Poster Category 4: Organismic Interactions

PR4.1

Co-cultivations of fungi: microscopic analysis and influence on protein production

Isabelle Benoit[1,2] Arman Vinck[1] Jerre van Veluw[1] Han A.B. Wösten[1] Ronald P. de Vries[2]
[1]*Utrecht University* [2]*CBS-KNAW*

During their natural life cycle most fungi encounter other microorganisms and live in mixed communities with complex interactions, such as symbiosis or competition. Industrial fermentations, on purpose or by accident, can also result in mixed cultures. Fungal co-cultivations have been previously described for the production of specific enzymes, however, little is known about the interactions between two species that are grown together. *A. niger* and *A. oryzae* are two of the most important industrial fungi worldwide and both have a long history of strain improvement to optimize enzyme and metabolite production.

Co-cultivation of these two Aspergilli with each other and with the ascomycete phytopathogen *Magnaporthe grisea*, and the basidiomycete white rot fungus *Phanerochaete chrysosporium*, has recently been described by our group (Hu et al, 2010). Total secreted protein, enzymatic activities related to plant biomass degradation and growth phenotype were analyzed from cultures on wheat bran demonstrating positive effects of the co-cultivation compared to the individual cultivations. In a follow-up study the morphology and mechanism of the interaction is addressed using microscopy and proteomics. Data from this study will be presented.

Reference
Hu *et al*. International Biodeterioration & Biodegradation 65 (2011)

PR4.2

A novel effector secreted by the anthracnose pathogen *Colletotrichum truncatum* is required for the transition from biotrophy to necrotrophy in fungal pathogens

Vijai Bhadauria[1] Sabine Banniza[1] Vandenberg Albert[1] Selvaraj Gopalan[2] Wei Yangdou[3]
1. Crop Development Centre, University of Saskatchewan, Saskatoon, SK Canada 2. Plant Biotechnology Institute, National Research Council of Canada, Saskatoon, SK Canada 3. Department of Biology, University of Saskatchewan, Saskatoon, SK Canada

The *in planta* transition from biotrophy to necrotrophy known as the biotrophy-necrotrophy switch (BNS) in hemibiotrophic fungal phytopathogens is critical in disease development. We herein report a novel effector gene *CtNU* from *Colletotrichum truncatum*, the causal agent of anthracnose on pulse crops that exclusively expresses precisely before the BNS and elicits severe hypersensitive response (HR)-like cell death in tobacco leaves transiently expressing the effector. Cell death triggered by CtNU requires its accumulation at the host cell plasma membrane, indicating that the effector may cause perturbation in cell surface dynamics. Overexpression of *CtNU* in *C. truncatum* and *Magnaporthe oryzae*, the rice blast pathogen resulted in incompatibility with host plants lentil and barley, respectively by causing a HR-like response during the biotrophic mode of fungal growth. These results provide compelling evidence that hemibiotrophic fungal phytopathogens deliver *CtNU* effectors to the host cell plasma membrane to promote pathogenesis by causing massive cell death during the *in planta* differentiation of necrotrophic hyphae from biotrophic hyphae.

PR4.3

Cryptococcus adeliensis the causative agent of stem canker of stone fruit trees

Behzad Borhani, Heshmatollah Rahimian, Valiollah Babaeizad, Mohammad Sayari
Faculty of Agronomic Sciences, Department of plant protection, SariAgricultural Sciences and Natural Resources University, Sari, Iran

In attempts made to isolate the previously recorded causative bacteria (Pseudomonas syringae pv. Syringae (PSS) or Xanthomonas arboricola pv. Pruni (Xap) from cankers on stone fruit trees in July 2011 in Khorasan province, yeast-like isolates were consistently recovered from the symptomatic branches. Dark brown to black sunken lesions, predominantly accompanied with exudation of gum turned to typical stem cankers following their expansion. The isolated yeast cell was circular and ca 6.5 µm in diameter and formed round, white to light-cream colored, mucoid colonies with entire margins on sucrose nutrient agar. The internal transcribed spacer (ITS) region of the rDNA of a representative isolate of the recovered yeast was amplified in PCR using primer pair ITS4 (5-TCCTCCGCTTATTGATATGC-3) and ITS5 (5-GGAAGTAAAAGTCGTAACAA-3) and the PCR product sequenced. The sequence was aligned and compared with the nucleotide sequences deposited in GenBank using CLUSTAL w software. The ITS-sequence(NCBI # JQ039907) showed 100% identity with those of Cryptococcus adeliensis isolates. Several recovered isolates of the yeast were inoculated on peach (Prunus persicae) and nectarine (P.persica var.nucipersica) budlings and typical cankers were produced on the inoculated branches. The respective strains were re-isolated from the inoculated plants. The isolates also produced a hypersensitive reaction on geranium (Pelargonium × hortorum). C. Adeliensis has been isolated from decaying algae in the Antarctica and from a patient suffering from meningitis. The stem canker caused by C. Adeliensis appears to be a newly emerged disease of stone fruit trees.

PR4.4

The ATF/CREP transcription factor Atf1 is essential for full virulence, deoxynivalenol production and stress tolerance in the plant pathogen *Fusarium graminearum*

Van Thuat Nguyen, Wilhelm Schäfer, Jörg Bormann
Biozentrum Klein-Flottbek, Universität Hamburg

In a previous study we characterized the stress-activated MAP-kinase FgOS-2 (*Saccharomyces cerevisiae* HOG1) as a central regulator in the life cycle of the cereal pathogen *Fusarium graminearum.* Grains infected with *F. graminearum* accumulate high amounts of mycotoxins, most prominent of which are deoxynivalenol (DON) and zearalenone (ZEA). We showed that FgOS-2 regulates DON- and ZEA-production to different extents depending on growth conditions. Here, we present data on the functional characterization of a putative downstream regulator, the ATF/CREP transcription factor Fgatf1. Like FgOS-2, Fgatf1 is mainly involved in osmotic stress response. Deletion mutants in Fgatf1 (ΔFgatf1) strains are sensitive to osmotic stress (e.g. mediated by NaCl) and less sensitive to oxidative stress mediated by H_2O_2 compared to wild type. Furthermore, sexual reproduction is delayed: perithecia develop slower and frequently remain immature. ΔFgatf1 strains show an increased DON-production under *in vitro* induction conditions compared to wild type. However, during plant infection, DON-production is strongly reduced. Expression of genes encoding for key enzymes in the DON-biosynthesis pathway are regulated accordingly. In infection assays on wheat and maize, the ΔFgatf1 strains show a reduced virulence compared to wild type. Interestingly, overexpression of atf1 ($atf1^{oe}$) leads to hypervirulence on wheat and *Brachypodium distachyon*. The infection proceeds faster and continues into the stalk. Moreover, overexpression of atf1 in ΔFgOS-2 partially complements ΔFgOS-2-phenotypes regarding growth on osmotic-stress medium and virulence towards wheat and maize. Taken together, these results provide new insights in the stress response signalling cascades of *F. graminearum* and assign the transcription factor Fgatf1 a central role in pathogenic development.

PR4.5

The stress-activated protein kinase FgOS-2 is a key regulator in the life cycle of the cereal pathogen *Fusarium graminearum*

Van Thuat Nguyen, Wilhelm Schäfer, Jörg Bormann
Biozentrum Klein-Flottbek, Universität Hamburg

Fusarium graminearum is an ascomycetous plant pathogen and the causal agent of Fusarium head blight disease in small grain cereals and of cob rot disease in maize. Infection with *F. graminearum* leads to yield losses and mycotoxin contamination. Zearalenone (ZEA) and deoxynivalenol (DON) are hazardous mycotoxins. The latter is necessary for virulence towards wheat. Deletion mutants of the *F. graminearum* orthologue of the *Saccharomyces cerevisiae* Hog1 stress-activated protein kinase, FgOS-2 (ΔFgOS-2) showed drastically reduced *in planta* DON and ZEA production. However, ΔFgOS-2 produced even more DON than the wild type under *in vitro* conditions, whereas ZEA production was similar to that of the wild type. ΔFgOS-2 showed a dramatically reduced pathogenicity towards maize and wheat. We constitutively expressed the fluorescent protein dsRed in the deletion strain and the wild type. Microscopic analysis revealed that ΔFgOS-2 is unable to reach the rachis node at the base of wheat spikelets. Vegetative growth was retarded upon osmotic treatment. Also the germination of mutant conidia on osmotic media was severely impaired. Germ tubes were swollen and contained multiple nuclei. The deletion mutants completely failed to produce perithecia and ascospores. Furthermore, FgOS-2 plays a role in reactive oxygen species (ROS)-related signalling. The transcription and activity of fungal catalases is modulated by FgOS-2. Among the genes regulated by FgOS-2 we found a putative calcium-dependent NADPH-oxidase (*noxC*) and the transcriptional regulator of ROS metabolism, *atf1*. The present study describes new aspects of stress-activated protein kinase signalling in *F. graminearum*.

PR4.6

Identification of candidate *AvrRvi1* effector genes from *Venturia inaequalis* by transcriptome analysis

Cecilia Deng[1] Fabienne di Gennaro[2] Carl Mesarich[3] Gio Broggini[2] Matthew Templeton[1] Joanna Bowen[1]
[1.] The New Zealand Institute for Plant & Food Research Limited, Auckland, New Zealand [2.] Swiss Federal Institute of Technology, Zurich, Switzerland [3.] The University of Auckland, Auckland, New Zealand

The fungus *Venturia inaequalis* infects members of the Maloideae, causing the economically important apple disease, scab. The genetics of the interaction between *Malus* and *V. inaequalis* follow the gene-for-gene model. Effectors (pathogen proteins required for infection) are secreted into the plant/pathogen interface early in the infection cycle to suppress defence/enhance infection. A subset of effectors can be recognised by plant resistance gene (*R*) products to induce resistance. For example, on host genotypes with the *R* gene *Rvi1, V. inaequalis* isolate MNH120 causes disease, inferring the mutation or absence of the cognate effector AvrRvi1, whereas isolate 1066 is incompatible, hence AvrRvi1 is functional. Previously the gene encoding this effector has been localised to a 330 kb BAC contig[a]. This contig has a suite of 54 predicted genes. Comparison of RNA sequencing reads from an early time-point of infection from compatible interactions with both isolates has enabled candidate *AvrRvi1* genes from this suite to be identified. The expression of four genes is up-regulated during 1066 infection compared with MNH120 infection. Two of these genes encode putative cytochrome p450 enzymes with a log-fold increase in expression of 6 and 5, respectively. A third gene has similarity to a putative phospholipase and the fourth is predicted to encode a small protein, lacking a putative signal peptide or similarity with known proteins, with a 1 and 5 log-fold increase in expression in 1066, respectively. Functional characterisation of these genes is currently being carried out.

[a]Broggini et al. (2007) *Fungal Genetics and Biology 44: 44-51*

PR4.7

Ammonium Transport in the Arbuscular Mycorrhiza Symbiosis

Matthias Ellerbeck, David Brucker, Friedemann Loos, Claudia Dafinger, Arthur Schüßler, Andreas Brachmann
Genetics LMU, München

Eighty percent of all land plants are presumed to undergo root symbiosis with obligate biotrophic arbuscular mycorrhizal (AM) fungi, which provide the often growth limiting inorganic nutrients phosphorous and nitrogen to the plant. In return, plants invest up to 20% of their photosynthetically fixed carbon to feed the fungal symbiont. While nutrient transporters on the plant side of AM are widely investigated, on the fungal side respective knowledge remains still in its infancies.

The best candidate for the nitrogen source being transferred to the plant is ammonium. To estimate the role of ammonium in the symbiosis, we focused on the identification and characterization of ammonium transporters (AMTs) in AM fungi. Six proteins with homology to fungal AMTs have been identified from two AM species, *Glomus intraradices*-like BEG195 (now: *Rhizophagus irregularis*) and *Geosiphon pyriformis*. While most of these proteins functionally complement ammonium uptake deficient yeast mutants, one AMT homolog does not seem to transport ammonium. We hypothesize that it might play a role in ammonium sensing at the symbiotic interface between fungus and plant instead.

To investigate the functional relevance of the AMT homologs we are performing *in situ* expression and localization analyses. By immuno localization studies in *R. irregularis*, we were able to detect all three AMT homologs inside plant roots in storage organs (spores and vesicles), but not in the arbuscular membrane. Therefore, the primary role of AMTs might be in the re-uptake of ammonium passively released across the fungal plasma membrane.

PR4.8

Functional characterization of early expressed *in planta* genes by the phytopathogenic fungus *Botrytis cinerea*

Jose Espino, Nora Temme, Anne Viefhues, Birgitt Oeser, Paul Tudzynski
Institut of Plant Biology and Biotechnology, Vienna University of Technology

Botrytis cinerea is a phytopathogenic fungus able to infect more than 200 plant species, causing the grey mould disease, and great economic losses in the agricultural sector.

New approaches to achieve an effective control of the disease involve the elucidation of the molecular mechanisms during host-plant interaction. Thanks to microarray studies, we have identified more than 150 fungal genes which are expressed during the early stages of infection (germination and penetration), but not in conidia. This fact could indicate that these genes could be important in the first steps of the disease. Moreover, most of the proteins encoded by them have unknown function, although transmembrane domains or signal peptides are predicted for several ones by bioinformatic analysis.

In a first step, their high expression was corroborated by real time PCR, showing, in some cases, a 10,000-fold upregulation at 12 hours post inoculation compared to the expression in conidia. We are currently working with 14 of these genes, knocking them out and checking the phenotype of the mutant strains. We are also considering knocking out gene clusters whose genes are expressed *in planta* during the same conditions. The results of these studies will be reported.

PR4.9

A *WOR1*- Like Protein Regulates Pathogenicity and Reproduction in the Phytopathogenic Fungus *Fusarium graminearum*

Wilfried Jonkers[1] Yanhong Dong[1] Karen Broz[2] H. Corby Kistler[2]
[1] *University of Minnesota* [2.] *USDA ARS*

WOR1 encodes a conserved fungal regulatory protein controlling the dimorphic switch and pathogenicity in *Candida albicans* and its ortholog *SGE1* in the plant pathogen *Fusarium oxysporum* is required for pathogenicity and expression of plant effector proteins. *F. graminearum*, an important toxigenic pathogen of cereals, is not known to employ switching or effector proteins during infection and so the potential role of this gene in pathogenesis was further tested. Deletion of the *WOR1* ortholog (called *FGP1*) in *F. graminearum* results in greatly reduced pathogenicity and loss of trichothecene toxin accumulation in infected wheat plants and *in vitro*. The loss of toxin accumulation alone is sufficient to explain the loss of pathogenicity to wheat. Under toxin-inducing conditions *in vitro* or *in planta*, expression of genes for trichothecene biosynthesis and many other genes are not detected or detected at lower levels in Δ*fgp1* strains. *FGP1* is also involved in the developmental processes of conidium formation and sexual reproduction and modulates a morphological change that accompanies mycotoxin production *in vitro*. The Wor1-like proteins in *Fusarium* species have highly conserved N-terminal regions and remarkably divergent C-termini. Interchanging the N- and C terminal portions of proteins from *F. oxysporum* and *F. graminearum* resulted in partial to complete loss of function. Wor1-like proteins are conserved but have evolved to regulate pathogenicity in a range of fungi, likely by adaptations to the C-terminal portion of the protein.

PR4.10

Uncovering the biotrophic and saprophytic proteomes of the plant pathogen *Verticillium longisporum*

Harald Kusch[1] Anika Kühn[1] Clara Hoppenau[1] Ivo Feussner[2] Burkhard Morgenstern[1] Birgit Voigt[3] Dörte Becher[3] Michael Hecker[3] Susanna A. Braus-Stromeyer[1] Gerhard H. Braus[1]
[1.] *Institut für Mikrobiologie und Genetik, Georg-August Universität Göttingen* [2.] *Abteilung Biochemie der Pflanze, Albrecht-von-Haller-Institut für Pflanzenwissenschaften, Georg-August Universität Göttingen, D-37077 Göttingen*
[3] *Institut für Mikrobiologie, Ernst-Moritz-Arndt-Universität Greifswald, D-17487 Greifswald*

The plant pathogenic fungus *Verticillium longisporum* is the causal agent of early senescence and ripening in rapeseed (*Brassica napus*) and other crucifer crops. Despite the significant economical importance of this pathogen, the factors for host specificity are still unknown and the set of virulence factors (effectors) is poorly analyzed. Therefore, we investigated the extra- and intracellular proteome compositions of *V. longisporum* grown in biotrophic medium (xylem sap of non-infected oilseed rape plants) in comparison to saprophytic growth medium (potato-dextrose broth (PDB) and simulated xylem sap medium (SXM)). Procedures for the isolation and purification of proteins were optimized for *Verticillium* samples. Protein extracts were separated by one- and two-dimensional gel electrophoresis and peptide samples were analyzed by MALDI-TOF and LC-MSMS. Using the draft genome sequence of *V. longisporum* 43 we are currently assembling and annotating, we demonstrate that proteomics experiments deliver valuable data for the improvement of genome annotation and serve as powerful screening techniques to identify potential effectors and factors for host specificity. Proteomic analyses of the intracellular compartment build the basis for the reconstruction of biochemical pathways. We show that exoproteomes vary to a great extent depending on growth phase and media composition. We could identify a broad range of putative effectors such as adhesins and different groups of carbohydrate active enzymes, which might be involved in the attachment to the plant and degradation of structurally complex cell wall molecules of the host. Furthermore potential LysM effectors, necrosis and ethylene inducing like proteins (NLPs) and cysteine-rich proteins were detected, which might be involved in pathogenicity. Comparison of the exoproteome of biotrophic and saprophytic growth demonstrated among others elevated protein abundance in xylem sap of metalloproteinases, NLPs and several conserved proteins with so far unknown function. Therefore resulting candidate genes and proteins might be of specific importance for the *Verticillium-B. napus* interaction and are currently analyzed using knockout and silencing mutants.

PR4.11

What have you been eating? A perspective on fungal nutrition during plant infection

Richard P. Oliver
ACNFP, Curtin University, Australia

Pathogens derive all their nutrients from their hosts. If such processes could be interrupted, a general approach to prevent disease would be at hand. A range of approaches have been used to identify the nutrients acquired by fungal pathogens from their plant hosts. These methods include infection tests on auxotrophic mutants, screening for metabolic genes in genome sequences and transcriptomic studies of infection.

Non-obligate pathogens can grow on artificial media and in most cases can grow on defined media containing only glucose, nitrate, oxidised P, N and S and some minerals. It was therefore reasonably assumed that fungi would use these substrates in planta. A combination of approaches has revealed a more complex and interesting picture. Nitrate assimilation seems to be unnecessary; instead the direct N-sources are mainly asparagine and glutamate and possibly GABA. We have recently shown that mutants in pantothenate biosynthesis are fully pathogenic indicating that the plant supplies and the fungus takes up pantothenate or a later intermediate in the CoA pathway. Furthermore although mutants in aminolevulinate (ALA) synthesis were non-pathogenic when infected onto intact plants they could infect wounded plants indicating that ALA or a later intermediate can be assimilated. Thus it appears that fungal pathogens can and do assimilate more complex molecules that was previously thought to be the case. Such an insight might open the door to a new approach for fungicide design.

Ipcho SVS, Hane JK, Antoni EA, Ahren D, Henrissat B, Friesen TL, Solomon PS & Oliver RP (2012) Transcriptome analysis of the wheat pathogen *Stagonospora nodorum;* gene model validation, effector candidate genes, intensive host regulation of metabolism and dispensability of pantothenate metabolism. Molecular Plant Pathology

PR4.12

Recognition and response to heterospecific non self: Podospora anserina as a model for the fungal immune response

Mathieu Paoletti[1] Bastiaans, Aanen, van Diepeningen, Debets[2] Clavé, Ness, Daskalov, Breton, Sicault-Sabourin, Saupe,[3]
[1.] CNRS, France [2.] Laboratory of genetics, University of Wageningen, The Netherlands [3.] Non-Self Recognition in Fungi, IBGC, Université de Bordeaux, France

The ability to detect and react to pathogens is essential to the development of any organism. In plants and mammals pathogen recognition relies on polymorphic Pattern Recognition Receptors (PRRs) belonging to the STAND class of proteins involved in signal transduction, essentially of the NB-LRR type. Pathogen driven evolution of PRR encoding genes can lead to auto-immune diseases. No such fungal immune systems have been described so far and *NB-LRR* encoding genes are absent from fungal genomes. In *Podospora anserina* Vegetative Incompatibility (VI), a conspecific non self recognition process (between individuals of the same species), leads to cell death and autophagy. VI is determined by interaction of *het-c*, encoding a glycolipid transfer protein, and members of the *hnwd* gene family encoding for STAND proteins. *hnwd* gene family members display the hallmarks of PRR encoding genes, including fast evolution promoting production of a repertoire of receptors and ability to initiate a cell death reaction. *het-c* is also showing signs of fast evolution. We recently hypothesized that these genes are involved in pathogen recognition and that the VI reaction is an autoimmune disease. This hypothesis gained experimental support. *P. anserina* WT strains initiate a strong reaction when confronted with another fungal species, *Epiccocum nigrum*. Crucially this reaction is not observed when mutants suppressed for the VI reaction are confronted to *E. nigrum*. We are investigating the function of HET-C, HNWD and additional STAND proteins in *P. anserina* response to *E. nigrum*. We also investigate the cellular response to heterospecific non self.

PR4.13

The functional characterization of candidate genes involved in host specialization of Mycosphaerella grass pathogens

Stephan Poppe, Petra Happel, Eva H. Stukenbrock
Max Planck Institute for Terrestrial Microbiology

The ascomycete fungus *Mycosphaerella graminicola* emerged as a new pathogen of cultivated wheat during crop domestication about 11.000 years ago. To understand the molecular basis of host specialization in this pathogen we have sequenced complete genomes of *M. graminicola* and closely related species infecting wild grasses.
Evolutionary genomic analyses allowed us to identify 17 genes that show strong evidence of positive selection between *M. graminicola* and the closely related sister species S1. We hypothesize these evolved in a co-evolutionary arms race with different hosts. None of the genes encode proteins with known function. In this project we focused on three candidate genes and investigated their role in *M. graminicola* and its two closest relatives S1 and S2 during host infection.

Quantitative Real time PCR experiments from the three fungal species infecting four different grass species show that the three genes are strongly up-regulated in planta and that candidate gene expression differs over a time course of 28 days supporting a role in host pathogen interaction. In addition we show that three different host species differentially induce gene expression in the fungi.

Confocal Laser Scanning Microscopy conducted at different time points also show clear differences between species during infection and fungal development. Deletion strains for each candidate gene have been created by *Agrobacterium tumefaciens* mediated transformation. The single deletion of two candidate genes led to a reduced virulence of *M. graminicola* on wheat. We show that genes involved in host specialization can be identified based on footprints of natural selection.

PR4.14

***Penicillium expansum* Glucose Oxidase-Encoding Gene*, GOX2,* a Key Factor for Gluconic Acid Production and Acidification During Pathogenicity of Deciduous Fruits**

Shiri Barad[1] Sigal Horowitz[2] Amir Sherman[3] Dov Prusky[1]
[1.] *Department of Postharvest Science, Agricultural Research Organization, Bet Dagan 50250, Israel*
[2.] *Department of Plant Pathology, Agricultural Research Organization, Bet Dagan 50250, Israel*
[3.] *Department of Genomics, Agricultural Research Organization, Bet Dagan 50250, Israel*

Penicillium expansum (Pe), the causal agent of blue mold rot, causes severe postharvest maceration of fruit, through secretion of total D-gluconic acid (GA). Two glucose oxidase-encoding genes – *GOX1*and *GOX2* – present in *P. expansum*, were analyzed. Glucose oxidase (GOX) activity and GA accumulation were strongly related to *GOX2* expression, which increased with pH to a maximum at pH 7.0, whereas *GOX1* was expressed at pH 4.0, where no GOX activity or extracellular GA was detected. This differential expression was also observed at the leading edge of the decaying tissue, where *GOX2* expression was dominant. The roles of the *GOX* genes in pathogenicity were further studied through: i. development of *goxRNAi* mutants exhibiting differential down-regulation of *GOX2*; ii. heterologous expression of the Pe- *GOX2* gene in the non-deciduous host-pathogen *P. chrysogenum*; and iii. modulation of GA production by $FeSO_4$ chelation. Interestingly, in *P. expansum* pH and GA production elicited opposite effects on germination and biomass accumulation: 26% of spores germinated at pH 7.0 when GOX activity and GA were highest, whereas in *P. chrysogenum* at the same pH, when GA did not accumulate, 72% of spores germinated. Moreover, heterologous expression of Pe-*GOX2* in *P. chrysogenum* resulted in enhanced GA production and reduced germination, suggesting negative regulation of spore germination and GA production. These results demonstrate that pH modulation, mediated by GA accumulation, is an important factor in generating the initial signal(s) for fungal development leading to host-tissue colonization by *P. expansum.*

PR4.15

Functional genomic tools to decipher the pathogenicity mechanisms of the necrotrophic fungus *Plectosphaerella cucumerina*, a natural pathogen of *Arabidopsis thaliana*

Brisa Ramos[1] Pablo González-Melendi[1] Andrea Sánchez-Vallet[2] Clara Sánchez-Rodríguez[3] Gemma López[1] Antonio Molina[1]

[1.] CBGP-UPM-INIA, Madrid, Spain [2.] Wageningen University, Wageningen, The Netherlands [3.] Max-Planck Institute for Molecular Plant Physiology, Postdam, Germany

The analysis of the interaction between *Arabidopsis thaliana* and adapted (*PcBMM)* and non-adapted (*Pc2127)* isolates of the necrotrophic fungus *Plectosphaerella cucumerina* has contributed to the identification of molecular mechanisms controlling plant resistance to necrotrophs. To characterise the initial stages of Arabidopsis colonization by *PcBMM* and *Pc2127* we used *Agrobacterium tumefaciens*-mediated transformation (ATMT) to generate fungal transformants constitutively expressing the Green Fluorescence Protein (*PcBMM-GFP* and *Pc2127-GFP*). Using confocal microscopy we found that *PcBMM-GFP* colonized *Arabidopsis* wild-type plants by successive degradation of leaf cell layers without forming appressorium or penetrating into host cells, as described for other necrotrophic fungi. By comparing *PcBMM-GFP* colonization process in wild-type plants, and hypersusceptible (*agb1*-1 and *cyp79B2cyp79B3)* and resistant (*irx1-6*) *Arabidopsis* mutants, we found that plant susceptibility to the fungus correlated with the time-course of spore germination and hyphal growth on leaf surface, and that the resistance response was established at 12-18 hours post inoculation (hpi). This result was supported by the observation that hyphal growth of the nonadapted *Pc2127-GFP* on the leaves of wild-type plants was arrested at 12-16 hpi. We generated a collection of random T-DNA insertional transformants by ATMT, and we screened a subset of them to test their virulence in *Arabidopsis* wild-type and *agb1-1* plants. In this screening we identified several fungal transformants with altered virulence in comparison with the wild-type *PcBMM.* The *P. cucumerina* functional genomics platform presented here will be a valuable tool to characterize the molecular bases of necrotrophic fungi pathogenicity.

PR4.16

A refined predicted protein secretome for the wheat leaf pathogen *Mycosphaerella graminicola*

Alexandre Amaral, John Antoniw, Kim Hammond-Kosack, Jason Rudd

Rothamsted Research, UK

Mycosphaerella graminicola infection of wheat leaves involves an initial extended period of symptomless intercellular colonisation prior to the development of disease lesions. Previous functional genomics and gene expression profiling studies have implicated the production of secreted virulence effector proteins as a key component facilitating the initial symptomless growth phase[1,2]. With a view to identifying further candidate virulence effectors, we have re-analysed the predicted protein secretome from this pathogen, by combining several bioinformatic approaches aimed to increase the probability of identifying truly secreted proteins. An initial secretome of 970 proteins was predicted. A refined prediction of 556 was made based upon further stringent selection criteria deriving from WolfPsort protein localisation prediction. Of these, 298 possess some functional annotation (based upon PFam; KOG or the CDD databases) leaving 258 with no functional annotation. Further characterisation of the un-annotated proteins included the analysis of features associated with known fungal effectors, for example, small size, cysteine-rich, and Blastp searches performed against other sequenced fungal genomes. Finally evidence in support of gene prediction was derived from gene expression profiling during fungal growth *in vitro* and *in planta*. Subsets of candidate genes are currently being subjected to sequence analysis, reverse genetics and BSMV-mediated overexpression in wheat leaves.

[1] Marshall et al., (2011). *Plant Physiol***.** 156**,** 756-769. [2] Rudd et al., (2010) *Fungal Genet Biol.* 47**,** p19-32.

PR4.17

Role of phenylalanine ammonia-lyase and Pathogenesis-related genes in defense mechanisms of some rice cultivars against *Rhizoctonia solani*

Mohammad Sayari, Valiollah Babaeizad, Mohammad Ali Tajick Ghanbari, Heshmatollah Rahimian
Faculty of Agronomic Sciences, Department Of Plant Protection, Sari Agricultural Sciences And Natural Resources University, Sari, Iran

The *Pathogenesis- related genes* (*PR genes*) are among the most important genes in rice in response to pathogens. In this study we focused on the role of *PR* genes and *PAL* in defense mechanisms of rice against *Rhizoctonia solani,* the causal agent of rice sheath blight. For this purpose Binam and Khazar cultivar (Cv) as resistant and susceptible cultivars, respectively, were used. In morphological assessment, the size of lesions was measured 2 weeks after inoculation with isolates of the fungus. The length of lesions in cv. Binam was half as high as those on Khazar cv. Analysis of data by Student's t test showed significant difference between two cultivars (P values of <0.05). In molecular studying, the expression rate of defense genes was evaluated in 2 weeks old seedling after challenging with *R. solani*. The expression patterns of *PR* gens (*PR3*, *PR10*, *PR13*), *PAL* and *NH1* were considered in seedlings by Quantative Real Time PCR method at different time courses (from 0 to 100 hours post inoculation). The expression rate of PAL, PR3, PR10 and PR13, 12 hour after inoculation (hai) in cv. Binam evaluated 10, 7.5, 10.5 and 10 times, respectively, compared to cv.Khazar. The peak of NH1 were 24 times, 24 hai in cv. Binam compared to cv. Khazar. The results of this study suggest that the genes understudy involving in defense mechanisms of rice against sheath blight agent, *R. solani*. Here with this results it seems that the expression of PAL and PR gens are independent of NH1.

PR4.18

Morphological and molecular characterization of *Rhizoctonia solani* AG2-1 the causal agent of canola stem canker in Iran

Valiollah Babaeizad, Sayari Mohammad, Mohammad Ali Tajick Ghanbari, Heshmatollah Rahimian
Faculty Of Agronomic Sciences, Department of Plant Protection, Sari Agricultural Sciences and Natural Resources University, Sari, Iran

In June 2011, Canker-like symptoms, a newly emerged disease were observed on canola (*Brassica napus*) in Mazandaran and Golestan provinces in Iran. Round to elongated, light to dark brown sunken lesion were formed on rapeseed stems. Canker were scattered all along the height of the stem but were more predominant on parts closer to the stem apices. *Rhizoctonia* -like fungi were isolated from the cankered areas about 5 days following plating surface-disinfected segments on Potato dextrose agar (PDA). All isolates were multinucleus and were identified as *Rhizoctonia solani* AG2-1 in anastomosis assay. The internal transcribed spacer (ITS) rDNA region was amplified using the primers ITS4 (5-TCCTCCGCTTATTGATATGC-3) and ITS5 (5-GGAAGTAAAAGTCGTAACAA-3) and the PCR product was sequenced. The sequence alignment of the 661 bp fragment was subjected to genetic distance analyse. After multiple sequence alignment with PHYLIP software the obtained sequences were compared with the other related sequences with the same region of *Rhizoctonia* genus in GenBank. Isolates from *canola* were all clustered with the representative isolates of AG2-1. The PCR products were digested with *Hinf*I, *Hin*cII, *Ava*I, and *TagI* restriction endonuclease enzymes, and different PCR-RFLP patterns were obtained for the isolates. To the basis of our knowledge this appears to be the first report of canola stem canker caused by R.solani AG 2-1 in the world.

PR4.19

Identification of the *Rhizoctonia solani* AG-4 the causal agent of safflower stem canker based on morphological and genetic characteristics

Valiollah Babaeizad, Mohammad Sayari, Mohammad Ali Tajick Ghanbari, Heshmatollah Rahimian
Faculty Of Agronomic Sciences, Department of Plant Protection, Sari Agricultural Sciences and Natural Resources University, Sari, Iran

A new disease symptoms was observed on safflower stems in Golestan province of Iran during 2010 and 2011. Linear brown lesions which gradually become canker was the main symptoms of this disease. The lesions may girdle the stem or large cankers can interfere with movement of nutrients from the leaves to the roots and eventually extending the canker can lead to seedling Damping-off. *Rhizoctonia* like fungus isolated from the collected samples cultured on Potato Dextrose Agar (PDA) medium after 4 days at 28°C. All isolates were multinucleus and were identified as *Rhizoctonia solani* AG4 in anastomosis reaction. The teleomorph stage of the isolated fungus was produced in vitro and identified *Thanatephorus cucumeris* based on morphological characteristics. After obtaining the genomic DNA of the isolates, an approximately 500 bp amplification product of the ITS4-5.8S-ITS5 region was obtained with PCR, using ITS4 and ITS5 universal primers. After multiple sequence alignment with MEGA5 software the obtained sequences compared with the other related sequences of *Rhizoctonia* genus in GenBank (NCBI). The PCR products were digested with *Mse*I, *Hin*cII, *Ava*II, and *MfeI* restriction endonucleases enzymes and different PCR-RFLP patterns were obtained. This is the first report of occurrence of safflower stem canker caused by *R .solani* in Iran and based on our knowledge this is the first research on genetically characterization of safflower stem canker agent in the world.

PR4.20

Phenylalanine Ammonia Lyase and Pathogenesis related genes involved in sheath blight disease resistance in Tarom, Iranian rice cultivar

Mohammad Sayari, Valiollah Babaeizad, Mohammad Ali Tajick Ghanbari, Heshmatollah Rahimian
Faculty of Agronomic Sciences, Department of Plant Protection, Sari Agricultural Sciences and Natural Resources University, Sari, Iran

Plants are encountered with the biotic and abiotic stresses in different ways. Among them PAL and PR genes plays a crucial role in plant microbe interactions. Tarom and Khazar as the Iranian resistant and susceptible cultivars (cv), respectively, were used to analyze the expression patterns of defense genes in response to *Rhizoctonia solani* the causal agent of rice sheath blight disease on 2 weeks old seedlings. The length of lesions was measured 2 weeks after inoculation with isolates of fungus. The size of lesions in cv.Tarom was half as high as those on Khazar. Analysis of data by Students t test showed significant difference between two cultivars (P values of < 0.05) . The role of NPR1, PAL, and Pathogenesis- related genes (PR3, PR10, PR13 in the defense mechanism were considered in inoculated plants using Quantative Real Time PCR technique. The expression rate of PAL, PR3, PR10, and PR13 evaluated at different time courses (from 0 to 100 hours post inoculation) and the peak of the genes understudy were 10, 7.5, 10.5 and 10 times 12 hours after inoculation (hai), respectively, in cv.Tarom compared to cv.Khazar.The expression level of *NPR1* rised 27 times in Tarom compared with Khazar at 24 hai. The results indicated that all of the considered genes involved in sheath blight disease resistance in Tarom cultivar, and the pathway of PR genes expression is independent of NPR1 in *R.solani*- rice interactions.

PR4.21

T-DNA-mediated insertional mutagenesis in *Botrytis cinerea* reveals less virulent mutants that are affected in light-dependent development

Julia Schumacher, Paul Tudzynski

Institut für Biologie und Biotechnologie der Pflanzen, Westf. Wilhelms-Universität, Hindenburgplatz 55, 48143 Münster, Germany

By using an *Agrobacterium tumefaciens*-mediated transformation (ATMT) approach in the plant pathogen *B. cinerea*, we generated a library with 2,350 transformants carrying random integrations of a hygromycin resistance cassette. A first virulence screen of all transformants on detached tomato leaves resulted in the identification of 560 less virulent strains (Giesbert et al., in press). 231 of these have been undergone a second screening on primary leaves of *Phaseolus vulgaris*, and the less virulent phenotype has been confirmed for 169 strains. The phenotypes of 30 less virulent ATMT mutants were further characterized by analysing the response to ROS, the formation of ROS and oxalic acid and the light-dependent differentiation. Interestingly, many of the less virulent mutants are affected in light-dependent development as they are either impaired in conidiation, sclerotia formation or both. One less virulent ATMT mutant that sporulates in the dark does not produce oxalic acid and carries the T-DNA insertion 1608 bp upstream of an ORF encoding a GATA transcription factor. Remarkably, two other less virulent ATMT mutants have been identified that are tagged in the same gene locus. Another less virulent ATMT mutant is severely reduced in growth, produces ROS in great quantities and shows a fluffy phenotype when incubated in the dark. TAIL-PCR analyses revealed that the T-DNA is inserted 1307 bp upstream of an ORF encoding a helix-loop-helix transcription factor. The detailed analysis of these transcription factors by analysing deletion and overexpression mutants is currently in progress.

PR4.22

The ectomycorrhizal fungus *Paxillus involutus* express a large diversity of peptidases when degrading protein substrates and plant litter material

Firoz Shah, Francois Rineau, Tomas Johansson, Anders Tunlid

Microbial Ecology Group, Department of Biology, Lund University, Ecology Building SE 223 62 Lund, Sweden

A majority of the nitrogen (N) present in forest soils is bound in organic forms including proteins. Though a poorly characterized process, this N is mobilized and becomes available to plants due to the activity of ectomycorrhizal (ECM) fungi. We have examined the extracellular proteolytic activity and the underlying gene expression patterns in the ECM fungus *Paxillus involutus* when degrading various protein sources (BSA, gliadin and pollen) and plant litter material. During N-deprived conditions, all substrates induced an extracellular proteolytic activity. The activity had acidic pH optimum (2.5-3.0), and it was mainly due to aspartic peptidases with minor contributions of metallo- and serine proteases. The activity was partly repressed by low concentrations of ammonium (1mg/L), but not nitrate in the medium. The transcriptomes expressed by the fungus was analyzed by 454 pyrosequencing and microarray experiments. The sequencing yielded 2,029,605 reads that were assembled into a set of 12,873 contigs. In total 232 of these contigs were annotated to peptidases including 90 metallo- , 26 aspartic-, 38 serine- and 23 cysteine- peptidases. In total, 61 of these transcripts (i.e. 26 %) were significantly upregulated (> 2 fold) during growth on the protein substrates and the litter extracts. Highest expression levels were found for transcripts of aspartic, metallo, and serine proteases, but the expression patterns differed depending on the medium. We suggest that the expression levels of the extracellular peptidases machinery of *P. involutus* can be tuned to different proteins sources and environmental conditions.

PR4.23

Antagonistic *Fusarium oxysporum*: pathogenicity gene expression modulated by ectosymbiont bacteria.

Anna Poli, Davide Spadaro, Maria Lodovica Gullino
University of Torino - Agroinnova

Fusarium oxysporum is a soilborne pathogen comprising over 120 *formae speciales*, responsible for vascular wilts in several plant species.

An antagonistic strain was isolated in Piedmont (northern Italy) from suppressive soils and named MSA35. The hyphae of this organism are colonized by an ectosymbiont bacterial consortium mostly including gamma proteobacteria (Minerdi et al., 2008). The removal of this consortium (CU: cured strain) re-establishes the pathogenicity of the organism. It was demonstrated that genes such as *fmk1*, *chsV* e *p/1* are repressed in the wild type strain (WT) and that volatile organic compounds with antimicrobial properties are produced.

In this work, the potential antagonistic activity of soluble compounds secreted by MSA35WT in the surrounding environment was evaluated. Experiments were performed in liquid cultures. Two pathogens (*F. oxysporum* f.sp. *lactucae* e *F. proliferatum*) were grown in a filtered medium were the antagonist strain was previously cultivated. Mycelial growth, measured as wet and dry weight, was significantly reduced in both cases as well as conidial germination. These results indicate that soluble compounds secreted by MSA30WT affect organisms which grow in close contact with the strain.

The expression of key genes was also evaluated on both MSA35WT and MSA35CU: *fmk1, fgb1*, *fga2* and *fhk1* are not expressed in the antagonist strain. Expression analysis of these genes on pathogens grown in the medium of MSA35WT and identification of secreted compounds are in progress.

PR4.24

Unraveling the effects of a bacterial metabolite in the model organisms *Saccharomyces cerevisiae* and *Neurospora crassa*

Danielle Troppens[1] Meiling Chu[2] Fergal O'Gara[3] Nick Read[2] John Morrissey[1]
[1.] Microbiology Department, University College Cork, Ireland [2.] Institute of Cell and Molecular Biology, University of Edinburgh, UK [3.] Biomerit Research Centre, Cork, Ireland

Naturally occurring antifungal compounds are abundant and very diverse and are mostly considered to be produced to regulate the growth of competing organisms in environments such as the rhizosphere of plants. However, in recent years the concept of antibiotics as signalling molecules has emerged and receives rising attention. In this study we are investigating the effects of the secondary metabolite 2,4-diacetylphloroglucinol (DAPG), which is produced by a few *Pseudomonas* spp.. This metabolite exhibits a broad spectrum of antimicrobial activity but little is known about its cellular targets or possible fungal resistance mechanisms. We are using two model organisms, *Saccharomyces cerevisiae* and *Neurospora crassa*, to address these questions. DAPG treatment impairs cell growth in both organisms and specifically causes loss of membrane potential in mitochondria suggesting that electron transport is a target. A screen of the yeast deletion library revealed that alterations of several different processes, such as protein biosynthesis and DNA repair, can confer resistance. We also found that in both *S. cervisiae* and *N. crassa,* DAPG induces a transient cytoplasmic Ca^{2+} signal. The relevance of this signal is part of our current investigations but it may indicate a possible role of DAPG as a signal. The outcomes of this study could facilitate understanding the mode of action of antifungals/antibiotics and their role in inter-and intra-species communication but also help exploitation of this metabolite for agri-biotech and other applications.

PR4.25

Development of a hemibiotroph within its host: Transcriptomic and histological studies of the *Colletotrichum graminicola*-maize pathosystem

Walter A. Vargas, José M. Sanz-Martín, Lina P. Rivera, Michael R. Thon, Serenella A. Sukno
Centro Hispanoluso de Investigaciones Agrarias (CIALE), Department of Microbiology and Genetics, University of Salamanca

Hemibiotrophic plant pathogens first establish a biotrophic interaction with the host plant, and later switch to a destructive necrotrophic lifestyle. The molecular mechanisms and the biochemical events involved in this process are poorly understood. Studies of biotrophic pathogens have shown that they actively suppress plant defenses after they have penetrated the host cell. To determine whether *C. graminicola* also suppresses host defenses during its biotrophic stage, we performed comprehensive transcriptomic, histological and biochemical studies of the early stages of *C. graminicola* infection of maize leaves, a model pathosystem for the study of hemibiotrophy. We identified novel putative fungal effectors differentially expressed during host colonization. Our findings also show the presence of a fungal respiratory burst in fungal tips during the transition from biotrophic into necrotrophic lifestyle. Additionally, time-course experiments revealed a strong induction of defense-related genes, as well as the accumulation of reactive oxygen species and antimicrobial compounds in host cells during the biotrophic stage. We demonstrate the production of maize-derived vesicular bodies containing H_2O_2 targeted to the fungal hyphae. These results demonstrate a strong induction of defense mechanisms occurring in maize cells during *C. graminicola* infection, even during the biotrophic development of the pathogen. Overall, these results demonstrate a complex molecular and metabolic interaction between *C. graminicola* and maize cells, with a strong induction on plant defense mechanisms at early stages of infection. We hypothesize that the switch into the necrotrophic lifestyle is an adaptive response by the fungus that enables it to evade the host immune system, kill the host cells and complete its life-cycle after host infection.

PR4.26

Identification and functional characterization of CgEP1, a novel pathogenicity factor from *Colletotrichum graminicola*

Walter A. Vargas, Jose M. Sanz-Martín, Michael R. Thon, Serenella A. Sukno
Centro Hispanoluso de Investigaciones Agrarias (CIALE), Department of Microbiology and Genetics, University of Salamanca

Fungi secrete effectors that alter the host's structure and function. *Colletotrichum graminicola* is the causal agent of maize anthracnose, which causes severe crop losses worldwide. To better understand disease development, we are studying novel effector proteins that are expressed during infection. We identified a gene encoding a putative effector protein by searching for proteins that contain secretion signal peptides as well as nuclear localization signals (NLS). In addition to the signal peptide and NLS, the protein has a highly basic isoelectric point and seven nearly identical internal repeated motifs. A BLAST search of the predicted protein sequence found no homologs in public databases, suggesting that it is unique to *C. graminicola*. We performed time-course assays for transcript abundance using RT-PCR as well as *in situ* experiments using transcriptional fusions of the promoter with *gfp* as a reporter gene. These studies revealed that the gene is expressed at early stages of host colonization, mainly in primary hyphae. We constructed null mutants by gene replacement and performed pathogenicity assays on maize seedlings. Anthracnose development was severely impaired on maize plants inoculated with mutant strains demonstrating that the gene is crucial for full pathogenicity of *C. graminicola* on maize. Based on our findings, we conclude that the gene encodes a novel fungal pathogenicity factor that we call CgEP1 (*C. graminicola* Effector Protein 1.) Results of *in planta* subcellular localization of the mature protein will be also presented.

PR4.27

Pathogen-caused release of poly-unsaturated free fatty acid suppresses plant defense by inhibition of callose synthesis in wheat

Antje Blümke[1] Cornelia Göbel[2] Christian Falter[1] Rainer Bode[2] Wilhelm Schäfer[1] Ivo Feußner[2] Christian Voigt[1]
[1.] Biocenter Klein Flottbek, University of Hamburg [2.] Dep. Plant Biochemistry, Georg August University, Göttingen

The precise function of callose in papillae has not been shown unequivocally. We demonstrate that upon infection of wheat spikes with the fungal plant pathogen *Fusarium graminearum*, callose synthase activity and callose deposition are suppressed, and wheat is susceptible to fungal spreading. The secreted lipase FGL1 is an important virulence factor for *F. graminearum*. In contrast to *F. graminearum* wild-type, the lipase-deficient *Δfgl1* mutant is unable to suppress wheat callose synthesis. Wheat spikes are resistant to colonization by this mutant. Long-chain unsaturated free fatty acids (FFA) inhibit plant callose synthesis in vitro and in planta; and the previously observed resistance of the wheat spike to *Δfgl1* is broken. The lipase-deficient fungal mutant is able to colonize the spike. Analysis of the FFA level in wheat spikes during infection revealed an elevated linolenic acid concentration during *F. graminearum* wild-type compared to *Δfgl1* infection.

We conclude that linolenic acid plays a decisive role in callose synthesis suppression during wheat - *F. graminearum* interaction. A proposed model explains this novel mechanism of plant defense suppression by pathogen-caused increase in FFA due to lipase secretion.

PR4.28

Discovering host specificity candidate genes of *Sporisorium reilianum* by genotyping mixed-variety offspring

Theresa Wollenberg, Jannik Donner, Katja Zuther, Lorena Stannek, Jan Schirawski
Georg-August-University Göttingen

Sporisorium reilianum is a biotrophic plant pathogenic basidiomycete that causes head smut of maize and sorghum. The fungus exists in two varieties with different host specificity. The sorghum variety (*SRS*) is fully virulent on sorghum. *SRS* infection of maize leads to weak symptoms, such as phyllody of the floral parts. The maize variety (*SRZ*) is fully virulent on maize, but does not show symptoms on sorghum inflorescences. Instead, *SRZ* infection of sorghum leads to the formation of red spots containing phytoalexins on leaves.
This different behavior challenged us to find factors responsible for host specificity. We analyze segregants of a mixed-variety infection both phenotypically and genotypically. Approximately 100 offspring of a cross of *SRZ* x *SRS* are tested for virulence on maize and sorghum. Strains that do not lead to disease symptoms on sorghum and those showing full virulence on sorghum are subjected to genotypic analysis by performing species-specific PCRs as well as an NGS approach. Genomic regions stemming from the *SRZ* parent in non-virulent offspring and from the *SRS* parent in virulent offspring are expected to contain candidate genes for host specificity. This way, we identified the beginning of chromosome 7 as one region of interest. This region harbors an *SRZ*-specific gene (*hsc1*) that, when introduced into *SRS*, was shown to positively contribute to the aggressiveness of the recombinant strains on maize and negatively on sorghum.

This shows that genotyping of mixed-variety offspring is a powerful tool to discover candidate genes involved in host specificity.

PR4.29

Isolation and characterization of *F. graminearum* mutants altered in virulence

Chunzhao Zhao[1] Theo van der Lee[2] Pierre J.G.M. de Wit[2] Cees Waalwijk,[2] Dingzhong Tang[1]
[1.] CAS [2.] Wageningen-UR

The ascomycete *Fusarium graminearum*, the major causal agent of Fusarium head blight, can infect many important grain cereals. To identify genes that are involved in virulence of *F. graminearum*, an effective *mimp1* transposable element-mediated mutagenesis approach was developed that allowed us to identify several mutants showing reduced virulence (Dufresne et al. FGB 2009). One of these transposon mutants is *ebr1*, which was disrupted in a novel Zn_2Cys_6 transcription factor, showing reduced radial growth and reduced virulence. Knocking out *EBR1* in *F. graminearum* strain PH-1 by homologous confirmed reduction of both radial growth and virulence. The conidia of knock-out strain PH-1*Δebr1* germinated faster than those of wild-type PH-1, but its conidiation was significantly reduced. Detailed analysis showed that the reduced radial growth might be due to reduced apical dominance of the hyphal tip leading to increased hyphal branching. Inoculation assays on wheat heads with a GFP-labeled PH-1*Δebr1* mutant showed that it was unable to penetrate the rachis of the spikelets. Protein fusion with GFP showed that EBR1 is localized in the nucleus of both conidia and hyphae. Knocking out the orthologous gene, FOXG_05408, in *F. oxysporum* f. sp. *lycopersici* caused a much weaker phenotype than the PH-1*Δebr1* mutant. Transformation of FOXG_05408 into PH-1*Δebr1* restored the mutant phenotype. Expression analysis by RNA-seq suggests that in the PH-1*Δebr1* mutant protein synthesis is reduced significantly.

PR4.30

***Piriformospora indica* small secreted proteins**

Robin Nostadt, Jörg Martin, Marcus Hartmann, Andrei Lupas, Alga Zuccaro
Max-Planck Insitute for Terrestrial Microbiology, Marburg

Transcriptional analysis provided first insights into *Piriformospora indica* root colonization strategies. This included the expression of small secreted proteins (SSP < 300 aa) during the early biotrophic colonization of barley. Among the SSPs several lectin-like proteins and *P. indica*-specific effector proteins were identified which represented about 10% of the fungal genes induced during biotrophy. In analogy to other fungi where effector genes are upregulated during host colonization, *P. indica* genes encoding SSPs are likely to play a role in determining the success of endophytic interaction which involves host cell penetration, suppression of plant immunity and growth within living cells. Most of the plant responsive SSPs are *P. indica* specific and poorly related to each other. Some of them are cysteine-rich, as describe for other effector molecules in mutualistic and pathogenic systems, other possess distinctive features such as a regular pattern of histidine and alanine residues. A search for motifs in the amino acid sequence of these histidine-alanine rich proteins identified a highly conserved pattern of seven amino acids "RSIDELD" at the C-terminus of 29 putative ORFs. *In planta* expression of two of the DELD proteins using the fusion plasmid 35S::SP_Dld1:mCherry has shown that these proteins are secreted and seem to localize in the apoplast. Bioinformatic and functional analyses of these effector-like proteins will be discussed.

PR4.31

Influence of indole-derivatives on the establishment of the biotrophic interaction of *Piriformospora indica* with barley roots.

Magdalena Zurawska[1] Yi Ding[1] Lars Voll[2] Karl Heinz Kogel[3] Alga Zuccaro[1]
[1.] Department of Organismic Interactions, [2.] Department of Biology University of Erlangen-Nürnberg, Germany,
[3]Institute of Phytopathology and Applied Zoology, Justus-Liebig-University of Giessen, Germany

Colonization of barley roots by *P. indica* is accompanied by changes in phytohormone homeostasis, such as induction of plant genes involved in ABA and auxin metabolism and signalling during the biotrophic colonization phase (Schäfer et al., 2009). *P. indica* produces the phytohormones indole-3-acetic acid (IAA) and indole-3-lactate (ILA), upon tryptophan feeding, through the intermediate indole-3-pyruvic acid (IPA) and indole-3-acetalaldehyde (IAD). Analysis of the underlying biosynthetic pathways for auxin identified the piTam1 gene as a key player. Transcriptional analysis in barley colonized roots showed that piTam1 gene is induced during the biotrophic phase. Congruent with previous reports that auxin renders the plant more susceptible to colonization by biotrophic microorganisms (Bari and Jones, 2009), *P. indica* transformants carrying the RNAi construct for the pi*Tam1* gene displayed decreased colonization of barley roots in the biotrophic phase, but not in the cell death associated phase. Local increased auxin levels in barley, measured by IC-MS/MS, at the early colonization phase additionally hints towards involvement of auxin in the establishment of early biotrophy.

PR4.32

Transcriptome of *Penicillium digitatum* During the Infection of Oranges

Ana-Rosa Ballester, Luis González-Candelas
IATA-CSIC

Penicillium digitatum is the causal agent of green mould rot of citrus fruit, and represents the major postharvest pathogen of citrus fruit in Mediterranean regions. In this study, with the aim of better understanding the infection process on oranges, we used massive parallel pyrosequencing with 454 Titanium technology to perform a global RNA-Seq transcriptomic analysis of *P. digitatum* in time series from 0 to 48 h after pathogen inoculation, where first symptoms of disease appeared. To identify the putative origin of the reads, two reference genomes were used: (i) the *Citrus sinensis* Genome Assembly (JGI v1.0; these sequence data were produced by the US Department of Energy Joint Genome Institute http://www.jgi.doe.gov/ in collaboration with the user community), and (ii) the first draft of the *P. digitatum* genome with a 20X genome coverage, elaborated in house. All sequence reads from a total of four libraries were assembled in a reference transcriptome containing 24410 contigs or putative genes. About 30% of those putative genes were assigned to *P. digitatum* and about 70% to *C. sinensis*. The number of *P. digitatum* putative genes increased as the infection progressed, whereas citrus genes showed the opposite trend. Quantitative reverse transcription PCR profiling of selected fungal genes revealed dynamic expression patterns during infection of orange fruits.

PR4.33

Experimental evidence for Crozier's paradox: somatic fusion in fungi as a model for the evolution of cooperation and kin recognition

Eric Bastiaans, Alfons J. M. Debets, Duur K. Aanen
Wageningen University

Cooperative behaviors, behaviors that benefit other individuals, are widespread. However, to understand cooperation we have to explain how cheating, i.e. profiting without contributing, is kept at low frequency. Kin selection is the predominant solution for this problem. Kin selection requires that cooperation is preferentially directed towards related individuals, and one way to achieve this is via genetic kin recognition. However, Crozier argued that in the short term positive frequency dependent selection will eliminate the genetic polymorphism required for such recognition, since common genotypes will experience more cooperation and thereby increase in frequency. Here we study somatic fusion as a model for cooperation and kin recognition. Sharing somatic tissue via fusion seems to be an extreme form of cooperation. The potential for such fusion is widespread, but the fitness consequences of fusion are unknown. In fungi, successful somatic fusion is usually restricted to clonally related individuals regulated by highly polymorphic recognition loci. We study somatic fusion between mycelia of the fungal species *Neurospora crassa*.

First we show that there is a highly significant positive correlation between total fitness and the degree of successful fusion. This result demonstrates that fusion between genetically identical mycelia is net beneficial (*i.e. B-C*>0) and thus cooperative. We then show experimental evidence for Crozier's theoretical prediction that, in the short term, positive frequency dependent selection acts against polymorphism of recognition alleles. With these findings we discuss which counteracting evolutionary forces maintain the extensive recognition polymorphism observed in nature.

PR4.34

Inhibition of ecto-phosphatase activity in conidia reduces adhesion and virulence of *Metarhizium anisopliae* on the insect host *Dysdercus peruvianus*

Walter O. Beys-da-Silva[1] Daniela Cosentino-Gomes[2] Nathália Rocco-Machado[2] Lucélia Santi[1] Leonardo Broetto[1] Marilene H. Vainstein[1] José R. Meyer-Fernandes[2] Augusto Schrank[1]

[1.] Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul [2.] Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro

Metarhizium anisopliae is an entomopathogenic fungus and one of the most important and best studied biological control agents in world. This fungus has the ability to infect a broad range of arthropods, from ticks and agricultural insect pests to vectors of human diseases. Entomopathogenic fungi have evolved distinct strategies for their attachment to hosts, varying considerably in their modes of action, virulence and degree of host specificity. In this work, we describe the characterisation of phosphatase activity directly on the conidia surface of *M. anisopliae* and its relevance in the host interaction process. The activity of this enzyme was linear with time and cell density, and only 20% of the total activity was secreted to the extracellular medium. The optimum pH was in the acidic range and divalent metals, such as Cu^{2+}, Cd^{2+} and Zn^{2+}, inhibited ecto-phosphatase activity, while Co^{2+}, Ca^{2+}, Sr^{2+} Mg^{2+} and Fe^{2+} had no effect. The activity was also reduced by sodium fluoride, sodium molybdate, sodium orthovanadate and inorganic phosphate. Importantly, the inhibition of phosphatase activity in conidia reduced the adhesion to *D. peruvianus* tegument and, consequently, *M. anisopliae* virulence. The results herein presented show, for the first time, the importance of ecto-phosphatase activity in *M. anisopliae* conidia and provide the first evidence of its direct involvement in adhesion and host infection.

PR4.35

Beyond nitrogen and pH: in search for new inducers of deoxynivalenol biosynthesis in F*usarium graminearum*

Jakob Bönnighausen, Peter Ilgen, Birgit Hadeler, Jörg Bormann, Wilhelm Schäfer

University Hamburg

The fungal pathogen *Fusarium graminearum* is the causal agent of Fusarium head blight (FHB) of small grain cereals and cob rot disease of maize. During infection, the fungus produces the trichothecene deoxynivalenol (DON). Contaminated grain is highly toxic to mammals and, thus, not suitable for food and feed production. The key enzyme in the biosynthesis of DON is the trichodiene synthase (Tri5), which is essential for virulence on wheat. We recently created a transgenic reporter strain (Ilgen *et al.* 2009), in which the promoter of the trichodiene synthase is fused to eGFP. Using this construct we are able to perform live cell imaging to check for putative DON-inductive conditions, especially during host invasion. Firstly, we were able to show that in axenic culture, ammoniumsulfate ($(NH_4)_2SO_4$) is strongly inducing eGFP, while, in contrast, sodiumnitrate ($NaNO_3$) elicited no visible eGFP fluorescence. However, this effect is rather due to a drop of pH in the $(NH_4)_2SO_4$ medium than due to the nitrogen source, since we were able to induce DON production in a $NaNO_3$ medium buffered to a low pH using citrate buffer. However, the question remains, how these *in vitro* results fit to the situation in the host-pathogen interaction. Thus, we started to investigate plant-derived substances for their pH-independent DON-inducing potential in a combined *in-planta* and *in-vitro* approach. Preliminary results indicate a supervisory inductive effect of certain plant compounds.

Ilgen, P., Hadeler, B., Maier, F.J., and Schäfer, W. 2009. MPMI 22:899-908.

PR4.36

Characterization of a high affinity ammonium transporter from the root endophytic symbiont *Piriformospora indica*

Yi Ding, Stefanie Pfiffi, Alga Zuccaro
Department of Organismic Interactions, Max-Planck Insitute for Terrestrial Microbiology

The root endophyte *Piriformospora indica* displays a biphasic lifestyle during colonization of barley roots with an early biotrophic phase followed by a cell death associated phase. The interaction of *P. indica* with barley roots results in growth promotion and in the induction of plant genes involved in nitrate and ammonium transport. Comparative analysis revealed the presence of two ammonium transporters (AMT) in the *P. indica* genome and the absence of nitrate transporters (NTR). One of the piAMT transporters was proven to be plant responsive by microarray and qPCR analysis. Its ammonium import function was verified by yeast complementation. *P. indicaAMT1* knock down mutants show reduced growth phenotype on media containing low amount of ammonium and display an altered colonization pattern in barley roots. Our results suggest that ammonium plays an important role in the interaction with barley. Further analyses are ongoing to better characterize the impact of different nitrogen sources on symbiosis.

PR4.37

Do secreted chorismate mutases have a conserved role as enzymatic effectors in the fungal kingdom?

Armin Djamei, Anupama Ghosh, Julien Dutheil, Regine Kahmann
Max Planck Institute for Terrestrial Microbiology

A successful colonization of plants by pathogens requires active effector-mediated suppression of defense responses. Recent findings showed that the biotrophic fungus *Ustilago maydis* secretes an enzymatically active, non-allosterically regulated chorismate mutase Cmu1. This enzyme is taken up locally by infected plant cells and then spreads to neighboring cells. Metabolic data of infected maize leafs indicate that Cmu1 is involved in the rechanneling of chorismate into the phenylpropanoid pathway away from the competing salicylic acid biosynthesis pathway. Secreted chorismate mutases were identified in many other fungi mostly associated with plants. Their sequence relationship indicates that several independent events led to the occurrence of secreted chorismate mutases. Using *U. maydis* we are currently testing by complementation of an *U. maydis cmu1* mutant whether other fungal secreted chorismate mutases predicted to be secreted have the potential to act as effectors in a Cmu1-like manner. If not other functions have to be postulated for the predicted secreted chorismate mutases which could indicate a neofunctionalization of this branching enzyme in the shikimate pathway.

PR4.38

Characterisation of the SAGA/ADA complex in *Aspergillus nidulans* by tandem affinity purification

Juliane Fischer, Hans-Wilhelm Nützmann, Volker Schroeckh, Axel A. Brakhage
Hans Knöll Institute Molecular and Applied Microbiology

The recent finding that the histone acetyltransferase (HAT) complex SAGA/ADA mediates the response of the fungus *Aspergillus nidulans* to the bacterium *Streptomyces rapamycinicus* (1) opens up a number of questions. It was shown that the SAGA/ADA complex is involved in the regulation of the orsellenic/ lecanoric acid biosynthesis gene cluster, as deletion of its HAT-encoding gene *gcnE* resulted in the lack of *orsA* transcription during co-cultivation.

In order to investigate the SAGA/ADA complex in *A. nidulans*, the complex subunits GcnE and AdaB were tagged and purified by tandem affinity purification (TAP). This method is especially suited for the purification of protein complexes under native conditions. Therefore, the TAP-tag method represents an appropriate system for the investigation and analysis of the SAGA/ADA complex composition under various conditions. The TAP-tag constructs were assembled by fusion PCR and transformed directly into *A. nidulans* via homologues recombination. Western blotting was performed to monitor the purification procedure. For *adaB-C-TAP* and *gcnE-N-TAP* bands of the expected size were detected. However, further optimisation of the purification procedure is required. Furthermore, the amount of purified target protein needs to be increased to allow detection by mass spectrometry.

In this study, a first step on the investigation of SAGA/ADA in *A. nidulans* was achieved by the successful tagging of the two subunits GcnE and AdaB with the TAP-tag. Furthermore, it is now possible to elucidate the structure and function of this complex during the interaction of *A. nidulans* and *S. rapamycinicus*.

(1) Nützmann et al. (2011) *PNAS*

PR4.39

In vitro Effect of Chitosan on Growth and Enzyme Production in Ganoderma sp.

Menaka Ganeson[1] Christina Supramaniam[2] Peter Alderson[3]
School of Bioscience, Faculty of Science, The University of Nottingham Malaysia Campus, Jalan Broga, 43500, Semenyih, Selangor, Malaysia

Ganoderma boninense is the causal agent of oil palm basal stem rot disease (BSR). Lignin degradation which involves the secretion of ligninolytic enzymes by Ganoderma plays an important role in pathogenesis of BSR. The aim of the study is to evaluate the efficacy of chitosan on the effect on the growth of *G. boninense* and production of ligninolytic enzymes. Four types of chitosan (low viscosity, high viscosity, low molecular weight, high molecular weight) at 1.0, 2.0 and 3.0 % concentrations were tested for their efficacy to control mycelia growth of the pathogenic GBLS isolate after 20 days of incubation *in vitro*. All of the concentrations of different chitosan tested significantly reduced mycelial growth compared with control treatment. Microscopic observations of the changes and alterations in surface morphology of *G. boninense* mycelium imputes possible mode of action of chitosan on fungal growth. GBLS produced a combination of laccase and Manganese proxidase (MnP) as lignin degrading enzymes (LDE) in semi solid state and liquid culture medium. Chitosan at the different concentrations 1.0, 2.0 and 3.0 % evaluated significantly inhibited the production of lignin degrading enzymes of laccase and MnP by GBLS isolate under solid state culture medium supplemented with rubber wood chips. This observation suggests that ability of chitosan to act as chelating agent facilitates the inhibition of LDE produced by GBLS.

PR4.40

Identification and analysis of *Penicillium digitatum* genes putatively involved in pathogenicity towards citrus fruits

Mario López-Pérez, Luis González-Candelas
IATA-CSIC

Penicillium digitatum, the causal agent of citrus green mould, is the major pathogen of citrus fruit during postharvest handling and storage in Mediterranean climate regions. It is a necrotrophic fungus that penetrates the fruit through wounds. Despite the economical relevance of this fungus our knowledge on its pathogenicity mechanisms is still very limited. In this communication we present the identification and analysis of genes putatively involved in pathogenicity.

We have obtained a subtractive cDNA library enriched in *P. digitatum* genes that are up-regulated during infection of citrus fruit, which in combination with macroarray hybridization, has allowed the identification of several fungal genes with a potential role in pathogenicity. Time course expression analysis of several selected genes confirmed that most of them are up-regulated during infection of citrus fruits. We have cloned and sequenced the coding and flanking sequences of several candidate genes that have been isolated from a genomic DNA library prepared in the fosmid PCC2FOS. In order to determine the role of these genes in pathogenicity we have employed *Agrobacterium tumefaciens*-mediated transformation to obtain *P. digitatum* null mutants lacking the genes coding for two different polygalacturonases, a pectin lyase and a Rieske protein. *P. digitatum* mutants lacking any of the three pectin degrading enzymes showed a reduction in infection capability when compared with either the wild type strain or an ectopic transformant, whereas the mutant strain lacking the Rieske protein was not affected in virulence.

PR4.41

Pathogenicity gene variations within the order Entomophthorales

Morten N. Grell[1] Annette B. Jensen[2] Lene Lange[1]
[1.] *Section for Sustainable Biotechnology, Aalborg University Copenhagen (AAU-Cph), Denmark*
[2.] *Department of Agriculture and Ecology, University of Copenhagen, Denmark*

Fungi within the order Entomophthorales (subphylum Entomophthoromycotina) are obligate biotrophic pathogens of arthropods with a remarkable narrow host range. Infection takes place through the cuticle when conidia hit a susceptible host, facilitated by enzymatic and mechanical mechanisms. In the hemolymph, they proliferate as hyphal bodies or cell wall-less protoplasts for easy nutrient uptake and host immune response avoidance. Entomophthoralean fungi often manipulate their host to seek an elevated position shortly before host death in order to optimize disease transmission. After host death, conidia are produced and discharged when humidity gets high—usually during night. In an earlier secretome study of field-collected grain aphids (*Sitobion avenae*) infected with entomophthoralean fungi, a number of pathogenesis-related, secreted enzymes were discovered (Fungal Genetics and Biology 2011, vol. 48, 343–352). Among these were cuticle degrading serine proteases and chitinases, involved in fungal penetration of the aphid cuticle, and a number of lipases most likely involved in nutrient acquisition. In the current study, we are investigating the distribution and variation of selected pathogenicity genes within genera *Entomophthora* and *Pandora*, using fungal genomic DNA originating from field-collected, infected insect host species of dipteran (flies, mosquitoes) or hemipteran (aphid) origin.

PR4.42

retro – a retrotransposon of Tricholoma vaccinum

Grunewald, Krause, Kothe
Friedrich Schiller University Jena

The basidiomycete *Tricholoma vaccinum* is an ectomycorrhizal fungus, forming symbiosis with spruce and pine. During symbiosis the mycelium colonizes the intercellular spaces of the root cortex to exchange nutrients and for the benefit of both individuals.

In differential display analyses using ectomycorrhiza a retrotransposon was identified showing gag and pol genes with protease, RTase, RNaseH and integrase domains. Transposition of retrotransposons occurs with an RNA intermediate.

In this work the retrotransposon *retro* of *Tricholoma vaccinum* is studied for its ability to induce its transposition by checking expression and copynumber under stressing conditions, like in mycorrhizal interaction of *Tricholoma vaccinum* and *Picea abies* in axenic dual cultures or using additives for *Tricholoma vaccinum* liquid cultures like butanol and ethanol.

Since laboratory strains and new isolates of *Tricholoma vaccinum, Tricholoma imbricatum*, *Tricholoma fracticum* and *Tricholoma fulvum* have developed under different conditions they will likely be different in their number of *retro* signals, which will be investigated in Southern blot analyses.

PR4.43

Ectomycorrhizal Symbiosis: Influenced by fungal aldehyde dehydrogenase

Catarina Henke[1,2] Katrin Krause[1] Theodore Asiimwe[1] Erika Kothe[1]
[1.] Friedrich Schiller University of Jena, Institute of Microbiology, Microbial Phytopathology, Neugasse 25, D-07743 Jena, Germany, [2] International Max Planck Research School, Max Planck Institute for Chemical Ecology, Hans-Knöll-Str. 8, D-07745 Jena che

In forests various organisms live in close interaction with other species and mycorrhiza is an ubiquitous kind of symbiosis in this habitat. The mutualistic symbiosis between the basidiomycete fungus *Tricholoma vaccinum* and the specific host spruce (*Picea abies*) is called ectomycorrhiza. Important role in ecosystem functioning is attributed, particularly improved plant growth by advanced nutrient and water supply and also a phytosanitory effect on the plant against pathogens by fungal activity was shown.

The molecular level of this association is so far slightly understood and we intent to investigate the molecular mechanisms of interaction.

Ald1 - coding for a fungal aldehyde dehydrogenase - was identified in differential display analyses using ectomycorrhiza. Ald1 has a function in the detoxification of alcohols and aldehydes occurring in mycorrhizal biotopes and is involved in phytohormone production.

Agrobacterium tumefaciens mediated transformation was used to produce Ald1 overexpressing transformants of *T. vaccinum* and functional analysis is investigated in ongoing experiments: we ask for the impact of different supplements on the phenotypic character of an *ald1* transformant and the wildtype in mycorrhiza.

PR4.44

Suppression of plant immunity by the *Ustilago maydis* effector protein Pep1

Christian Herrberger[1] Christoph Hemetsberger[1] Bernd Zechmann[2] Morten Hillmer[1] Gunther Doehlemann[1]
1. MPI Marburg, Germany 2. Karl-Franzens University, Graz, Austria

Plant-associated organisms secrete proteins and other molecules to modulate plant defenses and enable colonization of plant tissue. The colonization of maize plants by *Ustilago maydis* is initiated by a direct penetration of the cuticle and cell wall of the host epidermis. The secreted effector protein Pep1 is specifically expressed during pathogenic development of *Ustilago maydis*. *pep1* deletion mutants show no defect during saprophytic growth but are arrested upon the point of penetration and elicit a hypersensitive response of the plant. Thus, the establishment of a biotrophic interaction fails and the affected plant tissue shows various defense responses, particularly the production of reactive oxygen species (ROS).

We identified Pep1 being an efficient inhibitor of early plant defense response. In particular, the effector inhibits peroxidase activity and thereby suppresses the generation of ROS. Moreover we could show the direct interaction of Pep1 to a single peroxidase, which is upregulated during infection with the Δ*pep1*-mutant. Consequently, silencing of the maize peroxidase gene lead to partial complementation of the Δ*pep1*-virulence. Here, we present recent data from biochemical and microscopic approaches to specify the peroxidase interaction of Pep1 and the consequences of Pep1 function for early plant defense reactions.

PR4.45

Cell death suppression during the interaction of *Ustilago* and barley

Alexander Hof[1] Daniel Mathow[1] Daniela Aßmann[1] Daniela Schwammbach[1] Ruth Eichmann[2] Ralph Hückelhoven[2] Gunther Doehlemann[1]
1. Max Planck Institute for Terrestrial Microbiology, Department of Organismic Interactions, Karl-von-Frisch-Str. 10, D-35043 Marburg, Germany 2. Center of Life and Food Science Weihenstephan, Technische Universitaet Muenchen, Emil-Ramann-Str. 2, D-85350 Freising-Weihenstephan, Germany

In plants programmed cell death (PCD) is an essential defense mechanism during pathogen attack. Prevention of PCD is therefore essential to biotrophic plant pathogens such as *Ustilago hordei.* During the compatible interaction of this basidiomycetous fungus with its host plant barley, PCD is fully prevented and no macroscopic symptoms of infection are visible, whereas deletion mutants of the secreted effector protein Pep1 cause PCD comparable to the non-host resistance reaction after infection with *U. maydis.*

Microscopical analyses revealed that plants overexpressing the conserved cell death suppressor Bax Inhibitor-1 (BI-1) show an increased susceptibility to the non-host pathogen *U. maydis*. Interestingly, BI-1 seems to play no role in the interaction with the *U. hordei* or *U. maydis pep1* deletion mutant, respectively, indicating that the induced cell death reaction by the effector mutant is obviously mediated by a BI-1 independent pathway. Preliminary results point towards a role of apoptosis-like cell death during non-host responses, while autophagy might be performed during interactions independent from BI-1. With a combination of microscopic and enzymatic approaches, we try to dissect the different modes of programmed cell death triggered by the described fungal strains.

In a parallel approach, we are aiming to identify cell death suppressing proteins which enable the establishment of the biotrophic interaction of *U. hordei* and barley. Microarray experiments show differentially regulated genes during the early phase of *U. hordei* infection. Up-regulated secreted effectors serve as candidate genes to be used in different screening approaches for which recent progress will be presented.

PR4.46

Microbial communication comes to *Aspergillus fumigatus*: Activation of a fungal silent secondary metabolite gene cluster by *Streptomyces rapamycinicus*

Claudia König[1] Kirstin Scherlach[2] Volker Schroeckh[1] Hans-Wilhelm Nützmann[1] Christian Hertweck[2,3] Axel A. Brakhage[1,3]

[1.] Department of Molecular and Applied Microbiology, Hans-Knöll-Institute (HKI), Jena, Germany, [2.] Department of Biomolecular Chemistry, Hans-Knöll-Institute (HKI), Jena, Germany, [3.] Friedrich-Schiller-University, Jena, Germany

Aspergillus fumigatus is the most important air-borne human fungal pathogen. The genome of this filamentous fungus exhibits far more gene clusters predicted to encode secondary metabolites than compounds known. Because these unidentified metabolites could have important biological functions and possibly represent drug candidates, it is desirable to activate their often silent biosyntheses.

Our aim was to mimic physiological conditions under which secondary metabolite gene clusters could be activated. Previously, we demonstrated activation of a silent secondary metabolite gene cluster of *Aspergillus nidulans* by co-cultivation with *Streptomyces rapamycinicus* which led to formation of orsellinic and lecanoric acid. Interestingly, as shown here, the bacterium is also able to activate silent gene clusters in the human-pathogenic fungus *A. fumigatus*. Co-culturing of *A. fumigatus* with this streptomycete triggered the specific activation of a so far silent fungal secondary metabolite gene cluster leading to the production of a novel secondary metabolite.

Moreover, overexpression of a pathway-specific regulatory gene demonstrated its function as regulator of the newly identified gene cluster that also includes a polyketide synthase gene.

PR4.47

Identification and analysis of virulence factors in the *Colletotrichum higginsianum Arabidopsis thaliana* pathosystem

Martin Korn, Johannes Schmidpeter, Marlis Dahl, Christian Koch

University of Erlangen-Nuremberg; Department of Biology, division of Biochemistry

The hemibiotrophic ascomycete fungus *C. higginsianum* causes anthracnose disease on cruciferous plants. In order to identify genes involved in pathogenicity, we generated T-DNA insertion mutants of *C. higginsianum* using *Agrobacterium tumefaciens* mediated transformation. Over 7000 mutants were screened for virulence against the model host *A. thaliana*. 79 T-DNA insertion mutants (about 1%) reproducibly showed pathogenicity phenotypes. This collection contains mutants which are affected in appressoria formation, host penetration or hyphal growth. Mutants are currently being analyzed by Southern Blot and GenomeWalker PCR to determine their T-DNA insertion sites. For confirmation of the corresponding pathogenicity phenotypes, an approach for targeted gene knockout was implemented using a KU80 deficient strain impaired in the non-homologous end-joining pathway. This method was used to knockout several independent genes identified in the mutant screen, which are for example involved in metabolism, cell cycle or membrane transport. Initial results of these studies will be presented.

PR4.48

The Benzoate p-Hydroxylase of *Cochliobolus lunatus* – Search for an Antifungal Lead Compound

Nada Kraševec[1] Metka Novak[1] Neja Zupanec[1] Branka Korošec[1] Ljerka Lah[1] Barbara Podobnik[2] Group Gobec Stanislav[3] Sabina Berne[4] Jure Stojan[4] Radovan Komel[1,4]

[1.] National Institute of Chemistry, Ljubljana, Slovenia, [2.]Lek Pharmaceuticals d.d., Ljubljana, Slovenia, [3] Chair of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ljubljana, Slovenia, [4.]Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Slovenia

There is a constant need for new fungicides for (crop) disease control due to appearing resistances and new registration demands. Compounds with new mode of action and more favorable (eco) toxicological properties are necessary. Benzoate p-hydroxylase or CYP53A15, a cytochrome P450 identified in the pathogenic filamentous ascomycete *Cochliobolus lunatus*, is capable of hydroxylation of benzoate, a key intermediate in the metabolism of aromatic compounds in fungi which is basically toxic to the organism. CYP53 family is a promising antifungal target since it seems to be distributed in most of filamentous fungi and have no homolog in higher eukaryotes. Our experiments revealed that naturally occurring phenolic compounds inhibit CYP53A15. A very reliable structural homology model was built to perform initial virtual screening of library of compounds and molecular docking in active site. Compounds were selected from diverse commercial sources to construct a chemical library of over million compounds. A more manageable size of the library was generated by filtering for descriptors: molecular weight, number of ring systems, hetero atoms, H-bond donors or acceptors. Only solutions predicting interactions with the heme iron were considered. About 100,000 compounds were docked into the enzyme active site and ranked. From those, 40 compounds were obtained and evaluated in functional assays *in vitro*: CO-differential spectrum, substrate binding spectrum, HPLC analysis, and in growth inhibition assay *in vivo*.Results of this research have identified some inhibitors which could serve as lead compound for further development of (phyto) pharmaceuticals with different mode of action.

PR4.49

Mechanisms of detoxification in the ectomycorrhizal fungus *Tricholoma vaccinum*

Katrin Krause, Ines Schlunk, Theodore Asiimwe, Catarina Henke, Erika Kothe

University of Jena

Toxic substances, e. g. xenobiotics, heavy metals and aldehydes, are found in ectomycorrhizal habitats, like woods, gardens and parks. Mechanisms to prevent cells from toxins, mostly reported from yeast or filamentous ascomycetes, are the modification of compounds by enzymes, extracellular chelation by excreted ligands, cell wall binding, reduced influx across the plasma membrane, enhanced efflux, intracellular chelation by metallothioneins or glutathione, transport into subcellular compartments like the vacuole, protection against toxic metal-induced oxidative stress by thioredoxins and superoxide dismutases and filter function of the mycelial mantle.

Lab experiments were performed using axenic co-culture systems of the basidiomycete *Tricholoma vaccinum* and spruce, and genes *ald1* and *mte1,* upregulated in ectomycorrhiza, were identified. Both are involved in detoxification mechanisms and were investigated in more detail.

The fungal aldehyde dehydrogenase Ald1 catalyzes the conversion of different aldehydes to the corresponding carboxylic acids. By using competitive and real-time RT-PCR, *ald1* was shown to be induced in response to alcohol- and aldehyde-related stress. Ald1 overexpressing mutants of *T. vaccinum* showed increased ethanol stress tolerance in comparison to wildtype.

Mte1 of the multidrug and toxic compound extrusion (MATE) family exports different compounds. By heterologous expression in *Saccharomyces cerevisiae*, different metals, xenobiotics like DNA-intercalating dyes and fungicides were identified as substrates for this specific transporter.

PR4.50

The role of Stp1, a secreted effector of *Ustilago maydis* during pathogenesis

Liang Liang[1] Kerstin Schipper[2] Regine Kahmann[1]
1. Max-Planck-Institut für terrestrische Mikrobiologie 2. University of Duesseldorf

Secreted effectors play crucial roles during establishment of a successful biotrophic interaction. In the smut fungus *Ustilago maydis*, one of the essential effectors is *stp1*. *stp1* mutants are non-pathogenic and arrest shortly after penetration. Deletion analysis revealed that the N- and C-terminal domains of Stp1 are essential for protein function while the large central region is dispensable. In addition, co-expression of separated N- and C-terminal domains of Stp1 could restore pathogenicity of a *Δstp1* strain. To determine whether Stp1 acts in apoplast or is translocated into plant cells, we are performing uptake assays of Stp1-mcherry-NLS fusion protein. To elucidate the function and the localization of Stp1, we have identified interactors by yeast two-hybrid screening. 23 putative interactors from infected maize leaves were identified, coding for apoplastic as well as cytoplasmic plant proteins. Full length cDNA clones of 8 putative interactors were isolated and their interactions with full length Stp1 as well as truncated Stp1, N-terminal and C-terminal of Stp1 were tested. Stp1 protein purified from *E. coli* could inhibit the activity of a cysteine protease isolated as apoplastic interactor of Stp1. For other interesting interactors, we are verifying the interaction with Stp1 by other biochemical and functional assays and will present the latest results.

PR4.51

Identification of micro-organisms fighting *T. aggresivum* and other "Weed" fungi in mushroom compost

Nicole Jacobs[1] Maartje van Erp[1] Theo Goosen[1] Kees van den Hondel[2] Wim Arts[3] Christien Lokman[1]
1. HAN BioCentre 2. HiTeXacoat 3. CNC Grondstoffen BV

The cultivation of mushrooms is susceptible to unwanted growth of fungi, like *Trichoderma aggresivum* and *Penicillium*. The growth of these fungi cause substantial decrease of yields to the growers or even destroy the harvest completely. Successful methods to control damage of mushroom beds by these weed fungi are still rare. Microbes inhabiting the mature mushroom compost might play an important role in stimulating or disturbing growth of mushrooms. In this study we have identified micro-organisms present in the compost that are able to inhibit growth of fungi like *T. aggresivum* and *Penicillium* leaving the mycelial growth of the commercial mushroom *Agaricus bisporus* unaffected. Furthermore, we have shown that a *Pseudomonas* strain derived from natural soil is very effective in inhibiting weed fungi. Future research will be focused on the potential of these bacterial strains as biocontrol agents.

PR4.52

Eukaryotic translation initiation factor 5A is a central regulator in *Fusarium graminearum*

Ana Lilia Martinez-Rocha[1] Mayada Woriedh[2] Thuat Nguyen[1] Wilhelm Schäfer[1]

1. Biocenter Klein Flottbek, Molecular Phytopathology and Genetics, University of Hamburg 2. Cell Biology and Plant Biochemistry, University of Regensburg

Activation of the eukaryotic translation initiation factor 5A (eIF5A) requires a posttranslational modification, forming the unique amino acid hypusine. This activation is mediated by two enzymes, deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH). The activated protein transports mRNAs from the nucleus to the ribosomes, where it initiates protein biosynthesis. This system is conserved from archea to humans and has been shown to be instrumental in diseases as diverse as HIV infection, malaria, cancer and diabetes. For the first time, we evaluate its importance in a pathogenic fungus by over-expression of the enzymes that control hypusination of eIF5A. Over-expression of DOHH prevents infectivity of *Fusarium graminearum* to wheat and maize and leads to an over-production of reactive oxygen species (ROS). In addition, it reduces production of the mycotoxin deoxynivalenol *in vitro* and *in planta*. In contrast, over-expression of DHS leads to an increase of virulence to wheat and a decrease of ROS. Simultaneous over-expression of both enzymes results in infectivity and levels of ROS comparable to the wild type strain. Over-expression of DHS, as well as the simultaneous over-expression of both enzymes results in deoxynivalenol levels comparable to the wild type strain. We constitutively expressed the fluorescent protein GFP in the over-expressing mutants and the wild type. Analysis revealed that the over-expressed DOHH mutant is unable to reach the rachis node at the basis of wheat spikelets. Over-expressing DHS results in a faster wheat tissue invasion compared to wild type or the double over-expressing mutant. For the first time, we identified the signalling pathway controlling expression of DHS and DOHH. Our results suggest that transcriptional balance between the two activating genes is important for the correct function of eIF5A. This will be discussed in the context of results obtained in mamalian cells.

PR4.53

Antifungal potential and ecophysiological characterization of indigenous *Trichoderma* spp. isolated from Nile Valley (Egypt)

Marym Marzouk[1] Mohamed Ali[2] Manal Yasser[3] Ahmed Mousa[3] Irina Druzhinina[1]

1. Research Area Biotechnology and Microbiology, Working Group Microbiology, Institute of Chemical Engineering, Vienna University of Technology 2. Botany Department, Faculty of Science, Cairo University, Egypt 3. Botany Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt

Plant pathogenic fungi are the most devastating threats to crop production in Egypt. The need for effective and environmentally friendly control for agriculture (biocontrol) as an alternative to the use of chemical fungicides puts the genus of highly mycoparasitic filamentous fungus – *Trichoderma* in a special focus. The antagonistic potential of 62 Egyptian *Trichoderma* strains, which were identified by means of multiloci DNA barcodes, was assessed in dual confrontation tests with plant pathogenic fungi *Alternaria alternate*, *Botrytis cinrea*, *Fusarium solani*, *Macrophomina phaseolina*, *Rhizoctonia solani* and *Sclerotium rolfsii* . Antagonistic behavior of aggressive *Trichoderma* strains against each of the concerned pathogens was further studied on low nutrition medium and examined microscopically. Moreover, testes for volatiles and inhibitors were carried to emphasize the biological interaction. Among all tested *Trichoderma* strains, the dominant *T.* cf. *harzianum* phylotypes revealed the strongest inhibitory effect against all tested pathogens (66.3% - 100 % growth inhibition). These strains were able to completely overgrow the pathogens and were the best to inhibit the production of sclerotia by *R. solani* and *S. rolfsii*. The other tested species (*T. asperelloides*, *T. brevicompactum*, *T. ghanense, T. longibrachiatum*, *T. citrinoviride* and the two putatively new species) varied in their antagonistic potential depending on the host (prey) species. To understand the information stored in the genome of the highly mycoparasitic *T.* cf. *harzianum* strains we characterized their metabolism using BIOLOG Phenotype MicroArray (PM) technology.

PR4.54

Functional Analysis of *Ustilago maydis* effector Tin3 of Cluster 19A

Nina Neidig, Thomas Brefort, Regine Kahmann
Max-Planck Institut Marburg

Ustilago maydis is a biotrophic fungal plant pathogen that causes smut disease in its host plant maize. Previous genomic studies revealed that *Ustilago maydis* depends on a variety of novel secreted effector proteins to establish a compatible interaction with its host plant. With respect to tumor formation cluster 19A encoding 23 effectors is of special interest, as cluster mutants still proliferate inside the plant tissue but fail to produce tumors. Sub-deletions and single gene deletions of cluster 19A have shown that the major effectors reside in the leftmost half. Especially *tin3* (*tumor inducing 3*), a unique gene, contributes significantly to tumor formation. Using fluorescence microscopy and immunoblot-analysis it was shown that Tin3 is secreted into the apoplastic space and that it accumulates at hyphal tips and cell-to-cell passages. In Yeast-two hybrid approaches two interesting interaction partners for Tin3 were identified: Mir3, a plant defense related cysteine protease and Beclin1, an autophagy related protein of maize. We have present evidence that Tin3 is inhibiting the protease activity of Mir3 in in-vitro protease-assays and in plant lysates of infected maize plants. In addition, we are currently investigating the influence of Tin3 on autophagy induced by pathogen infection via yeast-complementation assays coupled with fluorescence microscopy. With these studies we hope to identify the role of Tin3 during pathogenic development of *U. maydis*.

PR4.55

Proteinaceous elicitor from ascomycete pathogen *Leptosphaeria maculans* induces resistance in oilseed rape

Miroslava Novakova[1] Vladimir Sasek[1] Phuong Kim Dinh[2] Olga Valentova[2] Lenka Burketova[1]
1. Laboratory of Plant Pathophysiology, Institute of Experimental Botany AS CR, Na Karlovce 1a, Praha 160 00, Czech Republic 2. Department of Biochemistry and Microbiology, Institute of Chemical Technology, Technická 5, 166 28 Praha 6, Czech Republic

During the interaction of pathogen and its host comes to considerable exchange of molecular information between both participants. Molecules originating from either pathogen or host capable of inducing resistance response of host are referred as elicitors. Different types of elicitor molecules, including oligosaccharides, glycoproteins and peptides, and phospholipids have been identified.

Studying interaction of Dothideomycete *Leptosphaeria maculans* with its natural host *Brassica napus* we are searching for elicitors produced during in vitro cultivation. Application of medium filtrate after 10 days of *L. maculans* cultivation protects *B. napus* plants from subsequent *L. maculans* infection. Using gene expression analysis by RT-qPCR the biological effect of the filtrate is supported by detection of elevated expression levels of defence marker genes in *B. napus*. Incubation of elicitor with proteinase K lowers the biological effect indicating the proteinaceous character of the elicitor. Moreover, the elicitor was precipitated with ammonium sulphate. Using the isoelectric focusing in combination with ion exchange chromatography we are identifying the elicitor.

PR5.56

Host-Induced Gene Silencing in Phytopathogenic Fungi Attacking Cereals

Daniela Nowara, Götz Hensel, Jochen Kumlehn, Patrick Schweizer
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

Functional genomics in obligate biotrophic phytopathogenic fungi are severly hampered by the fact that they cannot be grown on artificial media. This makes it difficult to generate, select and cultivate transformants or mutants which are affected in their ability to infect the host plant.

Recently we discovered host-induced gene silencing (HIGS) as a new method to knock down the transcript level of individual genes in *Blumeria graminis*. This was achieved by expressing RNA interference (RNAi) constructs targeting fungal sequences in the host plant. So far the mechanism how the RNAi mediating RNA molecules are transferred into the fungus and whether small interfering RNA or larger RNA would be responsible for the effect is not known.

To illustrate the effect we knocked down two different ß-1,3-glucanosyltransferase genes of *Blumeria graminis* by HIGS using transient systems as well as stably transformed plants. With this approach we could show that both genes may have different functions in cell-wall biology of *B. graminis* but are both important for the colonization of the host plant.

Currently we are also exploring HIGS in *Fusarium culmorum*, one of the causal organisms of the serious *Fusarium* head-blight disease.

PR4.57

ABC transporters in mycoparasitic fungi Trichoderma atroviride P1 and Trichoderma sp. BRM

Petra Olejnikova, Jana Pacekova, Zuzana Vihonska, Svetlana Krystofova
Departement of Biochemistry and Microbiology, Slovak University of Technology Radlinskeho 9, 81237 Bratislava Slovak Republic

Many Trichoderma spp. Have been applied in biological control of phytopathogenic fungi and used as biocontrol agents (BCA) in agriculture and forestry. Although mycoparasitic and biocontrol properties in Trichoderma spp. Have been under scrutiny for a while, the mocecular aspects of mycoparasitic activity and key genes remain largely unknown. Trichoderma spp produce secondary metabolites during mycoparasitic attack in order to harm a prey. In the same time, they increase the resistance against antibiotics of other fungal species. One of many genes responsible for resistance against antifungal compounds was recently identified as Taabc2, a gene encoding an ABC transporter in T. Atroviride P1. TAABC2 belongs to PDR transporters, an ABC transporter subfamily consisting of 8 genes of full-length and one gene of half-size. In our work we analyzed transcriptional changes of PDR transporters named TAPDR5 and TAPDR12 which are homologous to yeast Pdr5p and Pdr12p. The expression profiles of Tapdr5 and Tapdr12 were obtained for T. Atroviride P1, Trichoderma sp. BRM (benomyl-resistant mutant) and Trichoderma sp. T6, a strain prepared by protoplast fusion of T. Atroviride P1 and Trichoderma sp. BRM. The transcription profile was obtained under various culture conditions and in presence of antifungal compounds, organic acids, cell wall and mycelia of fungal pathogens. T6 strain exhibited significantly increased mycoparasitic abilities than parental strains. An individual gene expression 175rofile was observed for each Trichoderma strain.

PR4.58

Functional and molecular analysis of newly identified sulfate transporter in pathogenic *Fusarium* species with respect to their virulence and ability to infect potato.

Sebastian Pilsyk[1] Hanna Gawinska-Urbanowicz[2] Marzena Sienko[1] Renata Natorff[1] Urszula Perlinska-Lenart[1] Joanna Kruszewska[1]

[1.] Institute of Biochemistry and Biophysics, Polish Academy of Sciences [2.] The Plant Breeding and Acclimatization Institute (IHAR)

AstA protein (alternative sulfate transporter) represents a little known type of sulfate trasporter, belonging to an extensive and poorly characterized family of allantoate permeases Dal5. In *Aspergillus nidulans* the *astA* gene is under control of Sulfur Metabolite Repression (SMR). The closest homologs of *astA* are frequent in evolutionarily distant fungi belonging to the *Pezizomycotina* subphylum (orders *Sordariales* and *Eurotiales*) which exhibit similar plant pathogenicity. They are mostly crop pathogens and are represented by the following sequenced species: *Fusarium graminearum*, *F. verticillioides*, *F. oxysporum*, *Nectria haematococca*, *Verticillium albo-atrum*, *V. dahliae and Leptosphaeria maculans*. The AstA homolog is also present in: cellulolytic *Chaetomium globosum* detrimental to paper industry, opportunistic human pathogen *Neosartorya fischeri*, harmless *Podospora anserina* inhabiting cattle dung, as well as in entomobiocontrol fungi like *Cordyceps militaris* and *Metarhizium anisopliae*.

Fusarium sp. fungi, like *F. solani*, *F. oxysporum* and *F. sambucinum,* contribute to serious devastation of potato crops and increase the cost of cultivation due to application of pesticides. The main problem in the fight with plant pathogenic fungi lies in their metabolic and protein similarity with the host. The aim of this project is to investigate the function of AstA upon infection and colonization of plant host by fungal pathogens, like *Fusarium sambucinum*. The study will involve infection of potato tubers with *astA* deletion mutant of *F. sambucinum*.

PR4.59

Exploring the basidiomycete defensome using RNA sequencing

David F Plaza, Stefanie Schmieder, Silvia Bleuler-Martinez, Markus Aebi, Markus Künzler
Institute of Microbiology, ETH Zurich

Fungi undergo complex symbiotic and antagonistic interactions with other organisms in nature. As an example, fungal mycelium (M) and fruiting bodies (FBs) are preyed by fungivorous vertebrates and invertebrates. As defense against their predators, fungi produce secondary metabolites, peptides and proteins that interfere with the development or the growth of these organisms. In the inky cap mushroom *Coprinopsis cinerea*, several defense lectins and protease inhibitors have previously been found to be specifically and constitutively expressed in FBs. In this project, we aim at the identification of protein-encoding genes in *C. cinerea* whose expression is induced in the vegetative mycelium upon predation by fungal-feeding nematodes using RNA Sequencing by Oligonucleotide Ligation and Detection (SOLiD). As a first step towards this goal, we compared the transcriptomes of young FBs and unchallenged M to identify differentially expressed genes. The first striking observation was that most of the protein-encoding genes were expressed under these conditions (88% and 91% in FBs and M, respectively). Second, the fruiting body-specific expression of genes coding for previously identified defense proteins, including *C. cinerea* lectin 2 (CCL2), *Coprinopsis* galectin 2 (CGL2) and protease inhibitor of *Coprinopsis* 1 (PIC1), was confirmed. Third, several genes encoding cytoplasmic and secreted proteins with a predicted RicinB fold were shown to be differentially expressed. Currently, the transcriptomes of *C. cinerea* mycelium challenged with various fungivorous nematodes or subjected to various stress conditions, including oxidative stress, starvation, heat shock and mechanical damage, are sequenced. A preliminary analysis of this data will be presented.

PR4.60

Differential responses of sorghum to inoculation with two varieties of *Sporisorium reilianum*

Alana Poloni, Jan Schirawski
Uni Göttingen

Sporisorium reilianum is a biotrophic pathogen of maize and sorghum that causes head smut. This pathogen exists in two varieties (SRS and SRZ) that cause spore formation preferentially on sorghum (SRS), or only on maize (SRZ). To understand the different disease capacities, we investigated the infection process of the two varieties on sorghum. Microscopy of infection sites showed that both varieties are able to penetrate and ramify in leaves. Whereas SRS successfully spreads inside the plant from leaves to the floral meristems, hyphae of SRZ are only observed in leaf blades and leaf sheaths, but not in stems or meristems. To find out whether SRZ encounters more or stronger plant defense reactions, we investigated H_2O_2 production, callose deposition and phytoalexin generation. A strong H_2O_2 reaction could be detected at penetration sites of SRZ but not of SRS at one day after inoculation (dai). Callose was also found to accumulate in higher levels at 2 dai in plant cells that were colonized by SRZ than by SRS. Red pigmented phytolaexins were produced at 3 dai only when sorghum was inoculated with SRZ. Interestingly, in leaf regions containing phytoalexins, hyphae were short and displayed an unusual morphology, suggesting an important role of this defense mechanism against *S. reilianum*.

PR4.61

Characterization of two putative Salicylate hydroxylases from *Ustilago maydis*

Franziska Rabe, Armin Djamei, Volker Vincon, Ziba Ajami-Rashidi, Gunther Doehlemann, Regine Kahmann
Max Planck Institute for terrestrial Microbiology, Marburg

Plants have evolved a complex system to defend themselves against pathogens. One of the key regulators for the defense against pathogens is salicylic acid (SA). As a biotrophic pathogen the fungus *Ustilago maydis* must actively suppress the SA production of its host *Zea mays*. One effector involved in this is Cmu1, a secreted chorismate mutase. Cmu1 is translocated into the host cytosol where it interferes with the SA biosynthesis pathway of the plant.

This study focuses on the characterization of two putative *U. maydis* effectors, Um03408 and Um05230, which may also be involved in controlling SA levels. Both proteins are bioinformatically predicted to act as salicylate hydroxylases degrading salicylate to catechol. Real-time PCR analyses showed that *um03408* and *um05230* are strongly upregulated in the early phase of plant infection. By performing salicylate hydroxylase activity assays the SA hydroxylase activity of Um05230 could be verified. However, strains with a deletion of both genes showed no attenuation in virulence. As *um03408*, *um05230*, and *cmu1* could all contribute to virulence, the importance of these genes for the pathogenic development of *U. maydis* may become evident only when all three genes are deleted. Such a strain is under construction and first results on its phenotype will be presented.

PR4.62

A Novel Organ Specific Effector Involved in the *Ustilago maydis* – Maize Interaction

Amey Redkar[1] Ziba Ajami - Rashidi[1] Virginia Walbot[2] Gunther Doehlemann[1]

[1.] *Department of Organismic Interactions, Max Planck Institute for Terrestrial Microbiology, Marburg*

[2.] *Department of Biology, Stanford University, Stanford, California*

Ustilago maydis is a biotrophic smut fungus which causes formation of plant tumors in maize. The infection is commonly observed on all the vegetative and floral organs of the host plant. Given the fundamental differences between the different maize organs that are colonized by *U. maydis*, we hypothesized that the fungus deploys organ specific effectors to manipulate physiology and development of specific host organs (1). In the present study, we identified a novel secreted protein, termed See1 (Seedling efficient effector 1) that is organ-specifically regulated and is strongly induced in leaves and weakly in tassels. *U. maydis* deletion mutants for *see1* are found to show a strong reduction of tumor formation in maize seedlings. Mutant hyphae successfully enter the leave tissue but are arrested during the proliferation stage. In contrast, the *Δsee1* mutant induces normal tumor formation in tassels. To localize See1 during the disease progression we applied confocal microscopy using mCherry-tagged See1 protein. At present, we are aiming for the functional characterization of See1 to elucidate the organ-specific function of this effector.

(1) Skibbe D*, Doehlemann G*, Fernandes J, Walbot V. (2010) Maize tumors caused by *Ustilago maydis* require organ-specific genes in host and pathogen. Science 328:89-92.

PR4.63

Fungal-insect interactions with respect to secondary metabolism

Annika Regulin, Ulrike Fohgrub, Frank Kempken

Abteilung für Botanische Genetik und Molekularbiologie, Botanisches Institut und Botanischer Garten, Christian-Albrechts-Universität zu Kiel, Olshausenstr. 40, 24098 Kiel, Germany

Fungi synthesize an astonishing variety of secondary metabolites, some of which belong to the most toxic compounds in the living world. Even though little is known about the benefit of these metabolites, the ability to regulate the secondary metabolism might be seen as an evolutionary adaptation. Presumably fungi regulate secondary metabolites (e.g. mycotoxine) in response to confrontation with natural competitors like insects to guarantee efficient exploitation of environmental resources (1-3). Admittedly it should be mentioned that secondary metabolites are not the only defence mechanisms of fungi (4).

In order to enlighten the biological function of these secondary metabolites with reference to chemical defence reactions of insect-fungal interactions, we utilized complementary approaches of experimental ecology and functional genomic techniques. A further aspect was to investigate the influence of these competitors at trophic interactions.

In our current research the vinegar fly *Drosophila melanogaster* and its natural antagonist *Aspergillus nidulans* are used as an ecology model system. To analyse fungal up- or down regulated target genes in the interaction of *A. nidulans* with *Drosophila* larvae microarray analysis was performed. Quantitative RT-PCR confirms up-regulation of the global regulator *laeA* as well as of *aflR*. Moreover several other genes are up-regulated under competing conditions. Candidate genes are being used for reporter gene analysis and RNAi constructs are being used for competition experiments.

1. Rohlfs, M., Albert, M., Keller, N. P., and Kempken, F. (2007) *Biol Lett* 3**,** 523-25.
2. Kempken, F., and Rohlfs, M. (2010) *Fungal Ecol* 3**,** 107-14.
3. Rohlfs, M., Trienens, M., Fohgrub, U., and Kempken, F. (2009) *in* "The Mycota XV. Physiology and Genetics: Selected Basic and Applied Aspects" (Anke, T., Ed.), , Springer Heidelberg, New York, Tokyo, pp. 131-51
4. Kempken, F. (2011) *Mol Ecol* 20, 2876-77.

PR4.64

Chemical interaction in the mycorrhizosphere: impact of fungal hormones on *Tricholoma vaccinum* and its host

Katharina Reiher[1,2] Katrin Krause[1] Erika Kothe[1] Wilhelm Boland[2]

[1.] FSU Jena - Mikrobielle Phytopathologie, [2] MPI für chemische Ökologie, Jena

The ectomycorrhiza between the widespread basidiomycete *Tricholoma vaccinum* and its tree host spruce (*Picea abies*) represents a model system for mutualistic plant-fungus interaction. Besides the mycorrhiza forming basidiomycetes saprophytic fungi inhabit the soil and produce morphogenic substances, which modulate plant root development. Zygomycetes belonging to the *Mucorales* communicate *via* a system based on apocarotinoids. The earliest intermediate after the cleavage of β-carotene is a C18-ketone, called D'orenone, which was shown to stop root hair formation in *Arabidopsis thaliana*. If D'orenone modulates the morphology and physiology of *Picea abies* is still unknown. However, *T. vaccinum* produces the phytohormone indole-3-acidic acid (IAA) which also leads to an extensive hyphal branching and an increased and faster Hartig' net formation during mycorrhization. In this work the effect of D'orenone on the root system will be tested in axenic cultures and in co-cultivation with *T. vaccinum* - wildtype and IAA overexpressing mutants - *via* differential display. Moreover, the receptors for D'orenone perception in *T. vaccinum* will be studied with radioactive labeled D'orenone or FISH. Additionally, co-cultures of Zygomycetes with *T. vaccinum* and with the mycorrhized tree will be done to study the interaction of all three partners. This could improve the knowledge about communication between unrelated fungi which live closely together in the mycorrhizosphere and give hints to interacting "mycohormones", affecting tree morphology, mycorrhization rate, and, thereby, influence tree health and whole ecosystem fitness.

PR4.65

Investigation of unconventionally secreted proteins in *Ustilago maydis*

Stefanie Reissmann[1] Sina Krombach[1] Till Ringel[1] Florian Bochen[1] Kerstin Schipper[2] Thomas Brefort[1] Michael Mann[3] Regine Kahmann[1]

[1.] Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch Strasse 10, D-35043 Marburg, Germany

[2.] Heinrich-Heine University Düsseldorf, Institute for Microbiology, D-40204 Düsseldorf, Germany [3.] Max-Planck Institute for Biochemistry, D-82152 Germany

Secreted fungal proteins play a crucial role during the biotrophic interaction between the smut fungus *Ustilago maydis* and its host plant *Zea mays*. In the last decade it has been well established that proteins without a signal peptide can also be targeted to the outside of the cell in an ER/ Golgi independent manner. We want to identify such unconventionally secreted proteins in *U. maydis* and investigate their potential function as pathogenicity factors. Our approach is based on affinity purification of tagged candidate proteins, previously detected in the apoplastic fluid of infected maize leaves. Four of the twelve candidate proteins tested so far could be detected in culture supernatants. One candidate protein, Um02959, is a homolog to the *Dictyostelium discoideum* acyl-CoA-binding protein AcbA. In *D. discoideum* AcbA is unconventionally secreted as full-length protein and extracellularly processed into a small peptide (SDF-2) that triggers terminal spore differentiation upon interaction with a membrane receptor. We were able to locate the *U. maydis* protein Acb1 in the culture supernatant of fungal hyphae only in the presence of a protease inhibitor, suggesting extracellular processing. Trypsin digested purified UmAcb1 protein as well as hyphal culture supernatant triggered spore formation in *D. discoideum* indicating the presence of an SDF-2-like peptide in *U. maydis*. Interestingly deletion of the *acb1* gene in *U. maydis* resulted in a late virulence defect. We are currently attempting to rescue this phenotype by extracellular Um-SDF-2 peptide.

PR4.66

The Putative E3 Ubiquitin Ligase Ubl1 is a Central Regulator of Growth, Morphogenesis, and Virulence in *Fusarium* spp.

John Ridenour, Robert Hirsch, Hun Kim, Sandeep Sharma, Peter Horevaj, Burton Bluhm
Department of Plant Pathology, University of Arkansas

Fusarium verticillioides is a ubiquitous and destructive pathogen of maize. In addition to reducing yields, *F. verticillioides* produces fumonisins, a group of polyketide-derived toxins linked to cancer and birth defects in humans, as well as acute and chronic toxicoses in livestock. Recently, we identified *UBL1* in *Fusarium verticillioides* via a forward genetic screen aimed at identifying novel genes involved in pathogenesis. *UBL1* is predicted to encode a UBR1-like E3 ubiquitin ligase. Among eukaryotes, the UBR1-like E3 ubiquitin ligase is a broadly conserved component of the N-end rule proteolytic pathway; however, its role in filamentous fungi has not been extensively investigated. Targeted disruption of *UBL1* in the wild-type strain resulted in a highly pleiotropic phenotype, including reduced conidiation, altered hyphal morphology, increased pigment production, and impaired amylolitic activity. Additionally, disruption of *UBL1* led to a drastic reduction in virulence on maize kernels; however, fumonisin B1 biosynthesis per unit growth was not significantly different from the wild-type strain. Yeast two-hybrid assays revealed that Ubl1 interacts with components of G-protein signaling known to regulate pathogenesis. To investigate the possibility that *UBL1* plays a conserved role in fungal virulence, we deleted *UBL1* orthologs in the closely related pathogens *Fusarium graminearum* and *Fusarium oxysporum*. Interestingly, these mutants revealed that *UBL1* orthologs regulate diverse components of pathogenesis among *Fusarium* spp. This study directly implicates *UBL1* in growth and development in *Fusarium* spp. and provides one of the first links between a UBR1-like E3 ubiquitin ligase and virulence in plant pathogenic fungi.

PR4.67

Fungal ecology in a petri dish – establishment of a laboratory model system for assessing the role of fruiting body lectins in the fungal defense against predators

Stefanie S Schmieder, David F Plaza, Silvia Bleuler-Martinez, Markus Aebi, Markus Künzler
ETH Zürich

Lectins are proteins that specifically and reversibly bind carbohydrates. Among their diverse physiological roles, one universally described function is their participation as recognition and effector molecules in the innate defense of multicellular organisms against all kinds of predators.

In the fungal kingdom, a large number of lectins with different specificities have been isolated from fruiting and resting (sclerotia) bodies of multicellular representatives of the phyla Basidiomycota and Ascomycota. Various lines of evidence suggest that these lectins play a role as effector molecules in the defense against predators. Upon ingestion of the host cytoplasm, the fungal lectins bind to specific glycoconjugates in the digestive tract of the predators which leads to inhibition of development and eventually killing of the predator by a yet unknown mechanism. Toxicity of fruiting body lectins was so far assayed using model organisms e.g. *Caenorhabditis elegans* for nematodes.

To demonstrate the ecological significance of the observed toxicities, we established a laboratory model system for assaying the toxicity of these lectins towards fungivorous nematodes such as *Aphelenchus avenae*. In this system, we use the filamentous fungus, *Ashbya gossypii*, as a host. This fungus can easily be manipulated to express single or multiple exogenous lectins in the vegetative mycelium. Using this system, we could demonstrate a severe reduction in population growth of *Aphelenchus avenae* as a result of the expression of a single fruiting body lectin in the mycelium of the host fungus. Our results show that fungivores are susceptible to at least some of the fruiting body lectins.

PR4.68

Characterization of function and kinetics of Dicer proteins in the wheat pathogen *Mycosphaerella graminicola* during host infection

Klaas Schotanus, Eva H. Stukenbrock
Max Planck Institute for Terrestrial Microbiology

We investigate the role of RNA interference (RNAi) in the wheat pathogen *Mycosphaerella graminicola* during host infection by deletion of Dicer and Drosha proteins. A main goal is to determine if RNAi plays a role in pathogenicity and host specificity. For the transformation of *M. graminicola* we use an *Agrobacterium tumefaciens* mediated approach and we evaluate and compare wild type and mutant phenotypes in plant assays with the two hosts *Triticum aestivum* and *Brachypodium distachyon*. The transformation experiments are currently undergoing.
We have verified the computational predictions of microRNAs in the genome of *M. graminicola* by demonstrating the presence of small (26nt) RNAs using polyacrylamide gel electrophoresis. Small RNAs are expressed in rich medium as well as in plant tissue suggesting that RNAi is not restricted to the host-pathogen interaction but also play a role in the basic growth of the fungus. However, by describing the kinetics of the Dicer and Drosha encoding genes during three different time points of infection we show significant higher levels of transcript abundance during plant infection. Furthermore, we demonstrate that transcript abundance is dependent on host species indicating that RNAi in *M. graminicola* may affect both pathogenicity and host specificity.

PR4.69

Carbon Acquisition In The Ustilago/Maize Pathosystem

David Schuler[1] Ramon Wahl[1] Kathrin Wippel[2] Davide Sosso[3] Norbert Sauer[2] Jörg Kämper[1]
[1.] KIT, Institute for Applied Biosciences, Department of Genetics, Karlsruhe, Germany [2.] Friedrich-Alexander-University Erlangen-Nuremberg, Plant Physiology, Erlangen, Germany [3.] Carnegie Institution for Science, Stanford, CA, USA

Ustilago maydis is a fungal plant pathogen that infects maize plants. Investigating mechanisms for fungal carbon acquisition that are critical for the biotrophic interaction of *U. maydis* with its host, we identified Hxt1 (hexose transporter 1), a high affinity monosaccharide transporter. *Ustilago maydis Δhxt*-strains cause decreased disease symptoms, and growth in axenic culture is reduced on glucose, fructose and mannose. Surprisingly, these strains show increased growth on media containing xylose and galactose, the latter causing even a growth-inhibiting effects on wildtype strains.

In the *S. cerevisiae* hexose sensor proteins Snf3 and Rgt2, mutation of a conserved arginine residue results in a constitutively active signaling pathway. Interestingly, over-expression of a Hxt1-derivative carrying an analogous mutation decreased the virulence of *Δhxt1* strains even more.

In accordance with the significance of Hxt1 for pathogenic development we found expression of two maize glucose transporters up-regulated after infection with *Ustilago maydis*. These transporters belong to a recently identified family of sugar efflux carriers whose members in some cases can be induced by bacterial as well as fungal pathogens.

We propose that Hxt1 is required for uptake of glucose that is provided to the site of infection by plant efflux carriers, which are specifically induced after infection with *Ustilago maydis*. Further, we speculate on an additional sensor function of Hxt1 that could be most important to sense galactose and xylose levels within the plant that may be indicative for the physiological status of the host cells.

PR4.70

A surface hydrophobin in ectomycorrhiza interaction

Dominik Senftleben, Katrin Krause, Erika Kothe

Friedrich-Schiller-Universität, Institute for Microbiology, Microbial Phytopathology, Neugasse 25, D-07743 Jena, Germany

Hydrophobins are small-secreted proteins with low sequence homology. However, all proteins contain eight cysteines, which form disulfide bridges. They are divided into two classes, depending on their solubility and have a broad range of functions such as involvement in growth and development of filamentous fungi, *e.g.* formation of aerial structures. Mutual symbiosis like ectomycorrhiza is based on differential gene expression. Which was shown for hydrophobin *tthyd1*, that is up-regulated in the Hartig'net in the interaction of *Tricholoma terreum* with pine.

We investigate hydrophobins in *Tricholoma vaccinum*, a wide-spread basidiomycete (agaricales – tricholomataceae) which forms ectomycorrhiza with spruce. We want to show in our study in which stage of the life cycle respectively symbiotic interaction hydrophobins are produced, what kind of role they play with respect to function in the symbiotic tissue and whether they are regulated under heavy metal stress. Furthermore, the regulation of the four known hydrophobins (*tvhyd1*, *1b*, *2* and *3*) of *T. vaccinum* will be analyzed by RNAi experiments and heterologous expression in *Schizophyllum commune*.

PR4.71

Expression Profiling of *Solanum lycopersicoides* to Identify Mechanisms Underlying Resistance to *Botrytis cinerea*

Jonathon Smith[1] Bemnet Mengesha[2] Tesfaye Mengiste[2] Burton Bluhm[1]

[1.] Department of Plant Pathology, University of Arkansas, Fayetteville, AR, USA [2.] Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN, USA

The necrotrophic fungal pathogen, *Botrytis cinerea*, causes grey mold on a wide range of hosts, and is of major economic concern for tomato production. Tomato (*Solanum lycopersicum*; *Sl*) is highly susceptible to *B. cinerea*; however, *Solanum lycopersicoides* (*Slo*), a wild relative of tomato, is highly resistant. The overarching goal of this study is to characterize the molecular mechanisms underlying resistance to *B. cinerea* in *Slo* and identify novel target genes for improving resistance in tomato. To this end, we generated gene expression profiles from *Slo* 24 and 48 hours after inoculation with *B. cinerea*, as well as a pre-infection baseline, via high-throughput RNA-sequencing (Roche-454). Analyses of the transcriptomes revealed that numerous genes were differentially expressed in *Slo* in response to *B. cinerea*, including pathogenesis related proteins, peroxidases, osmotins, and genes involved in biosynthesis of secondary metabolites. These differentially regulated genes provide novel targets for expression analyses in tomato. Genes that are regulated differently between *Sl* and *Slo* in response to infection by *B. cinerea* are currently being characterized to determine their role in resistance. By identifying genes that confer resistance in Slo, this work will substantially refined the current understanding of defense against necrotrophic pathogens and provided targets for improving *B. cinerea* resistance in tomato.

PR4.72

Characterization Of Three Putative Transcription Factor-Coding Genes For Pathogenesis In The Plant-Pathogenic Fungus *Alternaria brassicicola*

Akhil Srivastava, Yangrae Cho
Plant and Environment Protection Sciences, University of Hawaii at Manoa

The necrotrophic fungus *Alternaria brassicicola* causes black spot disease of brassicaceous plants, including green cabbage (*Brassica oleracea*) and the oil-producing *B. napus*. Several transcription factors (TFs) have been associated with pathogenesis in phytopathogenic fungi. We know relatively little, however, about the mechanisms of regulating pathogenesis. To learn more about how pathogenesis is regulated in fungi, we have produced targeted gene knockout mutants for ~200 TF-coding genes and of research interests. We identified two genes whose mutants were putatively nonpathogenic, eleven genes whose mutants produced significantly smaller lesions compared to the wild type in preliminary pathogenicity tests, and one gene whose mutants unexpectedly produced lesions twice as large as the wild type. To clarify the functions of these genes, we studied the phenotypes of mutants for 3 of the 14 genes. The phenotypes of mutants for one gene, *Δabvf01*, were indistinguishable from the wild type in general, but mycelial growth after penetration was greatly reduced. Mutants of another gene, *Δabvf19*, grew slower with pectin as a major carbon source *in vitro* and expressed fewer transcripts than the wild type for a subset of genes that encode putative cell wall-degrading enzymes during pathogenesis. Conversely, mutants of the gene with increased virulence expressed more transcripts for these enzymes. Our study supports the importance of the coordinated regulation of genes putatively associated with using nutrients from their host plants during pathogenesis. A better understanding of how fungi regulate pathogenesis may identify specific transcription factors as targets for efficient disease management.

PR4.73

The role of secreted LysM domain proteins during the biotrophic development of *Ustilago maydis*

Nancy Stolle, Karin Münch, Regine Kahmann
Department of Organismic Interactions, Max Planck Institut for Terrestrial Microbiology

Previous studies revealed that *Ustilago maydis* depends on a variety of novel secreted effector proteins to establish a compatible, biotrophic interaction with its host plant maize. In this context, two putatively secreted LysM-containing proteins (*um11464*, *um05087*) have been identified. LysM-containing proteins are very common in pathogenic fungi and have been shown to contribute to virulence. Using homologous recombination in the solopathogenic strain SG200 we have generated deletion mutants of *um11464* and *um05087*. While the deletion of *um05087* did not show any phenotype in comparison to the progenitor strain SG200, *um11464* deletion mutants often formed cell chains, developed lateral buds and displayed bipolar growth in axenic culture. Surprisingly we observed that plant infections with *um11464* deletion mutants lead to hypervirulence. Since the mutant is able to form significantly more appressoria on the leaf surface we consider that hypervirulence is a result of the increased number of appressoria-forming filaments. *In situ* immunolocalization studies revealed that Um11464 is located predominantly at the cell surface. Therefore we assume that the protein accumulates at the fungal cell wall after secretion. Preliminary results indicate that a domain within the C-terminus of the protein might be responsible for the attachment to the fungal cell wall. Interestingly, the LysM domains which are supposed to bind carbohydrates are not involved in the binding. Currently, functional analyses are performed to investigate the biological role of Um11464 and how it affects pathogenesis negatively.

PR4.74

Functional analysis of a novel pathogenicity-associated gene *CoPRF1* in the anthracnose fungus *Colletotrichum orbiculare*

Kaoru Tanaka, Yasuyuki Kubo
Laboratory of Plant Pathology, Graduate School of Life and Environmental Science, Kyoto prefectural University, Kyoto 703-5101, Japan

Plant pathogens have co-evolved with their host plants which have evolved the defense system against their pathogens. It is known that plants express the basal immunity by the recognition of the pathogen-associated molecular patterns, but compatible pathogens suppress the plant basal defence by secreting the effector protein. *Agrobacterium tumefaciens* - mediated transformation (AtMT) was used to generate pathogenicity deficient insertional mutants in *Colletotrichum orbiculare* that causes anthracnose disease of cucumber, and a mutant named YK4524 was isolated which showed reduced pathogenicity. Genetic analysis of this mutant indicated that the insert was placed in a gene which presumably codes for an extracellular protein with signal peptide sequence, and significant homologous genes could not be recognized. So we named this gene *CoPRF1* (Pathogenesis-related factor 1). In this study, we performed functional analysis of *CoPRF1* to identify and characterize the novel pathogenicity-associated gene. Target gene disruption mutants obtained by AtMT showed significant reduction in virulence on the host leaves, while characteristics such as germination, appressorium formation and penetration hyphae formation of *coprf1* disruption mutants *in vitro* were normal, indicating that *CoPRF1* is not involved in the infection related morphogenesis. On the other hand, penetration ability of mutants was attenuated on intact cucumber cotyledons, and the elongation of its invasive hyphae was slower compared with the wild type. Thus, it was suggested that *CoPRF1* would engage in establishment of host infection.

PR4.75

Functional analysis of the tumor and anthocyanin-inducing effector protein Tin2 of Ustilago maydis

Shigeyuki Tanaka, Thomas Brefort, Regine Kahmann
Max Planck Institute for Terrestrial Microbiology

The fungus Ustilago maydis is the causal agent of smut disease in maize. The interaction with the host is governed by secreted effectors and many of the respective genes reside in clusters in the genome. Cluster19A is the largest of these clusters carrying 24 genes for putatively secreted effector proteins. Deletion mutants of the left half of cluster 19A (19A_1) show dramatic reduction of tumor formation and loss of anthocyanin induction, although the mutant retains the ability to grow inside the plant tissue. We demonstrate that the tin2 effector encoded in this region is secreted and expressed exclusively during biotrophic growth. Introduction of the tin2 gene into the 19A_1 mutant partially rescued tumor formation and fully restored anthocyanin induction. A Tin2 protein lacking the C-terminal 5 amino acids had lost this ability. In line with this, Tin2 mutant protein could not interact with cytoplasmic maize protein kinase ZmABP which was identified by yeast two hybrid screening as Tin2 interactor. Transient expression assays in Nicotiana benthamiana revealed that ZmABP was degraded proteasome-dependently. Interestingly, co-expression with Tin2 stabilized ZmABP. Tin2-binding region of ZmABP contains the phosphodegron-like motif DSGxS. When ZmABP carrying mutations in this motif was transiently expressed, the mutant protein proved more stable than the wild type protein. Therefore, it is likely that Tin2 effector masks the phosphodegron motif of ZmABP, which stabilizes functional full-length ZmABP kinase in plant cell, resulting in signal transduction leading to anthocyanin biosynthesys and tumor induction.

PR4.76

Molecular analysis of the regulation of the *stp1* effector gene in *Ustilago maydis*

Marie Tollot[1] Kerstin Schipper[2] Regine Kahmann[1]
1. MPI for terrestrial microbiology 2. University of Düsseldorf

The biotrophic maize pathogen *Ustilago maydis* relies on the secretion of effectors for successful colonization of plant tissues. *stp1* encodes an effector which is absolutely required for fungal development *in planta*. The expression of *stp1* starts during plant penetration and continues throughout all stages of biotrophic development.
The aim of this project is to identify the regulators responsible for the plant specific expression pattern of *stp1*. We first performed a promoter deletion analysis: *stp1* promoter sequences harboring various deletions were fused to the reporter *eGFP* and inserted into the *cbx* locus of strain SG200. The comparison of GFP expression levels in the resulting strains led to the identification of a short sequence potentially involved in *stp1* up-regulation *in planta*. This sequence contains two conserved elements, A1 and B, which are both required for full expression. A1 is related to the binding sites of two previously described transcription factors (TF), Mzr1 and Biz1, while B is unknown. When overexpressed, Mzr1 and Biz1 activate *stp1* expression (Zhen *et al.*, 2008; Flor-Parra *et al.,* 2006). Using a one-hybrid approach, we could show that both TFs bind to A1 but not B. As B is also required for *stp1* expression, this indicates that additional TFs are involved in the regulation of *stp1*.

To confirm these results and identify new TFs binding to the *stp1* promoter, we now plan to use magnetic beads coated with the target DNA to isolate TFs of interest from a nuclear extract of infected maize leaves. Initial results will be presented.

PR4.77

Biosynthesis of indole-3-acetic acid in basidiomycetes

Andrea Ulbricht
Friedrich-Schiller-Universität Jena

Mycorrhiza is a fundamental phenomenon in the interaction of fungi and plants. Most important for formation and functionality of this symbiosis is a vital communication of both symbionts. A key player in this process is indole-3-acetic acid (IAA), a well known growth hormone of plants that is also able to coordinate growth promotion and branching of hyphae in some fungi. Furthermore IAA can be synthezised in both interaction partners by several potential pathways. In preliminary work a tryptophane (Tryp) dependent pathway was postulated for *Tricholoma vaccinum*.

Since there are several potential ways to synthesize IAA in a Tryp dependent matter we want to get further insights into the cellular mechanism. Therefore we want to perform growth experiments with several plant interacting fungi (*Tricholoma vaccinum*, *Paxillus involutus, Armillaria mellea*), and saprophytes (*Schizophyllum commune*, *Heterobasidium annosum, Leucoagaricus leucothites, Lyophyllum loricatum*) in dependence of different potential intermediates of IAA-production (Tryp, indole-3-pryuvate [IPA], indole-3-acetaldehyde [IAAld]) as well as IAA itself. In case of *S. commune* a mono- and dikaryon and a IAA-synthesizing mutant are tested. To this aim length and branching degrees of hyphae will be analyzed.

In addition the expression of acetaldehyde-dehydrogenase will be tested by qPCR. This enzyme catalyzes the conversion of aldehydes to acids and in our case the of IAAld to IAA.

PR4.78

Functional analysis of MiSSPs (Mycorrhiza induced Small Secreted Proteins) from the mutualistic fungal symbion*t Laccaria bicolor.*

Claire Veneault-Fourrey[1] Yohann Daguerre[1] Annick Brun-Jacob[1] Jonathan Plett[1] Annegret Kohler[1] Minna Kemppainen[2] Alejandro Pardo[2] Francis Martin[1]

1. UMR1136, INRA-Lorraine Université, 54280 Champenoux, France 2. Micología Molecular, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes and CONICET, Roque Sáenz Peña 352, B1876 Bernal, Provincia de Buenos Aires, Argentina.

Boreal and temperate forest ecosystems depend on ectomycorrhizal symbiosis for trees nutrition, productivity and stress resistance. Ectomycorrhizal (ECM) symbioses appear several times during fungi evolution and ECM fungi likely derived from saprotrophic ancestors. Despite their ecological importance, very little is concerning the molecular dialogue that occurs between tree roots and ECM fungi to sustain the development of symbiosis. Recently, *L. bicolor* Mycorrhizal induced Small Secreted Protein7 (MiSSP7) has been proved to be the first symbiotic effector required for the development of symbiosis due to its targeting to the plant nucleus (Plett et al., 2011). Transcriptomic analyses reveal the presence of several MiSSPs within the genome of the symbiotic fungus *L. bicolor* (Martin et al., 2008). We have performed functional analysis of several MiSSPs in order to (i) demonstrate that MiSSPs are required for symbiosis development and (ii) to identify which plant compartment / proteins are targeted by MiSSPs. We will present and discuss our last results with regards to the putative role of MiSSPs as fungal effectors.

Plett et al. 2011, Current Biology, 21(14):1197-203 ; Martin et al., 2008 Nature 452, 88-92

PR4.79

Analysis Of The *Fusarium graminearum* Species Complex From Grain Crops Provides Evidence Of Species-Specific Differences In Host Preference

Anne-Laure Boutigny[1] Todd Ward[2] Gert Van Coller[3] Bradley Flett[4] Sandra Lamprecht[5] Kerry O'Donnell[2] Altus Viljoen[1]

1. Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa 2. Bacterial Foodborne Pathogens and Mycology Research Unit, Agricultural Research Service, United States Department of Agriculture, Peoria, IL 61604, USA 3. Institute for Plant Production, Western Cape Department of Agriculture, Private Bag X1, Elsenburg 7607, South Africa 4. Grain Crops Institute, Agricultural Research Council, Private Bag X1251, Potchefstroom 2520, South Africa 5. Plant Protection Research Institute, Agricultural Research Council, Private Bag X5017, Stellenbosch 7600, South Africa

Species identity and trichothecene toxin potential of 560 members of the *Fusarium graminearum* species complex (FGSC) collected from diseased wheat, barley and maize in South Africa was determined using a microsphere-based multilocus genotyping assay. Although three trichothecene types (3-ADON, 15-ADON and NIV) were represented among these isolates, strains with the 15-ADON type predominated on all three hosts. A significant difference, however, was identified in the composition of FGSC pathogens associated with Gibberella ear rot (GER) of maize as compared to Fusarium head blight (FHB) of wheat or barley ($P < 0.001$). *Fusarium graminearum* accounted for more than 85% of the FGSC isolates associated with FHB of wheat and barley ($N = 425$), and was also the dominant species among isolates from maize roots ($N = 35$). However, with the exception of a single isolate identified as an interspecific hybrid between *F. boothii* and *F. graminearum*, GER of maize ($N = 100$) was exclusively associated with *F. boothii*. The predominance of *F. graminearum* among FHB isolates, and the near exclusivity of *F. boothiii* among GER isolates, was observed across all cultivars, collection dates, and provinces sampled. Because these results suggest a difference in host preference among species of the FGSC, we hypothesize that *F. graminearum* may be less well adapted to infect maize ears than other members of the FGSC.

PR4.80

Symptom formation in *Sporisorium reilianum* is modulated by effector proteins

Yulei Zhao, Hassan Ghareeb, Habib Mohammad T, Jan Schirawski
Georg August-Universität Göttingen

Sporisorium reilianum and *Ustilago maydis* are closely related biotrophic pathogens that cause different symptoms on maize. Following fungal inoculation of seedlings, *U. maydis* induces tumors on leaves near the infection site within a few days, whereas symptoms of *S. reilianum* are visible only at flowering time, and include the formation of spores and leaf-like structures in inflorescences. To identify genes responsible for species-specific symptom generation, we compared the highly syntenic genomes of both fungi and discovered the presence of genomic regions of weakly conserved genes mainly encoding secreted proteins [1]. Deletion of the largest divergence region of about 30 genes in *S. reilianum* dramatically reduced virulence, and led to wilting of inoculated leaves. By subdeletion analysis we identified a region encoding three related secreted effector proteins as responsible for the early leaf wilting phenotype. Only two of the three genes are up-regulated during biotrophic growth of *S. reilianum* as determined by qRT-PCR analysis. Individual gene deletion indicated that both up-regulated genes contribute equally to symptom formation in *S. reilianum*. This shows that symptom formation of *S. reilianum* is modulated by fungal effector proteins.

[1] Schirawski et al., 2010. Science 330: 1546-1548.

PR4.81

High-resolution crystal structure of the LysM effector Ecp6 of the fungal tomato pathogen *Cladosporium fulvum*

Andrea Sanchez-Vallet [1] Raspudin Saleem Batcha [2] Anja Kombrink [1] Dirk-Jan Valkenburg [1] Jeroen R. Mesters [2] Bart P.H.J. Thomma [1]
[1.] Laboratory of Phytopathology, Wageningen University, 6708 PB Wageningen, Netherlands .[2.] Institute of Biochemistry, University of Lübeck, D-23538 Lübeck, Germany.

Plants induce defence responses upon recognition of chitin, the primary structural component of fungal cell walls. To prevent the induction of host defense responses, the plant pathogenic fungus *Cladosporium fulvum* secretes large amounts of ECP6 protein which binds chitin with high affinity and thus prevents their recognition by plant receptors. ECP6 is a Lysin motif (LysM)-containing effector protein with orthologs, known as LysM effectors, that are widely distributed in the fungal kingdom. LysM effectors of the Septoria tritici blotch pathogen *Mycosphaerella graminicola* and the rice blast pathogen *Magnaporthe oryzae* scavenge chitin molecules in a similar fashion as *C. fulvum* ECP6, demonstrating the importance of LysM effectors in fungal pathogenicity. LysM domains are highly conserved in many proteins produced by prokaryotes and eukaryotes which bind to peptidoglycan and chitin. However, the specific interactions of LysM domains with their substrates have not yet been elucidated. Here, we present a high-resolution crystal structure of the LysM effector ECP6. The structure revealed that each of the three LysM domains from ECP6 adopts an αββα tertiary structure, in which the chitin-recognition site is localized in the highly conserved region in the loop between the first β-sheet and the first α-helix. The close interaction that occurs between the first and the third LysM domain of an Ecp6 monomer forms a high-affinity binding site for single chitin molecule.

PR4.82

LysM effectors of fungal plant pathogens contribute to virulence in various manners

Anja Kombrink, Jason J. Rudd, Bart P.H.J. Thomma
Laboratory of Phytopathology, Wageningen University, 6708 PB Wageningen, Netherlands

LysM effector genes are found in the genomes of a wide range of fungal species. LysM effectors are secreted proteins that contain a varying number of LysM domains and no other recognizable protein domains. LysM domains are carbohydrate-binding modules that occur in various proteins that are produced by a variety of organisms. Ecp6 is the first characterized LysM effector that was isolated from the tomato leaf mould fungus *Cladosporium fulvum* and that is instrumental for fungal virulence. Carbohydrate binding assays demonstrated that Ecp6 specifically binds chitin, the major constituent of fungal cell walls that acts as microbial-associated molecular pattern (MAMP) that triggers immune responses upon recognition by the host. We demonstrated that the chitin-binding effector Ecp6 can compete with plant receptors for chitin binding, and thus prevents the activation of immune responses. Two orthologues of Ecp6 were identified in the fungal wheat pathogen *Mycosphaerella graminicola*, of which one suppresses chitin-induced immune responses in a similar fashion as Ecp6. Interestingly, unlike Ecp6, both *M. graminicola* LysM effectors were able to inhibit degradation of fungal hyphae by plant chitinases. Many fungal genomes carry multiple LysM effector genes that share only low sequence conservation and encode varying LysM domain numbers per molecule. Therefore, we hypothesize that different fungal LysM effectors are likely to bind different carbohydrate substrates, exert other functions, or are active in other stages of the fungal life cycle than plant infection. We will report on our most recent findings on LysM effector substrates and functions.

R4.83

The emergence of *Botryosphaeria* sp. as a wheat necrotrophic pathogen in Australia

Peter S. Solomon
Plant Sciences Division, Research School of Biology, The Australian National University, Canberra, ACT 0200, Australia.

Necrotrophic pathogens of wheat have a considerable impact on yield globally. The primary pathogens in this class include *Pyrenophora tritici-repentis*, *Stagonospora nodorum* and *Mycosphaerella graminicola*. Over the last decade, increasing levels of *Botryosphaeria* sp. have been identified on mature wheat grain in Australia. The infection of the grain by *Botryosphaeria* sp. results in slightly shrivelled grain with a pale colour closely resembling that of *Fusarium graminearum* – infected wheat. As a consequence of the infection, *Botryosphaeria* sp-infected grain is downgraded for animal feed only, resulting in considerable economic losses. Almost nothing is known about the pathogen or resulting disease and it is unclear though if the infected grain is actually harmful to human health. A project has recently started in the Solomon laboratory to look at the mycotoxin producing potential of *Botryosphaeria* sp. To do this, commercially grown infected wheat grain will be analysed using a variety of mass spectrometry techniques to determine the presence of known mycotoxins. In this poster, preliminary data will be presented on the growth and phenotyping of the *Botryosphaeria* sp. strains identified thus far as well some of the initial mass spectrometry analysis.

PR4.84

Fungal phenotypic plasticity in response to fungivores

Silvia Caballero, Marko Rohlfs

J.F Blumenbach Institute of zoology and Anthropology, Georg-August University of Göttingen, Germany

Secondary metabolites are natural products, principally synthesized by plants, bacteria and fungi among other organisms. Secondary metabolites are not essential for organism survival but may have other functions including adaptation to different environments. Although many beneficial as well as toxic secondary metabolites have been investigated intensively, their role in enabling fungi to explore and conquer new ecological niches remains elusive. A common threat fungi are exposed to in their natural habitat is the attack by fungivorous insects. In this context, we hypothesize that the model fungus *Aspergillus nidulans* produces secondary metabolites, e.g. the mycotoxins sterigmatocystin, as part of an adaptive response to ward off or harm fungivorous insects. We confront fungi with insects to investigate induction of changes in fungal development, secondary metabolite formation, and gene expression. By means of qRT PCR, we currently focus on changes in the expression of laeA (encodes a methyltransferase-domain protein that functions as a regulator of secondary metabolism and development) and aflR (encodes a Zn2Cys6-type sequence-specific DNA-binding protein that is thought to be necessary for expression of most of the genes in the sterigmatocystin gene cluster). On all levels of organization we observe significant alterations in fungal traits that finally modify the outcome of insect-fungus interactions.

PR4.85

Plant resistance inducer β-aminobutyric acid inhibits spore germination and growth of the ascomycete *Leptosphaeria maculans* by interfering with nitrogen metabolism

Vladimír Šašek [1] Miroslava Nováková [1,2] Petre Dobrev [3] Olga Valentová [2] Lenka Burketová [1]

1. *Laboratory of Plant Pathophysiology, Institute of Experimental Botany AS CR, Prague, Czech Republic*
2. *Department of Biochemistry and Microbiology, Institute of Chemical Technology, Prague, Czech Republic.*
3. *Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany AS CR, Prague, Czech Republic*

β-aminobutyric acid (BABA) is for decades known as an agent protecting plants from infection by broad range of pathogens. It is widely accepted that BABA has no antifungal activity and its effect is based solely on stimulation of plant immune system. Here we demonstrate that BABA displays strong *in vitro* antifungal activity against *L. maculans* with EC_{50} similar to the fungicide tebuconazole. Both spore germination and hyphal growth are affected. Unlike other resistance inducer benzothiadiazol, BABA reduced disease development on *Brassica napus* plants also when applied after inoculation. Suppression of disease progression in plants and antifungal activity *in vitro* was weaker for α-aminobutyric acid and negligible for γ-aminobutyric acid. These facts indicate that the mechanism by which BABA protects *B. napus* plants from *L. maculans* infection is based on antifungal activity. In contrast to standard antifungal assays, the medium used in our study contained no organic nitrogen. Trypton added into the medium at only 6 ppm completely reverted the effect of 2 μM BABA. Similar effect can be also achieved by addition of some proteinogenic amino acids. We hypothesised that BABA might inhibit inorganic nitrogen assimilation or might interfere with amino acid metabolism. While we have not conclusively demonstrated how BABA suppresses the disease progression, our results do indicate that antifungal activity is another mechanism by which BABA can protect plants from infection.

PR4.86

Sequential Delivery of Host-Induced Virulence Effectors by Appressoria and Intracellular Hyphae of the Phytopathogen *Colletotrichum higginsianum*

Jochen Kleemann[1] Linda J. Rincon-Rivera [1] Hiroyuki Takahara [2] Ulla Neumann [1] Emiel Ver Loren van Themaat [1] H. Charlotte van der Does [3] Stéphane Hacquard [1] Kurt Stüber [1] Isa Will [1] Wolfgang Schmalenbach [1] *Elmon Schmelzer [1] Richard J. O'Connell [1]*

[1]Department of Plant-Microbe Interactions, Max-Planck-Institute for Plant Breeding Research, Cologne, Germany [2]Department of Bioproduction Science, Faculty of Bioresources and Environmental Sciences, Ishikawa Prefectural University, Ishikawa, Japan [3]Department of Plant Pathology, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands

Phytopathogens secrete effector proteins to manipulate their hosts for effective colonization. Hemibiotrophic fungi must maintain host viability during initial biotrophic growth and elicit host death for subsequent necrotrophic growth. To identify effectors mediating these opposing processes, we deeply sequenced the transcriptome of *Colletotrichum higginsianum* infecting *Arabidopsis*. We found that expression of many effector genes is plant-induced and that distinct sets of effectors are deployed in successive waves by particular fungal cell-types. Using fluorescent protein tagging and TEM-immunogold labelling, effectors were localized to stage-specific compartments at the host-pathogen interface. Early-expressed effectors are focally secreted from appressorial penetration pores before host invasion. These proteins may function to suppress early plant defense responses, which we found to be activated before fungal entry. Later-expressed effectors accumulate in structures formed at the interface between biotrophic primary hyphae and living host cells, implicating these specialized hyphae in effector delivery. By transient expression in *Nicotiana benthamiana* leaves, we identified effectors either inducing or suppressing plant cell death. Our findings reveal new functions for appressoria and biotrophic hyphae and suggest that hemibiotrophy in *Colletotrichum* is orchestrated through the coordinated expression of antagonistic effectors supporting either cell viability or cell death.

PR4.87

Manipulation of trehalose biosynthesis in *Laccaria bicolor*

Sebastian Wittulsky, Uwe Nehls

Institute of Ecology, Botany, University of Bremen, Bremen, Germany

The disaccharide trehalose is a key regulator of glycolysis in higher plants, animals, and certain fungi. Based on gene expression and metabolite analysis, trehalose is also supposed to be a storage carbohydrate in ectomycorrhizal basidiomycotic fungi. Under symbiotic conditions, a crucial function of trehalose biosynthesis is carbohydrate sink formation, which is thought to enable a continuous fungal sugar support by a plant host. In this work manipulation of trehalose biosynthesis was initiated and a first glance of the impact of reduced trehalose formation on ectomycorrhizal fungal physiology will be given.

Successful gene knock out by homologous recombination has not been archived for higher basidiomycetes yet. Therefore, an RNAi strategy was followed to suppress genes involved in trehalose formation and break down using the ectomycorrhizal model fungus *Laccaria bicolor*. *Laccaria* transformation was performed using an *Agrobacterium*-based strategy. Monocaryotic mycelia were used to increase RNAi efficiency and the manipulation was performed with two different strains to compare the impact of the genetic background on fungal physiology. Suppression of trehalose-6-phosphate synthase gene expression was observed for all investigated transformants. Surprisingly, both transformed *Laccaria* strains differed in their growth behaviour. While the growth speed was increased by 10 % in one strain, it was inhibited by 10-45 % in the other. As trehalose is furthermore well known as stress protectant, temperature stress is also currently under investigation. These results together with metabolite content will be presented.

PR4.88

Expression studies of Candidate Effector Genes in different *Magnaporthe* species

Valerie Mogga, Rhoda Delventhal, Denise Weidenbach, Ulrich Schaffrath
Department of Plant Physiology, RWTH Aachen University, D-52074 Aachen, Germany

The causal agent of rice blast disease, *Magnaporthe oryzae,* is the economically most important threat in rice cultivation. While isolates of this species establish a host interaction with wheat or barley, isolates of *Magnaporthe grisea* derived from *Digitaria sanguinalis* establish a nonhost interaction with both crop plants.

Interestingly co-inoculation of barley plants with isolates of both species enabled the nonhost isolate to partly overcome nonhost resistance in epidermal cells simultaneously attacked by the adapted host isolate. Thereby providing evidence that host`s defence is actively manipulated by the adapted isolate.

Aiming at identifying pathogen-derived molecules orchestrating this scenario we performed transcription profiling in barley epidermal peels after inoculation with adapted and non-adapted isolates (24 h p.i.). Time course studies of candidate gene expression revealed that *HEGs* (hypothetical effector genes) can be grouped according to their maximal transcript abundance. A first group of *HEGs* was up-regulated during the early infection process when the fungus had not yet entered a host cell. Expression of those early *HEGs* could also be detected in fungal infection structures formed *in vitro*. A second group exhibited expression maxima during the biotrophic infection phase. At later stages, possibly correlated with the switch of the pathogen to necrotrophy, *HEG* transcript abundance was down-regulated. Currently functional analyses and localization studies of *HEGs* are in progress. Additionally we would like to investigate expression and function of *HEG* homologs in the interaction of different *Magnaporthe* species, e.g. *M. grisea*, with their cognate hosts.