuclear power plants, the risk of a nuclear disaster persists. Further, ionizing radiation present in cosmic rays introduces protective challenges for astronauts

traveling past Earth's magnetosphere. There is an unmet need for novel and effective radioprotective materials and prophylactic treatments that will protect humans from radiation exposure. Fungi, which are highly resistant to radiation and capable of surviving doses up to 1000x higher than mammals, are well-poised to serve as a model to investigate adaptation and resistance to ionizing radiation. In particular, melanized fungal species are often associated with highly irradiated environments, as melanin is considered an evolutionary derived trait that confers protection against radiation and extreme temperatures. However, recent studies suggest that fungal resistance to ionizing radiation is independent of melanin, implying the existence of unrealized adaptation and resistance mechanisms. To investigate these mechanisms, the melanized yeast *Exophiala dermatitidis* and the melanized filamentous fungus *Aspergillus niger* were adapted to various doses of γ-radiation. Whole-genome sequencing enabled the identification of genetic variations that had been selected for during directed evolution, which revealed "high-impact" frameshift and premature stop codon gain mutations in genes involved in DNA repair, transcriptional regulation, transmembrane transport, and protein ubiquitination. Genetic engineering was used to investigate the involvement of highly mutated genes on radiation resistance. These findings validate the use of melanized fungi as a model for studying adaptation and resistance to ionizing radiation and serve as a starting point for exploring novel radioprotective applications of such mechanisms.

Wood decay fungi studied under fermentative and oxygen-stress conditions

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Reactive oxygen species (ROS) play an essential role in wood decomposition reactions by fungi. White rot fungi decompose wood mainly via enzymatic reactions, by secreting carbohydrate-acting and lignin-attacking enzymes. In brown rot fungi wood decomposition occurs mainly non-enzymatically, via Fenton-chemistry reactions. In both decay types, hydrogen peroxide is needed for efficient decomposition of the wood. Under fermentative, oxygen-depleted conditions, however, the formation of ROS and hydrogen peroxide by fungi may be limited. Besides the fungal primary metabolism and metabolites, oxygen depletion and hypoxia conditions thus affect the wood decomposition processes and possibly, the production of fungal secondary metabolites. These compounds generally are biologically active and may act as redox mediators or ROS quenchers during the processes. However, a multitude of fungal bioactive compounds are unknown. Especially in brown rot fungi, it has been shown that specific secondary metabolites are involved in ROS mediated decomposition of the lignocellulose substrates but details of reactions

during aerobic or anaerobic wood decay are still rather unknown. This has relevance also from an ecological point of view, since in nature, the decomposition conditions vary and may become partially oxygen limited. In this presentation, we review our current study on the topic and present results of our research of the effects of ROS addition on fungal decomposition of lignocellulose biomass under fermentative conditions.

Analysis of self-assembly mechanism of hydrophobin RolA of Aspergillus oryzae using Langmuir- Blodgett method

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Hydrophobins are small secreted amphipathic proteins produced by filamentous fungi and contain eight conserved cysteine residues that form four intramolecular disulfide bonds. Hydrophobins are classified into two classes (class I and class II) according to their amino acid sequence, hydropathy profile, and the solubility of their self-assembled films. Class I hydrophobins adsorb to solid surfaces and are localized at interfaces between hydrophobic and hydrophilic phases, resulting in formation of self- assembled structures so called "rodlets" which are similar to β-amyloid fibrils. Class I hydrophobin RolA produced by *Aspergillus oryzae* attaches to solid surfaces, recruits polyesterase CutL1, and consequently promotes hydrolysis of polyesters. Because several positively charged residues in the N-terminal region of RolA face to water-phase and are involved in the interaction with CutL1, the orientation of RolA molecule on the solid surface is important. However, the mechanism by which RolA forms the self- assembled structure remains unclear. Using the Langmuir technique, we analyzed the process in which RolA formed the self-assembled structure at the air-water interface. We also transferred the assembled RolA structures onto hydrophobic or hydrophilic silicon basal-plates and observed the structures on the silicon plates by an atomic force microscopy. As a result, RolA formed the self-assembled films after two steps of phase transition, and different assembled structures of RolA were observed on the hydrophilic and hydrophobic silicon plates, respectively.

Timing of fungal spore release dictates survival during atmospheric transport

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The fungi disperse spores to move across landscapes and spore liberation takes different patterns. Many species release spores intermittently; others release spores at specific times of day. Despite intriguing evidence of periodicity, why (and if) the timing of spore release would matter to a fungus remains an open question. I will discuss our recent state-of-the-art numerical simulations of atmospheric transport with meteorological data, following the trajectory of many spores in the atmosphere at different times of day, seasons and locations across North America.

While individual spores follow unpredictable trajectories due to turbulence, in the aggregate patterns emerge: statistically, spores released during the day fly for several days, whereas spores released at night return to ground within few hours. Differences are caused by intense turbulence during the day and weak turbulence at night. The pattern is widespread but its reliability varies, for example, day/night patterns are stronger in southern regions.

Results provide testable hypotheses explaining both intermittent and regular patterns of spore release as strategies to maximize spore survival in the air. Species with short-lived spores reproducing where there is strong turbulence during the day, for example in Mexico, maximize survival by releasing spores at night. Where cycles are weak, for example in Canada during fall, there is no benefit to releasing spores at the same time every day.

Our data challenge the perception of fungal dispersal as risky, wasteful, and beyond control of individuals and suggest timing of spore liberation may be finely tuned to maximize fitness during atmospheric transport. The idea that fungi do control aspects of spore fate marries previous work demonstrating optimality of spore discharge. I will conclude with a plea for data to test the hypothesis that fungi choose timing of liberation to optimize spore survival.

Optimal strategies for fungal spores liberation

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Fungi rely on their spores for survival. From the perspective of an individual fungus, spores represent the chance to find fresh resources and broadcast genetic heritage. Each fungus takes care of its microscopic offspring up to their release in the surrounding environment, but what happens next? Environmental flows are unpredictable and parents are bound to lose control over spore's fate. However, statistical analysis of atmospheric transport shows that a good choice of timing for spore discharge can lead to optimized rates of survival. I will discuss what kind of information can fungi exploit to optimize survival of their progeny.

Bacterium endosymbiosis in *Ustilago maydis* infecting maize plants in nature

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Ustilago maydis is a phytopathogenic Basidiomycota fungus specific of maize and teozintle. Few years ago, it was described that laboratory strains of *U. maydis* were able to grow in a medium devoid of a nitrogen source. Data of acetylene reduction and 15N fixation, led us to conclude that this capacity was due to its ability to fix N2. Since it is known that eukaryotic organisms are unable to fix N2, we analyzed whether the fungus harbored a bacterium with this capacity. This hypothesis was confirmed by immunolocalization of bacteria in intact cells by a 16S rRNA FISH test. Additionally, sequencing of 16S rRNA of the endosymbiont confirmed that it belonged to the *Bacillus* genus(1). In the present study we proceeded to determine if this association was specific of the analyzed fungal strains, or a general characteristic of the species, since all the previously analyzed strains had the same origin(2). Thus, we isolated a large number of *U. maydis* strains from naturally infected cobs from different mexican locations and two from abroad. We observed that all the isolated strains were able to grow in

media without combined nitrogen, and harbored intracellular bacteria as determined by PCR amplification of the 16S rRNA gene. Nitrogenase activity measured by acetylene reduction and 15N2 fixation were detected in most of the strains. These data suggest that the existence of a symbiosis between *U. maydis* and bacteria with the capacity to fix nitrogen is a widely distributed characteristic that probably was acquired a long time ago, possibly a short time after the appearance of the species.

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The growth of marine fungi in complex substrates produces hydrophobic proteins with the ability to self-assembling

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The marine fungi are a diverse group of opportunistic and obligate organisms isolated from marine environments. They are often included in screening for new metabolites, and their ability to assimilate complex polymers such as alginate, ulvan, cellulose, and hemicellulose (1). Hydrophobins (HFBs) and Cerato-platanin proteins (CPPs) play an important role in the growth and development the filamentous fungi. These proteins have the capacity of self-assembles forming amyloid-like aggregates and form protein biofilms at air/water interfaces (2,3). In this study we analyse the marine fungi, *Dendryphiella salina* and *Penicilium pinophilum*. The identification of the genes involved in the production of HFBs and CPPs was performed and two class I HFBs were identified in *P. pinophilum* and one CPP in *D. salina*. Different treatments were tested to extract HFBs and CPPs from the mycelium and the culture broth in media with different carbon sources. The aggregation of these proteins was monitored by Thioflavin (ThT), circular dichroism (CD), infra-red spectroscopy (FT-IR) and atomic force microscopy (AFM). The best medium for improve the production of CPPs and HFBs in these fungi was the medium minimal with alginate 0,2% and from filtrated culture broths. The molecular weights of these proteins ranged less than 20 kDa. The analysis of CD, ThT, FT-IR and AFM show that these proteins form a structural amyloid β-sheet. Also, it is achievable to extract *D. salina's* CPPs and *P. pinophilum's* HFBs from a medium with waste of brown and green seaweed. This work shows that these fungi can feed with carbon sources

such as cheap seaweed biomass and produce extracellular CPPs and HFBs that can be used in pharmacy, nanotechnology and biomedicine.

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A genomic and transcriptomic study on the DDT-resistant *Trichoderma hamatum* FBL587: first genetic data into mycoremediation strategies

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Trichoderma hamatum FBL 587, isolated from DDT-contaminated agricultural soils, showed tolerance to DDT and ability to enhance DDT degradation process in soil, indicating potential applications in mycoremediation (1). In an attempt to provide molecular evidence for specific properties, WGS and RNA-Seq studies were performed. In the 38.9 Mb-genome, 10,944 protein-coding genes were predicted and annotated with the GO and the CAZy databases. Among the CAZymes identified, GH family enzyme genes were detected including cellulases (GH5, GH6), endo-1,4-ß-glucanases (GH5, GH7), xylanases (GH10), amylases (GH13_1, GH31), ß-glucosidases (GH1, GH3 GH5, GH30), β-galactosidases (GH35), exo-β-1,3-glucanases and endo-β-1,3-glucanases (GH55). Prediction of biosynthesis gene cluster was also carried out, highlighting genes potentially involved in the production of secondary metabolites (e.g., gliotoxin). RNA-Seq studies were performed on three replicates for both control and treatment (exposure to DDT). After the alignment of the reads against the genome, PCA showed that replicates cluster together for both control and treatment, and the treatment group is clearly distinct from the control group. When we focused on the expression of genes of specific metabolic pathways (e.g., metabolism of carbohydrates, organochlorine and aromatic compounds, gliotoxin), *T. hamatum* FBL 587 showed these genes were functional. Moreover, the analysis of the read counts to each annotated locus allowed

to identify differential expression under exposure to DDT (1,706 up-regulated and 1,770 down-regulated genes). The transcriptome analysis allowed the characterization of up-regulated genes of particular interest, e.g. those related to glutathione biosynthetic process (DDT promoted a high formation of ROS in this strain). Further investigation of transcriptome can help to define crucial aspects such as regulatory mechanisms.

1. Russo *et al*. 2019. Appl. Environ. Microbiol. doi:10.1128/AEM.01720-19.

Biological control of stem canker of royal poinciana caused by *Neoscytalidium dimidiatum* using endophytic actinobacteria able of producing ACC deamina

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Twenty-two endophytic actinobacterial isolates were isolated from inside the trunk of healthy royal poinciana trees. These endophytic isolates were tested for their capabilities to produce cell wall degrading enzymes (chitinase, ß-1,3-glucanase), siderophores, antifungal diffusible metabolites and volatile antibiotics active against *Neoscytalidium dimidiatum*, the causal agent of stem canker of royal poinciana in the United Arab Emirates (UAE). Stem canker symptoms can be determined by bark lesions, discoloration of xylem tissues, extensive gumming branch and leaf dryness, and longitudinal wood necrosis. A dieback signs were also detected leading to complete defoliation and the death of the trees in more advanced stages. Only five isolates out of the 22 endophytic isolates showed exceptional in vitro chitinolytic activity and antifungal metabolites and caused the lysis of N. dimidiatum hyphae. These five isolates were also tested for their potential to secrete the enzyme 1-aminocyclopropane-1-carboxylic acid deaminase (ACCD), the immediate precursor of the stress hormone ethylene. Under greenhouse conditions, the ACCD- producing isolates (ACCD+) were significantly more effective in reducing the incidence of stem canker compared to ACCD-non-producing isolates (ACCD-). The results clearly demonstrated that ACCD+ isolates could replace Cidely® Top, which is the currently recommended fungicide for the management of stem canker of royal poinciana in the UAE. This is the first study to demonstrate the ability of endophytic actinobacteria to control stem canker of royal poinciana. In addition, this is the first report to demonstrate the superiority of antagonistic endophytic actinobacteria to enhance their effectiveness as biocontrol agents by their ability to produce cell-wall degrading enzymes, antifungal metabolites and ACCD.

Poster Session 3.4
SYNTHETIC BIOLOGY AND BIOTECHNOLOGY

THURSDAY, FEBRUARY 20

14:00 - 15:30 | Location: **Frentani Convention Center**

Combining and improving phenotypic traits through the generation of synthetic two- and six-species yeast hybrids

David Peris[1,2,3], William G. Alexander[2,4], Kaitlin Fisher[2], Ryan V. Moriarty[2], Mira G. Basuino[4], Emily J. Ubbelohde[4], Lainy Ramírez-Aroca[1], Laura Pérez-Través[1], Eladio Barrio[5], Amparo Querol[1], Russell L. Wrobel[2], Chris Todd Hittinger[2]

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FLASH TALK - *Presenting author' e-mail: david.perisnavarro@gmail.com*

Polyploidy is a widespread phenomenon in biology, observed in plants, fungi, insects, and vertebrates. Polyploidy has been related with cellular differentiation in mammals and arthropods, and it promotes species diversification through evolutionary innovation. Additionally, polyploidy has important implications in tumorigenesis due to its implications in genome instability. Tools for testing polyploidy limits and their cellular consequences are lacking or scarce. Here, we have tested in an iterative way a methodology promoting *Saccharomyces* yeast hybridization, iHyPr (iterative Hybrid Production), for the generation of synthetic allotetraploids and higher-order yeast hybrids. By using independent hybridization schemes, we have successfully generated multiple allotetraploid, allohexaploid hybrids, and four six-species synthetic hybrids (6-species hybrids) of *Saccharomyces*, a genus important for industrial applications (beer, wine, and biofuels). To test the potential future industrial applications of our synthetic hybrids, we evolved allotetraploids in synthetic must and the six-species hybrids in a medium containing xylose, a sugar not utilized well by wild *Saccharomyces* strains. By a multiomic approach: metabolomics, whole genome sequencing and kinetic analyses of ancestral and evolved six-species hybrids, we demonstrated how iHyPr is a useful

tool for generating yeast diversity and better-adapted strains to new environments. We envision iHyPr as a tool to expand the *Saccharomyces* genus as a model to study the roles that polyploidy plays in organismal evolution, industry, and disease.

Characterization of gene regulatory networks of *Thermothelomyces thermophilus* to improve protein production

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Thermothelomyces thermophilus is a thermophilic, filamentous fungus, which is a very efficient producer of hydrolases (mainly lignocellulolytic enzymes) with high thermal resistance and therefore used by industry. The main advantage of industrial isolates of *T. thermophilus* are production of up to 100 g/L of protein while maintaining low viscosities during cultivation [1,2]. Nevertheless, detailed regulation processes for the expression of hydrolases are poorly characterized. In addition, strong natural secretion of lignocellulolytic enzymes has an influence on yield as well as the purity of proteins of interest. Therefore, reduction of the secretion of these hydrolases is of great interest for industry [3]. To target the above mentioned problems, we investigated these regulation processes via controlled chemostat bioreactor cultivation with different regulator knockout mutants, e.g. deletion of *clr2* (Cellulase regulator 2), followed by transcriptomic analysis under industrial relevant conditions.

1. Berka RM *et al*., 1997. Appl. Environ. Microbiol. 63: 3151-3157.

2. Visser H *et al*., 2011. Ind. Biotechnol. 7 : 214-223.

3. Haefner S *et al*., 2017. Patent: WO2017093450A1.

Expanding the molecular toolbox for the white-rot fungus *Dichomitus squalens*

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Dichomitus squalens is a promising reference species to investigate white-rot fungal plant biomass degradation, as it has a flexible physiology to utilize different types of biomass as sources of carbon and energy. Recent comparative (post-)genomics studies on *D. squalens* resulted in an increasingly detailed knowledge of the genes and enzymes involved in the lignocellulose breakdown in this fungus and showed complex transcriptional response in the presence of lignocellulose-derived compounds [1, 2]. To fully utilize this increasing amount of data, efficient and reliable genetic manipulation tools are needed e.g. to characterize function of certain proteins *in vivo* and facilitate construction of strains with enhanced lignocellulolytic capabilities. However, precise genome alterations are often very difficult in wild type basidiomycetes such as *D. squalens*, partially due to extremely low frequencies of homologous recombination and limited availability of selectable markers. To overcome these obstacles, we adapted co-targeting strategy based on pre-assembled Cas9-sgRNA ribonucleoproteins for selectable homology- and NHEJ-based gene editing in *D. squalens*. The preliminary results of this study will be presented.

1. Kowalczyk, J.E., *et al*., *The white-rot basidiomycete Dichomitus squalens shows highly specific transcriptional response to lignocellulose-related aromatic compounds.* Frontiers in Bioengineering and Biotechnology, 2019. 7(229).

2. Casado López, S., *et al*., *Induction of plant cell wall degrading CAZyme encoding genes by lignocellulose-derived monosaccharides and cellobiose in the white-rot fungus Dichomitus squalens.* Applied and Environmental Microbiology, 2018. 84: p. e00403-00418.

Imidazolium-labelled glycosides for the characterisation of enzymatic function during plant biomass degradation

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Enzymes involved in cellulose modification and degradation are ideal candidates for producing high value materials and chemicals from renewable biomass, thus contributing to a future bio-based economy.

Due to their evident economic importance, there is great demand for well-characterised,

effective Lytic Polysaccharide Monooxygenases (LPMOs) and cellulases. To assess the function of uncharacterized enzymes on cellulose we are developing substrates that are ideally suited for microliter scale reactions followed by quick, inexpensive and highly sensitive analysis via MALDI-ToF MS.

We identified an enzyme that is able to polymerise glucose into oligosaccharides with a degree of polymerization of up to 9. We employed this enzyme to generate oligosaccharides linked to ionic liquid-based imidazolium tags (ITag-) that ionise well in MS and thus vastly increase the signal of their conjugate [1]. We demonstrate that these oligosaccharides are substrates of both oxidative and hydrolytic cellulolytic enzymes. Sensitive detection of reaction products via MALDI-TOF MS enables identification of substrate / product range, and type of oxidative mechanism.

The flexible enzymatic synthesis of these substrates enables incorporation of modified building blocks, resulting in oligosaccharides with tailored properties. This can be exploited in a chemo-enzymatic strategy to generate I-tagged oligosaccharides that are blocked at their non-reducing termini. This resulted in protection against any exo-acting activity and allowed endo-acting cellulases to degrade the internal oligosaccharide chain, thus generating a endo-cellulase specific substrate that is highly suited for MS-based analysis.

In conclusion, we demonstrate a strategy for chemo-enzymatic synthesis of substrates for sensitive, MS based detection of oxidative and hydrolytic cellulosic enzyme activity, which is broadly applicable to glyco-enzyme characterisation.

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A putative methyltransferase TrMET involved in cellulase induction in *Trichoderma reesei*

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The ascomycete *Trichoderma reesei* is one of the main producers of cellulolytic enzymes for the biotechnology industry. The regulation machanism of the cellulase production is extremely anfractuous. Here, a putative methyltransferase TrMET was found to be involved in cellulase induction in *T.reesei*. The suppression of cellulase induction by this protein was discovered in Qm6a, QM9414 and the hypercellulase-producing strain Rut-C30 on avicel or lactose. Furthermore, TrMET was proved to interact with ACE1 which has been considered as a transcription repressor of cellulase induction by yeast two-

hibrid and bimolecular fluorescence complementation. The capacity of ACE1 binding on cellulase gene promoter was decreased after mutation of a putative site of methylation modification on ACE1. Due to the similar binding consensus sequence of ACE1 and the activator XYR1, the competition advantage of XYR1 consequently resulted in an increase in transcriptional activation effect. Based on these above data, we speculated that ACE1 underwent post-translational modification by TrMET and the methylation had a prominent effect on the activity of ACE1. Our finding is of great significance to understand the cellulase regulation network and would also provide a new strategy to improve cellulase production in *T.reesei*.

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Fungal host strains for the industrial enzyme or protein production

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Filamentous fungi from the genera *Aspergillus*, *Trichoderma* and *Penicillium* are being used for the production of a wide variety of enzymes and proteins. Their capacity to produce and secrete proteins at high levels has made them the organism of choice for a large number of industrial protein production processes. This makes these organisms also of interest as production platform for novel proteins or enzymes, for example enzymes for plastic remediation and proteins with other than enzymatic functionalities e.g. nutritional proteins.

At Dutch DNA Biotech, we develop protein production processes using *Aspergillus niger* as expression host. A suite of platform strains with industrially relevant properties, such as low proteolytic activity or low background protein production, is used to construct expression hosts for the production of homologous and heterologous proteins with high purity and yield. To design a collection of versatile mutant host strains, efficient genome editing techniques specifically adapted for use in fungal hosts have been adopted. Expression cassettes are introduced into the platform strains at high copy numbers by using various transformant selection approaches. Furthermore, the expression cassettes

carry promoters that were designed to ensure high constitutive expression levels of the gene of interest under industrially relevant fermentation conditions.

Protein production processes are developed by running lab scale fed-batch fermentations. Also here, targeted strain development for improved fermentation characteristics was addressed, more specifically aspects of fungal morphology in relation to protein production have been investigated. This research resulted in improved fermentation design and performance.

Based on our research in *Aspergillus*, also new fungal host strains with favourable protein production and fermentation characteristics are being explored, thereby further exploiting the immense biodiversity in the fungal kingdom.

FungalBraid: A GoldenBraid-based modular cloning platform for fungal synthetic biology

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The assembly of DNA pieces to obtain custom modules is a basic process in synthetic biology. Optimization of tools for the genetic manipulation of fungi is a relevant endeavor given the complexity and time required to obtain the specific gene constructs. Our group has successfully adapted the GoldenBraid (GB) technology developed for plant synthetic biology to filamentous fungi [1], taking advantage of *Agrobacterium tumefaciens* ability to transform both plants and filamentous fungi. GB is based on the use of IIS type restriction enzyme technology and a universal four-nucleotide barcode system that allows for the identification and ordered assembly of multiple genetic elements. We have adapted fungal-specific genetic elements such as promoters (*gpdA* and *trpC*), terminators (*tub* and *trpC*), as well as positive (hygromycin and geneticin) and negative (HSV*tk*) selection markers to the system. We have also developed specific GB structures for gene disruption in fungi through homologous recombination and dual selection. Higher order combinations of elements have been assembled; and the ectopic genetic transformation and/or gene disruption of *Penicillium digitatum*, *Penicillium expansum* and *Aspergillus niger* have been demonstrated. Fungal Braid (FB) is the name proposed for this new branch of GB technology that will contribute to the implementation of synthetic biology and genome edition in filamentous fungi [2]. Finally, we will also present the FB-specific section of the most recent version of the GB website and the software assisted tools for

cloning and assembling fungal-specific DNA parts.

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Newly designed modular carbohydrate-active enzyme to increase the efficiency of lignocellulose degradation

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Enzymatic degradation of abundant renewable lignocellulose containing biomass is a field that has the attention of both the industrial and scientific community. The polysaccharide polymers in lignocellulose are converted by cocktails of enzyme into simple sugars that can be used to produce value-added bio-products such as biofuels, chemicals, and foods. However, complete conversion of biomass to platform sugars is hindered primarily by recalcitrant substrate components which cannot be decomposed by available enzyme cocktails. One of the approaches to combat this limitation is improving carbohydrate active enzyme (CAZymes) capability for a more complete degradation. CAZymes are often organized in a modular manner including a catalytic domain connected to one or more carbohydrate-binding modules (CBM's). The CBM's are suggested to increase the proximity of the enzyme to its substrate, especially insoluble substrate. Therefore, in this research, we aim to investigate the diversity and activity of these modular CAZymes in order to improve fungal enzymes cocktails by expressing newly designed modular enzymes. Special emphasis is given to the CAZymes present in the filamentous microorganisms from the genera of *Aspergillus* and *Streptomyces* that are well known to have a great capacity for secreting a wide range of CAZymes. Interesting genes encoding specific cellulases such as GH9 endoglucanase, GH48 exoglucanase, and GH3 beta glucosidase, as well as small Laccase were analyzed in more detail. Development of newly designed enzyme configurations in *Aspergillus niger* are being explored for fungal cellulase cocktails improvement.

Keywords: Carbohydrate-binding module, CAZymes, enzyme domain modification,

Aspergillus, Streptomyces

CRISPR/Cas9 technology enables the development of the filamentous ascomycete fungus *Penicillium subrubescens* as a new industrial enzyme producer

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Penicillium subrubescens is an ascomycete filamentous fungus with an enriched content of specific carbohydrate-active enzyme families involved in plant biomass degradation. In fact, this fungus produces similar enzyme levels to the well-established and industrially relevant cell factory *Aspergillus niger,* making it a promising industrial cell factory for enzyme production. The development of tools that allow genetic manipulation of this species is crucial for further strain improvement and the functional characterization of its genes. In this context, the CRISPR/Cas9 system represents an excellent option for genome editing due to its high efficiency and versatility, being a simple and cost-efficient genome editing tool that allows the development of strains with improved properties, such as increased enzyme/metabolite production. To establish CRISPR/Cas9 genome editing in *P. subrubescens*, first a method for protoplast generation and transformation was developed, using hygromycin as a selection marker. Then the CRISPR/Cas9 system was established in *P. subrubescens* by successfully deleting the *ku70* gene, which is involved in the non-homologous end joining DNA repair mechanism. Phenotypic characterization of the mutants showed that *ku70* mutation did not affect *P. subrubescens* growth at its optimal temperature and *ku70* strains showed a similar protein production pattern to the wild type, allowing the use of these mutants as parental strains for subsequent transformation events.

With this work, we expand the repertoire of fungi where genetic engineering is possible and thus contribute to accelerate the study and exploitation of the potential of *P. subrubescens* as cell factory at biotechnological and industrial level.

Draft genome and annotation of *Aspergillus affinis* (*Circumdati*): first insights into a biotech perspective

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Aspergillus affinis (section *Circumdati*), a promising candidate for biomass bioconversion, produces ochratoxin A (OTA) that in possible contamination can be a threat to human health. However, little is known about its OTA biosynthetic genes. The whole-genome sequence of *A. affinis* may elucidate several aspects including its potential suitability in biomass bioconvesion at a pilot scale. Here, the nuclear and the mitochondrial (mt) genomes of *A. affinis* ATCC MYA-4773^T were sequenced, assembled and annotated. OTA-producing *Aspergillus* species of the section *Circumdati* (*A. ochraceus* fc-1, *A. persii* NRRL 35669, *A. steynii* IBT 23096, *A. westerdijkiae* CBS 112803) with available complete genome sequences on databases were used for comparison. The mt genome (31.7 Kb, GC % 25.3) contained core genes and accessory genes as well. Gene annotation for the nuclear genome (37 Mb, GC % 50.1) resulted in 11,386 genes, which were further classified according to GO terms and the CAZy databases. Among the 360 CAZymes identified, the GH family enzyme genes included cellulases (GH5, GH6), endo-1,4-ß-glucanases (GH5, GH7), xylanases (GH10), amylases (GH13_1, GH31), and inulinases (GH32), which may be of outstanding importance for white biotechnology. In general, the numbers of CAZy genes related to plant biomass degradation followed the phylogeny of *Aspergillus* section *Circumdati*. Among the SM biosynthetic gene clusters predicted, the OTA biosynthetic pathway cluster contained genes encoding a pks (*AaOTApks*), a nrps (*AaOTAnrps*), a cytochrome P450 monooxygenase (*AaOTAp450*), a halogenase (*AaOTAhal*), and a basic leucine zipper transcription factor (*AaOTAbZip*). In addition, genes encoding hypothetical proteins were present although the genetic synteny conservation found in relation to the *Aspergillus* species here examined. OTA gene cluster contains target genes suitable for developing new genetic tools for rapid and accurate control methods of ochratoxigenic *Aspergillus* strains.

Surface analysis tools identify how *Aspergillus niger* and its enzymes modify lignocellulose

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Fungal carbohydrate active enzymes are exploited on an industrial scale as biocatalyst

that convert plant lignocellulose to simple sugars, which can subsequently be converted to platform chemicals and biofuels. However, we have poor knowledge of the effect of fungi and their enzymes on the actual insoluble complex substrate, while such understanding underpins exploitation of enzymes to make designer polysaccharide materials, effective degradative enzyme cocktails and to engineer fungal production strains.

We investigated how exposure and accessibility of polysaccharides and lignin on lignocellulose surface changes during cultivation with industrially relevant fungus *Aspergillus niger*, with the aim to understand how this fungus and its enzymes interacts with lignocellulose.

Analysis of time-staged changes of lignocellulose using mass-spectrometry based imaging identified surface exposure of lignin was increased over time. We identified differential degradation of hemicellulose and pectin polysaccharides using immunohistochemistry and fractionation followed by ELISAs employing antibodies specific for carbohydrate epitopes. Degradation of specific polysaccharides was not always linked to presence of known corresponding degradative enzymes, suggesting lack of substrate access or absence of essential accessory enzymes.

Our results highlight that full understanding of fungal and enzymatic lignocellulose degradation requires a combination of enzyme biochemical data with identification of modifications in real, complex lignocellulose materials. The understanding underpins engineering of more effective biocatalysts and their exploitation in either break down of lignocellulose or modification to glyco-materials.

Exploring fungal genomes for novel natural products

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FLASH TALK - *Presenting author' e-mail: j.collemare@gmail.com*

Fungi compete and interact with other organisms, and respond to environmental conditions through producing myriads of compounds, so-called natural products or secondary metabolites (SMs). Many of them find application in industry and medicine due to their diverse biological activities, including antibiotics (penicillins, cephalosporins), immunosuppressants (cyclosporine A), carcinostatics (lentinan), antifungals (echinocandin B), cholesterol-lowering agents (statins). The genomic era has revealed that fungal genomes contain many more biosynthetic pathways than known

SMs, showing that the fungal kingdom has been an underexploited reservoir of bioactive compounds, even fungal classes that have been extensively screened in vitro. Here, we used a tailor-made bioinformatic pipeline to identify novel biosynthetic pathways in the genomes of four Lecanoromycetes that form lichens. A phylogenetic analysis of non-reducing polyketide synthetases (PKSs) identified a novel clade for which no SM has been assigned. Search in other fungal genomes revealed that this PKS is present in a few distant fungal species and comparative genomics predicted a putative gene cluster of 10 genes. Heterologous expression of the PKS gene in *Aspergillus oryzae* NSAR1 resulted in the production of a yellow compound. The compound produced by the transformants was analysed with liquid chromatography-mass spectrometry, and its chemical structure elucidated using nuclear magnetic resonance. Elucidation of the complete pathway using heterologous expression of all the genes from the predicted pathway is in progress. Biological activities are being tested and ecological implications are also discussed.

Deletion of the regulatory gene *ara1* or metabolic gene *xki1* in *Trichoderma reesei* leads to increased CAZyme gene expression on crude plant biomass

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Trichoderma reesei is one of the main producers of enzymes for the conversion of plant biomass to sustainable fuels and chemicals, but there is limited understanding of how the transcriptional response to crude plant biomass is regulated. Regulatory or catabolic deletion mutants showed a clear phenotype only during growth on simple substrates, but not on crude plant biomass. In addition, it is unknown whether induction on untreated recalcitrant crude plant biomass (with a large diversity of inducers) can be sustained for longer.

We investigated the transcriptomic response of *T. reesei* (focusing at CAZymes, C-catabolism and related regulators), to two industrial feedstocks which differ in composition, corn stover and soybean hulls, over time (4h, 24h and 48h), and its regulatory basis using deletion mutants of the two main regulators involved in this process (*xyr1*

and *ara1*). We also investigated whether deletion of a xylulokinase gene (Δ*xki1*) from the pentose catabolic pathway that converts potential inducers could lead to increased CAZyme gene expression.

Our data demonstrates the complexity of the regulatory system related to plant biomass degradation in *T. reesei* and the effect the feedstock composition has on this. Furthermore, this dataset provides leads to improve the efficiency of a *T. reesei* enzyme cocktail, such as by the choice of substrate or by deleting *xki1* to obtain higher production of plant biomass degrading CAZymes.

Biological importance of lytic polysaccharide monooxygenases and cellobiose dehydrogenase in *Aspergillus nidulans*

César Rafael Fanchini Terrasan, Marcelo Ventura Rubio, Jaqueline Aline Gerhardt, Mariane Paludetti Zubieta, Fernanda Lopes De Figueiredo, Fabiano Jares Contesini, Fernanda Lima Valadares, Andre Ricardo De Lima Damasio*

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Several AA9 lytic polysaccharide monooxygenases (LPMOs) and a AA3_1 cellobiose dehydrogenase were previously raised as targets due to their differential secretion during *A. nidulans* growth in different polymeric substrates. The genes encoding for these enzymes were selected as targets for studies aiming to evaluate their biological importance by gene deletion. Initial assays showed that when cultivated on agar plates supplemented with different substrates, no differences in mycelia or conidiation were observed, however, single deletion strains showed faster growth in some polymeric substrates such as polygalacturonic acid, Avicel and CMC, as compared to the WT. After cultivation in liquid medium with Avicel (condition in which the LPMOs and the CDH are concomitantly secreted), no differences in mycelia dry-weight or total protein secretion were observed, but the secretomes of LPMO single deletion mutants showed improved activity levels on some cellulosic substrates such as pNPC, pNPG and PASC. Analysis of the oligosaccharides profile by capillary electrophoresis, generated from reactions with PASC, displayed higher amount of small cellooligosaccharides released by the mutant secretomes in relation to the WT. To address the observed changes, further analysis of the secretomes by MS confirmed differential qualitative and quantitative secretion of enzymes acting on cellulose. In addition to the verified in the WT strain, the secretome of the mutants lacking one of the targets LPMO presented increased secretion of one beta-glucosidase and contained, additionally, one endoglucanase and one CBH. Overall, the sum of normalized peptide counts from enzymes with cellulolytic activity were increased by 10 - 20% in the delta LPMO strains, respectively.

Coupling cell communication and optogenetics: Implementation of a synthetic light-inducible intercellular system in yeast

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In recent years, the rise of synthetic biology has changed the way biological systems can be studied, as for example by constructing synthetic cell communication systems. Here, different biological components/activities are compartmentalized in multiple cell populations, allowing the study of specific behaviors at a community level. In that context, the budding yeast Saccharomyces cerevisiae performs mating between haploid cells using an efficient communication system based on peptide signaling molecules: MFα1.

In this work, we coupled yeast pheromone response with optogenetics to determine if the propagation of light signals, through the coordinated production of a pheromone, could synchronize several biological phenomena at a population level. In order to develop a synthetic intercellular system, different pheromone-responsive strains were generated by the insertion of a luciferase construct in a locus responsive to the pheromone. In addition, in other cells, we used the FUN-LOV optogenetic switch to control MFα1 gene expression in response to blue-light. In that context, supernatants obtained from yeast cultures grown in constant illumination induce a transcriptional activation only in strains that are hypersensitive to MFα1. We assessed the luciferase activity in co-cultures between opto-pheromone producer and hypersensitive reporter strains. Cell mixtures exposed to constant blue-light showed transcriptional activation, unlike cultures grown in darkness.

Thus, these results show the successful implementation of a two-component synthetic intercellular transcriptional-exocrine system where a cue, originally triggered by light, is propagated through extracellular signaling to control gene expression in different cells. We are currently working on implementing additional cellular steps to this system, to increase intercellular circuitry complexity and community dynamics. Funding: iBIO, FONDECYT 1171151 and HHMI International Research Scholar grant.

Practical guidance for the implementation of the CRISPR genome editing tool in filamentous fungi

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Within the last years, numerous reports described successful application of the CRISPR nucleases Cas9 and Cpf1 for genome editing in filamentous fungi. However, still a lot of efforts are invested to develop and improve protocols for the fungus and genes of interest with respect to applicability, scalability and targeting efficiencies. We present data for successful genome editing in the cell factory *Thermothelomyces thermophilus*, formerly known as *Myceliophthora thermophila*, using the three different nucleases SpCas9, FnCpf1 and AsCpf1 guided to different gene targets of our interest. CRISPR nucleases were either delivered to *T. thermophilus* on plasmids or preassembled with *in vitro* transcribed gRNA to form ribonucleoproteins (RNPs). All approaches enabled successful genome editing in *T. thermophilus*; however, with different success rates. In addition, we show that the success rate depends on the respective nuclease and on the targeted gene locus.

Investigation of secondary Metabolite Biosynthetic Pathwais in Endophytic Fungi Through Genomic Analysis

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Endophytic fungi isolated from medicinal plants have been widely studied due to their ability to produce secondary metabolites. However, these studies are commonly performed under laboratory conditions, which in most of the cases is not enough to explore all the metabolites endophytic fungi could possibly produce. Because most biosynthetic pathways are silenced under in vitro conditions, genomic approaches combined with heterologous expression have proven successful in activating the production of novel compounds. Our group previously reported the isolation of 1064 fungal endophytes from two medicinal plants from Pantanal and Cerrado Brazilian biomes. These isolates were grouped into 124 different groups based on the morphological characteristics. From this study 13 isolates were selected based on taxonomic diversity and production of bioactive secondary metabolites. Two new active compounds belonging to the carboxamide class were identified from two *Diaporthe* strains. The present study intends to explore the secondary metabolism of these 13 endophytes through genomic and expression analyses. In addition, modification of metabolite production by these species using different growth conditions, including using inhibitors of histone modifications is tested. This work will answer questions that were inferred in the previous study: which gene

clusters are responsible for the production of active metabolites? How conserved are these gene clusters in endophytes and more largely in the fungal kingdom? Is it possible to improve metabolite production using specific conditions?

Building *Trichoderma reesei* as an ideal chassis to produce valuable products

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The filamentous fungus *T. reesei* has the extremely high capacity for protein secretion and is potential production platform for heterologous protein as well as other useful moleculars. In order to construct a chassis strain of *T. reesei* suitable for highly efficient product for industry application we established a genome-editing system by using in vitro assembled Cas9-gRNA ribonucleoprotein in *T. reesei* and then applied this system to build a chassis strain by deleting ten genes encoding major lignocellulolytic enzymes and a protease regulator-coding gene pea1. To test this chassis strain, genes encoding different heterologous proteins including human serum albumin, fungal immunomodulatory protein, and a thermotolerant xylanase XYL7 derived from a gut metagenomics library of termite were heterologously expressed in it by replacing the cellobiohydrolase I (CBHI) individually. We obtain a relative high yield for all of the tested heterologous proteins. Besides, we are also test the chassis to synthesize small compounds. It seems we have successfully constructed *T. reesei* chassis with limited background of endogenous proteins and proteases.

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SATELLITE WORKSHOP

Asperfest 17

SUNDAY, FEBRUARY 16

LOCATION: **Sapienza University of Rome** | BUILDING: **CU022** | SIDE: **Botanica** | FLOOR: **Ground** | ROOM: **Giacomini**
Coordinated by **Ling Lu** | Nanjing Normal University, Poster Session Chair
Judging for Novozymes Student Poster Prize

17:00 - 20:00 **Registration and poster hang up, Poster and Welcome Reception** (sponsored by **Novozymes**)

17:00 - 18:30 **ODD-NUMBERED POSTERS**

18:30 - 20:00 **EVEN-NUMBERED POSTERS**

MONDAY, FEBRUARY 17

LOCATION: **Sapienza University of Rome** | BUILDING: **CU022** | SIDE: **Botanica** | FLOOR: **Ground** | ROOM: **Montalenti**

Session I

Chair: ***Richard Todd*** | Kansas State University & ***Norio Takeshita*** | University of Tsukuba

09:00 - 09:15 **Welcome, introductions and announcements**

09:15 - 09:30 **Transcriptional networks controlling asexual development in *Aspergillus nidulans*: An evolutionary perspective**
Oier Etxebeste | University of the Basque Country

09:30 - 09:45 **Iron sensing in *Aspergillus fumigatus***
Hubertus Haas - Innsbruck Medical University

09:45 - 10:00 **Fungal-host interactions: A duel to the (cell) death**
Neta Shlezinger | The Hebrew University of Jerusalem

10:00 - 10:15 ***Aspergillus terreus* itaconic acid fermentation: the physiology behind key technological parameters**
Levente Karaffa | University of Debrecen

10:45 - 11:15 **Coffee Break**

Session II

Chair: ***Richard Todd*** | Kansas State University

11:15 - 11:30 **Developing a high-throughput functional genomics platform for *Aspergillus flavus***
N. Louise Glass | UC Berkeley

11:30 - 11:45 **Genetic engineering of fungi exploiting pyrimidine salvage pathway-based self-encoded selectable markers**
Fabio Gsaller | Innsbruck Medical University

Flash Talks

CHAIR: ***Nancy Keller*** | University of Wisconsin & ***Michelle Momany*** | University of Georgia

11:45 - 12:15
Speed pitches
(5 min) to posters

1. **Identification of the guanine nucleotide exchange factor for SAR1 in the filamentous fungal model *Aspergillus nidulans***
   Ignacio Bravo-Plaza | Centro de Investigaciones Biológicas
2. **The nicotinic acid pathway of *Aspergillus nidulans* includes a reversible conversion to 6-hydroxynicotinic acid**
   Eszter Bokor | University of Szeged
3. **CreD ubiquitination required for endocytic degradation of the maltose transporter MalP in *Aspergillus oryzae***
   Shoki Fujita | Tohoku University
4. **Combinatorial control of transcription factors involved in sugar beet pulp utilization in the industrially relevant fungus *Aspergillus niger***
   Sandra Garrigues | Westerdijk Fungal Biodiversity Institute
5. **Identification of novel proteins for fungal cell-to-cell communication by localization screening from multicellularity-specific uncharacterized genes**
   Mamun Abdulla Al | The University of Tokyo
6. **A rapid CRISPR-mediated Tet-Off system reveals the phosphoinositide kinases Stt4 and Mss4 are essential for viability of *Aspergillus fumigatus***
   Hajer Alshraim Alshammri | The University of Manchester
7. **Comparative genomics of *Aspergillus fumigatus* and the influence of agriculture on ecology and azole resistance**
   Amelia Barber | University of Wuerzburg
8. ***Aspergillus fumigatus* elicits host-derived extracellular vesicles upon infection**
   Matthew Blango | Leibniz Institute for Natural Product Research and Infection Biology
9. **Rewiring metabolic pathways for organic acid production in the filamentous fungus *Aspergillus niger***
   Peter Punt | Dutch DNA Biotech & Leiden University

12:15 - 12:45 **Community directions discussion; Elections**
David Roos | FungiDB update

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SATELLITE WORKSHOP
Fusarium

MONDAY, FEBRUARY 17

LOCATION: **Sapienza University of Rome** | BUILDING: **CU022** | SIDE: **Botanica** | FLOOR: **Ground** | ROOM: **Giacomini**

08:45 **INTRODUCTION AND WELCOME**

Session I - Fusarium-host interaction (plants/animals/humans)

CHAIR: ***Claire Kanja*** & ***Pravin Khambalkar***

09:00 - 09:00 ***Fusarium oxysporum*** **effectoromes**
Martijn Rep | University of Amsterdam, the Netherlands

09:15 - 09:30 **DNA transposons drive adaptive evolution in the fungal cross-kingdom pathogen *Fusarium oxysporum***
Cristina López-Diaz | Universidad de Córdoba, Córdoba, Spain

09:30 - 09:45 **Functional characterisation of candidate *Fusarium graminearum* effectors**
Claire Kanja | Rothamsted Research, United Kingdom

09:45 - 10:00 **SIX6: A route to plant cell death**
Pravin Khambalkar | The Australian National University, Australia

10:00 - 10:15 **PHI-base, a multispecies phenotype database for pathogens, hosts and their interactions to enhance global food security and human health**
Martin Urban | Rothamsted Research, United Kingdom

10:15 - 10:45 **Coffee Break**

Session II - Evolution, taxonomy and genome dynamics

CHAIR: ***Alessandra Villani*** & ***Edoardo Piombo***

10:45 - 11:00 **Deciphering the effect of ambient pH on enniatins production by *Fusarium tricinctum***
Nadia Ponts | INRA, France

11:00 - 11:15 **Unveiling the *Fusarium graminearum* species complex: Global database of species and chemotypes**
Antonio Moretti | CNR-ISPA, Italy

11:15 - 11:30 **Mitochondrial genomes as phylogenetic backbone**
Balazs Brankovics | WUR Biointeractions & Plant Health, the Netherlands

11:30 - 11:45 **Using comparative genomics to identify genes involved in *Fusarium fujikuroi* pathogenicity**
Edoardo Piombo | University of Turin, Italy

11:45 - 12:00 **Variation in secondary metabolite production potential in the *Fusarium incarnatum-equiseti* species complex revealed by comparative analysis of 13 genomes**
Alessandra Villani | CNR-ISPA, Italy

12:00 - 12:45 Speed pitches (5 min) to posters

1. **Topographically triggered mycelial bundles in *Fusarium* species**
   Anne Van Diepeningen | WUR Biointeractions & Plant Health, The Netherlands
2. **FungiDB: integrating genomic data for pathogens and model organisms and providing advanced search capabilities and large-scale data analysis**
   Achchuthan Shanmugasundram | University of Liverpool, United Kingdom
3. **The specialized metabolite gene clusters of *Fusarium graminearum* species complex and their response during the cell-to-cell invasion of wheat**
   Sabina Moser Tralamazza | University of Neuchâtel, Switzerland
4. **Deciphering the effect of ambient pH on enniatins production by *Fusarium avenaceum***
   Florence Richard-Forget | INRA Bordeaux, France
5. **Genetic diversity and mycotoxin production among *Fusarium* head blight isolates belonging to the *Fusarium tricinctum* species complex from Italy**
   Maria Teresa Senatore | University of Bologna Italy
6. **Understanding the origin, diversity and evolution of the Panama Disease pathogen**
   David Torres Sanchez | Wageningen University, The Netherlands

12:45 - 14.00 **Lunch Break and poster viewing**

Session III - Regulation, signal detection and secondary metabolism

CHAIR: ***Slavica Janevska & Sabina Tralamazza***

14:00 - 14:15 **Microbial interactions of Fusarium oxysporum in the soil**
Corby H. Kistler | USDA, University of Minnesota, USA

14:15 - 14:30 **The Crz1 transcription factor regulates lipid metabolism and fumonisin production in *Fusarium verticillioides***
Marzia Beccaccioli | Sapienza University of Rome, Italy

14:30 - 14:45 **The fumonisin cluster gene *FUM18* encodes a functional ceramide synthase that confers self-protection against the produced sphingolipid inhibitor**
Slavica Janevska | Hans Knöll Institute, Jena, Germany

14:45 - 15:00 **Functional studies of the role of the RING-Finger protein CarS in *Fusarium fujikuroi***
Carmen M Limón | University of Seville, Spain

15:00 - 15:15 **Functional subregions of the endoplasmic reticulum of *Fusarium graminearum* upon induced Secondary Metabolism**
Marike Boenisch | University of Minnesota, USA

15:15 - 15:45 **Coffee break and poster viewing**

Session IV - Genetic exploitation in applied and industrial mycology
CHAIR: ***Trine Sørensen & Linda Brain***

15:45 - 16:00 **Genus-wide analysis of *Fusarium* polyketide synthases uncovers broad natural product potential**
Daren W. Brown | USDA Peoria, USA

16:00 - 16:15 **Characterisation of *Fusarium graminearum* chitin synthases**
Linda Brain | La Trobe University and University of Adelaide, Australia

16:15 - 16:30 **Novel nonribosomal peptides from *Fusarium graminearum***
Teis E. Søndergaard | Aalborg University, Denmark

16.30 - 16:45 **Solving the polyketide pigmentation puzzle in *Fusarium solani***
MR Nielsen | Aalborg University, Denmark

16.45 - 17:00 **Expansion of fungi enables high resolution in fluorescence microscopy**
Ulrich Terpitz | Julius Maximilian University, Germany.

17.00 Other business and Closing

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SATELLITE WORKSHOP

Trichoderma, Clonostachys and other biocontrol fungi

MONDAY, FEBRUARY 17

LOCATION: **Sapienza University of Rome** | BUILDING: **CU022** | SIDE: **Botanica** | FLOOR: **Ground** | ROOM: **D**

09:45 - 10:15 **Welcome Coffee**

10:15 - 10:30 **Welcome address and opening of the workshop**
Matteo Lorito | University of Naples Federico II, Italy

SESSION I - Invited talks: research results and industries point of view
CHAIR: ***Matteo Lorito*** | University of Naples Federico II, Italy

10:30 - 11:00 ***Trichoderma* and heritable plant responses**
Enrique Monte | University of Salamanca, Spain

11:00 - 11:30 **Whole-genus ecological genomics of *Trichoderma*: the first steps towards understanding the origin of environmental opportunism**
Irina Druzhinina | Nanjing Agricultural University, China

11:30 - 12:00 **Exploring genetic variation in *Clonostachys* to understand biological control mechanisms**
Magnus Karlsson | Swedish University of Agricultural Sciences, Uppsala, Sweden

12:00 - 12:30 **How root-colonizing endophytes promote plant performance and influence ecosystems**
Ralph Oelmüller | Schleiden Institute Biology, Jena, Germany

12:30 - 13:00 **Effect of plant species, pathogen, environmental factors and their interactions on *Trichoderma harzianum* strain INAT11**
Edith Ladurner | CBC Europe S.r.l, BIOGARD Division, Italy

13:00 - 13:30 **Present and future of *Trichoderma asperellum* + *Trichoderma gamsii* as biocontrol agent in the Isagro portfolio**
Riccardo Liguori | Isagro S.p.A. Novara Research Center, Italy

13:30 - 14:30 **Light Lunch**

SESSION II. Offered talks

CHAIR: ***Sabrina Sarrocco*** | University of Pisa, Italy

14:30 - 14:40 **_Trichoderma atroviride_ mycoparasitism and its regulation by the TOR signaling pathway**
Susanne Zeilinger | University of Innsbruck, Austria

14:40 - 14:50 **Uncovering essential mechanisms of chitin and chitosan remodeling in the cell wall of the mycoparasite _Trichoderma atroviride_**
Lisa Kappel | University of Innsbruck, Austria

14:50 - 15:00 **Testing the role of the transcription factor TvSom1 in adhesion of _Trichoderma virens_ germlings**
Benjamin Horwitz | Technion, Haifa, Israel

15:00 - 15:10 **Enhancing peptaibols production in the biocontrol fungus _Trichoderma longibrachiatum_ SMF2 by elimination of a putative glucose sensor**
Yu-Zhong Zhang | Shandong University, Qingdao, China

15:10 - 15:20 **Azaphilones biosynthesis in _Trichoderma harzianum_ benefits fungal survival to oxidative stress**
Jian Zhang | Nanjing Agricultural University, China

15:20 - 15:30 **Complete genome sequences reveals novel insights into chromosomal organization and evolution of different _Trichoderma_ species**
Ting-Fang Wang | Academia Sinica, Taipei, Taiwan

15:30 - 15:40 **Effects of _Trichoderma_ strains and metabolites on the growth, disease resistance, leaf transcriptome and metabolome of olive plants**
Roberta Marra | University of Naples Federico II, Italy

15:40 - 16:30 **Coffee Break**

SESSION III. Offered talks

CHAIR: ***Magnus Karlsson*** | Swedish University of Agricultural Sciences, Uppsala, Sweden

16:30 - 16:40 **Terpene synthases in _Trichoderma gamsii_ T6085**
Isabel Vicente Muñoz | University of Pisa, Italy

16:40 - 16:50 **Austrian _Trichoderma_ spp. impact mycotoxin production of the plant pathogen _Fusarium graminearum_**
Wolfgang Hinterdobler | Austrian Institute of Technology, Tulln, Austria)

16:50 - 17:00 ***Trichoderma* applications affect the physiological processes that improve strawberry production and quality**
Nadia Lombardi | University of Naples Federico II, Italy

17:00 - 17:10 **Valorization of by-products from oleaginous crops production using *Trichoderma* spp**
Laura Gioia | University of Naples Federico II, Italy

17:10 - 17:20 **LysM effectors regulate fungal development and required for hyphal protection and biocontrol traits in *Clonostachys rosea***
Mukesh Dubey | Swedish University of Agricultural Sciences, Uppsala, Sweden)

17:20 - 17:30 ***Pseudomonas chlororaphis* ToZa7 and *Clonostachys rosea* IK726, a successful biocontrol pair against Fusarium crown and root rot of tomato**
Anastasia Lagopodi | Aristotle University Thessaloniki School of Agriculture, Greece)

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SATELLITE WORKSHOP

Colletotrichum 2020

MONDAY, FEBRUARY 17

LOCATION: **Sapienza University of Rome** | BUILDING: **CU022** | SIDE: **Botanica** | FLOOR: **Ground** | ROOM: **C**
ORGANIZERS: ***Riccardo Baroncelli*** & ***Serenella Sukno*** | Universidad de Salamanca

09:00 - 09:15	**Arrival of participants and Welcome**
09:15 - 09:40	**Horizontal gene transfer contributes to virulence in *Colletotrichum*** **Michael Thon** | University of Salamanca
09:40 - 10:05	**Genome rearrangements drive evolution of virulence-related genes in the genomes of *Colletotrichum gloeosporioides* species complex** **Pamela Gan** | Riken
10:05 - 10:45	***Colletotrichum* species diversity on aromatic and ornamental plant hosts in Italy** **Vladimiro Guarnaccia** | University of Torino
10:45 - 11:15	**Coffee Break**
11:15 - 11:40	**Glycosylated flavonoids - fruit hidden arsenal against fungal pathogens** **Noam Alkan** | The Volcani Center
11:40 - 12:05	**Reactive Oxygen Species dosage in *Arabidopsis* chloroplasts improves resistance towards *Colletotrichum higginsianum* in a WRKY33-dependent fashion** **Lars Voll** | Philipps-University Marburg
12:05 - 12:45	**Investigating the role of a fungal oxidase-peroxidase tandem in plant pathogenicity** **Bastien Bissaro** | Aix-Marseille Université
12:45 - 14:00	**Lunch Break**
14:00 - 14:25	**The effect of fruit sugar level on the pathogenicity mechanism and host response during *Colletotrichum* infection of red tomatoes** **Carmit Ziv** | The Volcani Center
14:25 - 14:50	**Infectious process and intraspecific diversity of *Colletotrichum lupini*, a fungal pathogen responsible for lupin anthracnose** **Gaetan Le Floch** | University of Brest

14:50 - 15:15	**The olive anthracnose pathosystem as a case-study for fungal taxonomy, epidemiology and host-pathogen interactions towards sustainable disease resistance** **Pedro Talhinhas** | Universidade de Lisboa
15:15 - 15:50	***Colletotrichum* and *Citrus*, the Postbloom fruit drop studies advances** **Eduardo Goulin** | Instituto Federal de Educação, Ciência e Tecnologia de Santa Catarina
15:50 - 16:15	**Coffee Break**
16:15 - 16:35	**To have or not to have: A dispensable chromosome enables host colonization in the pathosystem *Colletotrichum higginsianum* – Arabidopsis thaliana** **Peter Plaumann** | Friedrich-Alexander-Universität Erlangen-Nürnberg
16:35 - 16:55	***Colletotrichum truncatum* effector repertoire revealed by comparative genomics and transcriptomics analyses** **Thaís Regina Boufleur** | University of São Paulo
16:55 - 17:15	**Genetic diversity within *Colletotrichum lupini*, the causal agent of lupin anthracnose, and its virulence on white lupin (*Lupinus albus*)** **Joris Alkemade** | Research Institute of Organic Agriculture (FiBL)
17:15 - 17:30	**Closure**

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SATELLITE WORKSHOP
Neurospora

MONDAY, FEBRUARY 17

LOCATION: **Sapienza University of Rome** | BUILDING: **CU022** | SIDE: **Botanica** | FLOOR: **First** | ROOM: **Marini Bettolo**
Neurospora Satellite Meeting | ***Neurospora crassa***

09:00 - 09:05 **Welcome**

09:05 - 09:25 **Habitat specific clock variation and its consequence on reproductive fitness**
Kwangwon Lee | Rutgers University, Camden

09:25 - 09:45 **Methylxanthines modulate the circadian period length independently of the action of phosphodiesterase**
Luis Larrondo | Pontificia Universidad Catolica de Chile

09:45 - 10:05 **Calcium signaling genes play a role in stress tolerance, thermotolerance, cellulose degradation, and circadian clock in *Neurospora crassa***
Ranjan Tamuli | Indian Institute of Technology, Guwahati

10:05 - 10:25 **Regulation of conidiation by the velvet complex in *Neurospora crassa***
Sara Cea-Sánchez | University of Seville

10:25 - 10:45 **GUL-1 mediates cell wall remodelling via the COT-1 pathway in *Neurospora crassa***
Inbal Herold | Hebrew University of Jerusalem

10:45 - 11:15 **Coffee Break**

11:15 - 11:35 **The role of the STRIPAK complex in sexual development of *Sordaria macrospora***
Stefanie Pöggeler | University of Göttingen

11:35 - 11:55 **SIP-1 is essential for germling fusion of *Neurospora crassa*, probably by mediating the initiation of cell-cell communication**
Anne Oostlander | University of Braunschweig

11:55 - 12:15 **BRO1 is required for cell-cell fusion in *Neurospora crassa* and localizes to a specific subpopulation of vesicles**
Hamzeh Hammadeh | University of Braunschweig

12:15 - 12:35	**A blueprint of the protein secretion machinery in *Neurospora crassa*** **Areejit Samal** | Homi Bhabha National Institute, Chennai
12:35 - 14:00	**Lunch Break**
14:00 - 14:20	**Crosstalk of cellulose and mannan signaling pathways during plant cell wall perception is inhibitive to cellulase expression** **Philipp Benz** | Technical University of Munich
14:20 - 14:40	**Fast forward genetics to associate *Neurospora crassa* mutant phenotypes with genes** **Scott Baker** | Pacific Northwest National Laboratory, Richland
14:40 - 15:00	**Developing a *tetO*/TetR system in *Neurospora crassa*** **Eugene Gladyshev** | Institut Pasteur, Paris
15:00 - 15:20	**Utilizing a set of fungal deletion and fusion tagging Golden Gate vectors for analyzing the function of regulatory and developmental proteins** **Ines Teichert** | University of Bochum
15:20 - 15:40	**Accessing cutting edge capability at the Environmental Molecular Sciences Laboratory** **Scott Baker** | Pacific Northwest National Laboratory, Richland
15:40 - 16:15	**Coffee Break**
16:15 - 16:35	**FungiDB: integrating genomic data for pathogens and model organisms and providing advanced search capabilities and large-scale data analysis** **Omar Harb** | FungiDB
16:35 - 17:30	**General Discussion**

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SATELLITE WORKSHOP

Magnafest 2020

MONDAY, FEBRUARY 17

LOCATION: **Sapienza University of Rome** | BUILDING: **CU022** | SIDE: **Botanica** | FLOOR: **Ground** | ROOM: **E**
Magnaporthe Meeting | Magnafest 2020

09:00 - 09:30	**Arrival and Coffee Break** **Introduction to meeting**
09:30 - 09:55	**FungiDB: integrating genomic data for pathogens and model organisms and providing advanced search capabilities and large-scale data analysis** **Evelina Basenko** | University of Liverpool
09:55 - 10:20	**Transcriptional Regulation of Effectors in the Rice Blast Fungus *Magnaporthe oryzae*** **Bozeng Tang** | The Sainsbury Laboratory
10:20 - 10:45	**Alternative splicing as an element of signal transduction in multi-step phosphorelay systems in fungi** **Sri Bühring** | Institute of Biotechnology and Drug Research
10:45 - 11:10	**Diversified modulation of transcriptome complexity by alternative splicing during rice- *Magnaporthe oryzae* interactions** **Jongbum Jeon** | Seoul National University
11:10 - 11:40	**Coffee Break**
11:40 - 12:05	**A rice/*Arabidopsis thaliana* glycosyl hydrolase gene displays ambivalent immunity with diverse types of phytopathogens** **Chi-Yeol Kim** | The Sainsbury Laboratory
12:05 - 12:30	**Understanding the Pmk1 MAP kinase signalling pathway during plant colonisation by the rice blast fungus *Magnaporthe oryzae*** **Neftaly Cruz-Mireles** | The Sainsbury Laboratory
12:30 - 13:30	**Lunch Break**
13:30 - 13:55	**Cellular Control of Proteostasis During Infection-Related Development by the Rice Blast Fungus *Magnaporthe oryzae*** **Takayuki Arazoe** | Tokyo University of Science

13:55 - 14:20	**Rapid adaptation of signalling networks in the fungal pathogen *Magnaporthe oryzae*** **Katharina Bersching** | Institute of Biotechnology and Drug
14:20 - 14:45	**The *Magnaporthe oryzae* circadian clock functions in determining rice blast pathogenicity** **Ciaran Griffin** | University of Plymouth
14:45 - 15:10	**Coffee Break**
15:10 - 15:40	**General Discussion and concluding comments**

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