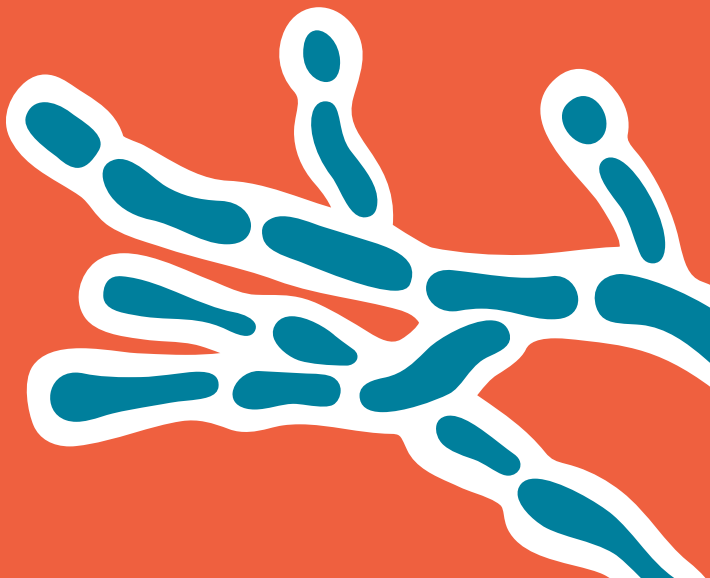
ECFG15
ROME • ITALY
2020

Fungal genetics, host pathogen interaction and evolutionary ecology

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

15TH EUROPEAN CONFERENCE ON FUNGAL GENETICS

SATELLITE WORKSHOPS



SUMMARY

ECFG15
ROME • ITALY 2020

SATELLITE WORKSHOP

Asperfest 17

SUNDAY, FEBRUARY 16

Location: **Sapienza University of Rome**
Building: **CU022** | Side: **Botanica** | Floor: **Ground** | Room: **Giacomini**

Registration and poster hang up, Poster and Welcome Reception (sponsored by **Novozymes Inc.**)

17:00 - 18:30	**Odd-numbered posters**
18:30 - 20:00	**Even-numbered posters**

Judging for Novozymes Student Poster Prize, Coordinated by **Ling Lu** | Nanjing Normal University, POSTER SESSION CHAIR
Poster take down

MONDAY, FEBRUARY 17

Location: **Sapienza University of Rome**
Building: **CU022** | Side: **Genetica** | 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 - 10:45	1. **Oier Etxebeste** | University of the Basque Country **Transcriptional networks controlling asexual development in *Aspergillus nidulans*: An evolutionary perspective**
	2. **Hubertus Haas** - Innsbruck Medical University **Iron sensing in *Aspergillus fumigatus***
	3. **Neta Shlezinger** | The Hebrew University of Jerusalem **Fungal-host interactions: A duel to the (cell) death**
	4. **Levente Karaffa** | University of Debrecen ***Aspergillus terreus* itaconic acid fermentation: the physiology behind key technological parameters**
10:45 - 11:15	**Coffee Break**

Session II

CHAIR: **Richard Todd,** Kansas State University

11:15 - 11:30 **N. Louise Glass** | UC Berkeley
Developing a high-throughput functional genomics platform for *Aspergillus flavus*

11:30 - 11:45 **Fabio Gsaller** | Innsbruck Medical University
Genetic engineering of fungi exploiting pyrimidine salvage pathway-based self-encoded selectable markers

Flash Talks

CHAIR: **Nancy Keller,** University of Wisconsin – **Michelle Momany,** University of Georgia

11:45 - 12:15

Speed pitches (3 min) to posters

1. **Ignacio Bravo-Plaza** | Centro de Investigaciones Biológicas
Identification of the guanine nucleotide exchange factor for SAR1 in the filamentous fungal model *Aspergillus nidulans*

2. **Eszter Bokor** | University of Szeged
The nicotinic acid pathway of *Aspergillus nidulans* includes a reversible conversion to 6-hydroxynicotinic acid

3. **Shoki Fujita** | Tohoku University
CreD ubiquitination required for endocytic degradation of the maltose transporter MalP in *Aspergillus oryzae*

4. **Sandra Garrigues** | Westerdijk Fungal Biodiversity Institute
Combinatorial control of transcription factors involved in sugar beet pulp utilization in the industrially relevant fungus *Aspergillus niger*

5. **Mamun Md. Abdulla Al** | The University of Tokyo
Identification of novel proteins for fungal cell-to-cell communication by localization screening from multicellularity-specific uncharacterized genes

6. **Hajer Alshraim Alshammri** | The University of Manchester
A rapid CRISPR-mediated Tet-Off system reveals the phosphoinositide kinases Stt4 and Mss4 are essential for viability of *Aspergillus fumigatus*

7. **Amelia Barber** | University of Wuerzburg
Comparative genomics of *Aspergillus fumigatus* and the influence of agriculture on ecology and azole resistance

8. **Matthew Blango** | Leibniz Institute for Natural Product Research and Infection Biology
***Aspergillus fumigatus* elicits host-derived extracellular vesicles upon infection**

	9. **Peter Punt** | Dutch DNA Biotech & Leiden University **Rewiring metabolic pathways for organic acid production in the filamentous fungus *Aspergillus niger***
12:15 - 12:45	**David Roos** | FungiDB update **Community directions discussion; Elections**
12:45 - 14:00	**Lunch**

Session III

CHAIR: **David Canovas,** University of Seville – **Nir Osherov,** Tel Aviv University

14:00 -14:15	**Sjoerd Seekles** | Leiden University **The effect of cultivation temperature on the heat resistance of *Aspergillus niger* conidia**
14:15 - 14:30	**Irene Picazo** | Centro de Investigaciones Biológicas **Effects of ambient alkaline pH on gene expression: a key regulatory role for the cation-homeostasis transcription factor SltA**
14:30 - 14: 45	**Danielle Weaver** | University of Manchester **Uncovering long non-coding RNA associated with drug response in *Aspergillus fumigatus***
14:45 - 15:00	**Gayan Abeysinghe** | University of Tsukuba **Exploring the variety of interactions between Fungi and Bacteria**
15:00 - 15:15	**Sayoko Oiki** | University of Tsukuba **Cellular response to farnesol and the role of nitric oxide production in *Aspergillus fumigatus***
15:15 - 15:30	**Paul Schäpe** | TU-Berlin **Harnessing transcriptomic data to predict the function of proteins in the microbial cell factory *Aspergillus niger***
15:30 - 15:45	**Anezia Kourkoulou** | National and Kapodistrian University of Athens **UapA-membrane lipid interactions are crucial for ER-exit, dimerization, function and expression of mammalian transporters in *A. nidulans***
15:45 - 16:15	**Coffee break**

Pontecorvo Lecture (sponsored by Zymergen)
CHAIR: **Norio Takeshita,** University of Tsukuba – **Benjamin Knox**, Zymergen, Inc.

16:15 - 17:00	**Katsuya Gomi** | Tohuku University **How the koji mold *Aspergillus oryzae* produces amylolytic enzymes?**
17:00 - 17:30	**Election results; Novozymes student poster prizes; other discussion items**
17:30	**Dismiss**

Presenter indicated in bold type* denotes a student poster presenter

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

Mamun Md. Abdulla Al[1], Katayama Takuya[1], Cao Wei[2], Nakamura Shugo[2], Maruyama Junichi[1]

[1] *Department of Biotechnology, The University of Tokyo, Bunkyo-ku, Tokyo, Japan*
[2] *INIAD, Toyo University, Bunkyo-ku, Tokyo, Japan*

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

2. Analysis of self-assembly mechanism of hydrophobin RolA of *Aspergillus oryzae* using Langmuir- Blodgett method

Keietsu Abe[1], Yuki Terauchi[1], Takumi Tanaka[1], Masaya Mitsuishi[1], Hiroshi Yabu[2],

Akira Yoshimi[3], Fumihiko Hasegawa[3]

[1] *Graduate School of Agricultural Science, Department of Microbial Biotechnology, Sendai, Miyagi, Japan*
[2] *Tohoku University, WPI-Advanced Institute for Materials Research, Sendai Miyagi Japan*
[3] *Tohoku University, New Industry Creation Hatchery Center, Sendai Miyagi Japan*

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.

*3. Exploring the variety of interactions between Fungi and Bacteria

Gayan Abeysinghe[1,2], Momoka Kuchira[1], Akihiro Ninomiya[1], Nozomu Obana[1,3], Masuo Shunsuke[1], Naoki Takaya[1], Akira Nakamura[1], Norio Takeshita[1]

[1] *Microbiology Research Center for Sustainability (MiCS), Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan*
[2] *Department of Biological Sciences, Faculty of Applied Sciences, Rajarata University of Sri Lanka*
[3] *Transborder Medical Research Center, Faculty of Medicine, University of Tsukuba, Japan*

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.

Keywords: Fungi, bacteria, co-culture, secondary metabolites, SMGC, phylogeny

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

Mario Aguiar, Anna-Maria Dietl, Matthias Misslinger, Thomas Orasch, Hubertus Haas

Institute for Molecular Biology, Medizinische Universität Innsbruck, Austria

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.

*5. A rapid CRISPR-mediated Tet-Off system reveals the phosphoinositide kinases Stt4 and Mss4 are essential for viability of *Aspergillus fumigatus*

Hajer Alshraim Alshammri, Catrin Bailey, Riba Thomas, Jorge Amich and Michael Bromley

Manchester Fungal Infection Group, Institute of Infection, Immunity and Respiratory Medicine, University of Manchester, Manchester, UK

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*.

6. *In vitro* enzyme evolution of Purine Hydroxylase I (HxA) and Purine Hydroxylase II (HxnS)

Judit Ámon[1], Eszter Bokor[1], Csaba Vágvölgyi[1], Claudio Scazzocchio[2], Zsuzsanna Hamari[1]

[1] *Department of Microbiology, University of Szeged, Faculty of Science and Informatics, Hungary*
[2] *Department of Microbiology, Imperial College London, UK*

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.

This work was supported by NKFI-K16 119516 and 20391-3/2018/FEKUSTRAT.

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

Mark Arentshorst[1], Jos Reijngoud[1], Ian Reed[2], Ebru Alaz[3], iPeter Punt[3], Adrian Tsang[2], Arthur Ram[1]

[1] *Leiden University, Institute of Biology Leiden, Leiden, The Netherlands*

[2] *Concordia University, Centre for Structural and Functional Genomics, Montreal, Canada*
[3] *Dutch DNA Biotech, Utrecht, The Netherlands*

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*.

8. Comparative genomics of *Aspergillus fumigatus* and the influence of agriculture on ecology and azole resistance

Amelia Barber[1], Jennifer Born[2], Tongta Sae-Ong[1], Kang Kang[1], Gianni Panagiotou[1], Holger Deising[2], Oliver Kurzai[3]

[1] *Leibniz HKI – Jena, Germany*
[2] *Martin Luther University, Haale-Wittenberg, Halle (Saale), Germany*
[3] *University of Wuerzburg, Wuerzburg, Germany*

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.

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

Evelina Basenko[1], Omar Harb[2], Achchuthan Shanmugasundram[1], Mark Caddick[1] and David Roos[2]*

[1] *Institute of Integrative Biology, Functional and Comparative Genomics, University of Liverpool, Liverpool UK*
[2] *Department of Biology, The University of Pennsylvania, Philadelphia, Pennsylvania, USA*
** Presented on behalf of the entire VEuPathDB team.*

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.

FungiDB is supported in part by NIH HHSN272201400030C and 75N93019C00077 and the Wellcome Trust #WT108443MA and Wellcome Biomedical Resources #212929/Z/18/Z grants.

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

Cindy Meister, Anna Maria Köhler, **Emmanouil Bastakis** and Gerhard Braus

Georg August University Göttingen, Institute for Microbiology and Genetics, Göttingen, Germany.
E-mails: cmeiste@gwdg.de, akoehle3@gwdg.de, emmanouil.bastakis@uni-goettingen.de, gbraus@gwdg.de

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)

11. The lysine deacetylase RpdA is essential for virulence in *Aspergillus fumigatus*

Ingo Bauer[1], Matthias Misslinger[1], Yana Shadkchan[2], Anna-Maria Dietl[1], Verena Petzer[3], Thomas Orasch[1], Beate Abt[1], Stefan Graessle[1], Nir Osherov[2], Hubertus Haas[1]

[1] *Institute of Molecular Biology, Biocenter, Medical University of Innsbruck, Innsbruck, Austria*
[2] *Aspergillus and Antifungal Research Laboratory, Department of Clinical Microbiology and Immunology, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel*
[3] *Department of Internal Medicine II, Infectious Diseases, Immunology, Rheumatology, Pneumology, Medical University of Innsbruck, Innsbruck, Austria*

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*.

12. *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]

[1] *Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena, Germany*
[2] *Friedrich Schiller University Jena, Jena, Germany*

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.

*13. The nicotinic acid pathway of *Aspergillus nidulans* includes a reversible conversion to 6-hydroxynicotinic acid

Eszter Bokor[1], Judit Ámon[1], Mónika Varga[1], András Szekeres[1], Claudio Sccazzochio[2], Csaba Vágvölgyi[1], Zsuzsanna Hamari[1]

[1] *University of Szeged, Faculty of Science and Informatics, Department of Microbiology, Szeged, Hungary*
[2] *Department of Microbiology, Imperial College, London, England*

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.

This work was supported by NKFI-K16 119516 and 20391-3/2018/FEKUSTRAT.

*14. Structural homology function predictions for fungal nicotinate catabolising enzymes

Eszter Bokor[1], Judit Ámon[1], Claudio Sccazzochio[2], Csaba Vágvölgyi[1], Zsuzsanna Hamari[1]

[1] *University of Szeged, Faculty of Science and Informatics, Department of Microbiology, Szeged, Hungary*
[2] *Department of Microbiology, Imperial Collage, London, England and Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France*

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.

This work was supported by NKFI-K16 119516 and 20391-3/2018/FEKUSTRAT.

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

Ignacio Bravo-Plaza[1], Miguel Hernández-González[2], Mario Pinar[1], J. Fernando Díaz[1], Miguel A. Peñalva[1]

[1] *Centro de Investigaciones Biológicas CSIC, Ramiro de Maeztu 9, Madrid 28040, Spain*
[2] *The Francis Crick Institute, 1 Midland Road, London NW1 1AT, UK*

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}.

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

Michael Bromley[1], Can Zhao[1], Narjes Al-furaiji[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]

[1] *University of Manchester, Manchester Fungal Infection Group, Manchester, UK*
[2] *HKI, Jena, Germany*

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 agenome-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-codingRNAs. 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*.

*17. Imidazolium-labelled glycosides for the characterisation of enzymatic function during plant biomass degradation

Gregory S. Bulmer[1], Ashley P. Mattey[1], Fabio Parmeggiani[1], Ryan Williams[2], Andrea Marchesi[1], Lisa S. Seibt[1], Peter Both[1], Kun Huang[1], M. Carmen Galan[2], Sabine L. Flitsch[1], Anthony P. Green[1] and Jolanda M. van Munster[1*]

[1] *Manchester Institute of Biotechnology (MIB) & School of Natural Sciences, The University of Manchester, Manchester, UK*
[2] *School of Chemistry, University of Bristol, Cantock's Close, Bristol, UK*

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.

1. Sittel L.; Galan M.C . Org Biomol Chem 2017

18. Fungal host strains for the industrial enzyme or protein production

Wouter de Bonte, Jean-Paul Meijnen, Pascal van Alphen, Sylvia Segers, Peter J. Punt.

Dutch DNA Biotech B.V., Utrecht, The Netherlands

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.

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

Sofia Dimou, Olga Martzoukou, Mariangela Dionysopoulou, Vangelis Bouris, Sotiris Amillis and George Diallinas

Department of Biology, National and Kapodistrian University of Athens, Panepistimioupolis, Athens, Greece

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.

1. Zanetti G, Pahuja KB, Studer S, Shim S, Schekman R, COPII and the regulation of protein sorting in mammals. Nat. Cell. Biol. 20111 14, 20-28.
2. FeydeS, De Craene LO, Bär S, Bertazzi DL, Friant S, Membrane trafficking in the yeast Saccharomyces cerevisiae model. Int. J. Mol. Sci. 2015 16, 1509-1525.
3. Diallinas G. Dissection of Transporter Function: From Genetics to Structure. Trends Genet. 2016 Sep;32(9):576-590.
4. Bouris V, Martzoukou O, Amillis S, Diallinas G. Nutrient transporter translocation to the

plasma membrane via a Golgi-independent unconventional route. bioRxiv 540203; doi: https://doi.org/10.1101/540203

*20. Sorting of the UapA purine transporter to the plasma membrane is COPII/actin-dependent, but Golgi/microtubule-independent

Sofia Dimou[#], Mariangela Dionysopoulou[#], Sotiris Amillis, Olga Martzoukou, Vangelis Bouris and George Diallinas[*]

Department of Biology, National and Kapodistrian University of Athens, Panepistimioupolis 15784, Athens, Greece
** Correspondence to: diallina@biol.uoa.gr, tel. +30(210)7274649; fax +30(210)7274702*
[#] Equal contribution

UapA, a xanthine-uric acid/H^+ symporter in *Aspergillus nidulans*, is one of the best characterized eukaryotic transporters [1]. This allows us to use UapA as a model cargo for identifying the trafficking routes and molecular mechanisms involved in membrane protein sorting and regulated turnover [2-4]. Previously, we showed that UapA endocytic turnover is non-polar, clathrin-dependent, but surprisingly AP-2 independent [5]. Here, we focus specifically on the mechanism by which *de novo* synthesized UapA traffics to the PM. We provide strong evidence that after translocation into the ER network, newly made UapA localizes into ER-exit sites (ERes) and requires functional Sec23/24 and actin for translocation, in a non-polar fashion, to the PM. Surprisingly, we find that UapA can still be targeted to the PM when the expression of AP-1, AP-3, clathrin light-chain (ClaL), SedV, GeaA, HypB, $RabE^{Rab11}$, $RabA/B^{Rab5}$ is repressed or microtubule polymerization is blocked. In line with these findings, UapA does not show co-localization with Golgi and post-Golgi molecular markers. Overall, our results strongly suggest that UapA traffics to the PM via a novel unconventional secretory route. In the meeting, we will present current experiments addressing the role of other sorting proteins, often involved in specific unconventional sorting in other systems, such GRASP, Sec16, conventional myosins and autophagy-related Atg5 and Atg7, and present the 'design' of an unbiased genetic screen for identifying factors involved in UapA sorting the PM.

1. Diallinas G. Trends Genet. 2016, 32:576-590.
2. Karachaliou M, Amillis S, Evangelinos M, Kokotos AC, Yalelis V, Diallinas G.
3. Mol Microbiol. 2013, 88:301-17.
4. Evangelinos M, Martzoukou O, Chorozian K, Amillis S, Diallinas G. Mol Microbiol. 2016, 100:893-911.
5. Martzoukou O, Karachaliou M, Yalelis V, Leung J, Byrne B, Amillis S, Diallinas G. J Mol Biol. 2015, 427:2679-96.
6. Martzoukou O, Amillis S, Zervakou A, Christoforidis S, Diallinas G. Elife. 2017, 6. pii: e20083.

*21. Functional analysis of the tRNA-ome of *Aspergillus fumigatus*

Lauren Dineen[1], Ressa Lebedinec[1], Connor Stonall[1], Marcin G Fraczek[2], Can Zhao[1], Daniela Delneri[2], Paul Bowyer[1], Michael Bromley[1]

[1] *Manchester Fungal Infection Group , Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK*
[2] *Division of Evolution & Genomic Sciences, Faculty of Life Sciences, University of Manchester, Manchester, UK*

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.

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

Mariangela Dionysopoulou[1], Anezia Kourkoulou[1], Carlo Marobbio[2], Luigi Palmieri[2] , George Diallinas[1]

[1] *Department of Biology, National and Kapodistrian University of Athens, Panepistimioupolis, Athens, Greece*
[2] *Dipartimento di Bioscienze Biotecnologie e Biofarmaceutic, Università degli studi di Bari, Bari, Italy*

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.

23. Formation of a new intron within an extant intron: how can stwintronisation happen?

Norbert Ág[1], Napsugár Kavalecz[1], Fruzsina Pénzes[1], Levente Karaffa[1], Claudio Scazzocchio[2], Michel Flipphi[1], **Erzsébet Fekete**[1]

[1] *Dept. of Biochemical Engineering, University of Debrecen, Hungary*
[2] *Department of Microbiology, Imperial College London, London, UK*

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)(*). 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'.

Acknowledgements: 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] & by the [EFOP-3.6.1-16-2016-00022] project co-financed by the EU and the European Social Fund. NK and EF are supported by the "New National Excellence Program" [ÚNKP-19-3 / ÚNKP-19-4] (Hungarian Ministry of Innovation and Technology).

(*) Kavalecz *et al.* (2019) Sci Rep 9: 9940

24. Involvement of spliceosomal twin introns in instances of alternative splicing in Aspergillus

Napsugár Kavalecz[1], Norbert Ág[1], Levente Karaffa[1], Claudio Scazzocchio[2], Michel Flipphi[1], **Erzsébet Fekete[1]**

[1] *Dept. of Biochemical Engineering, University of Debrecen, Hungary*
[2] *Department of Microbiology, Imperial College London, London, UK*

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.

Acknowledgements: 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. NK and EF are supported by the "New National Excellence Program" [ÚNKP-19-3 / ÚNKP-19-4] (Hungarian Ministry of Innovation and Technology).

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

Shoki Fujita[1], Hinako Tada[1], Mizuki Tanaka[2], Takahiro Shintani[1], Katsuya Gomi[1]

[1] *Tohoku University, Graduate School of Agricultural Science, Sendai, Japan*
[2] *University of Shizuoka, School of Food and Nutritional Sciences, Shizuoka, Japan*

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.

26. Combinatorial control of transcription factors involved in sugar beet pulp utilization in the industrially relevant fungus *Aspergillus niger*

Sandra Garrigues, Roland S. Kun and Ronald P. de Vries

Fungal Physiology, Westerdijk Fungal Biodiversity Institute & Fungal Molecular Physiology, Utrecht University, Utrecht, The Netherlands

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*.

27. Expression profiles of amylolytic genes in the black koji-mold *Aspergillus luchuensis*

Wataru Hashimoto[1], Hiraku Arai[1], Osamu Mizutani[2], Osamu Yamada[3], Takahiro Shintani[1], **Katsuya Gomi[1]**

[1] *Graduate School of Agricultural Science, Tohoku University, Sendai, Japan*
[2] *Department of Bioscience and Biotechnology, Faculty of Agriculture, University of the Ryukyus, Nishihara, Japan*
[3] *National Research Institute of Brewing, Higashi-Hiroshima, Japan*

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.

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

Lukas Birštonas[1], Alex Dallemulle[1], Manuel S. López-Berges[1], Ilse D. Jacobsen[2], Martin Offterdinger[3], Beate Abt[1], Maria Straßburger[4], Ingo Bauer[1], Oliver Schmidt[5], Bettina Sarg[6], Herbert Lindner[6], Hubertus Haas[1], **Fabio Gsaller[1]**

[1] *Medical University of Innsbruck, Institute of Molecular Biology Division/Biocenter*
[2] *Leibniz Institute for Natural Product Research and Infection Biology-Hans Knoell Institute, Research Group Microbial Immunology*
[3] *Medical University of Innsbruck, Institute of Neurobiochemistry/Biocenter*
[4] *Leibniz Institute for Natural Product Research and Infection Biology-Hans Knoell Institute, Transfer Group of Antiinfectives*
[5] *Medical University of Innsbruck, Institute of Cell Biology/Biocenter*
[6] *Medical University of Innsbruck, Institute of Clinical Biochemistry/Biocenter*

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.

29. Characterization of azole resistant *Aspergillus fumigatus* strains isolated from imported tulip bulbs that were purchased in Japan

Daisuke Hagiwara

University of Tsukuba, Faculty of Life and Environmental Sciences, Tsukuba Ibaraki Japan

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.

30. Complete mitochondrial genome sequences of *Aspergillus luchuensis, Aspergillus parasiticus* and *Aspergillus pseudoglaucus*

Kap-Hoon Han[1], Jongsun Park[2], Woochan Kwon[2], Bohan Zhu[1], Anbazhagan Mageswari[3], In-Beom Heo[3], Jong-Hwa Kim[1], Seung-Beom Hong[3]

[1] *Woosuk University, Pharmaceutical Engineering, Wanju, Jeonbuk, South Korea*
[2] *InfoBoss Research Center, Seoul, South Korea*
[3] *National Institute of Agricultural Science, Korean Agricultural Culture Collection, Wanju, Jeonbuk, South Korea*

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.

31. *vosA*-dependent ascospore gene expression in *Aspergillus nidulans*

Kap-Hoon Han[1], Min-Ju Kim[2], Mi-Kyung Lee[3], Huy Quang Pham[4], Myeong Ju Gu[2], Dongyup Hahn[2], Jae-Ho Shin[2], Jae-Hyuk Yu[5], Hee-Soo Park[2]

[1] *Woosuk University Pharmaceutical Engineering, Wanju, Jeonbuk, South Korea*
[2] *Kyungpook National University, School of Food Science and Biotechnology, Daegu, South Korea*
[3] *Korea Research Institute of Bioscience and Biotechnology, Biological Resource Center, Jeongeup, Jeonbuk, South Korea*
[4] *Kyungpook National University, School of Applied Biosciences, Daegu, South Korea*
[5] *University of Wisconsin-Madison, Departments of Bacteriology and Genetics, Madison, Wisconsin, USA*

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*.

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

Taishi Inoue, Takahiro Shintani, Katsuya Gomi

Tohoku University, Sendai, Japan

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*.

*33. Phenotypic analyses of *Aspergillus niger* strains originated from the Korean fermentation starter

Eun Ji Jeong, Jeong-Ah Seo

Soongsin University, Seoul, South Korea

The Korean fermentation starter, nuruk, has been known as a mixed culture of yeasts and filamentous fungi that enable various flavors and tastes of the traditional rice wine. We obtained two isolates of *Aspergillus niger* (KYF3 and KJC3) from nuruk, which have been widely used in cell factory. For investigating the characteristics of Koreanisolates, ten well-known strains of *A. niger* were tested for comparison. We tested the growth conditions on each different solid medium and temperature. The optimum growth conditions of two isolates were at 37°C on the complete medium with approximately 7.5mm per day. The enzyme activities of alpha-amylase, glucoamylase,cellulase and acid protease on solid-state and liquid media were measured. *A. niger* KYF3 and KJC3 showed higher cellulase and acid protease activities than other strains on solid state. Additionally, most of tested *A. niger* strains produced a variety of levels of fumonisin which is a well-known mycotoxin. On the other hand, we sequenced the whole genomes

of two Korean *A. niger* isolates for the reference genome construction and will be further analyze two genomes to compare gene ontology and expression levels of the genes encoding hydrolytic enzymes. Results in this study will be helpful to understand the high activities of enzymes and apply to industrial purpose.

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

Sascha Jung, Norman Paege, Vera Meyer

Department of Applied and Molecular Microbiology, Institute of Biotechnology, Technische Universität Berlin, Berlin, Germany

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.

35. Comparative performance of *Aspergillus terreus* itaconic acid fermentations on D-xylose and xylitol

Levente Karaffa[1], István S. Kolláth[1], Erzsébet Fekete[1], Christian P. Kubicek[2]

[1] *University of Debrecen, Department of Biochemical Engineering, Debrecen, Hungary*
[2] *TU Wien, Institute of Chemical, Environmental and Bioscience Engineering, Vienna,Austria*

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.

36. Occurrence, distribution, multiplicity and origins of the divalent metal/proton symporter (NRAMP/DMT) in the Ascomycota

Michel Flipphi[1], Balázs Fejes[1], Erzsébet Fekete[1], T.T. Minh Nguyen[2], Adrian Tsang[2], Christian P. Kubicek[3] and **Levente Karaffa**[1]

[1] *Department of Biochemical Engineering, University of Debrecen, Debrecen, Hungary*
[2] *Centre for Structural and Functional Genomics, Concordia University, Montreal, Quebec, Canada*
[3] *Institute of Chemical, Environmental and Bioscience Engineering, TU Wien, Vienna, Austria*

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.

37. Diversity of metabolic profiles and evolutionary forces acting in secondary metabolism gene clusters of *Aspergillus nidulans*

Nancy Keller[1], Mickey Drott[1], Rafael Bastos[2], Gustavo Goldman[2], Claudio Greco[1]

[1] *University of Wisconsin, Medical Microbiology and Immunology, Madison, WI, USA*
[2] *University of São Paulo, Ciencias Farmaceuticas, São Paulo, Brazil*

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.

Financial support: R01GM112739 USA, FAPESP and CNPq, Brazil

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

Jong-Hwa Kim, Sang-Cheol Jun, Bohan Zhu, Kap-Hoon Han

Woukosuk University, Pharmaceutical Engineering, Wanju, Jeonbuk, South Korea

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.

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

Anezia Kourkoulou[1], Pothos Grevias[1], George Lambrinidis[2], Emmanuel Mikros[2] and George Diallinas[1]

[1] *Department of Biology, National and Kapodistrian University of Athens, Panepistimioupolis, Athens, Greece*
[2] *Department of Pharmacy, National and Kapodistrian University of Athens, Panepistimioupolis, Athens, Greece*

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.

40. Functional studies of nigerolysins in *Aspergillus niger*

Nada Kraševec[1], Maruša Novak[2], Urška Čepin[3,4], Vesna Hodnik[2], Mojca Narat[5], Maja Jamnik[1], Gregor Anderluh[1], Kristina Sepčić[2]

[1] *Department of Molecular Biology and Nanobiotechnology, National Institute of Chemistry, Ljubljana, Slovenia*
[2] *Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia*
[3] *BioSistemika Ltd, Ljubljana, Slovenia*

[4] National Institute of Biology, Ljubljana, Slovenia
[5] Department of Animal Science, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

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

*41. Regulation of wheat bran utilization in the industrially relevant filamentous fungus *Aspergillus niger*

Roland S. Kun[1], Sandra Garrigues[1], Marcos Di Falco[2], Adrian Tsang[2], Ronald P. De Vries[1]

[1] Westerdijk Fungal Biodiversity Institute, Fungal Physiology, Utrecht The Netherlands
[2] Concordia University, Center for Structural and Functional Genomics, Montreal Quebec Canada

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*.

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

Ling Lu, Yeqi Li

Nanjing Normal University, Nanjing, Jiangsu, China

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.

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

Ronnie J.M. Lubbers[1], Adiphol Dilokpimol[1], Jorge Navarro[2], Mao Peng[1], Mei Wang[3], Anna Lipzen[3], Vivian Ng[3], Igor V. Grigoriev[3], Jaap Visser[1], Kristiina S. Hildén[4] and Ronald P. de Vries[1*]

[1] *Fungal Physiology, Westerdijk Fungal Biodiversity Institute & Fungal Molecular Physiology, Utrecht University, Utrecht, The Netherlands*
[2] *Fungal Natural Products, Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands*
[3] *US Department of Energy Joint Genome Institute, Walnut Creek, California 94598, USA*
[4] *Department of Microbiology, University of Helsinki, Viikinkaari 9, Helsinki, Finland*
E-mail: R.lubbers@wi.knaw.nl

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.

*44. GalR, GalX and AraR co-regulate D-galactose and L-arabinose utilization in *Aspergillus nidulans*

Jiali Meng[1], Tania Chroumpi[1], Sandra Garrigues[1], Roland S. Kun[1], Miia R. Mäkelä[2], Ronald P. de Vries[1]

[1] *Fungal Physiology, Westerdijk Fungal Biodiversity Institute & Fungal Molecular Physiology, Utrecht University, Utrecht, The Netherlands*
[2] *Fungal Genetics and Biotechnology, Department of Microbiology, University of Helsinki, Helsinki, Finland*

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*.

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

Giulia Mirabile[1], Maryen Alberto Vazquez[2], Patrizia Bella[1], Selene Giambra[1], Gaetano Conigliaro[1], Salvatore Davino[1], Livio Torta[1]

[1] *Università degli Studi di Palermo, Dipartimento Scienze Agrarie, Alimentari e Forestali, Palermo, Italy*
[2] *Instituto de Ciencia Animal, Departamento de Bioquimica, Cuba*

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.

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

Matthias Misslinger[1], Mareike Scheven[2], Peter Hortschansky[2], Manuel Sanchez Lopez-Berges[1], Katharina Heiss[1], Nicola Beckmann[1], Thomas Heigl[1], Martin Hermann[3], Thomas Krüger[2], Olaf Kniemeyer[2], Axel Brackhage[2], Hubertus Haas[1]

[1] *Medical University of Innsbruck, Institute of Molecular Biology / Biocenter, Innsbruck, Austria*
[2] *Hans Knöll Institute (HKI), Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany*
[3] *Medical University of Innsbruck, Department of Anaesthesiology and Critical Care Medicine, Innsbruck, Austria*

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*.

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

Ken Miyazawa[1], Akira Yoshimi[2], Tasuku Nakajima[1], Keietsu Abe[1,2]

[1] *Gradraduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan*
[2] *NICHe, Tohoku University, Sendai, Japan*

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.

1. Miyazawa et al. Front. Microbiol. (2019) 10:2090

48. Septins coordinate cell wall integrity and lipid metabolism

Michelle Momany, Alex Mela

University of Georgia, Fungal Biology Group and Plant Biology Department, Athens, Georgia, USA

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.

49. *Aspergillus flavus* metabolic chemistry and its influence on the efficacy of pre-harvest biocontrol

Geromy Moore, Matthew Lebar, Carol Carter-Wientjes

Agricultural Research Service, United States Department of Agriculture, New Orleans, Louisiana, USA

Use of atoxigenic *Aspergillus flavus* strains as pre-harvest biocontrol is an effective strategy to inhibit mycotoxin contamination. The exact mechanism(s) employed by these atoxigenic strains is not fully understood. The potential for atoxigenic *A. flavus* metabolites to elicit an inhibitory response from toxigenic strains was investigated through secreted (extrolite) and volatile organic compound (VOC) studies. Findings indicate that at least one unknown extrolite, and several VOCs, are produced by atoxigenic *A. flavus* that significantly reduce mycotoxin levels, including aflatoxin and cyclopiazonic acid (CPA). This suggests that *A. flavus* biocontrol strains may produce one or more chemicals that turn off mycotoxin production in neighboring toxigenic strains. The goal is

to identify *A. flavus* compounds that can be used to improve biocontrol efforts, either by screening candidate biocontrol strains for increased production of beneficial extrolites and VOCs, or by using these chemicals to supplement pre- and post-harvest biocontrol efforts through foliar sprays or fumigation.

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

Akihiro Ninomiya[1], Syun-ichi Urayama[1, 2], Daisuke Hagiwara[1, 2]

[1] *Faculty of Life and Environmental Sciences, University of Tsukuba, Japan*
[2] *Microbiology Research Center for Sustainability, University of Tsukuba, Japan*
Corresponding author: ninomiya.akihiro.gb@u.tsukuba.ac.jp

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.

51. How McrA regulates secondary metabolism

Berl Oakley[1], Tomohiro Akashi[2], Elizabeth Oakley[1]

[1] *University of Kansas, Molecular Biosciences, Lawrence, Kansas, United States of America*
[2] *Nagoya University, Division of OMICS Analysis, Nagoya, Japan*

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.

52. Cellular response to farnesol and the role of nitric oxide production in *Aspergillus fumigatus*

Sayoko Oiki[1], Akihiro Ninomiya[1], Syun-ichi Urayama[1,2] and Daisuke Hagiwara[1,2]

[1] *Faculty of Life and Environmental Sciences, University of Tsukuba, Japan*
[2] *Microbiology Research Center for Sustainability, University of Tsukuba, Japan*

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.

*53. Identification and characterization of *Aspergillus nidulans ΔflbB* mutants showing an aconidial phenotype under phosphate stress

Ainara Otamendi[1], Oier Etxebeste[1], Eduardo A. Espeso[2]

[1] *Laboratory of Biology, Department of Applied Chemistry, Faculty of Chemistry, University of The Basque Country (UPV/EHU), San Sebastian, Spain*
[2] *Department of Cellular and Molecular Biology, Centro de Investigaciones Biológicas (CSIC), Madrid, Spain*

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.

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

Georgia F. Papadaki[1], George Lambrinidis[2], Emmanuel Mikros[2] and George Diallinas[1]

[1] *Department of Biology, National and Kapodistrian University of Athens, Athens, Greece*
[2] *Department of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece*

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.

55. CreA regulation was observed at low free monosaccharide level during *Aspergillus niger* grown on crude plant biomass

Mao Peng[1], Ronnie Lubbers[1], Paul Daly[1], Ronald de Vries[1], Igor Grigoriev[2], Miia Mäkelä[3]

[1] *Westerdijk Fungal Biodiversity Institute Fungal Physiology Utrecht Utrecht Nederland*
[2] *US Department of Energy Joint Genome Institute, Walnut Creek, California, United States*
[3] *University of Helsinki Food and Environmental Sciences, Helsinki, Finland*

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.

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

Giancarlo Perrone[1], Massimo Ferrara[1], Antonia Gallo[2], Donato Magistà[1], Scott E. Baker[3,4]

[1] *Institute of Sciences of Food Production (ISPA) National Research Council (CNR), Bari, Italy*
[2] *Institute of Sciences of Food Production (ISPA) National Research Council (CNR), Lecce, Italy*
[3] *Joint Bioenergy Institute, Emeryville, CA, United States of America,*
[4] *Biosystems Design and Simulation Group, Environmental Molecular Sciences Division, Pacific Northwest National Laboratory, Richland, WA, United States of America*

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.

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

Irene Picazo[1], Oier Etxebeste[2], Aitor Garzia[3], Eduardo A. Espeso[1]

[1] *Centro de Investigaciones Biológicas (CSIC)*
[2] *University of The Basque Country (UPV/EHU)*
[3] *The Rockefeller University*

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.

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

Abeer H. Hossain[1,2], Roy van Gerven[1], Aiko Hendrickx[1], Wouter de Bonte[1] and

Peter. J. Punt[1,3]

[1] *Dutch DNA Biotech B.V., Utrecht, The Netherlands*
[2] *University of Amsterdam, Amsterdam, The Netherlands*
[3] *Leiden University, Leiden, The Netherlands*

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*.

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

Barbara Ramšak[1], Jessica Markau[1], Tim Dahlmann[1], Sven Krappmann[2], Ulrich Kück[1]

[1] *Ruhr-University Bochum, Bochum, Germany*
[2] *Institute of Microbiology – Clinical Microbiology, Erlangen, Germany*

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].

1. Latge J.P., Clin Microbiol Rev, 1999
2. O'Gorman C. M., et al, Nature, 2009
3. Szewczyk E. & Krappmann S., Eukaryot Cell, 2010
4. Monteiro M. C., Mycoses, 2018; [5] Becker K., et al, Mol Microbiol, 2015

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

Luis Enrique Sastré Velásquez, Alex Dallemulle, Fabio Gsaller

Medical University of Innsbruck, Institute of Molecular Biology, Innsbruck, Austria

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*.

61. Comparative genomics between an industrially important species, *Aspergillus sojae*, and harmful one, *Aspergillus parasiticus*

Atsushi Sato[1], Inge Kjærbølling[2], Mikael R. Andersen[2], Toutai Mituyama[3], Yasuji Koyama[4], Yasuo Ohnishi[5]

[1] *Kikkoman Corporation Research & Development, Noda, Chiba, Japan*
[2] *Technical University of Denmark, Department of Biotechnology and Bioengineering, Søltoft Plads, Kongens Lyngby, Denmark*
[3] *National Institute of Advanced Industrial Science and Technology, Artificial Intelligence Research Center, Koto-ku Tokyo, Japan*
[4] *Noda Insititute for Scientific Research, Noda, Chiba, Japan*
[5] *The University of Tokyo, Department of Biotechnology, Graduate School of Agricultural and Life Sciences, Bunkyo-ku Tokyo, Japan*

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.

1. Kurtzman et al. 1986. Mycologia, 78: 955-959.
2. Yuan et al. 1995. Appl Environ Microbiol., 61: 2384-2387.
3. Keller et al. 2008. BMC Bioinformatics, 9: 278.
4. Blin et al. 2019. Nucleic Acids Res. 47:W81.

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

Paul Schäpe, Timothy C. Cairns, Vera Meyer

Technische Universität Berlin, Chair of Applied and Molecular Microbiology, Berlin, Germany

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.

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

Kevin Schmitz[2]*, Dominik Schäfer[1]*, Dirk Weuster-Botz[1], J. Philipp Benz[2]*

[1]*Technical University of Munich, Department of Mechanical Engineering, Institute of Biochemical Engineering, Garching, Germany*
[2]*Technical University of Munich, Holzforschung München, Wood Bioprocesses, TUM School of Life Sciences Weihenstephan, Freising, Germany*
** These authors contributed equally to this work*

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.

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

Sjoerd J. Seekles[1,2,*], Tom van den Brule[1,3], Maarten Punt[1,4], Jan Dijksterhuis[1,3], Jos Houbraken[1,3], Han A. B. Wosten[1,4] and Arthur F.J. Ram[1,2]

[1]TiFN, Top Institute of Food and Nutrition, Wageningen, The Netherlands
[2]Leiden University, Institute of Biology Leiden, Leiden, The Netherlands
[3]Westerdijk Fungal Biodiversity Institute, Applied and Industrial Mycology, The Netherlands
[4]Utrecht University, Fungal Microbiology, Utrecht, The Netherlands
* E-mail: s.j.seekles@biology.leidenuniv.nl

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.

65. ROS-dependent and independent host-induced fungal regulated cell death in defense against invasive aspergillosis

Neta Shlezinger and Tobias Hohl

Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel

Human fungal pathogens are major drivers of devastating morbidity and mortality accounting for a staggering 1.6 million deaths annually. *Aspergillus fumigatus* is a leading cause of infectious mortality in immune compromised individuals. Oxidative burst, which generates reactive oxidative species (ROS) via NADPH oxidase plays a pivotal role in the defense against fungal pathogens and was long thought of as the primary effector system against *A. fumigatus*. However, we observed that NADPH-deficient leukocytes have significant residual conidiacidal activity in vivo. Mitochondria are major sites of ROS production in most cells; however, mitochondrial ROS (mROS) have traditionally been regarded as byproducts of oxidative respiration.

To determine whether mROS was required for host defense against *A. fumigatus*

infection, we infected transgenic mice that express a mitochondrial-targeted catalase (mCAT) in absence and presence of NADPH-oxidase. Using functional reporters of fungal regulated cell death, we show that mROS regulates macrophage conidiacidal activity independent of NADPH oxidase-derived ROS. Nonetheless, mROS presence did not affect infectious outcomes, arguing for the presence of alternate non-oxidative antifungal effector mechanisms. To explore this hypothesis, we employed genome-wide dual omic approaches to simultaneously profile transcriptional changes of host leukocytes and fungal cells in response to host-fungal encounters in order to identify additional host inducers of fungal RCD and the corresponding fungal pathways. Using mixed chimeric mice that contain both WT (p91phox +/+) and -deficient (p91phox -/-) neutrophils we identified infection-specific alterations in several murine and fungal metabolic pathways upon fungal internalization.

Our study illustrates the efficacy of dual RNA sequencing in unraveling the virulence and defense mechanisms of neglected, genetically intractable fungal pathogens. Thus, our findings provide molecular insights into RCD that may extend beyond the *A. fumigatus* pathosystem and will lay the groundwork for the development of innovative, fungal-selective, pan-antifungal drugs.

*66. Newly designed modular carbohydrate-active enzymes to increase the efficiency of lignocellulose degradation

Andika Sidar[1,3*], Erica D Albuquerque[1], Gerben P Voshol[1], Arthur FJ Ram[1], Erik Vijgenboom[1], Peter J Punt[1,2]

[1] *Leiden University, Institute Biology of Leiden, The Netherlands*
[2] *Dutch DNA Biotech, Zeist, the Netherlands*
[3] *Gadjah Mada University, Department of Food and Agricultural Product Technology, Indonesia*

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

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

Maria Cristina Stroe[1], Tina Netzker[2], Kirstin Scherlach[1], Vito Valiante[1], Christian Hertweck[1], Axel A. Brakhage[1]

[1] *Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute (HKI), Jena, Germany*
[2] *McMaster University, Department of Biology, Hamilton, Canada*
Corresponding author: maria.stroe@leibniz-hki.de

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.

68. Fungal highway and bacterial toll

Norio Takeshita

University of Tsukuba Microbiology Research Center for Sustainability, MiCS, Tsukuba, Japan

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.

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

Syun-ichi Urayama[1], Yuka Iwahashi[1], Shunsuke Masuo[1], Shusaku Kanematsu[1], Hiromitsu Moriyama[2], Naoki Takaya[1], Nobuhiko Nomura[1], Norio Takeshita[1], Masanori Toyofuku[1], Daisuke Hagiwara[1]

[1] *University of Tsukuba, Faculty of Life and Environmental Sciences, Tsukuba, Ibaraki, Japan*
[2] *Tokyo University of Agriculture and Technology, Laboratory of Molecular and Cellular Biology, Fuchu, Tokyo, Japan*

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.

70. Surface analysis tools identify how *Aspergillus niger* and its enzymes modify lignocellulose

Jolanda M. van Munster[1#], Tue V. Vuong[2], Sue E. Marcus[3], Paul Knox[3], Emma Master[2]

[1] *Manchester Institute of Biotechnology & School of Chemistry, University of Manchester, UK*
[2] *Department of Chemical Engineering and Applied Chemistry, University of Toronto, Canada*
[3] *Centre for Plant Sciences, Faculty of Biological Sciences, University of Leeds, UK*
[#] *Corresponding and Presenting author jolanda.vanmunster@manchester.ac.uk*

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.

71. Discovery of the biosynthetic pathway for the antifungal hymeglusin in *Scopulariopsis candida*

Clay Wang

University of Southern California, Pharmacology and Pharmaceutical Sciences, Los Angeles, CA, USA

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.

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

Hongchen Wang, Qiuyi Chen, Shizhu Zhang, Ling Lu

University of Nanjing normal, Institute of life sciences, Nanjing, China

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°C, 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.

73. Uncovering long non-coding RNA associated with drug response in *Aspergillus fumigatus*

Danielle Weaver[1], Takanori Furukawa[1], Fabio Gsaller[2], Daniela Delneri[1], Michael Bromley[1], Paul Bowyer[1]

[1] *University of Manchester, Faculty of Biology, Medicine & Health, Manchester, UK*
[2] *University of Innsbruck, Division of Molecular Biology, Innsbruck, Austria*

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.

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

Bo Yuan[1], Johannes W. A. van Dijk[1], Ying Ye[2], David H. Sherman[2,4,5,6], Clay C. C. Wang[1,3]

[1] *Department of Pharmacology and Pharmaceutical Sciences, USC Pharmacy School, Los Angeles, California, US*[2]*Life Sciences Institute, University of Michigan, Ann Arbor, Michigan, US*
[3] *Department of Chemistry, University of Southern California, Dornsife College of Letters, Arts, and Sciences*
[4] *Department of Medicinal Chemistry, University of Michigan,US*
[5] *Department of Chemistry, University of Michigan, US*
[6] *Department of Microbiology and Immunology, University of Michigan, US*

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.

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

Shizhu Zhang, Yuan Chen, Ling Lu

Nanjing Normal University, School of Life Sciences, Nanjing, Jiangsu, China

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.

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

Yuanwei Zhang, Jialu Fan, Ling Lu

Nanjing Normal University, College of Life Sciences, Nanjing, Jiangsu, China

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.

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

Can Zhao, Najes Alfuraiji, Hajer Alshammri, Elaine Bignell, Michael Bromley

Manchester Fungal Infection Group, University of Manchester, Manchester, UK

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.

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

Abdulrahman Kelani[1], Matthew G. Blango[1], Flora Rivieccio[1], Florian Mock[2], Manja Marz[2], Axel A. Brakhage[1,2]

[1] *Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena, Germany*
[2] *Friedrich Schiller University Jena, Jena, Germany*

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.

79. Genome sequencing of evolved Aspergilli populations reveals robust genomes, transversions in *A. flavus*, and sexual aberrancy in non-homologous end-joining mutants

Isidro Álvarez-Escribano[1,6], Christoph Sasse[2,6], Jin Woo Bok[3,6], Hyunsoo Na[4], Mojgan Amirebrahimi[4], Anna Lipzen[4], Wendy Schackwitz[4], Joel Martin[4], Kerrie Barry[4], Gabriel Gutiérrez[1], Sara Cea[1], Ana T. Marcos[1], Igor V. Grigoriev[4,5], Nancy P. Keller[3], Gerhard H. Braus[2], **David Cánovas[1]**

[1] *Department of Genetics, Faculty of Biology, University of Seville, Spain*
[2] *Department of Molecular Microbiology and Genetics and Göttingen Center for Molecular Biosciences (GZMB), Georg-August-University, Göttingen, Germany*
[3] *Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, Wisconsin, USA; Department of Bacteriology, University of Wisconsin-Madison, Madison, Wisconsin, USA*
[4] *US Department of Energy Joint Genome Institute, Walnut Creek, California, USA*
[5] *Department of Plant and Microbial Biology, University of California Berkeley, Berkeley, CA*
[6] *These authors have equally contributed to this work*

Aspergillus spp. comprises a very diverse group of lower eukaryotes with a high relevance for industrial applications and clinical implications. These multinucleate species are

often cultured for many generations in the laboratory, which can unknowingly propagate hidden genetic mutations. To assess the likelihood of such events, we studied the genome stability of aspergilli by using a combination of mutation accumulation (MA) lines and Whole Genome Sequencing.

We sequenced the whole genomes of 30 asexual and 10 sexual MA lines of three *Aspergillus* species (*A. flavus*, *A. fumigatus*, and *A. nidulans*), and estimated that each MA line accumulated mutations for over 4,000 mitoses during asexual cycles. We estimated mutation rates of 4.2 x 10^{-11} (*A. flavus*), 1.1 x 10^{-11} (*A. fumigatus*) and 4.1 x 10^{-11} (*A. nidulans*) per site per mitosis, suggesting that the genomes are very robust. Unexpectedly we found a very high rate of GC→TA transversions only in *A. flavus*. In parallel 30 asexual lines of the non-homologous end-joining (NHEJ) mutants of the three species were also allowed to accumulate mutations for the same number of mitoses. Sequencing of these NHEJ MA lines gave an estimated mutation rate of 5.1 x 10^{-11} (*A. flavus*), 2.2 x 10^{-11} (*A. fumigatus*) and 4.5 x 10^{-11} (*A. nidulans*) per base per mitosis, which is slightly higher than in the wild-type strains and some ~5-6 times lower than in the yeasts. Additionally, in *A. nidulans* we found a NHEJ-dependent interference of the sexual cycle, that is independent of the accumulation of mutations.

We present here direct counts of the mutation rate of filamentous fungal species, and find that *Aspergillus* genomes are very robust. Deletion of the NHEJ machinery results in a slight increase in the mutation rate, but at a rate we suggest is still safe to use for biotechnology purposes. Unexpectedly we found GC>TA transversions predominated only in the species *A. flavus*, which could be generated by the hepatocarcinogen secondary metabolite aflatoxin. Lastly, a strong effect of the NHEJ mutation in self-crossing was observed and an increase in the mutations of the asexual lines was quantified.

Reference: Álvarez-Escribano I, et al. Genome sequencing of evolved aspergilli populations reveals robust genomes, transversions in *A. flavus*, and sexual aberrancy in non-homologous end-joining mutants. BMC Biol. 2019 Nov 11;17(1):88.

80. Enhanced genome editing in *Thermothelomyces thermophilus* using CRISPR-Cpf1

Tabea Schuetze and Vera Meyer

TU Berlin, Department of Applied and Molecular Microbiology, Berlin, Germany

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.

ECFG15
ROME • ITALY 2020

SATELLITE WORKSHOP
Fusarium

MONDAY, FEBRUARY 17

Location: **Sapienza University of Rome**
Building: **CU022** | Side: **Botanica** | Floor: **Ground** | Room: **Giacomini**

Session I - Fusarium-host interaction (plants/animals/humans)

CHAIR: **Claire Kanja** & **Pravin Khambalkar**

08:45 - 09:00	**Introduction and welcome**
09:00 - 09:15	**Martijn Rep** | University of Amsterdam ***Fusarium oxysporum* effectoromes**
09:15 - 09:30	**Cristina López-Diaz** | Universidad de Córdoba **DNA transposons drive adaptive evolution in the fungal cross-kingdom pathogen *Fusarium oxysporum***
09:30 - 09:45	**Claire Kanja** | Rothamsted Research **Functional characterisation of candidate *Fusarium graminearum* effectors**
09:45 - 10:00	**Pravin Khambalkar** | The Australian National University **SIX6: A route to plant cell death**
10:00 - 10:15	**Martin Urban** | Rothamsted Research **PHI-base, a multispecies phenotype database for pathogens, hosts and their interactions to enhance global food security and human health**
10:15 - 10:45	**Coffee Break**

Session II - Evolution, taxonomy and genome dynamics

CHAIR: **Allesandra Villiani** & **Edoardo Piombo**

10:45 - 11:00	**Nadia Ponts** | INRA **Deciphering the effect of ambient pH on enniatins production by *Fusarium tricinctum***
11:00 - 11:15	**Antonio Moretti** | CNR-ISPA **Unveiling the *Fusarium graminearum* species complex: Global database of species and chemotypes**

11:15 - 11:30	**Balazs Brankovics** | WUR Biointeractions & Plant Health **Mitochondrial genomes as phylogenetic backbone**
11:30 - 11:45	**Edoardo Piombo** | University of Turin **Using comparative genomics to identify genes involved in *Fusarium fujikuroi* pathogenicity**
11:45 - 12:00	**Alessandra Villani** | CNR-ISPA **Variation in secondary metabolite production potential in the *Fusarium incarnatum-equiseti* species complex revealed by comparative analysis of 13 genomes**
12:00 - 12:45 Speed pitches (5 min) to posters	1. **Anne Van Diepeningen** | WUR Biointeractions & Plant Health **Topographically triggered mycelial bundles in *Fusarium* species** 2. **Achchuthan Shanmugasundram** | University of Liverpool **FungiDB: integrating genomic data for pathogens and model organisms and providing advanced search capabilities and large-scale data analysis** 3. **Sabina Moser Tralamazza** | University of Neuchâtel **The specialized metabolite gene clusters of *Fusarium graminearum* species complex and their response during the cell-to-cell invasion of wheat** 4. **Florence Richard-Forget** | INRA Bordeaux **Deciphering the effect of ambient pH on enniatins production by *Fusarium avenaceum*** 5. **Maria Teresa Senatore** | University of Bologna **Genetic diversity and mycotoxin production among Fusarium head blight isolates belonging to the *Fusarium tricinctum* species complex from Italy** 6. **David Torres Sanchez** | Wageningen University **Understanding the origin, diversity and evolution of the Panama Disease pathogen**
12:45 - 14.00	**Lunch Break and poster viewing**

Session III - Regulation, signal detection and secondary metabolism
CHAIR: **Janevska Slavica** & **Sabine Tralamazza**

14:00 - 14:15	**Corby H. Kistler** | USDA, University of Minnesota **Microbial interactions of *Fusarium oxysporum* in the soil**

14:15 - 14:30	**Marzia Beccaccioli** | Sapienza University of Rome **The Crz1 transcription factor regulates lipid metabolism and fumonisin production in *Fusarium verticillioides***
14:30 - 14:45	**Slavica Janevska** | Hans Knöll Institute **The fumonisin cluster gene *FUM18* encodes a functional ceramide synthase that confers self-protection against the produced sphingolipid inhibitor**
14:45 - 15:00	**Carmen M Limón** | University of Seville **Functional studies of the role of the RING-Finger protein CarS in *Fusarium fujikuroi***
15:00 - 15:15	**Marike Boenisch** | University of Minnesota **Functional subregions of the endoplasmic reticulum of *Fusarium graminearum* upon induced secondary metabolism**
15:15 - 15:45	**Coffee break and poster viewing**

Session IV - Genetic exploitation in applied and industrial mycology
CHAIR: **Jens Laurids Sørensen** & **Linda Brain**

15:45 - 16:00	**Daren W. Brown** | USDA Peoria **Genus-wide analysis of *Fusarium* polyketide synthases uncovers broad natural product potential**
16:00 - 16:15	**Linda Brain** | La Trobe University and University of Adelaide **Characterisation of *Fusarium graminearum* chitin synthases**
16:15 - 16:30	**Teis E. Søndergaard** | Aalborg University **Novel nonribosomal peptides from *Fusarium graminearum***
16.30 - 16:45	**MR Nielsen** | Aalborg University **Solving the polyketide pigmentation puzzle in *Fusarium solani***
16.45 - 17:00	**Ulrich Terpitz** | Julius Maximilian University **Expansion of fungi enables high resolution in fluorescence microscopy**
17.00	**Other business and Closing**

01. Expansion of fungi enables high resolution in fluorescence microscopy

Ulrich Terpitz[1], Ralph Götz[1], Sabine Panzer[1], Nora Trinks[1], Johannes Wagener[1], David Turrà[2], Antonio Di Pietro[2] and Markus Sauer[1]

[1] *Julius Maximilian University, Würzburg, Germany*
[2] *University of Córdoba, Cordoba, Spain*
Email: ulrich.terpitz@uni-wuerzburg.de

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 basidiomycetes - 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.

02. Functional subregions of the endoplasmic reticulum of *Fusarium graminearum* upon induced Secondary Metabolism

Marike Boenisch, Karen Broz and Corby Kistler

University of Minnesota, Saint Paul, Minnesota, USA
Email: mboenisc@umn.edu

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.

03. Characterisation of *Fusarium graminearum* chitin synthases

Linda Brain[1], Marilyn Anderson[1], Mark Bleackley[1] and Vincent Bulone[2]

[1] *La Trobe University, Australia and 2University of Adelaide, Australia*
Email: 18686311@students.latrobe.edu.au

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 cells. Furthermore, this polysaccharide 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 important 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 express them in yeast for biochemical characterisation. This study has identified two FgCHSs that are functional homologs of

Saccharomyces cerevisiae chitin synthases and are also expressed during corn, wheat and barley infections. FgCHS3b and FgCHS4 complemented a chs double knockout in S. cerevisiae 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.

04. First genome sequence resource for *Fusarium lateritium*, causal agent of nut gray necrosis

Alessandro Grottoli[1], Silvia Turco[1], Angelo Mazzaglia[1]

[1] *Department of Agricultural and Fores Sciences (DAFNE), University of Tuscia, 01100 Viterbo, Italy*
Email: alessandro.grottoli@unitus.it

Corylus avellane L. (hazelnut) is a nut species cultivated in Italy on an area of about 70,000 ha. The cultivated areas are mainly located in four Italian's regions: Campania, Latium, Sicily and Piedmont. Italy is among the largest world hazelnut producers together with Turkey, Spain, USA and Greece. In early summer 2000, a new symptomatology, a severe fruit drop, was observed in several hazelnut orchards located in the Latium Region in central Italy. The symptomatic fruits were characterized by a brown greyish necrotic spot/patch on the nut shell, bracts and less often on the petioles. Based on the symptoms observed on affected fruits the disease was named nut grey necrosis (NGN). In early 2010, *Fusarium lateritium* Nees (*Fl*) was first reported as the causal agent of NGN on hazelnut in Italy; moreover, *Fl* has been reported also as agent of hazelnut twig cankers and fruit rot on walnut and olive. Although several *Fl* pathogenicity tests were conducted, supporting the speculation of the involvement of *Fl* in the NGN disease and hazelnut twig canker, molecular and transcriptional analysis of *Fl* mechanisms, involved during the early and late stages of infection, were not been conducted. Here we report the first *Fl* genome draft assembly of an Italian *Fl* strain isolated from nut shell coming from a hazelnut orchard located in Latium Region. This genome assembly could represent a first important step to understand the infection mechanisms of *Fl*; furthermore, this resource could be a milestone to arrange a focused and specific control disease strategy.

05. Supernumerary chromosomes in Italian *Fusarium verticillioides* strains

Alessandro Grottoli[1,2], Luigi Faino[1], Marzia Beccaccioli[1,2], Valeria Scala[3], Massimo Reverberi[1,2]

[1] *SARA EnviMob s.r.l., Via dei Castani, 116, 00172 Rome, Italy*
[2] *Department of Environmental Biology, University of Rome "Sapienza", P.le Aldo Moro, 00185 Rome, Italy*

[3] *Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Centro di Ricerca Difesa e Certificazione, Via C.G, Bertero, 22, Roma, 00156, Italy*
Email: grottoliphd@gmail.com

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.

06. SIX6: A route to plant cell death

Pravin Khambalkar, Daniel Yu, Simon Williams and David Jones

The Australian National University, Canberra, Australia
Email: pravinkhambalkar88@gmail.com

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.

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

Maria Teresa Senatore[1], Todd J. Ward[2], Kerry O'Donnell[2], Mark Busman[2] and Antonio Prodi[1]

[1] *University of Bologna, Italy*
[2] *U.S. Department of Agriculture, Agricultural Research Service, Peoria, USA*
Email: mariateresa.senatore@unibo.it

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.

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

M. Beccaccioli[1], A. Cacciotti[1], S. Vitale[2], A. Di Pietro[2], D. Turrà[2], V. Scala[3], M. Reverberi[1]

[1] *Department of Enviromental Biology, "Sapienza" University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy*
[2] *Department of Genetics, Campus de Excelencia Internacional Agroalimentario ceiA3, Universidad de Córdoba, Córdoba, Spain*
[3] *CREA-DC, Via Carlo Giuseppe Bertero 22, 00156 Roma, Italy*
Email: marzia.beccaccioli@uniroma1.it

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*.

References:
1. Dall'Asta, C., Falavigna, C., Galaverna, G., & Battilani, P. (2012). Role of maize hybrids and their chemical composition in Fusarium infection and fumonisin production. Journal of Agricultural and Food Chemistry, 60(14), 3800-3808.
2. Scala, V., Giorni, P., Cirlini, M., Ludovici, M., Visentin, I., Cardinale, F., ... & Galaverna, G. (2014). LDS1-produced oxylipins are negative regulators of growth, conidiation and fumonisin synthesis in the fungal maize pathogen Fusarium verticillioides. Frontiers in microbiology, 5, 669.
3. Thewes, S. (2014). Calcineurin-Crz1 signaling in lower eukaryotes. Eukaryotic cell, 13(6), 694-705.

09. Novel nonribosomal peptides from *Fusarium graminearum*

Teis Esben Søndergaard[1], Klaus Ringsborg Westphal[1], Simone Backleitner[2], Matthias Brundtø[1], Trine Sørensen1, Lena Studt[2], Reinhard Wimmer[1] and Jens Laurids Sørensen[1]

[1] *Aalborg University, Aalborg, Denmark and*
[2] *BOKU, Tulln, Austria*
Email: tes@bio.aau.dk

Filamentous fungi present a rich source of natural bioactive metabolites that are used in the battle of survival in their natural habitats and as infection facilitators during host pathogenesis. Several of these are small nonribosomal peptides with bioactivity like penicillin. The end product from the majority of fungal clusters harboring nonribosomal peptide synthetases still need to be elucidated, but a major obstruction is the tight regulation of secondary metabolite clusters in fungi and difficulties to predict downstream modifications. Here, manipulating the fungi *Fusarium graminearum*, we purified and elucidated two peptides. i) a novel hexapeptide, Fusahexin and ii) an octa-peptide Fusaoctaxin A that is tandemly synthesized by two nonribosomal peptide synthetases and modified during transport by a cluster specific ABC transporter with peptidase activity. Some fungal clusters hold two nonribosomal peptide synthetases and several secondary metabolite clusters include ABC transporters and having insight into tandem synthetases mechanism and downstream peptidase modifications could enlighten the discoveries of novel nonribosomal peptides including new obtainable antibiotics.

10. DNA transposons drive adaptive evolution in the fungal cross-kingdom pathogen *Fusarium oxysporum*

Cristina López-Díaz[1], Dilay Hazal-Ayhan[2], Isabel Okeke[1], Li-Jun Ma[2], Antonio Di Pietro[1]

[1] *Departamento de Genética, Universidad de Córdoba, Córdoba, Spain*
[2] *Department of Biochemistry and Molecular Biology, University of Massachusetts, Amherst, USA*

Fungal pathogens pose a severe threat to food security and human health. These organisms often show exquisite host adaptation, but also undergo rapid evolution leading to shifts or expansions in the host range. The soil-inhabiting ascomycete *Fusarium oxysporum* causes vascular wilt disease on more than a hundred different crops and disseminated infections in immunocompromised humans. Remarkably, a single field isolate of this fungus can kill tomato plants, immunodepressed mice and the model insect host *Galleria mellonella*. How this fungus has evolved to infect hosts across different kingdoms of life is currently unknown. Similar to other fungal pathogens, *F. oxysporum* carries accessory genomic regions, which are rich in repeats and transposable elements (TEs) and have been associated with host range. Here we used experimental evolution to study adaptation of *F. oxysporum* to different environments. Fungal populations obtained after 10 serial passages through tomato plants or different types of media displayed significant alterations in growth, development and virulence compared to the original clonal isolate. Sequencing of the evolved populations revealed changes both at the nucleotide and chromosome level, many of which became fixed in the population. Strikingly, more than 60% of the detected variants were DNA transposon insertions, with the two hAT and pogo subfamily elements hormin and Fot, respectively, accounting for more than 90% of the detected transposition events. Interestingly, lineages evolved under analogous conditions carried independent events at the same locus. For example, 4 of 5 lineages passaged through YPG medium plates had TE insertions in a predicted gene with unknown function, and 3 of these subsequently acquired additional non-synonymous mutations in a gene encoding a subunit of the velvet regulatory complex, further increasing their competitiveness under plate growth conditions. Importantly, plate-adapted populations exhibited significantly attenuated virulence on tomato plants and Galleria larvae. Our results suggest that DNA transposons and chromosome plasticity act as a major short-term evolutionary forces in *F. oxysporum,* and that host adaptation involves trade-offs between developmental programs favouring invasion versus proliferation.

11. The *Fusarium graminearum* FGSG_03624 xylanase primes plant immunity

S. Tundo[1], M.C. Paccanaro[1], I. Moscetti[2], R. D'Ovidio[3], F. Faoro[4], F. Favaron[1], L. Sella[1]

[1] *Department of Land, Environment, Agriculture and Forestry (TESAF), University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy*
[2] *Department of Ecology and Biology (DEB), University of Tuscia, Via S. Camillo de Lellis snc, 01100 Viterbo, Italy*
[3] *Department of Agricultural and Forestry Sciences (DAFNE), University of Tuscia, Via S. Camillo de Lellis snc, 01100 Viterbo, Italy*

[4] *Department of Agricultural and Environmental Sciences, University of Milano, Via Celoria 2, 20133 Milano, Italy.*
Email: silvio.tundo@unipd.it

Plants activate defense responses against pathogens once pattern recognition receptors (PRRs) detect pathogen-associated molecular patterns (PAMPs). Apart from the role of degrading cell wall components, some CWDEs also function as PAMPs independently from their enzymatic activity, as shown for EIX and Xyn11A xylanases of *Trichoderma viride* and *Botrytis cinerea*, respectively, and recently for the *Fusarium graminearum* FGSG_03624 xylanase. Aim of the work was to investigate the ability of FGSG_03624 to modulate plant immunity. Our results indicate that exogenous treatments with FGSG_03624 increase durum wheat resistance against *F. graminearum* by priming plant immune response. Indeed, callose deposition was detected in infected spikes previously treated with the xylanase. Conversely, callose deposits were not detected in infected control plants. To deeper investigate the molecular mechanism underlying the capacity of FGSG_03624 to induce plant defense responses and to verify the specificity of this effect, we exogenously treated *Arabidopsis* with FGSG_03624 and we transiently and constitutively expressed an enzymatically inactivated form of this xylanase in tobacco and *Arabidopsis,* respectively. Afterwards, these plants were challenged with *Pseudomonas syringae* pv *maculicola* and *B. cinerea*. Effectiveness in reducing symptoms caused by the bacterial pathogen was evident both in *Arabidopsis* and in tobacco, whilst no symptoms reduction was observed after *B. cinerea* infection. The increased resistance of *Arabidopsis* plants to *P. syringae* was associated to a faster and stronger activation of jasmonate/ethylene and salicylate pathways during pathogen infection. In conclusion, our results highlight the ability of FGSG_03624 to prime defense responses in plants and to confer plant disease resistance.

12. *fcrav2*, a gene with ROGDI domain involved in virulence, trichothecene biosynthesis, fungicide sensitivity and resistance to osmotic and oxidative stress in *Fusarium culmorum*

Safa Oufensou[1,2], Francesca Spanu[1], Barbara Scherm[1], Virgilio Balmas[1], Wiem Chtioui[1], Giovanna Delogu[3], Matias Pasquali[4], Quirico Migheli[1]

[1] *Dipartimento di Agraria, Università degli Studi di Sassari, Viale Italia 39, I-07100 Sassari, Italy*
[2] *Faculté des Sciences de Bizerte, 7021 Zarzouna Bizerte, Tunisie*
[3] *DEFENS- Università di Milano, via Celoria 2, 20233 Milano, Italy*
[4] *Istituto CNR di Chimica Biomolecolare, Traversa La Crucca 3, I-07100 Sassari, Italy*

Fusarium culmorum is a soil-borne fungal pathogen able to cause foot and root rot and Fusarium head blight on small grain cereals, particularly on wheat and barley. It causes significant yield and quality loss and results in the contamination of kernels with type B trichothecene mycotoxins. Knowledge on pathogenicity factors of this fungus is still limited. A transposon tagging approach based on the *mimp1/impala* double component

system has allowed us to select a mutant altered in multiple metabolic and morphological processes, trichothecene production and virulence. The flanking regions of *mimp1* were used to seek homologies in the *F. culmorum* genome and revealed that *mimp1* had reinserted within the last exon of a gene encoding a hypothetical protein of 318 amino which contains a ROGDI like leucine zipper domain, supposedly playing a protein-protein interaction or a regulatory role. By functional complementation and bioinformatic analysis we characterize the gene as yeast Rav2 homologue, acknowledging the high level of divergence in multicellular fungi. Deletion of *FcRav2* or its orthologous gene in *F. graminearum* highlighted its ability to influence a number of functions including virulence, trichothecene type B biosynthesis, resistance to azoles and resistance to osmotic and oxidative stress. Our results indicate that the FcRav2 protein (and possibly the RAVE complex on the whole) may become a suitable target for new antifungal drug development or plant-mediated resistance response also in filamentous fungi of agricultural interest. This work represents the first characterization of the role of RAV2 in filamentous fungi.

13. Functional characterisation of candidate *Fusarium graminearum* effectors

Claire Kanja, Kim Hammond-Kosack, Martin Urban, Ana Machado Wood

Rothamsted Research, Department of Biointeractions and Crop Protection, Harpenden, UK
Email: claire.kanja@rothamsted.ac.uk

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[1]

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[2], finding candidates suitable for functional studies from a pool of almost 300 secreted proteins with unknown functions remains problematic[3].

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[4].

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.

References

1. Steiner, B., Buerstmayr, M., Michel, S., Schweiger, W., Lemmens, M. & Buerstmayr, H. 2017. Breeding strategies and advances in line selection for Fusarium head blight resistance in wheat. *Tropical Plant Pathology,* 42**,** 165-174
2. Brown, N. A., Antoniw, J. & Hammond-Kosack, K. E. 2012. The predicted secretome of the plant pathogenic fungus *Fusarium graminearum*: a refined comparative analysis. *PLoS One,* 7**,** e33731.
3. Sperschneider, J., Dodds, P. N., Gardiner, D. M., Manners, J. M., Singh, K. B. & Taylor, J. M. 2015. Advances and challenges in computational prediction of effectors from plant pathogenic fungi. *PLoS Pathog,* 11**,** e100480
4. Saunders, D. G., Win, J., Cano, L. M., Szabo, L. J., Kamoun, S. & Raffaele, S. 2012. Using hierarchical clustering of secreted protein families to classify and rank candidate effectors of rust fungi. *PLoS One,* 7**,** e298476

14.Solving the polyketide pigmentation puzzle in *Fusarium solani*

Nielsen MR[1], Kaniki SEK[1], Pedersen TB[2], Holzwarth AKR[1], Wimmer R[2], Sørensen JL[1]

[1] *Aalborg University, Department of Chemistry and Bioscience, Esbjerg, Denmark*
[2] *Aalborg University, Department of Chemistry and Bioscience, Aalborg, Denmark*

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.

15. Mitochondrial genomes as phylogenetic backbone

Balázs Brankovics, Meixin Yang, Hao Zhang, Cees Waalwijk, Theo van der Lee & Anne van Diepeningen

WUR B.U. Biointeractions & Plant Health, Wageningen, the Netherlands, CAAS, Institute of Plant Protection, Beijing, China

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. asiaticum* 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.

16. Unveiling the *Fusarium graminearum* species complex: Global database of species and chemotypes

Del Ponte EM, GM Moreira, Nicolli CP, TJ Ward, C Waalwijk, TAJ van der Lee, H, Zhang, SN Chulze, DJ Tessmann, SA Stenglein, D Pan, S Vero, K O'Donnell, LJ Vaillancourt, A Moretti and A Logrieco

Fusarium graminearum is ranked among the five most dangerous fungal pathogens that affect agro-ecosystems. It causes floral diseases in small grains including wheat, barley and oats, as well as summer crops such as maize and rice. Infected kernels are lightweight and may be contaminated with mycotoxins of concern to human and animal health, mainly trichothecenes and zearalenone. The increased availability of *F. graminearum* strains collected worldwide, from different hosts/substrates, together with advances in taxonomy based on genealogical concordance, led to the recognition of species, as part of the *Fusarium graminearum* species complex (FGSC), during the 2000s. Surveys of FGSC strains have been conducted and reported worldwide for different crops/substrates, with strains being accurately identified to species, including novel species within the complex, using DNA barcoding methods. In addition, the potential to produce mycotoxins, mainly B-trichothecenes, is determined using either chemical methods or, more commonly, time and cost-effective PCR-based assays that target portions of genes of the trichothecene biosynthetic (TRI) cluster. We conducted a systematic review of peer-reviewed studies reporting lineages/species within FGSC. Two main data tables were created. The first contained data at the article level including bibliographic, scientometric, geographic, methodological (ID methods), host of origin and FGSC species. A second data table contained information at the strain level such as article, code(s), host/substrate, year, geographical coordinates, species and trichothecene genotype. In this talk, analyses of the bibliographic and scientometric data obtained from 100 peer-reviewed articles (2000 to 2019) authored by 514 unique authors and published in 34 journals will be presented. In all these articles, information was available for 24,004 strains, but species and chemotype ID data at the strain level were available for 63% of total strains (15,161 strains), of which 75% were obtained from the authors and the rest extracted from supplemental materials. The database and interactive interface will be publicly available allowing for searches, summarization and mapping of strains according to several criteria including article, country, host, species and trichothecene genotype. The database will be updated continuously and may be useful for guiding future surveys and exploring factors associated with species distribution such as climate and land use. Authors are encouraged to submit data at the strain level to the database. This work is supported by CNPq and MycoKey project.

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

Marike J. Boenisch[1], Karen Broz[2], Anna Lipzen[3], Kerrie Barry[3], Igor Grigoriev[3], H. Corby Kistler[1,2]

[1] *University of Minnesota, St. Paul, MN, USA*
[2] *USDA ARS Cereal Disease Laboratory, St. Paul, MN, USA*
[3] *DOE Joint Genome Institute, Berkeley, CA, USA*

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.

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

Slavica Janevska[1#], Iuliia Ferling[2], Julia Rautschek[1], Sandra Hoefgen[1], Robert H. Proctor[3], Falk Hillmann[2], Vito Valiante[1*]

[1] *Biobricks of Microbial Natural Product Syntheses, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Jena, Germany*
[2] *Evolution of Microbial Interactions, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Jena, Germany*
[3] *National Center for Agricultural Utilization Research, United States Department of Agriculture, Peoria, USA*

[#] *Present address: Molecular Plant Pathology, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, Netherlands*
** Corresponding author: vito.valiante@leibniz-hki.de*

Sphingolipid inhibitors are promising pharmaceuticals for treatment of severe human diseases, such as cancer, Alzheimer's and schizophrenia. Fumonisin (FUM) mycotoxins are efficient inhibitors of ceramide synthase (CS), and FB1 is the major derivative produced by *Fusarium verticillioides*.

We localized the early FUM biosynthetic enzyme Fum8 (aminotransferase) 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.

19. The endophytic behaviour of *Fusarium odoratissimum*, the causal agent of Panama disease, in weeds and ornamentals

Meijer HJG, Salacinas MA, Zuniga Burgos VA, Martinez de la Parte E and Kema GHJ

Wageningen University and Research Center, Wageningen, The Netherlands
Email: harold.meijer@wur.nl

Panama disease in Cavendish banana is caused by the fungal pathogen *Fusarium odoratissimum,* which is colloquially called Tropical race 4 (TR4) and causes havoc in the global banana industry. This soil-borne disease is often suggested to survive in the soil for decades in combination with a saprophytic lifestyle and the presence in alternative hosts.

In this study, we evaluated the capacity of non-banana plants to function as alternative hosts for TR4 survival and as vehicle for the spread of TR4. We sampled aerial parts of asymptomatic weeds in the Philippines from abandoned and existing banana farms with Panama disease infestation. In eight weed species, belonging to seven botanical families, TR4 presence was confirmed. In addition, the spread of TR4 was monitored in an array of weeds, ornamentals and model plants for research, following inoculation in the greenhouse. In many plant species tested, infestation by TR4 was confirmed in aerial parts while being asymptomatic. TR4 infested plants with soil-cleaned roots, placed with physical distance to Cavendish plants provided sufficient inoculum to rapidly infect banana plants. In addition, TR4 colonies, derived from aerial parts of infested plants, are capable to infect Cavendish plants without showing significant loss of aggressiveness. These results show that TR4 is capable to sustain itself within an array of alternative hosts and maintain its pathogenicity towards Cavendish banana.

20. Structural dynamics of chromosomes and its role in genome plasticity of *Fusarium oxysporum*

Lucía Gómez Gil[1], Cristina López Díaz[1], Dilay H. Ayhan[2], Li-Jun Ma[2], Antonio Di Pietro[1]

[1] *Department of Genetics, University of Córdoba, Córdoba, Spain*
[2] *Department of Biochemistry & Molecular Biology, University of Massachusetts Amherst, MA, USA*

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).

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.

Reference:
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)

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

Javier Pardo-Medina, Obdulia Parra-Rivero, Gabriel Gutiérrez Pozo, M Carmen Limón, Javier Avalos

Universidad de Sevilla, Spain
Email: avalos@us.es, jpardo6@us.es

The pathway of neurosporaxanthin synthesis in *Fusarium* is stimulated by light, a regulation investigated in detail in *Fusarium fujikuroi* that involves the transcriptional activation of the structural genes, linked in a coregulated cluster. The pathway is down-regulated by the RING-finger protein CarS, as indicates the carotenoid-overproducing phenotype of the mutants. A detailed examination of the region upstream to *carS* in RNA-seq analyses identified a putative 1.2-kb non-coding transcript, whose levels increased in *carS* mutants. This nonannotated gene, *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*. The re-introduction of the wild *carP* sequence in the *carP* deleted strain 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 to synthesize carotenoids in *Fusarium*, possibly controlling CarS by an unknown mechanism currently under investigation.

22. *Fusarium oxysporum* effectoromes

Martijn Rep

Molecular Plant Pathology, Green Life Sciences, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, the Netherlands

Proteins that are secreted by plant-invading microbes during host colonisation and that promote colonisation and/or disease symptom development are commonly called

effectors. Effectors of plant pathogenic strains of *Fusarium oxyporum* (Fo) have first been identified through xylem sap proteomics of infected tomato plants and were called SIX (Secreted in xylem) proteins. The first genome sequence of a tomato-infecting strain Fo revealed that the genes for these SIX proteins lie on a single chromosome. This 'pathogenicity chromosome' can be lost without affecting growth or metabolism and can also be transferred to another genetic background, thereby conferring the ability to cause disease in tomato. Pathogenicity chromosomes containing homologs of *SIX* genes and other putative effector genes have now also been identified in other *formae speciales* of Fo, and in a few other Fusarium species. For unknown reasons, a miniature impala non-autonomous transposable element (mimp) is consistently present upstream of *SIX* genes. By this feature, effectoromes can be identified for any Fo strain. These effectoromes, which include avirulence factors (effectors that trigger immunity) are predictive of whether a strain is potentially pathogenic, and if so on which plant species.

23. Exploring the role of plant vesicle trafficking during *Fusarium graminearum* infection

Luca Steel[1], Panwar V[1], Grimwade-Mann M[1,2], Kanyuka K[1], Hammond-Kosack K[1]

[1] *Rothamsted Research, Harpenden, UK*
[2] *Bangor University, Bangor, UK*

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.

References: Nomura *et al.,* 2006. Science **313**.5784:220-223.

24. Using comparative genomics to identify genes involved in *Fusarium fujikuroi* pathogenicity

Edoardo Piombo[1,2], Marco Rosati[2] and Davide Spadaro[1,2]

[1] *AGROINNOVA – Centre of Competence for the Innovation in the Agro-environmental Sector, University of Turin, Largo Braccini 2, 10095 Grugliasco, TO, Italy*
[2] *Department of Agricultural, Forestry and Food Sciences (DISAFA), University of Turin, Largo Braccini 2, 10095 Grugliasco, TO, Italy*

Bakanae, a seedborne rice disease, is caused by *Fusarium fujikuroi*. Most strains of this fungus induce symptoms as elongation and thinning of internodes, abnormal height and frail stems. However, the phenotype of affected plants can vary depending on the fungal genotype involved. There are reports of isolates inducing stunting, chlorosis, yellowing and root and crown rots. Rotting of seedlings prior to emergence is common, and there are also strains that, while having a complete gibberellin gene cluster, seem to be incapable of inducing any symptoms. To be able to discriminate among the many potential resistance genes identified on rice cultivars, it is important to deepen our knowledge of how *F. fujikuroi* can provoke the disease, understanding what genes are responsible for the differences among the strains. Five isolates were sequenced, with avirulent, elongation-inducing and stunting-inducing pathotypes, discovering that minimal changes in the genome can induce radical phenotypic differences, depending on the temperature. A combination of *de novo* gene prediction, SNP calling and structural variant analysis allowed to identify 13 putative factors of virulence, together with 3 genes whose function could be involved in shifting from the stunting-inducing to the elongation-inducing phenotype. The results are complemented by pathogenicity trials showing correlation between high temperature and virulence of the strains. Global warming, along with the decreasing availability of chemical seed-dressing products, will therefore cause increasing incidence of bakanae disease, making it a priority to get a more precise picture of the complex interactions between *F. fujikuroi* and rice.

25. Improvement of a lux-system that detect new antifungals

M Carmen Limón[1], Najar-García A[1], Castilla-Alcantara JC[1], Saez-Moreno R[1], Van Den Hondel CAMJJ[2]

[1] *University of Seville, Department of Genetics, Seville, Spain*
[2] *University of Leiden, Molecular Microbiology and Biotechnology, Leiden, Netherlands*
Email: carmenlimon@us.es

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 growing in the presence of different concentrations of different commercially available antifungals. Our current data about the detection system and its optimization will be presented.

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

M Carmen Limón, Julia Marente, Marta Franco-Losilla, Ismael Borrego-Serrano, Carmen Vega and Javier Avalos

University of Seville, Department of Genetics, Seville, Spain
Email: carmenlimon@us.es

Fusarium fujikuroi produces a large variety of secondary metabolites in response to different environmental factors. Light stimulates the synthesis of carotenoids such as neurosporaxanthin (NX), 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 pathway. Evidence of the role of CarS as a repressor was obtained by increasing *carS* mRNA using a constitutive *gpdh* promoter and the Tet-on system. Both mutants have 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-terminus have been isolated. 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 WT.

On the other hand, mutations in *carS* are pleiotropic, since affect not only carotenoid biosynthesis but also conidiation, germination, and antibiotic sensitivity to 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 correlates with enhanced mRNA levels of structural *car* genes and reduced levels of *carS* mRNA.

27. The specialized metabolite gene clusters of *Fusarium graminearum* species complex and their response during the cell-to-cell invasion of wheat

Sabina Moser Tralamazza[1,2], Leen Abraham[1], Ursula Oggenfuss[1], Liliana Oliveira Rocha[3], Benedito Corrêa[2], Daniel Croll[1]

[1] *Laboratory of Evolutionary Genetics, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland*
[2] *Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil.*
[3] *Department of Food Science, Food Engineering Faculty, University of Campinas, Campinas, Brazil*

In fungi, specialized (or secondary) metabolites encode key functions to exploit plant hosts or environmental niches. Promiscuous exchange among species and frequent reconfigurations make gene clusters some of the most dynamic elements of fungal genomes. We analyzed the genomes of the *Fusarium graminearum* species complex (FGSC) composed of very closely related plant pathogens and causal agents of Fusarium head blight on cereals. Although many studies have focused in *F. graminearum sensu stricto*, the dynamics of infection of FGSC as a group were rarely investigated. We mined for specialized metabolite gene clusters in eight distinct species of the group. We identified 54 well defined specialized metabolites gene clusters within the FGSC, seven which were absent from the *F. graminearum* reference. The gene cluster diversity has dynamic and diverse origins including independent gene losses, horizontal transfer and chromosomal rearrangements. Next, we wanted to identify both conserved and variable transcriptomic responses of gene clusters upon infection of wheat. For this, we generated RNAseq datasets of both in vitro culture conditions and coleoptile infections for five FGSC members. Heterogeneity in the responsiveness of specialized gene clusters may underlie key differences in host adaptation across the species complex.

28. FungiDB: integrating genomic data for pathogens and model

organisms and providing advanced search capabilities and large-scale data analysis

Achchuthan Shanmugasundram[1], Evelina Basenko[1], Omar Harb[2], Mark Caddick[1] and David Roos[2]... *on behalf of the entire VEuPathDB team.*

[1] *Institute of Integrative Biology, Functional and Comparative Genomics, University of Liverpool, Liverpool UK*
[2] *Department of Biology, The University of Pennsylvania, Philadelphia, Pennsylvania, USA*

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:

1. ***Browsing*** of genomes and gene pages in an encyclopedic manner to explore all available information and data.
2. ***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.).
3. ***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.
4. ***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.

FungiDB is supported in part by NIH HHSN272201400030C and 75N93019C00077 and the Wellcome Trust #WT108443MA and Wellcome Biomedical Resources #212929/Z/18/Z grants.

29. *Fusarium graminearum* molecular chemotypes and populations in

Finland, Norway and Russia

Tapani Yli-Mattila[1], Tatiana Gagkaeva[3], Heidi Aamot[3], Taha Hussien[1], Todd Ward[4]

[1] *Molecular Plant Biology, Department of Biochemistry, University of Turku, Turku, Finland, tel. +358-44-0560700.*
[2] *All-Russian Institute of Plant Protection (VIZR), St. Petersburg-Pushkin, Russia*
[3] *NIBIO – Norwegian Institute for Bioeconomy Research, Pb 115, NO-1431 Ås, Norway*
[4] *Mycotoxin Prevention and Applied Microbiology USDA-ARS, Peoria, USA*
E-mail tymat@utu.fis

The 3ADON chemotype of *F. graminearum* predominates in northern Europe, whereas the 15ADON chemotype is predominant in Central and southern Europe. In the present work, molecular chemotypes and variable number tandem repeat (VNTR) markers were used to assess population structure and diversity among *Fusarium graminearum* isolates from four regional locations: Finland and northwestern Russia (F+NWR; *N* = 40), south Russia including central European Russia (SR; *N* = 54), Russian Far East (RFE; *N* = 96), and Norway (NOR; *N* =106). Trichothecene genotype composition was significantly different across the sampling locations. The 3ADON type was predominant in F+NWR+NOR, the 15ADON type was predominant in SR, and RFE had a balanced composition of these two trichothecene genotypes. The NIV genotype was not observed among the studied collection of *F. graminearum*. Analyses of population structure and relatedness indicated that the *F. graminearum* population in F+NWR are closely related to the NOR population and they can be considered as a unified population. However, significant differentiation was observed between the northern European F+NWR+NOR population and those from the other sampled regions. The F+NWR+NOR population had substantially less genetic diversity than in the other regions. It may be more specialized to oats, because only the 3ADON genotype has been found in oats in Europe. This idea is also supported by the preliminary qPCR results according to which Finnish 3ADON isolates were more effective in infecting oats than 15ADON isolates from south Russia, while 15ADON isolates were more effective in infecting spring wheat.

30. Understanding the origin, diversity and evolution of the Panama Disease pathogen

D. E. Torres Sanchez, N. Ordóñez, N. Maryani, F. García-Bastidas, H.J.G. Meijer, M.F. Seidl, G.H.J. Kema

Wageningen University, The Netherlands

Spatiotemporal origins and factors impacting dissemination remain elusive for many epidemics. Bananas are the world's most popular fruit and represent crucial food commodities. Global banana production is dominated by Cavendish monocultures, the remedy to manage the Panama disease epidemic in Central America in the last century.

Here we will discuss the most recent results of our ongoing efforts to study the diversity and dissemination of the causal agents of Panama disease worldwide. We genotyped a global collection of fungal *Fusarium* isolates and traced the origins of Panama disease to Southeast Asia, bananas' center of origin and a biodiversity hotspot for banana-infecting *Fusarium* species. While the previous epidemic was caused by a suite of genetically diverse *Fusarium* species, we show that the current epidemic that devastates Cavendish is caused by a single, genetically distinct new *Fusarium* species, and ongoing ingression into banana-growing regions of Asia, the Middle East, the Indian subcontinent, Africa and most recently Latin America seriously threatens worldwide banana production.

31. *In vitro* and in field response of different fungicides against *Fusarium* species causing ear rot sisease of maize

Masiello Mario, Stefania Somma, Veronica Ghionna, Antonio Francesco Logrieco and Antonio Moretti

Institute of Sciences of Food Production, Research National Council (ISPA-CNR), Via Amendola 122/O, 70126 Bari, Italy

Fusarium graminearum, a deoxynivalenol producer, the fumonisin-producing species *F. proliferatum* and *F. verticillioides*, and *Aspergillus flavus*, the main aflatoxin B1 producing fungal species, are the main toxigenic fungi that colonize maize. Several strategies are available to control toxigenic fungi and related mycotoxins, such as chemical control. However, there is poor knowledge on the efficacy of fungicides on maize plants since few molecules are registered. The sensitivity of *F. graminearum*, *F. proliferatum*, *F. verticillioides*, and *A. flavus* to eleven fungicides, selected based on their different modes of action, was evaluated in both *in vitro* assays and, after selection, in the field. *in vitro*, demethylation inhibitors (DMI) showed excellent performances, followed by thiophanate-methyl and folpet. Among the succinate dehydrogenase inhibitors (SDHI), isopyrazam showed a higher effectiveness against Fusarium species than boscalid, which was ineffective against Fusarium, like the phenyl-pyrrole fludioxonil. Furthermore, both SDHIs and fludioxonil were more active against *A. flavus* than *Fusarium* species. In field trials, prothioconazole and thiophanate-methyl were confirmed to be effective to reduce *F. graminearum* (52% and 48%) and *F. proliferatum* contamination (44% and 27%). On the other hand, prothioconazole and boscalid could reduce *A. flavus* contamination at values of 75% and 56%, respectively.

Keywords: Aflatoxins, deoxynivalenol, fumonisins, Succinate Dehydrogenase Inhibitors

32. Identification of *Fusarium* toxigenic fungal species associated with

maize ear rot: calmodulin as single informative gene

Antonia Susca, Alessandra Villani, Antonio Moretti, Gaetano Stea and Antonio Logrieco

Institute of Sciences of Food Production, National Research Council (CNR), Via Amendola 122/O, 70126 Bari, Italy

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 STUDY, 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 *Fusarium* mycobiota possibly occurring on maize, identifying calmodulin gene as single gene taxonomically informative; (ii) designed 7 set of primers for rapid identification of 14 *Fusarium* species, and finally (iii) tested specificity of the 7 set of primers, in combination with 3 additional set previously developed.

Keywords: Mycotoxins, species-specific primers, barcoding

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

Alessandra Villani[1], Robert H. Proctor[2], Hye-Seon Kim[2], Daren W. Brown[2], Antonio F. Logrieco[1], Maria Teresa Amatulli[1,3], Antonio Moretti[1] and Antonia Susca[1]

[1] *Institute of Sciences of Food Production, National Research Council, Bari, Italy*
[2] *Department of Agriculture Peoria, National Center for Agricultural Utilization Research, U.S., Peoria, IL, USA*

[3] *Thales Alenia Space Italia, Torino, Italy.* [4] *Institute of Sciences of Food Production, National Research Council, Bari, Italy.*
Email: antonio.moretti@ispa.cnr.it

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.

Keywords: *Fusarium incarnatum-equiseti* species complex, comparative genome analyses, Secondary metabolite genes, Phylogeny, Horizontal gene transfer

34. Subcellular compartmentalization of sesquiterpene mycotoxin synthesis in Fusarium

H. Corby Kistler and colleagues

USDA ARS Cereal Disease Laboratory, University of Minnesota, St. Paul, MN, USA

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 cellular and developmental processes that allow for the expression of the fungal terpenome. 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

35. Topographically triggered mycelial bundles in *Fusarium* species

Anne van Diepeningen[1], Colin Ingham[2], Laura Caton[2] and Jan Dijksterhuis[3]

[1] *Wageningen Plant Research, Wageningen*
[2] *Hoekmine BV, Utrecht*
[3] *Westerdijk Institute, Utrecht, The Netherlands*
Email: anne.vandiepeningen@wur.nl

Growing microorganisms in new ways can produce surprising changes in morphology. One such novel method is the use of Micro-Cultivation Chips (MCC) that can support the cultivation of fungi as micro-colonies. MCC comprise an array of microwells (from 20 to 300 microns diameter) with a porous ceramic base. A number of *Fusarium* species (including *F. oxysporum*) produce mycelial bundles when cultured on MCC. These bundles maintain the diameter of the wells, even when they are beyond the limitations of the well – they appear to be templated by the early confinement in the microwells. We are working to understand how these structures form, what they do and how they relate to the natural situation of these fungi.

36. Biomanagement of *Fusarium oxysporum* f. sp. *cubense* (Foc) TR4 in banana using chitosan and endophytic biocontrol agents

Luis V. Lopez-Llorca[1], Federico Lopez-Moya[1], Jose Angel Lopez- Jimenez[2], Daniel Gonzalez-Silvera[2], Marta Suarez-Fernandez[1], Ana Lozano-Soria[1], Cristina Mingot Ureta[1]

[1] *Laboratory of Plant Pathology, Department of Marine Sciences and Applied Biology, Multidisciplinary Institute for Environmental Studies (MIES) Ramon Margalef, University of Alicante, 03080 Alicante, Spain*
[2] *Department of Physiology , University of Murcia, 30003 Murcia, Spain*
Email: lv.lopez@ua.es

Foc TR4 is currently causing an epidemics worldwide which is threatening banana production worldwide. In our laboratory, we have previously shown that chitosan is a potent antifungal that permeabilizes the membrane of sensitive fungi. This process

leads to a massive activation of oxidative stress which leads to fungal death in sensitive fungi. We also showed that some plant pathogenic Fusaria (*F. oxysporum* f sp. *radicis-lycopersici, Forl*) and other pathogens are sensitive to chitosan. On the contrary endophytic beneficial fungi such as nematophagous fungi (*Pochonia chlamydosporia*, Pc) and entomopathogenic fungi are resistant to chitosan. We have recently shown that Foc TR4 is sensitive to chitosan and that chitosan can reduce panama disease under laboratory conditions. Likewise Pc which is known to produce a large array of secondary metabolites with biological activity causes partial inhibition of Foc TR4. All these results together open a new and sustainable way to manage the current epidemics of the pathogen.

37. Mycotoxin production from *Fusarium proliferatum* isolated from garlic in Italy

A. Infantino[1], M. Beccaccioli[2], M. Aragona[1], C. Costa[3], M. Reverberi[2]

[1] *CREA-DC of Rome, Research Center for Plant Protection and Certification, Via C.G. Bertero 22, 00156 Roma, Italy*
[2] *Sapienza University of Rome, Dept. Environmental Biology, Piazzale Aldo Moro 5, 00185 Roma, Italy*
[3] *CREA-IT, Research Center for Engineering and Agro-Food processing, Via della Pascolare, 16, 00015 Monterotondo (RM)*
Email: alessandro.infantino@crea.gov.it

Fusarium proliferatum has been reported affecting garlic (*Allium sativum* L.) in Italy and worldwide, both in the open field and during storage. This species is known to produce several mycotoxins, such as fumonisins, moniliformin, beauvericin, fusaric acid, and fusaroproliferin. On commercial garlic samples, fumonisins content seems not to be a risk for human consumption. Nevertheless, *in vitro* production of several mycotoxins, other than fumonisins, from *F. proliferatum* isolated from garlic has been demonstrated. The role of these mycotoxins in the behaviour of this species (host range, virulence, radial growth) has been only partially elucidated. In this study, the production of Fusaric Acid (FA), Beauvericin (BEA), Fumonisin (FB1 and FB2), and moniliformin (MON) of 32 *F. proliferatum* isolates from symptomatic garlic bulbs and from other cultivated species, has been evaluated both *in vitro* and *in vivo*. In the first case, isolates were grown for 4 weeks on autoclaved rice kernels, while for *in vivo* mycotoxin production, disinfected bulbils of a susceptible garlic ecotype were artificially inoculated with a plug of each isolate grown for one week on PDA. In both cases, mycotoxins were evaluated by HPLC-MS/MS. A Principal Component Analysis, conducted on two quantitative variables (radial growth, virulence) evidenced, on the positive side of PC1, a positive and higher contribute of FB1, FB2 and FA produced *in vitro* for samples derived from garlic. Analyses of mycotoxins produced *in vivo* are in progress. The overall results will shed lights on the possible role of the analyzed mycotoxins in determine differences among isolates for virulence or host range.

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

Daren W. Brown, Hye-Seon Kim, Mark Busman and Robert H. Proctor

US Department of Agriculture, Agriculture Research Service, NCAUR, Peoria, IL USA 61604

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.

39. Cytological and signaling aspects of a *Fusarium solani* – legumes association

Vasiliki Skiada[1], Marianna Avramidou[1], Paola Bonfante[2], Andrea Genre[2], Kalliope K. Papadopoulou[1]

[1] *Department of Biochemistry and Biotechnology, University of Thessaly, Biopolis, Larissa, 41500, Greece*
[2] *Department of Life Sciences and Systems Biology, University of Torino, Torino, 10125, Italy*

Fusaria may thrive as plant pathogens, saprotrophs on plant debris, or as asymptomatic endophytes within plant tissues. Pathogenic, or endophytic *Fusarium* isolates associating with legume plants have been reported. Emerging evidence by multiple research groups demonstrate that there is a continuum in plant-microbe interactions: plant-interacting microbes (symbiotic, mutualistic, pathogenic) utilize similar signaling traits and trigger common cellular responses in order to be recognized by the host-plant, and perhaps other molecular players, and/or the niche, and/or the physiological status of the plant define the outcome of the association.

The endophytic isolate *Fusarium solani* strain K (FsK), protects tomato plants against

root and foliar pathogens (Kavroulakis *et al.*, 2007), spider mites (Pappas *et al.*, 2018), zoophytophagous insects (Garantonakis *et al.*, 2018) and drought (Kavroulakis *et al.*, 2018). FsK also colonizes *Lotus japonicus* and upon colonization, distinct plant and fungal cellular responses occur, reminiscent of responses reported in symbiotic and/or pathogenic plant-microbe interactions (Skiada *et al.*, 2019). At the molecular level, genes acting upstream, within, and downstream the so-called Common Symbiotic Signaling Pathway (CSSP; the single pathway used upon symbiont recognition and accommodation in symbiont-interacting host plants), are also regulated during FsK-*Lotus* association. Results are complemented by analysis of nuclear calcium elevations (spiking) in FsK-legume associations, a so far considered symbiont-specific plant response, lying at the center of the CSSP. FsK exudates trigger nuclear calcium spiking in *M. truncatula* Root Organ Culture epidermis, and this response is CSSP-specific, as depicted by mutant analysis. This is also shown for other fungal exudates derived from pathogenic or mutualistic fungi.

In all, we demonstrate that there is an evolutionary continuity in how microbes are recognized by the host plant, and discuss the possible alternative routes for microbial recognition, by focusing on FsK-legumes system.

- Garantonakis et al.,. 2018. Tomato Inoculation With the Endophytic Strain *Fusarium solani* K Results in Reduced Feeding Damage by the Zoophytophagous Predator *Nesidiocoris tenuis*. *Frontiers in Ecology and Evolution* 6: 126
- Kavroulakis et al., 2018. Tolerance of tomato plants to water stress is improved by the root endophyte *Fusarium solani* FsK. *Rhizosphere* 6: 77–85.
- Kavroulakis et al., 2007. Role of ethylene in the protection of tomato plants against soil-borne fungal pathogens conferred by an endophytic *Fusarium solani* strain. *J.Experiment. Botany* 58: 3853–3864.
- Pappas et al., 2018. The Beneficial Endophytic Fungus *Fusarium solani* Strain K Alters Tomato Responses Against Spider Mites to the Benefit of the Plant. *Frontiers in Plant Science* 9: 1603.
- Skiada et al., 2019. Colonization of Legumes by an Endophytic *Fusarium solani* strain FsK Reveals Common Features to Symbionts or Pathogens. *Fungal Genetics and Biology* 127: 60–74.

40. Exploring the nuclear and mitochondrial genomes of *Fusarium tricinctum*

Nadia Ponts[1], Jérôme Gouzy[2], Charlotte Gautier[1], Laetitia Pinson-Gadais[1], Marie Foulongne-Oriol[1], Christine Ducos[1], Florence Richard-Forget[1], Jean-Michel Savoie[1], Chen Zhao[1], Gérard Barroso[1,3]

[1] *Centre de Recherche INRA Bordeaux-Aquitaine, UR1264 MycSA, Villenave d'Ornon, France*
[2] *LIPM, Université de Toulouse, INRA, CNRS, Castanet-Tolosan, France*

[3] University of Bordeaux, Bordeaux, France
Email: nadia.ponts@inra.fr

Fusarium tricinctum is a phytopathogenic fungus increasingly encountered during episodes of ear blight affecting straw cereals and maize in Europe and Asia. This species can also produce "emerging" mycotoxins, such as enniatins and beauvericin, that could be at the origin of future health crises. The genome of the strain INRA104 was recently sequenced at over 500X fold-coverage leading to a final assembly of 23 scaffolds, including one scaffold for the mitochondrial genome, for a total size of 42.8 Mb and 14,212 predicted genes. Among the annotated genes, we identified the complete 9.4 kb-long sequence *esyn1*, encoding the key enzyme Enniatin synthase 1 of the enniatin and beauvericin biosynthetic pathway. The genetic polymorphism of *esyn1* was explored in 14 *F. tricinctum* strains and 11 strains of the closely related *Fusarium avenaceum*. Moreover, the complete mitochondrial genomes of the two species were annotated and compared. Mitochondrial genetic variability is mainly located within intergenic regions, including SNPs, length variations mediated by InDels, and length mutations generated by DNA sliding events in microsatellite type sequences. Moreover, the intraspecific and interspecific variations in the presence/absence of large group I introns of the conserved mitochondrial genes suggest high mobility of these introns, resulting from several events of gain and loss occurring during short evolution periods. Phylogenetic analyses of intron orthologous sequences suggest that most of these introns could have originated from lateral transfers from phylogenetically close as well as distant species belonging to various Ascomycota genera and even to the distant Basidiomycota fungal division.

Keywords: *Fusarium tricinctum*, *Fusarium avenaceum*, diversity, comparative genomics

41. Deciphering the effect of ambient pH on enniatins production by *Fusarium avenaceum*

Charlotte Gautier, Nathalie Ferrer, Sylvain Chéreau, Enric Zehraoui, Nadia Ponts, Florence Richard-Forget

Centre de Recherche INRA Bordeaux-Aquitaine, UR1264 MycSA, Villenave d'Ornon, France
Email: charlotte.gautier@inra.fr

Little is known as regards to the molecular mechanisms by which the biosynthesis of enniatins can be regulated. This lack of knowledge hinders the development of control tools to ensure minimum levels of contamination in cereal harvests. The aim of this study was to decipher the effect of ambient pH. First, growth and toxin production of 12 *Fusarium avenaceum* strains were tested in FDM media buffered at various pH values. For all strains, the highest mycelium biomass was obtained at pH 7. As regards enniatin yields, our results showed that these toxins can be produced at a wide range of pH values. The optimal pH value for their yield was strain-dependent: while in most

strains, the highest amounts of toxins were quantified at pH 4-5, pH values higher than 6 were shown to promote the production of enniatins in a sub-set of four strains. Second, we investigated the role of the PacC homologue from *F. avenaceum*, FavPac1, in the regulation of enniatins production. FavΔPac1 deletion mutants were constructed in three *F. avenaceum* strains characterized by contrasted responses to variations in environmental pH. Fungal development, expression of the peptide synthetase gene (*esyn*) coding for the biosynthesis of enniatins and the accumulation of toxin at different pH were tested in the mutants and in their corresponding wild strains. The resulting data shed light on the mechanisms by which FavPac1, a transcription factor that regulates pH homeostasis, could be involved in the modulation of the production of enniatins by *F. avenaceum*.

Keywords: *Fusarium avenaceum*, enniatins, pH, Pac1

42. Microbial interactions of *Fusarium oxysporum* in the soil.

Nick LeBlanc, Adil Essarioui, Zixun Zhong, Michael Fulcher, Linda Kinkel, and **H. Corby Kistler**

University of Minnesota, St. Paul, MN 55108 USA

This project is part of a long-term field experiment to characterize the impacts of plant species diversity on microbial community structure and function in prairie soil. Our work focuses on the role of microbial species interactions in mediating soil community functional characteristics, including microbial nutrient cycling and pathogen suppression, and on the ways that plant community diversity influences the co-evolutionary dynamics of soil microbes. We have documented the significant effects of plant diversity on soil fungal and bacterial populations, and on the functional characteristics of Streptomyces and Fusarium populations in soil. We have generated a large collection of these coevolved bacterial and fungal isolates from replicated soil samples associated with the prairie grass *Andropogon gerardii* and the prairie legume *Lespedeza capitata* grown in monoculture and in 16-species plant communities polyculture. Carbon utilization phenotypes, the ability to inhibit growth of other microbes from sympatric and allopatric populations, and antibiotic resistance capacities have been previously characterized for every isolate. We will describe the whole genome sequencing of ten soil *Fusarium oxysporum* and ten *Streptomyces* strains and their interaction transcriptomics and metabolomics. There have been few if any comparable studies of genomic, transcriptomic, or metabolic data among naturally-coevolved populations for which significant phenotypic effects of coevolution have already been documented. We predict our studies will address potential means for active management of microbial evolutionary and co-evolutionary dynamics in soil. The genomic data will provide a resource for researchers studying whole genome metabolic modeling, in particular to provide information needed to expand existing models to include secondary metabolic interactions and to accommodate larger, more complex fungal genomes.

ECFG15
ROME • ITALY 2020

SATELLITE WORKSHOP

Trichoderma, Clonostachys and other biocontrol fungi

MONDAY, FEBRUARY 17

Location: **Sapienza University of Rome**
Building: **CU022** | Side: **Botanica** | Floor: **First** | Room: **Marini Bettolo**

09:45 - 10:15	**Welcome Coffee**
10:15 - 10:30	**Matteo Lorito** | University of Naples Federico II **Welcome address and opening of the workshop**

SESSION I - Invited talks: research results and industries point of view

CHAIR: **Matteo Lorito**, University of Naples Federico II

10:30 - 11:00	**Enrique Monte** | University of Salamanca ***Trichoderma* and heritable plant responses**
11:00 - 11:30	**Irina Druzhinina** | Nanjing Agricultural University **Whole-genus ecological genomics of *Trichoderma*: the first steps towards understanding the origin of environmental opportunism**
11:30 - 12:00	**Magnus Karlsson** | Swedish University of Agricultural Sciences **Exploring genetic variation in *Clonostachys* to understand biological control mechanisms**
12:00 - 12:30	**Ralph Oelmüller** | Schleiden Institute Biology **How root-colonizing endophytes promote plant performance and influence ecosystems**
12:30 - 13:00	**Edith Ladurner** | CBC Europe S.r.l, BIOGARD Division **Effect of plant species, pathogen, environmental factors and their interactions on *Trichoderma harzianum* strain INAT11**
13:00 - 13:30	**Riccardo Liguori** | Isagro S.p.A. Novara Research Center **Present and future of *Trichoderma asperellum* + *Trichoderma gamsii* as biocontrol agent in the Isagro portfolio**
13:30 - 14:30	**Light Lunch**

SESSION II. Offered talks

CHAIR: **Sabrina Sarrocco**, University of Pisa

14:30 - 14:40	**Susanne Zeilinger** | University of Innsbruck ***Trichoderma atroviride*** **mycoparasitism and its regulation by the TOR signaling pathway**
14:40 - 14:50	**Lisa Kappel** | University of Innsbruck **Uncovering essential mechanisms of chitin and chitosan remodeling in the cell wall of the mycoparasite *Trichoderma atroviride***
14:50 - 15:00	**Benjamin Horwitz** | Technion, Haifa **Testing the role of the transcription factor TvSom1 in adhesion of *Trichoderma virens* germlings**
15:00 - 15:10	**Yu-Zhong Zhang** | Shandong University **Enhancing peptaibols production in the biocontrol fungus *Trichoderma longibrachiatum* SMF2 by elimination of a putative glucose sensor**
15:10 - 15:20	**Jian Zhang** | Nanjing Agricultural University **Azaphilones biosynthesis in *Trichoderma harzianum* benefits fungal survival to oxidative stress**
15:20 - 15:30	**Ting-Fang Wang** | Academia Sinica **Complete genome sequences reveals novel insights into chromosomal organization and evolution of different *Trichoderma* species**
15:30 - 15:40	**Roberta Marra** | University of Naples Federico II **Effects of *Trichoderma* strains and metabolites on the growth, disease resistance, leaf transcriptome and metabolome of olive plants**
15:40 - 16:30	**Coffee Break**

SESSION III. Offered talks

CHAIR: **Magnus Karlsson,** Swedish University of Agricultural Sciences

16:30 - 16:40	**Isabel Vicente Muñoz** | University of Pisa **Terpene synthases in *Trichoderma gamsii* T6085**
16:40 - 16:50	**Wolfgang Hinterdobler** | Austrian Institute of Technology **Austrian *Trichoderma* spp. impact mycotoxin production of the plant pathogen *Fusarium graminearum***

16:50 - 17:00	**Nadia Lombardi** | University of Naples Federico II ***Trichoderma* applications affect the physiological processes that improve strawberry production and quality**
17:00 - 17:10	**Laura Gioia** | University of Naples Federico II **Valorization of by-products from oleaginous crops production using *Trichoderma* spp**
17:10 - 17:20	**Mukesh Dubey** | Swedish University of Agricultural Sciences **LysM effectors regulate fungal development and required for hyphal protection and biocontrol traits in *Clonostachys rosea***
17:20 - 17:30	**Anastasia Lagopodi** | Aristotle University Thessaloniki School of Agriculture ***Pseudomonas chlororaphis* ToZa7 and *Clonostachys rosea* IK726, a successful biocontrol pair against Fusarium crown and root rot of tomato**

Trichoderma and heritable plant responses

M.E. Morán-Diez, M.B. Rubio, A.E. Martínez de Alba, R. Hermosa and **E. Monte**

Spanish-Portuguese Institute for Agricultural Research (CIALE), University of Salamanca, Villamayor, Salamanca, Spain

A huge amount of *Trichoderma* strains is being applied in plant protection around the world. Most of them are used and commercialized because of their biocontrol activity against plant pathogenic fungi, oomycetes, and even nematodes. However, the versality of *Trichoderma* is astonishing since strains from several species have evolved from a genetic predisposition towards mycotrophy to colonize roots and adopt an endophytic lifestyle. As a result, other plant beneficial effects of *Trichoderma* began to emerge. This is the case of their abilities to promote plant growth, to elicit plant defenses against pathogen attack and environmental stress, the attraction of pest natural enemies and their use in the improvement or maintenance of soil productivity. In addition to their direct biocontrol action, these more recently described *Trichoderma* capabilities give us opportunities to register and commercialize the most effective strains as bioprotectants or as biostimulants. However, it would be a mistake to believe that *Trichoderma* are not able to display both skills at the same time, since induction of systemic defenses and plant growth promotion are *Trichoderma* capacities determined by the host, depending on which one the plant wants to tap. In this sense, a recent study carried out on tomato plants (Medeiros et al., 2017) has demonstrated that plant growth promotion induced by *Trichoderma* is inherited without compromising the level of resistance to the nematode *Meloidogyne javanica* as the progeny of *Trichoderma*-primed plants displayed increased size and resistance to the nematode without fitness costs. Moreover, gene expression results from the defense inductions in the offspring of *Trichoderma*-primed plants, suggest that an auxin-induced reactive oxygen species production promoted by *Trichoderma* may act as a major defense strategy during plant growth.

Medeiros *et al.* (2017). *Sci Rep.* 7: 40216.

Whole-genus ecological genomics of *Trichoderma*: the first steps towards understanding the origin of environmental opportunism

Irina S. Druzhinina[1,2], Feng Cai[1,2], Mingyue Ding[1], Komal Chenthamara[2], Mohammad Rahimi[2], Renwei Gao[1], Priscila Chaverri[3], Alfredo Lopez De Leon[4], Scott Baker[5], Marie-Claude Moisan[6], Ronald de Vries[7], Adrian Tsang[8], Igor V. Grigoriev[5], Randy Berka[9]

[1] *Fungal Genomics Group, Nanjing Agricultural University, Nanjing, P.R. China*
[2] *Microbiology and Applied Genomics Group, Institute of Chemical, Environmental and Bioscience*

Engineering (ICEBE), TU Wien, Vienna, Austria
[3] *Department of Plant Science and Landscape Architecture, University of Maryland, Maryland, USA*
[4] *Novozymes Inc., Davis, CA, USA*
[5] *US DOE Joint Genome Institute, Berkeley, CA, USA*
[6] *Center for Structural and Functional Genomics, Concordia University, Montreal, Quebec, Canada*
[7] *Microbiology, Utrecht University, Utrecht, The Netherlands*
[8] *Centre for Structural and Functional Genomics, Concordia University, Montreal, Quebec, Canada*
[9] *Archer Daniels Midland Company, Davis, CA, USA*

Trichoderma (Hypocreales, Ascomycota) exhibits unique nutritional versatility forming biotrophic interactions with fungi, animals, plants, and efficiently degrading lignocellulose and other natural and synthetic polymers. The genus includes up to four hundred molecularly defined species, most of which are rare and ecologically restricted. However, there is also a group of phylogenetically unrelated species with a remarkable potential of environmental opportunism. The characteristic feature of strains from this "active minority" might be their ability to establish in soil and rhizosphere, which is the rare habitat for *Trichoderma* if the whole taxonomic diversity of the genus is considered. Interestingly, the soil isolates of *Trichoderma*, including the industrial cellulase producer *T. reesei*, are usually the common, cosmopolitan species with the broadest impact on humankind. To understand the biological and biochemical mechanisms underlying these characteristics of *Trichoderma* spp. and further enable transformational applications of these fungi and their products, we initiate the whole genus comparative genomic and transcriptomic analyses of at least 300 isolates from taxonomically defined clades, species and phylogenetic lone linages rePresenting the broad spectrum of infrageneric ecological versatility and biogeographic distribution. Here we will present the first results obtained from the analysis of 30 newly sequenced and 15 previously available *Trichoderma* genomes, including species with different ecology and biogeography. The analysis of intraspecific genomic polymorphism compared to interspecific differences will shed light on the evolution of *Trichoderma* species and clades and reveal the impact of sexual recombination and interfungal DNA exchanges (lateral gene transfer) on the architecture and functionality of fungal genomes.

Exploring genetic variation in *Clonostachys* to understand biological control mechanisms

Martin Broberg[1], Mukesh Dubey[1], Mudassir Iqbal[1], Maria Viketoft[2], Mikael Brandström Durling[1], Dan Funck Jensen[1], **Magnus Karlsson[1]**

[1] *Swedish University of Agricultural Sciences, Department of Forest Mycology and Plant Pathology*
[2] *Swedish University of Agricultural Sciences, Department of Ecology*

Large-scale analyses of genetic variation between species (phylogenomics) and within species (population genomics) can provide important clues to the mechanisms involved

in biocontrol interactions. A comparative genomic analysis of six different *Clonostachys* species identified several gene families evolving under selection for gene gains ($P \leq 0.05$) in the ancestral lineage leading to *Clonostachys*, including polyketide synthases (PKS), non-ribosomal peptide syntethases (NRPS) and different membrane transporters. A genome-wide association study (GWAS) approach was then conducted to investigate if these long-term genomic targets were also responsible for short-term, adaptive changes. Fifty-three whole-genome re-sequenced *Clonostachys rosea* strains were screened for their ability to control fusarium foot rot disease in wheat and for *in vitro* antagonism against plant-parasitic nematodes. Strains displayed a large variation in both traits with a normal distribution, indicating a polygenic inheritance. The GWAS identified 189 and 279 single nucleotide polymorphism (SNP) markers significantly (local false sign rate ≤ 1e-10) associated with the respective traits. One PKS gene (*pks10*) and two NRPS genes (*nps4* and *nps5*) were present in the genomic regions associated with foot rot control and nematicidal activity, respectively. Gene deletion strains of *pks10*, *nps4* and *nps5* were generated and displayed reduced ($P \leq 0.05$) *in vitro* antagonism against *Fusarium graminearum* and *Pratylenchys penetrans*, respectively. Gene deletion strains of *nps4* and *nps5* strains also showed reduced biocontrol efficacy in a naturally nematode infested soil in a wheat pot experiment. In summary, our comparative genomic and GWAS analyses identified PKS, NRPS and various membrane transporters as targets of selection that emphasize the role of biosynthesis of, and protection against, secondary metabolites for the evolution of the *Clonostachys* genus.

How root-colonizing endophytes promote plant performance and influence ecosystems

Ralf Oelmüller

Schleiden Institute, Biology, Jena, Thuringia, Germany

Root-colonizing endophytic fungi manipulate plant performance and participate in shaping ecosystems by connection the roots via hyphal networks. Based on studies with model organisms, I will describe molecular mechanisms of how beneficial symbioses are established. Benefits for the plants can only be achieved if the fungus-induced alterations in the roots are distributed within the entire plant body. There is increasing evidence that systemic distribution of information is an important feature of the endophyte-root symbioses. Finally, very little is known about the molecular mechanisms which transfer information from one plant to a neighboring plant via hyphal networks. I will discuss molecular tools which might shine light on the information propagation in an ecosystem. Understanding the mechanisms of endophyte-root symbioses provides important information for agricultural applications and influence of the microbes on the ecosystems.

Effect of plant species, pathogen, environmental factors and their interactions on *Trichoderma harzianum* strain INAT11

Edith Ladurner[1], Fabio Fiorentini[1], Massimo Benuzzi[1], Alessandro Raiola[2], Roberto Causin[2], Davide Ferrigo[2]

[1] *CBC (Europe) S.r.l. - BIOGARD Division, Grassobbio (BG), Italy)*
[2] *University of Padova - Dept. TESAF, Sect. Plant Pathology AGRIPOLIS, Legnaro (PD), Italy*

In preliminary studies conducted by University of Padova, *Trichoderma harzianum* strain INAT11 had shown promising activity against *Fusarium graminearum*, henceforth Fg, causing Fusarium Head Blight of Wheat and *Fusarium verticillioides*, henceforth Fv, responsible of and Pink Ear Rot of maize. Therefore, in vitro studies, studies on potted plants, and field studies were performed to investigate the effect of plant species (maize and wheat), pathogen (Fg and Fv) and environmental factors (temperature, pH, and water availability) on the survival, growth and root colonization ability of strain INAT11. The in vitro studies on buffered growth media showed that the effects of temperature, pH and water availability on strain INAT11 were similar to those on other *T. harzianum* strains. However, in the subsequent studies on potted plants and in the field studies, extremely complex interactions among plant species, soil pH, water availability, pathogens and strain INAT11 emerged. Root colonization resulted to be influenced by a combination of factors and not just one single factor. Furthermore, under open-field conditions, the negative effects of alkaline pH values on strain INAT11 were considerably less pronounced than those expected based on the in vitro studies. The impact of these findings on the development of the antagonist as a seed treatment for crop protection are discussed.

Present and future of *Trichoderma asperellum* and *Trichoderma gamsii* as biocontrol agents in the Isagro portfolio

Riccardo Liguori, Giovanni Venturini, Giulio Testa

Isagro SpA, Novara Research Center, via Fauser 28, 28100, Novara, Italy

A mixture composed by two different species of antagonistic *Trichoderma*, *T. asperellum* and *T. gamsii,* is marketed by Isagro among its biocontrol solutions under different trade names (Remedier®, Ecofox Life®, Radix Soil®, Blindar®). The product is a success of the cooperation between the agrochemical company Isagro and the Italian Universities, where the two *Trichoderma* strains have been isolated and tested for their ability in controlling fungal soilborne diseases. The combination of the two strains provides the commercial product with a better fitness and competitiveness in different environmental and soil conditions. The product gained the official registration and authorization for sale in the Italian market already in the year 2006. The activity spectrum includes several

plant pathogenic fungi *e.g*. *Armillaria, Pythium, Phytophthora, Rhizoctonia*, *Sclerotinia* and *Verticillium*. Recently, driven by the growing market need, it has been developed and registered also for the use as foliar spray to prevent the esca disease of grapevine in young vineyard plantations. The technical positioning of the *Trichoderma*-based product in grapes is the result of more than five years of laboratory and field studies carried out in collaboration between Isagro and research institutes all over Italy. Although present since many years on the market, *Trichoderma* spp. as biocontrol agents still show some open questions to be answered as well as many new opportunities. Formulation optimization and new uses, shelf-life and activation, crop enhancement, interaction with new target pathogens, regulatory compliance, are some of the tasks for the future research to guarantee the longest possible life of *Trichoderma* spp. strains in sustainable agriculture.

Trichoderma atroviride mycoparasitism and its regulation by the TOR signaling pathway

Rossana Segreto[1], Hoda Bazafkan[1], Julia Millinger[1], Maria Doppler[2], Christoph Blueschl[2], Rainer Schuhmacher[2], Lea Atanasova[1], **Susanne Zeilinger[1]***

[1] *University of Innsbruck, Department of Microbiology, Innsbruck, Austria*
[2] *University of Natural Resources and Life Sciences Vienna, Department of Agrobiotechnology, Center for Analytical Chemistry, Tulln an der Donau, Austria*

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 Δ*are1* mutants produced less biomass than the wild type on most of the 95 tested nitrogen sources, while Δ*npr1* and Δ*rhe2* 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.

Uncovering essential mechanisms of chitin and chitosan remodeling in the cell wall of the mycoparasite *Trichoderma atroviride*

Lisa Kappel[1,2], Sabine Gruber[1,2]

[1] *Institute of Microbiology, University of Innsbruck, Innsbruck, Austria,*
[2] *Department of Bioengineering, FH-Campus Wien, Vienna, Austria*

The ability to invade host organisms during fungal parasitism mandates adaptive remodeling of the cell wall to prevent host recognition and defense reactions. In particular, cell wall chitin has been identified as a promising target to combat fungal diseases. Strategies to escape the chitin-triggered and chitin-targeted immune system of the host are shared by all plant and human pathogens, but mechanisms in mycoparasites had not been investigated so far. Here we provide the first complete description of the enzymes involved in chitin and chitosan metabolism and their contribution to a dynamic cell wall architecture in *Trichoderma atroviride*. We show that they serve as protective components in self-defense reactions during the mycoparasitic attack and are therefore essential for biocontrol. We identified a set of more than 20 enzymes, which are involved in chitin and chitosan synthesis, and characterized their concerted interplay during the mycoparasitic attack and evasion of host defense mechanisms. Five out of eight chitin synthases, including two enzymes containing myosin motor head domains, are non-redundant, play critical roles in chitin biosynthesis during vegetative development and are indispensable for mycoparasitism. Intriguingly, six chitin deacetylases are differentially regulated in mycoparasitism, and the formation of cell wall chitosan appears to be required to scavenge reactive oxygen species from the host. We further provide evidence for the excessive production of six novel chitosanases during the mycoparasitic attack, which implies that the chitinous backbone of the host cell wall is one of the first targets of *T. atroviride*. Our results contribute significantly to understanding the molecular mechanism of chitin and chitosan in mycoparasites during biocontrol with the overarching goal to selectively exploit the discovered strategies.

Testing the role of the transcription factor TvSom1 in adhesion of *Trichoderma virens* germlings

Ariella Alperovitch-Lavy[1], Tri-Thuc Bui[2], Rebekka Harting[2], Gerhard H. Braus[2]

Benjamin A. Horwitz[1]

[1] *Faculty of Biology, Technion – Israel Institute of Technology, Israel and*
[2] *Department of Molecular Microbiology and Genetics, Institute of Microbiology and Genetics, University of Göttingen and Göttingen Center for Molecular Biosciences, Germany*

Trichoderma-root interactions prime the plant immune response, attenuating disease upon later challenge with a pathogen. Relatively little is known about the molecular details of this opportunistic fungal-plant symbiosis. Attachment of hyphae or germlings to the root, however, is likely to be a critical early step. Trichoderma may adhere to the plant host with adhesive molecules found on the hyphal surface. In the soilborne pathogen *Verticillium dahliae*, three transcription factors controlling the network underlying adhesion have been isolated by a yeast expression strategy [1]. TvSom1 is a candidate *T. virens* ortholog of one of these, Som1. The 2668 bp predicted coding region of TvSom1 in the *T. virens* database (Joint Genome Institute [2]) is interrupted by 4 introns and encodes a 795 amino acid protein. Alignment of TvSom1 with *V. dahliae* Som1 gives 63.9% identity and 82.4% similarity. The sequences of the LisH domain, nuclear localization signal (NLS) in the N-terminal half of the protein, and SSDP domain are well-conserved in the alignment, while the SnAPC domain is less so with disconnected regions of identity, and the NLS in the C-terminal region differs at 3 residues. TvSom1 is expressed at low levels in germinating conidia. Progress in expression of TvSom1 in yeast for functional complementation of adhesion and in the construction of deletion mutants in the fungus will be presented. Adhesion-related genes can eventually be targeted for both understanding of, and agriculturally-relevant manipulation of, the Trichoderma-root interaction. New insights can be obtained into biocontrol of fungal pathogens in the soil, and the trade-off between fungal-fungal and fungal-root interactions in the rhizosphere. This balance is agriculturally relevant.

1. Bui et al. (2018) New Phytol. 221(4):2138-2159.
2. Grigoriev et al. (2014) Nucleic Acids Res. 42(1):D699-704.

Enhancing peptaibols production in the biocontrol fungus *Trichoderma longibrachiatum* SMF2 by elimination of a putative glucose sensor

Yu-Zhong Zhang, Yan-Rong Zhou, Wei-Xin Zhang, Xiu-Lan Chen

Shandong University, State Key Laboratory of Microbial Technology, Qingdao, China
Corresponding author: zhangyz@sdu.edu.cn

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 increate 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.

Azaphilones biosynthesis in *Trichoderma harzianum* benefits fungal survival to oxidative stress

Guan Pang, Irina S. Druzhinina, Tingting Sun, Hong Zhu, **Jian Zhang**

Plants nutrition department, College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing, Jiangsu province, China

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.

Complete genome sequences reveals novel insights into chromosomal organization and evolution of different *Trichoderma* species

Ting-Fang Wang

Academia Sinica, Institute of Molecular Biology, Taipei, Taiwan

Single-molecule real-time (SMRT) sequencing developed by Pacific BioSciences (PacBio) offers three major advantages compared to second-generation sequencing: long read length and high consensus accuracy, and a low degree of bias. Together with high sequencing coverage, these advantages overcome the difficulty of sequencing genomic regions such as long AT-rich islands and repeated regions (e.g., ribosomal DNA) in the genomes of three Trichoderma reesei wild isolates, i.e., QM6a, CBS999.97(MAT1-1) and CBS999.97(MAT1-2). In this study, we report the high quality complete genomes sequence of two biocontrol Trichoderma species isolated from Taiwan. Comparative genomic analyses of these complete genomes have provided not only better understandings to their biological properties (e.g., genome-wide chromosome architectures and genome evolution) but also new potential applications.

Effects of *Trichoderma* strains and metabolites on the growth, disease resistance, leaf transcriptome and metabolome of olive plants

Roberta Marra[1], Mariangela Coppola[1], Angela Pironti[1], Nadia Lombardi[1], Giada d'Errico[1], Andrea Sicari[2], Sergio Bolletti Censi[2], Sheridan Lois Woo[3,4], Rosa Rao[1], Francesco Vinale[4,5]

[1] *University of Naples Federico II, Department of Agricultural Sciences, Portici, Naples, Italy*
[2] *Linfa S.c.a r.l., Napoli*
[3] *Department of Pharmacy, University of Naples Federico II, Naples, Italy*
[4] *National Research Council, Institute for Sustainable Plant Protection, Portici, Naples, Italy*
[5] *University of Naples Federico II, Department of Veterinary Medicine and Animal Productions, Naples, Italy*

Biocontrol fungi belonging to the genus *Trichoderma* may help plants to overcome various biotic stress and promote growth by different mechanisms of action, such as direct antagonism, competition, induction of plant resistance and/or production of secondary metabolites (SMs). In this work, we analyzed the effects of selected *Trichoderma* strains

or SMs on young olive (*Olea europaea* cv. Carolea) trees to stimulate plant development and resistance response to olive leaf spot disease caused by *Fusicladium oleagineum*. The number of naturally infected leaves was found to be significantly decreased (p<0.05) in treated plants (up to 60% reduction), compared to control. Moreover, transcriptomic and metabolomic analyses of olive leaves were carried out by real-time PCR and LC-MS Q-TOF, respectively, to compare the treatments at a molecular level. The induction of defense-related genes, as well as the activation of those involved in the synthesis of the phenolic compound oleuropein, indicated that gene expression varied significantly in treated plants according to the fungal strain or the metabolite applied. The phenolic composition of olive leaves was also affected by *Trichoderma* applications whereby a total of 104 phenolic compounds were detected and 20 of them were putatively identified. Targeted and untargeted approaches were used to analyze and discriminate the metabolites produced by the plant following the different microbial treatments. Statistical analyses revealed differences both in the number and in the type of phenolic compounds accumulated in olive leaves after *Trichoderma* applications. Some secoiridoids were less abundant in the treated plants in comparison to the untreated controls, whereas the accumulation of flavonoids (including luteolin and apigenin derivatives) increased following the application of different *Trichoderma* strain. This is the first work demonstrating the beneficial effects exerted by *Trichoderma* strains or SMs on olive plants.

Terpene synthases in *Trichoderma gamsii* T6085

I. Vicente[1,2,*], R. Baroncelli[2], M.E. Morán-Díez[2], M.E. González-Rey[3], I.G. Collado[3], R. Hermosa[2], E. Monte[2], G. Vannacci[1] and S. Sarrocco[1]

[1] *Department of Agriculture, Food and Environment, University of Pisa, Pisa (Italy)*
[2] *Department of Microbiology and Genetics, Spanish-Portuguese Institute for Agricultural Research, University of Salamanca, Villamayor (Spain)*
[3] *Department of Organic Chemistry, University of Cádiz, Puerto Real (Spain)*
** Email i.vicentemu@gmail.com*

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*.

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

Wolfgang Hinterdobler[1], Julia Scholda[1], Guofen Li[1], Stefan Böhmdorfer[2] and Monika Schmoll[1]

[1] *AIT-Austrian Institute of Technology GmbH, Center for Health & Bioresources, Bioresources, Tulln, Austria*
[2] *University of Natural Resources and Life Sciences (BOKU), Department of Chemistry, Tulln, Austria*

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.

Trichoderma applications affect the physiological processes that improve strawberry production and quality

Nadia Lombardi[1], Andrea Scaloni[2], Simonetta Caira[2], Anna Maria Salzano[2], Antonio Dario Troise[1], Paola Vitaglione[1], Sheridan Lois Woo[3,4,5]

[1] *University of Naples Federico II, Department of Agricultural Sciences, Portici, Naples, Italy*
[2] *National Research Council, Proteomics & Mass Spectrometry Laboratory, ISPAAM, Naples, Italy*
[3] *University of Naples Federico II, Department of Pharmacy, Naples, Italy*
[4] *University of Naples Federico II, Task Force on Microbiome Studies, Naples, Italy*
[5] *National Research Council, Institute for Sustainable Plant Protection, Portici, Naples, Italy*

Many selected *Trichoderma* strains are active ingredient of biological products for agriculture, due to their ability to protect plants to biotic and abiotic stresses, plus improve plant growth and quality. This study examined the effects of three *Trichoderma* strains (T22, TH1 and GV41) on the productivity and the quality of strawberry grown in pots. *Trichoderma* treatments improved plant growth, increased strawberry yield and favored the accumulation of anthocyanins and other antioxidants in the ripened fruits. Proteomic analysis of *Trichoderma* treated fruits ascertained 333 differentially represented proteins (DRPs) associated with various *Trichoderma* treatments. DRPs were mostly related to the functional category of: i) protein metabolism (components involved in protein biosynthesis, protein degradation and protein translocation); ii) stress response (components associated with redox homeostasis, external stimuli response and protein modification); iii) carbon and energy metabolism (enzymes related to carbohydrate metabolism, energy and photosynthesis); iv) vesicle trafficking; v) secondary metabolism (enzymes catalyzing biosynthesis/degradation of secondary metabolites and phytohormones). These results indicated that the beneficial effect of the *Trichoderma*-based products to the developing plant are also transferred to fruits, thus modulating different physiological processes that positively influence food quality and consumer health. This improvement in fresh fruit production is also associated to an

increase in crop yield obtained by using an agriculturally sustainable approach.

Valorization of by-products from oleaginous crops production using *Trichoderma* spp.

Laura Gioia[1], Stefania Lanzuise[1], Assunta Bottiglieri[1], Ernesto Comite[1], Andrea Sicari[2], Enrique Monte[3], Rosa Hermosa[3], Francesco Vinale[5,1], Matteo Lorito[1,5], Sheridan Lois Woo[4,5]

[1] *Department of Agricultural Sciences, University of Naples Federico II, Portici (NA), Italy*
[2] *Santa Rita srl, Naples, Italy*
[3] *Spanish-Portuguese Centre of Agricultural Research (CIALE), Departament of Microbiology and Genetics, University of Salamanca, Salamanca, Spain*
[4] *Department of Pharmacy, University of Naples Federico II, Naples, Italy*
[5] *Institute for Sustainable Plant Protection, National Research Council, Portici (NA), Italy*

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 $10^{\wedge 9}$ 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.

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

Mukesh Dubey, Martin Broberg, Dan Funck Jensen, and Magnus Karlsson

Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden. mukesh.dubey@slu.se

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.

Pseudomonas chlororaphis ToZa7 and *Clonostachys rosea* IK726, a successful biocontrol pair against Fusarium crown and root rot of tomato

Anastasia L. Lagopodi[1], Nathalie N. Kamou[1], Francisco Cazorla[2], and Giannis Kandylas[1]

[1] *Aristotle University of Thessaloniki, Laboratory of Plant Pathology, School of Agriculture, Greece*
[2] *University of Malaga, Department of Microbiology, Spain*

Pseudomonas chlororaphis ToZa7 is a biological control agent producing phenazine-

1-carboxamide, hydrogen cyanide, proteases and siderophores (Kamou et al., 2015). *Clonostachys rosea* IK726 has biocontrol ability, against several plant pathogens (Knudsen et al. 1995; Jensen et al. 2000). Both strains reduce tomato foot and root rot severity caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*, while *C. rosea* IK726 colonizes tomato roots during such interaction (Kamou et al., 2015; Karlson et al., 2015). As previously reported, the combination of the two biological control agents although proved deleterious for *C. rosea* IK726, *in vitro*, it was successful in reducing tomato foot and root rot severity, *in planta* (Kamou et al., 2016). Induction of defense responses in tomato after individual or combined application of ToZa7 and IK726, challenged with the pathogen, was studied through the expression patterns of the defense related genes *PR-1a*, *GLUA* and *CHI3*, using qRT-PCR. Expression of *PR1-a* was noteworthy 48 h after challenge inoculation, when IK726 alone or in combination with ToZa7 was pre-inoculated on tomato roots (38.53 and 53.74-fold, respectively), while 72 h after challenge inoculation, it was the highest in ToZa7. Expression of *CHI3* was much lower while upregulation of *GLUA* was overall not observed. However, after individual application of *P. chororaphis* ToZa7, for 120 hours *PR-1a* and *GLUA* were up-regulated (15.22 and 13.11-fold, respectively), as compared to the untreated control, without challenge inoculation by the pathogen. Confocal laser scanning microscopy of intact tomato roots and bacterial counts of disinfected roots revealed that *P. chororaphis* ToZa7 colonizes the exterior and the internal tissues.

Jensen et al. 2000. Eur J Plant Pathol 106: 233–242

Kamou et. al., 2015. Bioc Sci Tech 25: 928–949

Kamou et. al., 2016. Arch Microbiol 198: 369-377

Karlson et al., 2015. Genome Biol Evol 7(2):465–480

Knudsen et al., 1995. Plant Pathol 44: 467–477

ECFG15
ROME • ITALY 2020

SATELLITE WORKSHOP

Colletotrichum 2020

MONDAY, FEBRUARY 17

Location: **Sapienza University of Rome**
Building: **CU022** | Side: **Botanica** | Floor: **Ground** | Room: **C**

ORGANIZERS: **Riccardo Baroncelli,** Universidad de Salamanca & **Serenella Sukno,** Universidad de Salamanca

09:00 - 09:15	**Arrival of participants and Welcome**
09:15 - 09:40	**Michael Thon** | University of Salamanca **Horizontal gene transfer contributes to virulence in *Colletotrichum***
09:40 - 10:05	**Pamela Gan** | Riken **Genome rearrangements drive evolution of virulence-related genes in the genomes of *Colletotrichum gloeosporioides* species complex**
10:05 - 10:45	**Vladimiro Guarnaccia** | University of Torino ***Colletotrichum* species diversity on aromatic and ornamental plant hosts in Italy**
10:45 - 11:15	**Coffee Break**
11:15 - 11:40	**Noam Alkan** | The Volcani Center **Glycosylated flavonoids - fruit hidden arsenal against fungal pathogens**
11:40 - 12:05	**Lars Voll** | Philipps-University Marburg **Reactive Oxygen Species dosage in Arabidopsis chloroplasts improves resistance towards *Colletotrichum higginsianum* in a WRKY33-dependent fashion**
12:05 - 12:45	**Bastien Bissaro** | Aix-Marseille Université **Investigating the role of a fungal oxidase-peroxidase tandem in plant pathogenicity**
12:45 - 14:00	**Lunch Break**
14:00 - 14:25	**Carmit Ziv** | The Volcani Center **The effect of fruit sugar level on the pathogenicity mechanism and host response during *Colletotrichum* infection of red tomatoes**
14:25 - 14:50	**Gaetan Le Floch** | University of Brest **Infectious process and intraspecific diversity of *Colletotrichum lupini*, a fungal pathogen responsible for lupin anthracnose**

14:50 - 15:15	**Pedro Talhinhas** | Universidade de Lisboa **The olive anthracnose pathosystem as a case-study for fungal taxonomy, epidemiology and host-pathogen interactions towards sustainable disease resistance**
15:15 - 15:50	**Eduardo Goulin** | Instituto Federal de Educação, Ciência e Tecnologia de Santa Catarina ***Colletotrichum* and *Citrus*, the Postbloom fruit drop studies advances**
15:50 - 16:15	**Coffee Break**
16:15 - 16:35	**Peter Plaumann** | Friedrich-Alexander-Universität Erlangen-Nürnberg **To have or not to have: A dispensable chromosome enables host colonization in the pathosystem *Colletotrichum higginsianum* – Arabidopsis thaliana**
16:35 - 16:55	**Thaís Regina Boufleur** | University of São Paulo ***Colletotrichum truncatum* effector repertoire revealed by comparative genomics and transcriptomics analyses**
16:55 - 17:15	**Joris Alkemade** | Research Institute of Organic Agriculture (FiBL) **Genetic diversity within *Colletotrichum lupini*, the causal agent of lupin anthracnose, and its virulence on white lupin (Lupinus albus)**
17:15 - 17:30	**Closure**

Horizontal gene transfer contributes to virulence in *Colletotrichum*

Michael R. Thon[1,*], José M. Sanz-Martín[1], Riccardo Baroncelli[1] and Serenella A. Sukno[1]

[1] *Universidad de Salamanca, Instituto Hispano-Luso de Investigaciones Agrarias (CIALE), Villamayor, Salamanca, Spain*
** Presenting author: mthon@usal.es*

Horizontal gene transfer (HGT) is the stable transmission of genetic material between organisms by means other than vertical inheritance. HGT has an important role in the evolution of prokaryotes but is relatively rare in eukaryotes. We studied the importance of HGT in plant pathogenic fungi by identifying horizontally transferred genes in the genomes of members of the genus *Colletotrichum*. We identified eleven HGT events from bacteria and one from plants to *Colletotrichum* spp. or their ancestors. The horizontally transferred genes from bacteria encode proteins involved in amino acid, lipid and sugar metabolism as well as lytic enzymes. Four of the eleven genes have homology to known virulence factors, suggesting that HGT may be important for niche adaptation and virulence. The putative minimal dates of the HGT events were calculated using a time calibrated phylogenetic tree, revealing a constant flux of genes from bacteria to fungi throughout the evolution of subphylum Pezizomycotina. HGT appears to be a constant, albeit rare phenomenon in the Pezizomycotina, occurring at a steady rate during their evolution. The gene acquired from plants encodes a protease which we call CPLS (*Colletotrichum* plant-like serine protease). Pathogenicity assays of CPLS null mutants show that CPLS is expressed at the early stages of pathogenesis and has a role in virulence. Transcriptional profiling shows that several independent defense mechanisms are suppressed in the presence of CPLS including NLR receptors, phenylpropanoid and flavonoid biosynthetic pathways. Thus, CPLS contributes to virulence by modulating host defense responses to promote plant susceptibility. Our results suggest that HGT is an important evolutionary process in fungi that contributes to the evolution of plant pathogens.

Genome rearrangements drive evolution of virulence-related genes in the genomes of *Colletotrichum gloeosporioides* species complex

Pamela Gan[1,*], Ryoko Hiroyama[1], Ayako Tsushima[1,2], Sachiko Masuda[1], Naoyoshi Kumakura[1], Trinh Xuan Hoat[3], Takeshi Suzuki[4], Mari Narusaka[5], Yoshihiro Narusaka[5], Yoshitaka Takano[6] and Ken Shirasu[1,2]

[1] *RIKEN Center for Sustainable Resource Sciences, Yokohama, Kanagawa, Japan*
[2] *Graduate School of Science, The University of Tokyo, Bunkyo, Tokyo, Japan*
[3] *Plant Protection Research Institute, Ha Noi city, Viet Nam*
[4] *Chiba Prefectural Agriculture and Forestry Research Center, Chiba, Japan*
[5] *Research Institute for Biological Sciences, Okayama Prefectural Technology Center for Agriculture, Forestry, and Fisheries, Okayama, Japan*
[6] *Graduate School of Agriculture, Kyoto University, Kyoto, Japan*

* Presenting author: pamela.gan@psc.riken.jp

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.

Colletotrichum species diversity on aromatic and ornamental plant hosts in Italy.

Vladimiro Guarnaccia[1,2,*], Giovanna Gilardi[2], Ilaria Martino[2], Maria Lodovica Gullino[1,2] and Angelo Garibaldi[1,2]

[1] *DiSAFA, University of Torino, Largo Paolo Braccini, 2, 10095 Grugliasco, TO, Italy;*
[2] *Center of Competence Agroinnova, University of Torino, Largo Paolo Braccini, 2, 10095, Grugliasco, Italy.*
* *Presenting author: vladimiro.guarnaccia@unito.it*

Species of *Colletotrichum* are considered important plant pathogens, saprobes, and endophytes on a wide range of plant hosts. In Italy, several *Colletotrichum* species have been reported in open field and greenhouse environment. In this study, the occurrence, diversity and pathogenicity of *Colletotrichum* spp. associated with aromatic belonging to the family Lamiaceae and several ornamental plants were explored. Surveys were carried out during 2011-2019 in Northern Italy. A total of 45 *Colletotrichum* strains were isolated from symptomatic leaves and twigs of 14 host species. Two multi-locus phylogenies were established based on four genomic loci (ITS, *GAPDH*, *ACT* and *TUB2*). Preliminary pathogenicity tests were performed with representative isolates. *Colletotrichum* strains were identified as members of four major species complexes: *C. acutatum*, *C. dematium*, *C. destructivum* and *C. gloeosporioides*. Totally, ten *Colletotrichum* species were found in association with leaf or twig lesions on the investigated hosts. The pathogenicity of one representative isolate for each combination *Colletotrichum* species/host was tested on seedlings or rooted cuttings kept in a growth chamber. All the tested strains were pathogenic and reproduced symptoms identical to those observed in natural conditions. The present study improves our understanding of *Colletotrichum* species associated with several hosts largely cultivated in Italy, and provides useful information for effective disease management.

Glycosylated flavonoids - fruit hidden arsenal against fungal pathogens

Kumar S Pradeep [1], Oleg Feygenberg[1], Dalia Maurer[1] and **Noam Alkan[1,*]**

[1] *Department of Postharvest Science, Agricultural Research Organization, the Volcani Center, Israel*
** Presenting author: noamal@agri.gov.il*

Fruit defense against pathogens relies on induced and preformed mechanisms. In this work, we evaluated performed resistance of red and green mango fruit against the fungal pathogen *Colletotrichum gloeosporioides* and identified the main active antifungal components. HPLC analysis of non-hydrolyzed mango peel extracts identified major glycosylated-anthocyanin and glycosylated-flavonol, which were more abundant on the 'red side' of red mango fruit. Organic extracts of red mango peel were more efficient in inhibiting *C. gloeosporioides* than green mango peel. *Transcriptome analysis of the mango – C. gloeosporioides interaction showed increased expression of* glucosidase *genes related to both fungal pathogenicity and host defense.* Glucosidase treatment on organic peel extract increased its antifungal activity. Additionally, quercetin and *cyanidin had* significantly higher antifungal activity than their glycosylated derivatives. *The* volatiles of peel extract treated with glucosidase had antifungal activity and included 15 volatiles, 7 of them present only in red fruit. *These results suggest that the fruit obtains a concealed arsenal of glycosylated flavonoids in its peel when they are hydrolyzed by* β-glucosidase *that is induced by both fungus and host during infection process, they become more toxic to the fungal pathogen, inhibiting decay development.*

Deficiencies in the mitochondrial electron transport chain affect redox poise and resistance towards *Colletotrichum higginsianum*

Christopher McCollum[1], Sonja Geißelsöder[1], Timo Engelsdorf[2], Anna-Maria Voitsik[1] and **Lars Voll[2,*]**

[1] *Division of Biochemistry, Department Biology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany*
[2] *Molecular Plant Physiology, Department of Biology, Philipps-University Marburg, Marburg, Germany*
[*] *Presenting author: lars.voll@biologie.uni-marburg.de*

To investigate if and how the integrity of the mitochondrial electron transport chain (mETC) influences susceptibility of Arabidopsis towards *C. higginsianum*, we have selected previously characterized mutants with defects at different stages of the mETC, namely the complex I mutant ndufs4, the complex II mutant sdh2-1, the complex III mutant ucr8-1 and a mutant of the uncoupling protein, ucp1-2. Relative to wild type, the selected complex I, II and III mutants showed decreased total respiration, increased alternative respiration as well as increased redox charge of the NADP(H) pool and decreased redox charge of the NAD(H) pool in the dark. In the light, mETC mutants accumulated free amino acids, albeit to varying degrees. Glycine and serine, which are involved in carbon recycling from photorespiration, and N-rich amino acids were predominantly increased in mETC mutants compared to the wild type. Taking together the physiological phenotypes

of all examined mutants, our results suggest a connection between the limitation in the re-oxidation of reducing equivalents in the mitochondrial matrix and the induction of nitrate assimilation into free amino acids in the cytosol, which seems to be engaged as an additional sink for reducing power. Taking metabolite profiling data from all investigated mETC mutants, a strong positive correlation of resistance towards *C. higginsianum* with NADPH pool size, pyruvate contents and other metabolites associated with redox poise and energy charge was evident, which fosters the hypothesis that limitations in the mETC can support resistance at post-penetration stages by improving the availability of metabolic power.

Investigating the role of a fungal oxidase-peroxidase tandem in plant pathogenicity

Bastien Bissaro[1,*], Sayo Kodama[2], Mireille Haon[1], David Ribeaucourt[1,3], Michael Lafond[3], Harry Brumer[4], Richard O'Connell[5], Yasuyuki Kubo[2] and Jean-Guy Berrin[1]

[1] *INRA, Aix-Marseille Université, UMR Biodiversité et Biotechnologie Fongiques, Polytech'Marseille, Avenue de Luminy, Marseille, France*
[2] *Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto, Japan*
[3] *Aix Marseille Univ., CNRS, Centrale Marseille, iSm2, Marseille, France*
[4] *Michael Smith Laboratories, University of British Columbia, East Mall, Vancouver, Canada*
[5] *INRA, UMR BIOGER, AgroParisTech, Université Paris-Saclay, Thiverval-Grignon, France*
* *Presenting author: bastien.bissaro@inra.fr*

New members of the Copper-Radical Oxidases (CROs), from the Auxiliary Activity family 5_2 of the Carbohydrate-Active enZymes classification (CAZy – AA5_2, www.cazy.org), were recently found to catalyze the oxidation of aliphatic and aromatic alcohols to their corresponding aldehydes (Yin et al. 2015). Strikingly, these enzymes (named "AlcOx") are almost exclusively found in phytopathogenic ascomycete fungi and their biological function remains unknown. To tackle this question, using biochemical and biological approaches, we investigated the function of the AlcOx from different *Colletotrichum* species. At the transcriptomic level, analyses of *C. orbiculare, C. fructicola and C. graminicola* during infection revealed co-regulation of a pair of adjacent genes coding for an AlcOx and a putative peroxidase, only during the *appressorium* phase. At the enzyme level, work done on other CROs families point at an activating role of peroxidases on AlcOx, *via* a mechanism that remains to be elucidated. Recombinant production of AlcOx and peroxidases from *Colletotrichum* species is in progress to biochemically characterize their substrate preference and degree of synergism *in vitro*. At the biological level, the function of the AlcOx and peroxidase from the cucumber anthracnose fungus *C. orbiculare* is currently investigated *in vivo*. Preliminary results show that the pathogenicity of knock-out mutants decreased compared to the wild type strain, suggesting a role of the AlcOx and the peroxidase in the cucumber infection process by *C. orbiculare*.

Yin, D., Urresti, S., Lafond, M., Johnston, E.M., Derikvand, F., Ciano, L., Berrin, J.-G., Henrissat, B., Walton, P.H., Davies, G.J., Brumer, H., 2015. Structure–function characterization

reveals new catalytic diversity in the galactose oxidase and glyoxal oxidase family. *Nature Communications* 6. https://doi.org/10.1038/ncomms10197

The effect of fruit sugar level on the pathogenicity mechanism and host response during *Colletotrichum* infection of red tomatoes

Carmit Ziv[1,*], Dilip Kumar[1], Arthur A. Schaffer[2] and Dov Prusky[1]

[1] *Department of Postharvest Science, Agricultural Research Organization, the Volcani Center, Israel*
[2] *Plant Sciences, Vegetable and Field Crops, Agricultural Research Organization, the Volcani Center, Israel*
[*] *Presenting author: carmit.ziv@volcani.agri.gov.il*

The destructive phytopathogen *Colletotrichum* causes postharvest losses and secrete ammonia, as a mechanism to modulate environmental pH and regulate gene expression that contribute to differential pathogenicity. Previous observations indicated that the differential pH modulation is strongly dependent on carbon availability causing acidification or alkalization of the host tissue in the presence of carbon excess or its limitation, respectively. The natural increase in total soluble solids (TSS) in ripening fruits suggested a dynamic process of pH modulation occurring in harvested fruit. In the present work the response of two tomato lines having similar genetic background, but differential level of TSS content, were compared. *C. gloeosporioides* showed enhanced colonization of the LowSC (Sugar Content) line compared to the colonization of the HighSC. During enhanced colonization of LowSC tomato fruits, fungal transcription profile indicated activation of macromolecules metabolic processes and down regulation of carbohydrate metabolic process. Furthermore, the expression of glycosyl hydrolases, glucanases and several MFS transporters by *C. gloeosporioides* were observed. The host response of reduced colonized HighSC line by *C. gloeosporioides* was accompanied by an increase expression of glucosyltransferases with UDP-glucosyltransferase activity, which regulate the activity of compartmentalized fungitoxic secondary metabolites. These enzymes play important role in modulating fruit defense mechanism against pathogens. In addition, tomato sugar transporters were highly induced during pathogenic inoculation, suggesting a role for sugar uptake in the arms race between the fruit and the fungal pathogens. While the increased colonization include the activation of specific pathogenicity factors by the pathogen, the level of sugar present in the host may as well differentially modulate colonization patterns by activating specific fungus-pathogenic and host-response factors.

Infectious process and intraspecific diversity of *Colletotrichum lupini*, a fungal pathogen responsible for lupin anthracnose

Gaetan Le Floch[1,*], Guillaume Dubrulle[1], Flora Pensec[1], Adeline Picot[1], Patrice Nodet[1], Stephanie Madec[1] and Riccardo Baroncelli[2]

[1] *University of Brest, Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, ESIAB,*

F-29280 Plouzané, France.
[2] *Instituto Hispano-Luso de Investigaciones Agrarias (CIALE), University of Salamanca, Calle 10 del Duero, 12; 37185 Villamayor (Salamanca), Spain*
[*] *Presenting author: gaetan.lefloch@univ-brest.fr*

Lupin anthracnose is a major threat caused by the filamentous fungus *Colletotrichum lupini*. The genus *Colletotrichum* includes a wide number of phytopathogen species distributed worldwide. They were classified into 14 species complexes, with *C. lupini* belonging to the *C. acutatum* species complex. To date, studies performed on *C. lupini*, aimed at better understanding its phylogenetic classification and infectious lifecycle. But additional studies seemed necessary to increase our knowledge on (i) intraspecific diversity within the *C. lupini* species and (ii) the molecular determinants of pathogenicity. To address the first objective, a study was performed on strains from official collections and from isolates recently collected in the west of France. A low intra-specific phylogenetic diversity was found compared to the phenotypic diversity, notably characterized by contrasted aggressiveness between strains. The second objective was evaluated by determining fungal gene expression and protein synthesis during lupin infection by *C. lupini* using, respectively, a RNAseq-based transcriptomic approach and a mass spectrometry-based proteomic approach with a nLC Q-exactive Orbitrap. Taken together, our results highlighted that the dynamics of symptoms, gene expression and protein synthesis shared similarities to those of hemibiotrophic pathogens. In addition, few genes of unknwown or poorly-described functions were found to be specifically associated to the early or late stages of infection, suggesting that they may be of importance for pathogenicity. Functional validation will be discussed to confirm their role in promoting *C. lupini* pathogenicity.

The olive anthracnose pathosystem as a case-study for fungal taxonomy, epidemiology and host-pathogen interactions towards sustainable disease resistance

Pedro Talhinhas[1,*], Andreia Loureiro[1] and Helena Oliveira[1]

[1] *LEAF, Instituto Superior de Agronomia, Universidade de Lisboa. Tapada da Ajuda, 1349-017 Lisboa, Portugal*
[*] *Presenting author: ptalhinhas@isa.ulisboa.pt*

Colletotrichum fungi are interesting models to study fungal biology. However, many of the economically-relevant diseases caused by *Colletotrichum* occur on perennial hosts, often with limited availability of target organs due to specificities of the host life cycle. Far from easy or fast, nevertheless such pathosystems are real-life cases that require attention and may provide important information. The olive anthracnose pathosystem is such an example. Olive anthracnose shows up on olive fruits as they mature, causing fruit drop (yield loss) or deteriorated oil, with the pathogen persisting mostly asymptomatically on leaves and branches until fruits mature in the next autumn. Dissecting the olive-*Colletotrichum* interaction using histopathology and epidemiology tools has enabled a more detailed characterization of this pathosystem, prompting crop protection

strategies. Additionally, olive anthracnose pathogens are diverse, with least 12 genetically distinct fungal populations causing olive anthracnose, and have in part contributed to the current taxonomic framework of the the acutatum species complex, where most of them cluster. While *Colletotrichum nymphaeae* and *C. acutatum* s.s. are the most virulent, the occurrence of interaction in virulence levels between *Colletotrichum* spp. and olive cultivars have been identified, prompting the need for further selection studies. Species of *Colletotrichum* causing olive anthracnose vary according to geography. Recent data suggests that *C. acutatum* s.s. may be replacing the less virulent *C. godetiae* in the central Mediterranean area, thus rePresenting a regional scale epidemiology case study. Olive production, harvest and processing systems are experiencing profound changes and stricter rules concerning pesticide use are likely to have a strong impact on control strategies. A detailed knowledge of pathogen diversity, population dynamics and host-pathogen interactions is thus basal for the deployment of durable and effective disease control strategies.

Colletotrichum and *Citrus*, the Postbloom fruit drop studies advances.

Eduardo H. Goulin[1,2,*] and Marcos A. Machado[2]

[1] *Instituto Federal de Educação, Ciência e Tecnologia de Santa Catarina, Canoinhas, Santa Catarina, Brazil*
[2] *Centro de Citricultura "Sylvio Moreira" (CCSM), Instituto Agronômico (IAC), Cordeirópolis, São Paulo, Brazil*
[*] *Presenting author: eduardo.goulin@gmail.com*

Since ancient times citrus is an economically important culture for countries worldwide. Phytopathogens are responsible for significant losses in citrus production and fruit quality. Postbloom fruit drop (PFD) is one of the fungal diseases that can attack the citrus crop. The disease is characterized by lesions on the petals that can extend to young fruits, inducing their early drop. One of the causal agents of this disease is *Colletotrichum abscissum*, which belongs to the *Colletotrichum acutatum* species complex. During the past years, there were several changes in the taxonomy of *Colletotrichum* species. And, one species that cause disease in citrus named *C. acutatum* was divided in 32 different species. Many of them have their genomes and transcriptome sequenced, which is an advantage for its studies. The control of PFD is based in chemical applications that can induce the resistance development over the time, besides other parallel damages caused by fungicides. New technologies are being applied every year to better understand the pathogens biology, it can contribute to plant diseases control as an alternative to chemical control, such as the RNA interference technology. Furthermore, the mechanisms to test the new control strategies became necessary. In this scenario, many advances were performed to address answers to those questions. We compared our isolates from PFD lesion in citrus with all type strains from *C. acutatum* complex (described by Damm), and all isolates from PFD that affect flowers of sweet orange, as well as our isolates in Brazil, belongs to the species of *C. acutatum* complex named *C. abscissum*. Irrespective of crops and fungicides used, development of fungicide-

resistant pathogen populations is among the most severe problems in agriculture. Therefore, we investigate the functionality of the RNAi machinery in *C. abscissum* and test genetically whether the chemically pre-defined fungal SDHi target may represent a promising target gene in host-induced gene silencing (HIGS) plants. The *C. abscissum* RNAi machinery was functionally proven by silencing of gene report. Then, the silencing of SDH subunits were induced and verified. the RNA interference is an important tool that can be exploited to post bloom fruit drop disease control and also the chemical fungicide target are still useful in the new technologies control strategies. We develop a reliable alternative *in vitro* system for symptom induction of *C. abscissum* infection in detached citrus flowers. Inoculated citrus flowers were placed on Petri dishes with a water-agar substrate and typical symptoms of PFD after 72 hours in BOD chambers. This is an undoubtedly effective large-scale screening system, especially because it is fast and reliable and requires less space than do greenhouses or open field experiments. These previous results conducted us to test and successfully silence the fungal genes by double-strand RNAi. And, more recently, HIGS plants were generated and will be tested for gene silencing efficiency against *C. abscissum*. In conclusion, is notable the advances with the PFD pathogen and promising control strategies would be available during the next years.

To have or not to have: A dispensable chromosome enables host colonization in the pathosystem *Colletotrichum higginsianum* - *Arabidopsis thaliana*

Peter-Louis Plaumann[1,*], Evelina Satzinger[1], Marlis Dahl[1] and Christian Koch[1]

[1] *Division of Biochemistry, Department of Biology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany*
** Presenting author: peter-louis.plaumann@fau.de*

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.

Colletotrichum truncatum effector repertoire revealed by comparative genomics and transcriptomics analyses

Thaís R. Boufleur[1], Nelson Sidnei Massola Júnior[1], Flávia Rogério[1], Maísa Ciampi-Guillardi[1], Serenela A. Sukno[2], Michael R. Thon[2], Riccardo Baroncelli[2]

[1] *Department of Plant Pathology and Nematology, University of São Paulo, Piracicaba, SP, Brazil; and*
[2] *Universidad de Salamanca, Instituto Hispano-Luso de Investigaciones Agrarias (CIALE), Villamayor, Salamanca, Spain.*

** Presenting author: thaisboufleur@usp.br*

Soybean anthracnose is mainly associated with the hemibiotrophic fungus *Colletotrichum truncatum*, but recently several new species of *Colletotrichum* have been reported in commercial soybean fields. In order to better understand the adaptation of anthracnose causing *Colletotrichum* spp to soybean we sequenced and analyzed the genome of 4 representative isolates belonging to the pathogenic species *C. truncatum, C. plurivorum, C. sojae* and *C. musicola*. The genomes were assembled, and gene prediction and annotation were performed to identify and characterize protein encoding genes. The proteomes and secretomes of the newly sequenced genomes along with those of an additional 8 species representing the diversity of the genus and not pathogenic to soybean were classified into protein families using a variety of bioinformatic approaches. Comparative genome analyses of small secreted proteins identified complex-specific and species-specific proteins but did not identify proteins that are shared only between the 4 soybean-pathogenic species. Identified lineage specific effector protein candidates (LSECs) where further characterized by the predicted localization of the mature peptides. A transcriptomic analysis was carried out at different timepoints of infection to investigate the expression profiles of *C. truncatum* genes in planta. Based on these analyses, four species specific effector protein candidates (SSECs) were identified that are predicted to be secreted, targeting the nucleus overexpressed in planta at 12 hours post inoculation (early-penetration stage). These specific extracellular proteins may be effectors, proteins that have important roles in modulating the plant's immune system and in host specificity. These results represent a new resource that will be useful for further research into the biology and evolution of these key pathogens and in the management of soybean anthracnose.

Genetic diversity within *Colletotrichum lupini,* the causal agent of lupin

anthracnose, and its virulence on white lupin (*Lupinus albus*)

Joris Alkemade[1,2,*], Monika M. Messmer[1], Ralf T. Voegele[2], Maria R. Finckh[3], Peter Hohmann[1]

[1] *Department of Crop Sciences, Research Institute of Organic Agriculture (FiBL), Switzerland*
[2] *Institute of Phytomedicine, University of Hohenheim, Germany*
[3] *Department of Ecological Plant Protection, Kassel University, Germany*
[*] *Presenting author: joris.alkemade@fibl.org*

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.

Arnoldi, A., Boschin, G., Zanoni, C. & Lammi, C. 2015. The health benefits of sweet lupin seed flours and isolated proteins. *Journal of Functional Foods,* 18**,** 550-563.

Damm, U., Cannon, P. F., Woudenberg, J. H. C. & Crous, P. W. 2012. The Colletotrichum acutatum species complex. *Studies in Mycology,* 73**,** 37 - 113.

Dubrulle, G., Pensec, F., Picot, A., Rigalma, K., Pawtowski, A., Nicolleau, S., Harzic, N., Nodet, P., Baroncelli, R. & Le Floch, G. 2019. Phylogenetic Diversity and Effect of Temperature on Pathogenicity of Colletotrichum lupini. *Plant Disease***,** 1-24.

Lambers, H., Clements, J. C. & Nelson, M. N. 2013. How a phosphorus-acquisition strategy based on carboxylate exudation powers the success and agronomic potential of lupines (Lupinus, Fabaceae). *American Journal of Botany,* 100**,** 263-288.

Lucas, M. M., Stoddard, F. L., Annicchiarico, P., Frias, J., Martinez-Villaluenga, C., Sussmann, D., Duranti, M., Seger, A., Zander, P. M. & Pueyo, J. J. 2015. The future of lupin as a protein crop in Europe. *Frontiers in Plant Science,* 6**,** 705-711.

Nirenberg, H. I., Feiler, U. & Hagedorn, G. 2002. Description of Colletotrichum lupini comb. nov. in modern terms. *Mycologia,* 94**,** 307-320.

Pecchia, S., Caggiano, B., Da Lio, D., Cafà, G., Le Floch, G. & Baroncelli, R. 2019. Molecular Detection of the Seed-Borne Pathogen Colletotrichum lupini Targeting the Hyper-Variable IGS Region of the Ribosomal Cluster. *Plants,* 8**,** 222-238.

Talhinhas, P., Baroncelli, R. & Le Floch, G. 2016. Anthracnose of lupins caused by Colletotrichum lupini: a recent disease and a successful worldwide pathogen. *Journal of Plant Pathology,* 98**,** 5-14.

ECFG15
ROME • ITALY 2020

SATELLITE WORKSHOP
Neurospora

MONDAY, FEBRUARY 17

Location: **Sapienza University of Rome**
Building: **CU022** | Side: **Botanica** | Floor: **Ground** | Room: **D**

09:00 - 09:05	**Welcome**
09:05 - 09:25	**Kwangwon Lee** | Rutgers University, Camden **Habitat specific clock variation and its consequence on reproductive fitness**
09:25 - 09:45	**Luis Larrondo** | Pontificia Universidad Catolica de Chile **Methylxanthines modulate the circadian period length independently of the action of phosphodiesterase**
09:45 - 10:05	**Ranjan Tamuli** | Indian Institute of Technology, Guwahati **Calcium signaling genes play a role in stress tolerance, thermotolerance, cellulose degradation, and circadian clock in *Neurospora crassa***
10:05 - 10:25	**Sara Cea-Sánchez** | University of Seville **Regulation of conidiation by the velvet complex in *Neurospora crassa***
10:25 - 10:45	**Inbal Herold** | Hebrew University of Jerusalem **GUL-1 mediates cell wall remodelling via the COT-1 pathway in *Neurospora crassa***
10:45 - 11:15	**Coffee Break**
11:15 - 11:35	**Stefanie Pöggeler** | University of Göttingen **The role of the STRIPAK complex in sexual development of *Sordaria macrospora***
11:35 - 11:55	**Anne Oostlander** | University of Braunschweig **SIP-1 is essential for germling fusion of *Neurospora crassa*, probably by mediating the initiation of cell-cell communication**
11:55 - 12:15	**Hamzeh Hammadeh** | University of Braunschweig **BRO1 is required for cell-cell fusion in *Neurospora crassa* and localizes to a specific subpopulation of vesicles**

12:15 - 12:35	**Areejit Samal** | Homi Bhabha National Institute, Chennai **A blueprint of the protein secretion machinery in *Neurospora crassa***
12:35 - 14:00	**Lunch Break**
14:00 - 14:20	**Philipp Benz** | Technical University of Munich **Crosstalk of cellulose and mannan signaling pathways during plant cell wall perception is inhibitive to cellulase expression**
14:20 - 14:40	**Scott Baker** | Pacific Northwest National Laboratory, Richland **Fast forward genetics to associate *Neurospora crassa* mutant phenotypes with genes**
14:40 - 15:00	**Eugene Gladyshev** | Institut Pasteur, Paris **Developing a *tetO*/TetR system in *Neurospora crassa***
15:00 - 15:20	**Ines Teichert** | University of Bochum **Utilizing a set of fungal deletion and fusion tagging Golden Gate vectors for analyzing the function of regulatory and developmental proteins**
15:20 - 15:40	**Scott Baker** | Pacific Northwest National Laboratory, Richland **Accessing cutting edge capability at the Environmental Molecular Sciences Laboratory**
15:40 - 16:15	**Coffee Break**
16:15 - 16:35	**Omar Harb** | FungiDB **FungiDB: integrating genomic data for pathogens and model organisms and providing advanced search capabilities and large-scale data analysis**
16:35 - 17:30	**General Discussion**

Habitat specific clock variation and its consequence on reproductive fitness

Kwangwon Lee

Rutgers University, Camden, NJ, USA

The circadian clock controls daily activities at the cellular and organismic level allowing an organism to anticipate incoming stresses and to utilize resources accordingly. The circadian clock has been attributed as a fitness trait in multiple organisms.However, the mechanism of how circadian clock variation influences organismal reproductive fitness is still not well understood. We found habitat-specific-clock-variation (HSCV) in *Neurospora discreta*, a species that is adapted to two different habitats, under or above tree bark. African (AF) *N. discrete* strains, whose habitat is above the tree bark, have higher fitness under a light/dark cycling condition relative to North American (NA) strains, whose habitat is under the tree bark in constant dark. However, NA strains gained fitness under their habitat of constant dark by losing their clock regulation of asexual development. Our further investigations have shown that the organismal reproductive fitness appear to be modulated by cellular ROS levels. Our results demonstrate a mechanism by which local adaptation involving circadian clock regulation influences reproductive fitness.

Methylxanthines modulate the circadian period length independently of the action of phosphodiesterase

Consuelo Olivares-Yañez, Loreto Salas, Pilar Alessandri, **Luis F. Larrondo**

iBIO, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile

In *Neurospora* the use of caffeine and others methylxanthines, inhibitors of phosphodiesterase (PDE), lead to an increase in circadian period length and in the cAMP levels. Using *in vivo* luminescence reporter systems, we evaluated the mechanism of action of these drugs and the role of cAMP signaling pathway in the modulation of circadian period. We observed that, as previously reported by race tube assays, all these drugs led to period lengthening. Remarkably, this phenotype was observed in all of the analyzed cAMP signaling pathway mutants, suggesting that these PDE inhibitors lead to circadian phenotypes through mechanisms different from the canonical PDE-cAMP-PKA signaling axis.

Funding: iBio and FONDECYT 1171151.

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

Ranjan Tamuli

Indian Institute of Technology, Guwahati, India

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*.

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

Sara Cea-Sánchez, Maria Corrochano Luque, Guilherme TP Brancini, Gabriel Gutiérrez, N. Louise Glass, David Cánovas and Luis María Corrochano Peláez

Department of Genetics, University of Seville, Spain

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 Δ*ve-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.

Supported by European funds (European Regional Development Fund) and the Spanish Ministerio de Ciencia, Innovación y Universidades (BIO2015-67148-R) to LMC and DC. The work of Guilherme TP Brancini at the University of Seville was supported by a BEPE short-term fellowship from FAPESP (2018/00355-7), Brazil.

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

I. Herold[1], D. Kowbel[2], D. L. Delgado-Álvarez[3], M. Garduño-Rosales[3], R. R. Mouriño-Pérez[3] and O. Yarden[1]

[1] *The Hebrew University of Jerusalem, Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, Rehovot, Israel*
[2] *University of California Berkeley, Plant & Microbial Biology, Berkeley, CA, USA*
[3] *CICESE (Centro de Investigación Científicay Educación Superior de Ensenada), Departamento de Microbiología, Ensenada, Mexico*

Impaired function of the NDR kinase COT-1 results in markedly thickened cell walls. This effect is partially suppressed by inactivation of *gul-1* (an 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. GUL-1 affects transcript abundance of at least 25 genes involved in cell-wall remodeling via the COT-1 pathway. 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. GUL-1 physically interacts with protein components of the translational machinery as well as with stress granule and P-body proteins. Taken together, we have demonstrated that GUL-1 is a bona fide RNA-binding protein involved in cell wall remodeling and stress response.

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

Stefanie Pöggeler, Sabrina Erb, Anika Gibron

Genetics of Eukaryotic Microorganisms, University of Göttingen, Germany

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.

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

Anne Oostlander, Marcel Schumann, Ulrike Brandt, and André Fleißner

Institute for Genetics, Technische Universität Braunschweig, Braunschweig, Germany

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.

BRO1 is required for cell-cell fusion in *Neurospora crassa* and localizes to a specific subpopulation of vesicles

Hamzeh H. Hammadeh, Marcel R. Schumann, Ulrike Brandt and André Fleißner

Institut für Genetik, Technische Universität Braunschweig, Braunschweig, Germany

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 recently identified BRO-1, the homolog of the mammalian ALG-2-interacting protein X (ALIX), as a new factor essential for 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,* BRO1 is essential. Subcellular localization and live cell imaging revealed that BRO1-GFP localizes to the cytoplasm and in vesicles in non-interacting germlings and in the mature hyphae. The co-localization of BRO1-GFP and different vesicles markers indicated that BRO1 marks a sub- population of vesicles, which are probably specifically involved in cell fusion. BRO1-GFP accumulates at the tips of the interacting germlings in a dynamic, oscillating manner, such that high signal intensity of BRO1-GFP in one tip correlates with low signal intensity at the tip of the fusion partner. Suppression of *bro1* 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 *bro1* 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 BRO1 fail to stably maintain the polarity center, which leads to the establishment of additional, new germ tubes.

We hypothesize that BRO1 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.

A blueprint of the protein secretion machinery in *Neurospora crassa*

R.P. Vivek-Ananth[1,#], Evanjalee Albert Arokyaraj[1,2,#], Shri Vishalini Rajaram[1,2], M. Karthikeyan[1] and **Areejit Samal**[1,2,*]

[1] *Computational Biology Group, The Institute of Mathematical Sciences (IMSc), Homi Bhabha National Institute (HBNI), Chennai, India*
[2] *Center for Biotechnology, Anna University, Guindy, Chennai, India*
[#] *Equal Contribution*

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 genome-scale 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.

Sugars „in-sight" – towards a new analyses of *Neurospora crassa*

Philipp Benz[1], Maria A. C. Horta[1], Nils Thieme[1], Gustavo Goldman[2]

[1] *TUM School of Life Sciences, Technical University of Munich, Germany*
[2] *Faculdade de Ciências Farmacêuticas, University of São Paulo, Brazil*

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 phospho-proteomics 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.

Fast forward genetics to associate *Neurospora crassa* mutant phenotypes with genes

Scott Baker

Pacific Northwest National Laboratory, Richland, WA, USA

We have sequenced the genomes of ~550 *Neurospora crassa* strains, the majority of which have mutant phenotypes which have not yet been associated with genes. We have identified candidate gene loci for "anonymous" genes in over 250 strains. Included among these strains are temperature sensitive, auxotrophic and morphological mutants. Analysis of these sequences is ongoing and collaboration is welcome.

Developing a *tetO*/TetR system in *Neurospora crassa*

Tinh-Suong Nguyen & **Eugene Gladyshev**

Group Fungal Epigenomics, Department of Mycology, Institut Pasteur, Paris, France

We describe the successful development of a regulatable *tetO*/TetR system in *Neurospora crassa*. The system includes (i) a codon-optimized gene expressing a chimeric TetR-GFP variant, and (ii) a standard full-length *tetO* array integrated, as a proof of principle, near the *his-3* gene. Interestingly, while the *tetO* array remains genetically stable in vegetatively growing cells, it triggers potent Repeat-Induced Point mutation (RIP) during the sexual phase. Both the RID-dependent and the DIM-5/DIM-2 dependent pathways of RIP become strongly activated. Importantly, this process occurs in the absence of TetR-GFP and thus reveals the innate ability of this widely used DNA construct to engage in recombination-independent homologous self-pairing.

Utilizing a set of fungal deletion and fusion tagging Golden Gate vectors for analyzing the function of regulatory and developmental proteins

Tim Dahlmann, Dominik Terfehr, Susanne Witfeld, Kordula Becker, **Ines Teichert**

Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, Germany

Functional analysis of gene products is mostly achieved by gene deletion and subsequent complementation, coupled to downstream analysis such as localization studies and identification of protein-protein interaction partners. Fast and easy generation of the corresponding plasmids is of high value. We previously generated deletion vectors by conventional restriction / ligation, In-Fusion, and yeast recombination cloning. However, all these systems have drawbacks. In restriction / ligation, two consecutive reactions are performed to integrate 5' and 3'-flanking regions, In-Fusion cloning is rather expensive, and yeast recombination is time-consuming and does not allow for repetitive sequences. This is of special importance when using the previously described FLP/*FRT* marker recycling system, which employs repetitive *FRT* sites for site-specific recombination. Here, we present a set of fungal deletion cassettes and fusion tagging constructs for Golden Gate cloning. This method utilizes type IIs restriction endonucleases that enable free choice of the cloning sites at the nucleotide scale. Further, Golden Gate cloning is performed in a one-step reaction overnight in a PCR cycler and thus very fast. We generated five deletion cassette donor vectors conveying resistance to three different antibiotics, thus making these vectors applicable for a wide variety of fungi. Two cassettes allow for later recycling of the marker through FLP/*FRT* recombination. Further, we present a set of Golden Gate vectors for translational fusions. The vectors enable C- and N-terminal tagging with EGFP, mRFP and the 3xFLAG-tag. As a proof of principle, we applied these vectors to functional gene analysis in the two ascomycetes *Penicillium chrysogenum* and *Sordaria macrospora*. Among others, we study genes from a comparative genomics approach of *P. chrysogenum* wild type and industrial strains as well as transcription factor target genes from *S. macrospora* and found that *S. macrospora idc3* is required for hyphal fusion and fruiting body development. Overall, our results imply our vector set as an efficient tool for robust plasmid cloning for diverse applications. Importantly, the vector set can easily be adapted to specific requirements of further fungal species.

Accessing cutting edge capability at the Environmental Molecular Sciences Laboratory

Scott Baker

Pacific Northwest National Laboratory, Richland, WA, USA

The Environmental Molecular Sciences Laboratory is a DOE National User Facility which provides access for researchers to a variety of capabilities that can accelerate

and amplify the impact for user science. Of particular relevance to the Neurospora research community are our integrated research platforms, Functional Omics, Molecular Bioimaging and Cellular Dynamics. Details of scientific instrumentation and expertise available to users and information on how to access the EMSL will be discussed.

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

Evelina Basenko[1], **Omar Harb[2]**, Achchuthan Shanmugasundram[1], Mark Caddick[1] and David Roos[2]

[1] *Institute of Integrative Biology, Functional and Comparative Genomics, University of Liverpool, Liverpool UK*
[2] *Department of Biology, The University of Pennsylvania, Philadelphia, Pennsylvania, USA*

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:

1. ***Browsing*** of genomes and gene pages in an encyclopedic manner to explore all available information and data.
2. ***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.).
3. ***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.
4. ***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.

FungiDB is supported in part by NIH HHSN272201400030C and 75N93019C00077 and the Wellcome Trust #WT108443MA and Wellcome Biomedical Resources #212929/Z/18/Z grants.

* Presented on behalf of the entire VEuPathDB team.

ECFG15
ROME • ITALY 2020

SATELLITE WORKSHOP

Magnafest 2020

MONDAY, FEBRUARY 17

Location: **Sapienza University of Rome**
Building: **CU022** | Side: **Botanica** | Floor: **Ground** | Room: **E**

09:00 - 09:30	**Arrival and Coffee Break** Introduction to meeting
09:30 - 09:55	**Evelina Basenko** | University of Liverpool **FungiDB: integrating genomic data for pathogens and model organisms and providing advanced search capabilities and large-scale data analysis**
09:55 - 10:20	**Bozeng Tang** | The Sainsbury Laboratory **Transcriptional Regulation of Effectors in the Rice Blast Fungus *Magnaporthe oryzae***
10:20 - 10:45	**Sri Bühring** | Institute of Biotechnology and Drug Research **Alternative splicing as an element of signal transduction in multi-step phosphorelay systems in fungi**
10:45 - 11:10	**Jongbum Jeon** | Seoul National University **Diversified modulation of transcriptome complexity by alternative splicing during rice- *Magnaporthe oryzae* interactions**
11:10 - 11:40	**Coffee Break**
11:40 - 12:05	**Chi-Yeol Kim** | Seoul National University **A rice/ Arabidopsis thaliana glycosyl hydrolase gene displays ambivalent immunity with diverse types of phytopathogens**
12:05 - 12:30	**Neftaly Cruz-Mireles** | The Sainsbury Laboratory **Understanding the Pmk1 MAP kinase signalling pathway during plant colonisation by the rice blast fungus *Magnaporthe oryzae***
12:30 - 13:30	**Lunch Break**
13:30 - 13:55	**Audra Mae Rogers** | Tokyo University of Science **Cellular Control of Proteostasis During Infection-Related Development by the Rice Blast Fungus *Magnaporthe oryzae***
13:55 - 14:20	**Katharina Bersching** | Institute of Biotechnology and Drug **Rapid adaptation of signalling networks in the fungal pathogen *Magnaporthe oryzae***

14:20 - 14:45	**Ciaran Griffin** | University of Plymouth **The Magnaporthe oryzae circadian clock functions in determining rice blast pathogenicity**
14:45 - 15:10	**Coffee Break**
15:10 - 15:40	**General Discussion and concluding comments**

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

Evelina Basenko[1], Omar Harb[2], Achchuthan Shanmugasundram[1], Mark Caddick[1] and David Roos[2]

[1] *Institute of Integrative Biology, Functional and Comparative Genomics, University of Liverpool, Liverpool UK*
[2] *Department of Biology, The University of Pennsylvania, Philadelphia, Pennsylvania, USA*

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:

1. ***Browsing*** of genomes and gene pages in an encyclopedic manner to explore all available information and data.
2. ***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.).
3. ***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.
4. ***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.

FungiDB is supported in part by NIH HHSN272201400030C and 75N93019C00077 and the Wellcome Trust #WT108443MA and Wellcome Biomedical Resources #212929/Z/18/Z grants.

* Presented on behalf of the entire VEuPathDB team.

Transcriptional Regulation of Effectors in the Rice Blast Fungus *Magnaporthe oryzae*

Bozeng Tang[1,2], Xia Yan[1], Darren Soanes[2], Lauren Lyder[1], Vincent Were[1], Michael Kershaw[2] and Nick Talbot[1]

[1] *The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, NR4 7UH, UK.*
[2] *School of Biosciences, University of Exeter, EX4 4QD, United Kingdom*

To cause rice blast disease, *Magnaporthe oryzae* secretes effectors to suppress plant immunity. Many effector genes are only expressed in plant tissue after invasive growth, and have very low expression in conidia and mycelium. To date, very little is known about how invasive-specific fungal effector gene expression is regulated. To investigate this mechanism, we have screened mutants showing constitutive expression of fluorescence-labelled effector gene fusions in conidia and mycelium. We then employed bioinformatics analysis and bulked segregant analysis to identify potential regulators of effector gene expression. We identified Rgs1 (Regulator of G-protein Signalling 1) as a regulator of effector gene expression that acts as a repressor of effector gene expression during the pre-penetration phase of development, prior to plant infection. Genetic complementation experiments and phenotyping suggest that we have identified a loss function allele of RGS1 that leads to de-repression of effector gene expression throughout the life cycle of the fungus.

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

Sri Bühring[1], Dr. Alexander Yemelin[1], Dr. Karsten Andresen[2], Michael Becker[1] and Dr. Stefan Jacob[1]

[1] *Institute of Biotechnology and Drug Research gGmbH [IBWF], Erwin-Schrödinger-Str. 56, D-67663 Kaiserslautern*
[2] *Johannes Gutenberg-University Mainz, Microbiology and Wine Research, Institute of Molecular Physiology, Johann-Joachim-Becherweg 15, D-55128 Mainz*

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.

Diversified modulation of transcriptome complexity by alternative splicing during rice-*Magnaporthe oryzae* interactions

Jongbum Jeon[1], Gobong Choi[1], Jaeho Ko[2], Gir-Won Lee[3], Sook-Young Park[4], Ki-Tae Kim[2], Hyunjun Lee[2], Seongbeom Kim[2], and Yong-Hwan Lee[1,2,5,6,7,8]

[1] *Interdisciplinary Program in Agricultural Genomics, Seoul National University, Seoul 08826, Korea*
[2] *Department of Agricultural Biotechnology*
[3] *Macrogen Inc., Seoul 08511, Korea*
[4] *Department of Plant Medicine, College of Life Science and Natural Resources, Sunchon National University, Suncheon 57922, Korea*
[5] *Center for Fungal Genetic Resources*
[6] *Plant Genomics and Breeding Institute*
[7] *Plant Immunity Research Center*
[8] *Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 08826, Korea.*

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.

Characterising the Pmk1 MAP Kinase signalling pathway using quantitative proteomic approaches in the rice blast fungus *Magnaporthe oryzae*

Frank L.H. Menke, Neftaly Cruz-Mireles, Miriam Oses-Ruiz, Lauren S. Ryder, Paul Derbyshire, Jan Sklenar and Nicholas J. Talbot

The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, Norwich, NR4 7UH, UK

The rice blast fungus *Magnaporthe oryzae* uses specialised dome-shaped infection structures called appressoria to penetrate host cells. The formation of an appressorium is initated when a germinating spore comes into direct contact with the hydrophobic surface of a host leaf. Mitogen activated protein kinases (MAPKs) are well known for having an important role in signalling pathways of pathogenic fungi. The Pmk1 MAPK signalling pathway is well characterised in *M. oryzae,* with early activation of Pmk1 acting as an important prerequisite for appressorium formation. How the presence of a suitable surface is perceived to activate the Pmk1 signaling cascade, and what the immediate Pmk1 targets are that are required for appressorium formation is largely unknown. To complement the molecular genetics approaches in identifying upstream and down stream components required to initiate appressorium formation, we have deployed a number of proteomics approaches, including quantitative phospho-proteomics and co-immunoprecipitation coupled with Liquid Chromatography-Mass Spectrometry. Pmk1 is also required for penetration peg formation and cell-to-cell movement *in planta*. To characterise its role during these stages of infecton-related-development, we are using a combination of chemical genetics and proteomic approaches to elucidate the role of Pmk1 signalling during plant infection. I will highlight the latest technological advances in proteomics, as well as pitfalls and challenges, to address these questions.

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

Neftaly Cruz-Mireles, Miriam Oses-Ruiz, Wasin Sakulkoo, Paul Derbyshire, Frank Menke and Nicholas J. Talbot

Nicholas J. Talbot Lab
The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, Norwich, NR4 7UH, UK. (1385/2000)

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 pmk1*^{AS} 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.

Ectopic recombination between solo-long terminal repeats triggered pathogenic changes and genome rearrangement in the rice blast fungus

Takayuki Arazoe[1], Shuichiro Murochi[1], Daiki Hiraoka[2], Kana Kiguchi[3], Toshiki Tanaka[3], Tetsushi Sakuma[4], Takashi Yamamoto[4], Tsutomu Arie[5], Shigeru Kuwata[3], Izumi Chuma[6], Shuichi Ohsato[3], Yukio Tosa[2] and Takashi Kamakura[1]

[1] *Tokyo University of Science, Japan*
[2] *Kobe University, Japan*
[3] *Meiji University, Japan*
[4] *Hiroshima University, Japan*
[5] *Tokyo University of Agriculture and Technology, Japan*
[6] *Obihiro University of Agriculture and Veterinary Medicine, Japan*

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*.

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

Katharina Bersching[1], Dr. Stefan Bohnert[1], Christiane Grünewald[2], Dr. Alexander Yemelin[1], Dr. Karsten Andresen[2] and Dr. Stefan Jacob[1]

[1] *Institute of Biotechnology and Drug research gGmbH [IBWF], Erwin-Schrödinger-Str. 56, D-67663 Kaiserslautern*
[2] *Johannes Gutenberg-University Mainz, Microbiology and Wine Research, Institute of Molecular Physiology, Johann-Joachim-Becherweg 15, D-55128 Mainz*

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*.

The *Magnaporthe oryzae* circadian clock functions in determining rice blast pathogenicity

Griffin C.[1], Foster A.J.[2], Soanes D.M.[3], Plessis A.[1], Hanley M.E.[1], Talbot N.J.[2], Littlejohn G.R.[1,4]

[1] *School of Biological and Marine Sciences, University of Plymouth, UK*
[2] *Sainsbury Laboratory, Norwich, UK*
[3] *School of Biosciences, College of Life and Environmental Sciences, University of Exeter, UK*
[4] *For correspondence, george.littlejohn@plymouth.ac.uk*

Magnaporthe oryzae strongly exhibits a diurnal conidiation phenotype, where in natural infections, spores are produced at night and distributed as fresh inoculum by wind and dewdrop in the early morning. This behavior is easily seen in the characteristic and rhythmic banding patterns of conidiation observed in *M. oryzae* cultures grown on complete medium agar plates in a light-dark photoperiodic cycle. *M. oryzae* cultures entrained in a 12h light - 12h dark photoperiod prior to transfer to constant, free-running, conditions maintain rhythmic conidiation for a number of days, which suggests the presence of a functional circadian clock in *M. oryzae*. Amino acid sequence homology between *M. oryzae* and *Neurospora crassa* has allowed us to identify and generate CRISPR-Cas9 mutants of several core clock genes and photoreceptors in the rice blast fungus.

We are using these mutants to test the role of entrainment and clock function in rice blast disease outcomes and so far have found a mutant in the *M. oryzae* core clock component wc2 to exhibit reduced pathogenicity. We have also identified a strong effect of time of inoculation, relative to dawn, in *M. oryzae* pathogenicity, with plants inoculated at dusk giving rise to fewer and smaller lesions than seen in dawn infections. Our results

to date raise several fundamental questions about the nature and importance of the circadian clock in rice blast disease, including; Why do dusk inoculations not give rise to normal lesions? Why does *Magnaporthe oryzae* maintain a circadian clock? Are there fitness costs associated with disruption of the *M. oryzae* circadian clock?

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

Audra Mae Rogers and Martin Egan

University of Arkansas, USA

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.

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

Momotaka Uchida[1], Kotetsu Kita[1], Ayaka Fujigasaki[1], Takahiro Konishi[1], Tsutomu Arie[2], Tohru Teraoka[2], Takayuki Arazoe[1], Takashi Kamakura[1]

[1] *Tokyo University of Science*
[2] *Tokyo University of Agriculture and Technology*

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 clustered 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.

A rice/ *Arabidopsis thaliana* glycosyl hydrolase gene displays ambivalent immunity with diverse types of phytopathogens

Chi-Yeol Kim[1], Ju-Young Park[1], Gobong Choi[2], Seongbeom Kim[1] and Yong-Hwan Lee[1,2,3,4,5*]

[1] *Department of Agricultural Biotechnology*
[2] *Interdisciplinary Program in Agricultural Genomics*
[3] *Center for Fungal Genetic Resources*
[4] *Plant Immunity Research Center*
[5] *Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Korea*

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.

Keywords *Arabidopsis thaliana,* rice, *Magnaporthe oryzae,* ambivalent immunity

ECFG15
ROME • ITALY
2020

DESIGNED BY
PINGO SCSI

FEBRUARY

17-20

2020

ROME

SAPIENZA
UNIVERSITY
OF ROME

www.ecfg15.org

info@ecfg15.org