Poster Category 10: Other Fungal Features and Oddities

PR10.1

Effect of fungicide application on the occurrence of *Fusarium culmorum* and mycotoxin production in wheat grain determined using Real-Time PCR

Anna Baturo-Ciesniewska, Aleksander Lukanowski, Czeslaw Sadowski
University of Technology and Life Sciences, Department of Phytopathology and Molecular Mycology, Bydgoszcz, Poland

Numerous analyses show that the presence of fungi of *Fusarium* genus in cereal grain is associated not only with decreases in the yield and technological quality, but also poses a threat to human and animal health because of the mycotoxins produced by these fungi. The amount of mycotoxins is related to the degree of grain contamination by fungi. Fungicides significantly reduce *Fusarium* species, but their application in some conditions may cause the higher incidence of toxic metabolites in grain.

The aim of the study carried out at experimental field in Lisewo Malborskie in Poland was to determine if azoxystrobin, metconazole and prothioconazole with t*ebuconazole* used for the control of wheat FHB at half, full, and quarter more the recommended dose rate may affect in differentiated way on the occurrence of *Fusarium* spp. and their ability to mycotoxin production in harvested grain, in wheat ears artificially inoculated with two DON-producing isolates of *F. culmorum*. After DNA isolation from harvested grain the presence of *F. culmorum* was determined using traditional SCAR-PCR with species specific primers and with Real-Time PCR technique using a LightCycler 480II (Roche) and SYBR Green I dye. Also the deoxynivalenol (DON) content was determined by GC-ECD. We revealed that there is correlation of gene copy number with actual concentration of mycotoxins and that improper use of fungicides may increase the concentration of toxins in the grain.

PR10.2

Fusarium Ear Rot Pathogens And Their Mycotoxins Associated With South African Maize

Anne-Laure Boutigny[1] Ilze Beukes[1] Ian Small[1] Sebastian Zühlke[2] Michael Spiteller[2] Belinda Janse Van Rensburg[3] Bradley Flett[3] Altus Viljoen[1]
1. University of Stellenbosch, Department of Plant Pathology, Private Bag X1, Matieland 7602, South Africa
2. Institute of Environmental Research, Dortmund University of Technology, Otto-Hahn-Str. 6, 44221 Dortmund, Germany 3. Grain Crops Institute, Agricultural Research Council, Private Bag X1251, Potchefstroom 2520, South Africa

Maize is the most important agricultural crop produced in Southern Africa, and is consumed daily by millions of Africans as a staple food. In South Africa, the crop is often affected by ear rot pathogens belonging to the genus *Fusarium* and their mycotoxins. To determine the prevalence of *Fusarium* species and their toxins, samples were collected from two susceptible maize cultivars at 14 localities in South Africa during 2008 and 2009. *Fusarium* species was quantified by real-time PCR and their mycotoxins by multi-toxin analysis using HPLC-MS. In 2008, *F. graminearum* was the predominant species in the eastern Free State, Mpumalanga and KwaZulu-Natal provinces, while *F. verticillioides* was predominant in the Northwest, the western Free State and the Northern Cape provinces. In 2009, maize ear rot infection was higher and *F. graminearum* became the predominant species found in the Northwest Province. *Fusarium subglutinans* was associated with maize ear rot in both years at most of the localities, while *F. proliferatum* was not detected from any of the localities. Deoxynivalenol and zearalenone correlated well with the amount of *F. graminearum* found in maize grain, fumonisins with *F. verticillioides*, and moniliformin and beauvericin with *F. subglutinans*. Our findings suggest a shift in the occurrence of *Fusarium* species and their mycotoxins in South African maize, which could be contributed to changing agricultural practices and climatic changes in production areas.

PR10.3

Chemically Induced Haploinsufficiency Screens to Identify Drug Mechanism of Action in *Aspergillus Fumigatus*

Darel Macdonald[1] Anna Johns[1] Max Eberle[2] Paul Bowyer[1] David Denning[1] Mike Bromley[1]
[1]*University of Manchester* [2]*University of Karlsruhe*

Current options for the treatment of Aspergillus infections are limited and suffer from a variety of shortcomings. Despite the discovery of numerous promising drug targets, few lead compounds have been discovered by target based approaches. This can be explained, in part, by the 'druggability' of a target as some compounds which demonstrate promising activity against an enzyme are not active against the whole cell or are toxic. A solution to this problem is to employ techniques to identify gene targets from compounds that already show antifungal activity and have clean toxicity profiles.

Chemical genetic profiling aids identification of drug mechanism of action as diploid strains lacking a single copy of a drug's target are hypersensitive to that drug. Heterozygote *S. cerevisiae* and *C. albicans* libraries have been used to identify the mechanism of action of several promising compounds; however, this has been hindered in *A. fumigatus* by the complexity in generating an adequate set of heterozygous strains. A high-throughput targeted gene KO method for *A. fumigatus* has been established by employing fusion-PCR to generate targeted gene disruption cassettes, optimizing the common transformation protocol for *A. fumigatus* high-throughput gene disruption, and utilising a diploid *Ku80*$^{-}$*/Ku80*$^{-}$mutant to facilitate more reliable homologous recombination. Preliminary efforts have produced 46 heterozygous KO strains and subsequently, the feasibility of chemical genetic haploinsufficiency studies in filamentous fungi has been demonstrated. This enables high-throughput methods for surveying the genome of *A. fumigatus* for new drug targets and supports unveiling the mechanisms of action of antifungal drugs.

PR10.4

Intracellular Proteome Response of *P. Chrysogenum* To The Addition Of Polyamines 1,3 Diaminopropane And Spermidine

Carlos García-Estrada[1] Jorge Martín[1] Carlos Barreiro[1] Rebeca Domínguez-Santos[1,2] Ricardo V. Ullán[1] Marta Fernández-Aguado[1,2] Juan Francisco Martín[1,2]
[1.]*Instituto de Biotecnología de León (INBIOTEC),* [2.]*Área de Microbiología, Universidad de León*

Penicillium chrysogenum is a filamentous fungus industrially used for the production of the beta-lactam antibiotic penicillin. It has been recently reported that the addition of polyamines 1,3-diaminopropane and spermidine induces penicillin gene expression and leads to an increase of the penicillin titers. In order to characterise the metabolic processes that have been modified by these polyamines, the intracellular proteome of *P. chrysogenum* Wisconsin 54-1255 grown in the presence of either of these two polyamines was analysed by means of two-dimensional electrophoresis followed by protein identification by MALDI TOF-TOF.

Protein changes related to some mechanisms that have a positive effect on penicillin production were found after the addition of 1,3-diaminopropane or spermidine. One of them is the visualization of a novel isoform of the isopenicillin N acyltransferase (the last enzyme of the penicillin pathway). Another modification has a positive effect on the biosynthesis of beta-alanine. This aminoacid is an intermediate in the biosynthesis of panthotenic acid, which is converted to 4´-phosphopantetheine. The latter is an essential prosthetic group for several enzymes, including the alpha-aminoadipyl-L-cysteinyl-D-valine synthetase, which is the first enzyme of the penicillin biosynthetic pathway. A decrease of the enzymes for the catabolism of phenylacetic acid (the side chain precursor of benzylpenicillin) is induced by those polyamines. This mechanism may also explain the increase in penicillin titers since more amounts of the side chain precursor would be available during penicillin biosynthesis.

These proteomics studies offer us a global vision of the effects that the polyamines 1,3-diaminopropane and spermidine have on *P. chrysogenum* primary and secondary metabolism.

PR10.5

The Regulatory Factor PcRFX1 (CPCR1 Ortholog) Controls Penicillin Biosynthesis And Sporulation In *Penicillium Chrysogenum*

Rebeca Domínguez-Santos[1,2] Carlos García-Estrada[1] Katarina Kosalková[1] Ricardo V. Ullán[1] Marta Fernández-Aguado[1,2] Juan-Francisco Martín[1,2]

[1]*Instituto de Biotecnología de León (INBIOTEC),* [2] *Área de Microbiología, Universidad de León*

Penicillium chrysogenum is a filamentous fungus mainly known for being the industrial producer of the β-lactam antibiotic penicillin. No penicillin pathway-specific regulators have been found in the amplified region containing the penicillin gene cluster so far and therefore, penicillin biosynthesis seems to be controlled directly by global regulatory factors.

The transcriptional factor CPCR1 has been recently identified in another β-lactam producer, *Acremonium chrysogenum,* where it acts as a positive regulator of the cephalosporin C biosynthesis and it seems to be involved in morphological development

The CPCR1 ortholog in *P. chrysogenum* has been characterized in our group. The gene encoding this transcription factor (*Pcrfx*1) was identified in the *P. chrysogenum* genome (Pc20g01690).

The promoter region of the penicillin biosynthetic genes was analysed to search for putative PcRFX1 DNA binding sites. Two binding sites in *pcbAB*, one in *pcbC* and another in the promoter region of *penDE* were found. The putative PcRFX1 DNA binding sequences located on the penicillin biosynthetic gene promoters were proven to be functional.

Gene silencing of *Pcrfx*1 decreased the production of isopenicillin N and penicillin G in the knock-down mutant after 48 and 72 h of culture. In this mutant, the steady-state levels of the penicillin biosynthetic genes transcripts were reduced.

Finally, the effect of gene silencing on hyphae morphology and sporulation was analysed both in the wild-type and in the knock-down mutant strains. A more abundant sporulation has found in the wild-type regarding the knock-down mutant. These results indicate that PcRFX1 acts as a global regulator in *P. chrysogenum*.

PR10.6

Phylogeny of *Penicillium* species based on ß-tubulin gene sequences.

Deena Errampalli, Matthew Czerwinski

AAFC

Many *Penicillium spp*., including *Penicillium expansum,* cause blue mold, an important postharvest disease of apples world wide. To determine the identity of the species*,* 33 *Penicillium* isolates collected from floatation tanks in three apple packinghouses in Ontario, Canada and six reference isolates were selected for DNA sequencing. Sequencing was performed using forward primer Bt-T2M-Up (5'-CAACTGGGCTAAGGGTCATT-3') and reverse primer Bt-LEV-Lo1 (5'-GTGAACTCCATCTCGTCCATA-3') from the ß-tubulin gene. The partial ß -tubulin gene sequences of the test isolates were compared with known reference isolates. The phylogenetic analysis of ß -tubulin gene sequence data of 33 isolates, led to the identification of 3 isolates as *P. solitum*, and the remaining 30 isolates as *P. expansum*. The fungal colony morphology and pathogenicity were correlated with the species. A very low genetic diversity was observed.

Some isolates of *Penicillium spp*. have developed resistance to thiabendazole (TBZ, Mertect™), a fungicide registered for the control of blue mold. The DNA sequence at codon 198 of the ß -tubulin gene corresponds to thiabendazole resistance if the GAG sequence had a substitution. Based on the analysis of DNA sequence of codon 198, six *P. expansum* isolates and three *P. solitum* isolates were found to be TBZ-resistant. The remaining 18 *P. expansum* isolates were sensitive to TBZ. The TBZ-amended media studies correlated with the sequence data of the isolates identified either as TBZ-resistant or -sensitive. This information, the identification of *Penicillium* species and their resistance to TBZ, is important in developing postharvest disease management strategies for stored apples.

PR10.7

A naphthopyrone synthase-like PKS from *Aspergillus terreus* produces phytotoxins

Markus Gressler [1] Christoph Zaehle [2] Sabrina Boldt [2] Christian Hertweck [2,3] Matthias Brock [1]

[1]Research Group Microbial Biochemistry and Physiology; [2]Department of Biomolecular Chemistry, HKI Jena, Beutenbergstraße 11A, 07745 Jena, Germany; [3]Friedrich-Schiller-University, 07743 Jena, Germany

Aspergillus terreus is a saprophytic filamentous fungus with its natural habitat in soil, compost or associated with decaying fruit. *A. terreus* has a large potential to produce a wide variety of different secondary metabolites. However, it lacks a polyketide synthase (PKS) gene conserved in all related *Aspergillus* species that produces a naphthopyrone derivative responsible for colouration of conidia. Here, we discovered that in *A. terreus* the PKS most closely related to naphthopyrone synthases produces a phytotoxin. Analysis of HPLC profiles from a PKS deletion mutant revealed that it is required for the synthesis of at least 15 different metabolites, among them the major metabolite terrein. This well-known phytotoxin is a strong antioxidant that shows weak toxicity to mammalian cells but potently harms the surface of several fruits. Using a beta-galactosidase reporter strain we observed a weak expression of the gene cluster on minimal media and moderate activation on complex media. Interestingly, expression strongly increased in presence of plant derived compounds such as malt extract or different fruit juices. This indicates a specific recognition of yet unknown plant compounds resulting in phytotoxin production. Further analyses of the gene cluster and its metabolites are currently under investigation. Additionally, the potential of metabolites in inhibiting root growth of plants is addressed.

PR10.8

Sodiomyces alkalinus – a New Holomorphic Alkaliphilic Ascomycete from Soda Soils Is a Member of Plectosphaerellaceae

Alexey Grum-Grzhimaylo[1] Alfons Debets[1] Elena Bilanenko[2]

[1.] Lab of Genetics, Wageningen University [2.] Faculty of Biology, Lomonosov Moscow State University

The majority of fungi are considered to have optimal growth around slightly acidic or neutral pH. Fungi optimally growing at high alkaline pH values (>9) are rare and have been poorly described. Among probable reasons for that are relative remoteness and low abundance of potential habitats where these fungi could be possibly isolated. Only few filamentous alkaline fungi have been reported to date. In the present study, we characterize a new alkaliphilic holomorphic ascomycete isolated from soda soils. Growth experiments in a wide pH range have confirmed an alkaline nature of this new fungus. Scanning electron microscopy images have revealed morphological features which possess adaptive defensive properties in order to cope with harsh external environments. Originally this fungus was assigned to the genus Heleococcum (order Hypocreales) based on morphology but molecular taxonomy shows that our fungus represents a new genus in a Plectosphaerellaceae family clade. Sequences of four genes (RPB2, nSSU rRNA, nLSU rRNA and 5.8S rRNA) were used in a Bayesian approach in order to pinpoint the taxonomic position of this alkalophilic fungus. Representing an extreme case of adaptive evolution to alkaline conditions, this species offers a great potential for studying exocellular alkaline enzymes which may also be of interest to industry.

PR10.9

Discovery of novel basic helix-loop-helix (bHLH) transcription factors regulating development in *Aspergillus oryzae*

Feng Jie Jin, Yasuji Koyama
Noda Institute for Scientific Research

The basic helix-loop-helix (bHLH) family of proteins comprises a group of transcriptional factors that are often important in development and differentiation. In our previous report, we identified a *sclR* gene encoding a bHLH transcription factor. We constructed *sclR*-disruptant and *sclR*-overexpressing strains, finding that there was hardly any sclerotium-like body to be observed in the *sclR*-disruptant strain, whereas *sclR*-overexpressing strain produced less conidia and more sclerotium-like body on the malt agar medium.

In this study, we identified another bHLH transcription factor-encoding gene, *ecdR*. The *ecdR* gene disruptant hardly produced conidia. Conversely, the overexpression of *ecdR* resulted in the formation of a large number of conidia at an early stage. Additionally, when serially diluted conidia were spread-cultivated onto malt agar medium, we found that conidial number of the control strain depended on the cultivated conidium density, while the *ecdR*-overexpressing strain showed no significant change in conidiation. These phenotypes of the *ecdR*-disruptant and *ecdR*-overexpressing strains are partially similar to those of the *sclR*-overexpressing strain and *sclR*-disruptant, respectively. Yeast two-hybrid assays indicated that EcdR interacted with SclR to form heterodimer and simultaneously they could also form homodimer. Interestingly, although EcdR interacted with SclR, their expression patterns were completely different. From these results, we concluded that EcdR and SclR have opposite roles in development. By competitively interacting with each other according to culture conditions, they form heterodimer and may result in a mutual inhibition of function.

PR10.10

Investigation of Antimicrobial Effect of *Neosartorya fischeri* Antifungal Protein (NFAP)

Laura Kovács, Zoltán Karácsony, Máté Virágh, Zsuzsanna Hamari, Csaba Vágvölgyi, László Galóczy
Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Hungary

Objectives: Small molecular mass, cysteine-rich antifungal protein was isolated and characterized from an ascomycetous fungus *Neosartorya fischeri*. *Neosartorya fischeri* antifungal protein (NFAP) shows remarkable antimicrobial effect against other filamentous fungi. In this study, we investigated the antimicrobial effect of NFAP with heterologous expression of the *nfap* gene in an NFAP-sensitive fungus, *Aspergillus nidulans*.

Methods: Heterologous expression of nfap gene was carried out in *A. nidulans* (pyrG89) using a pAMA-based autonomously replicating vector construction. Effect of the produced NFAP on the germination of *A. nidulans* conidia was investigated with 4'-6-Diamidino-2-phenylindole- (DAPI, Serva) and calcofluor white-staining (CFW, Sigma-Aldrich). Annexin V-FITC Apoptosis Detection Kit (Sigma-Aldrich) was used for revealing the possible apoptotic effect.

Results: The *nfap* gene was expressed in the *A. nidulans* transformant strains. Macroscopic observations revealed the reduction of hyphal growth in case of the transformants expressing the nfap gene compared to the untransformed *A. nidulans* strain. Transformants displayed abnormal and delayed germination: conidiospores formed very short, swelled hyphae with multiple branches. The germination tubes were destructed after 8 hours of cultivation. Later, membrane damage and accumulation of nuclei in the broken hyphal tips were detected by DAPI- and CFW-staining. Apoptotic events were also detected in case of NFAP-producing A. nidulans strains.

Conclusion: Manifestation of antifungal effect of NFAP on a sensitive fungus is similar to those described previously for the related peptides of *Aspergillus giganteus* (AFP) and *Penicillium chrysogenum* (PAF).

This work was supported by the Hungarian Scientific Research Fund (OTKA; grant reference number PD 83355).

PR10.11

Cloning and Functional Analysis of Genes Coding for Some Enzymes of the Mevalonate Pathway in Trichoderma

Patrycja Zembek, Sebastian Pilsyk, Urszula Perlinska-Lenart, Wioletta Gorka-Niec, Katarzyna Gawarecka, Ewa Swiezewska, Joanna S Kruszewska
Institute of Biochemistry and Biophysics PAS

The mevalonate pathway leads to synthesis of many biologically active molecules such as terpenes or quinines. Furthermore, dolichyl phosphate, a carrier of carbohydrate residues in glycosylation processes, is also produced in this pathway. In this study we decided to clone and analyze the function of rer2Tr and erg20Tr genes coding, respectively, for cis-prenyltransferase and farnesyl diphosphate synthase (FPPS) in Trichoderma. We cloned and expressed both genes in the appropriate S. cerevisiae mutants. Expression of the rer2Tr gene in the S.cerevisiae SRT1/Δrer2 mutant resulted in a very high amount of the rer2Tr transcript and only 19% higher activity of cis-prenyltransferase. Since dolichols isolated from the S.cerevisiae SRT1/Δrer2/rer2Tr mutant were synthesized by yeast Srt1p and the RERII protein from Trichoderma, we expected a mixture of dolichols characteristic for both organisms. HPLC analysis of dolichols isolated from the strain revealed only the yeast type. This result suggests that either the Trichoderma enzyme is not active or it produces the yeast type of dolichols. To elucidate this problem we expressed the rer2Tr gene in the Δrer2/Δsrt1 double mutant. Cloning and analysis of the Trichoderma erg20Tr gene in the S. cerevisiae Δerg20 mutant showed that the yeast gene could not be suppressed by the Trichoderma one. To analyze erg20Tr gene function we overexpressed it in T. reesei. The transformants exhibited higher activity of FPPS.

PR10.12

Antifungal susceptibility of *Aspergillus* spp. under hypoxic growth conditions

Elisabeth Maurer, Ulrike Binder, Cornelia Lass-Flörl
Division of Hygiene and Medical Microbiology, Innsbruck Medical University

Invasive aspergillosis is a major life-threatening disease in immunocompromised patients, with mortality rates up to 90 %. The most common species is *Aspergillus fumigatus* (90 % of infections), followed by *A. flavus,* and *A. terreus*.

During infection, fungal pathogens must adapt to microenvironmental stresses, including hypoxia as well as high CO_2 levels. Such oxystress conditions are usually not taken into account in current models of infection and assessment of antifungal sensitivities. As antifungal test systems, such as Etests, we compared the *in vitro* activity of amphotericin B, various azoles, and echinocandines in hypoxic conditions (1 % O_2, 5 % CO_2) to their activity in normoxic conditions against 47 isolates of *Aspergillus* spp. belonging to *A. flavus (n=9)*, *A. terreus (n=16),* and *A. fumigatus (n=22)*. We found that in hypoxic conditions similar to those that might occur in *aspergillus*-infected tissue, a reduction in the *in vitro* MIC of amphotericin B for all three species occurred. Similar MIC reduction effects were found for azoles, especially for *A. flavus* species, while for echinocandines differences where less significant and the phenomenon of trailing was also persistent in hypoxic conditions, which makes determination of MIC rather difficult. Further tests are currently in progress to find out if similar results can be obtained with microbroth dilution assays, where not only gas concentrations are regulated to mimic host environments, but also other parameters such as pH, iron limitation or the provision of host cell components can be manipulated.

PR10.13

Evolution of necrotrophic effectors within *Phaeosphaeria nodorum* and close relatives

Megan C. McDonald[1] Patrick C. Brunner[1] Timothy L. Friesen[2] Zhaohui Liu[3] Richard P. Oliver[4] Bruce A. McDonald[1]

[1] ETH Zuerich [2.] USDA-ARS [3.] North Dakota State University [4.] Curtin University

The genetic relationships among *Phaeosphaeria* species that infect wheat are not well understood. This study expanded on earlier work using sequence data to define the species infecting wheat in a global sample. We sequenced 1,542 bp across 3 loci in 355 isolates from around the world, including many isolates from Iran, near the host center of origin. We were able to differentiate *P. nodorum* and two previously defined taxonomic groups of *Phaeosphaeria avenaria tritici* (Pat), called Pat1 and Pat3. We identified three new closely related *Pat* groups from grass hosts, named *Pat4, Pat5* and *Pat6.* We present two new species from Iranian wheat, tentatively named P1 and P2. We found evidence of incomplete lineage sorting between *P. nodorum* and Pat1. We propose these 9 groups as distinct phylogenetic clades.

The main virulence mechanisms identified in *P. nodorum* are host selective toxins (HSTs), which interact in a gene-for-gene manner with toxin sensitivity genes in wheat to cause lesion formation. We found that global populations of wheat-infecting *P. nodorum* carried *SnTox3, SnToxA* and *SnTox1* with widely varying frequencies. In a global sample of over 1000 isolates, the multi-toxin genotypes did not differ significantly from frequencies expected under random mating. Furthermore, the distribution of toxin sequence diversity did not coincide with the distribution observed for neutral markers in *P. nodorum.* By combining the species phylogeny with data on toxin distribution, we could elucidate the evolutionary timescales over which host selective toxins evolved to become major contributors to this disease complex in the wheat agro-ecosystem.

PR10.14

Molecular identification of clinically important *Bipolaris* species

Krisztina Krizsán, Gábor Nagy László Nagy, Eszter Tóth, Tamás Papp, Csaba Vágvölgyi

University of Szeged, Faculty of Science and Informatics, Department of Microbiology, Szeged, Hungary

The anamorphic ascomycetous genus, *Bipolaris* includes several plant pathogenic species. Three of them, namely *Bipolaris australiensis*, *B. hawaiiensis* and *B. spicifera*, are also able to cause infections in both immunocompromised and immunocompetent humans. Using the conventional morphology-based identification methods, which involves the examination of the septation, shape and size of the conidia, their differentiation is unreliable and time consuming.

In the present study, we examined the phylogentic relationships, among the three human pathogenic *Bipolaris* species, based on this analysis, we tried to establish useful markers for molecular identification of species and strains. For this reason, 35 isolates of *B. australiensis*, *B. hawaiiensis* and *B. spicifera* were obtained from human keratomycosis and from international strain collections and the internal transcribed spacer (ITS) and the intergenic spacer (IGS) regions of the nuclear ribosomal RNA gene cluster, the β-tubulin and the translational elongation factor EF-1 alpha genes were sequenced and analysed. The sequences were investigated in a phylogenetic context also. Earlier molecular phylogenetic studies involving determined the taxonomic position of the *Bipolaris* and related genera, but within the genus, the position of the clinically important species remained ambiguous. The joint analysis of the above mentioned four sequences with Bayesian method completed with the morphological data of the studied isolates resolved the taxonomic questions in connection with the clinically important *Bipolaris* species. Based on the determined sequences, a rapid molecular identification method could be.

PR10.15

Post-transcriptional suppression against potential transposable elements by cryptic splicing and premature polyadenylation in *Aspergillus oryzae*

Hironobu Ogasawara[1] Saori Takahashi[1] Katsuya Gomi[2]

1. Akita Res. Inst, Food and Brewing, Akita, Japan 2. Graduate School of Agricultural Science, Tohoku University, Sendai, Japan

An active DNA transposon *Crawler* isolated from the genome of industrially important fungus *Aspergillus oryzae* transposes under extreme stress conditions [1]. A stress-fluctuation cDNA browser with DOGAN-DB was constructed to survey transposon-like genes such as *Crawler*. Full length of DNA sequences encoding transposable elements were frequently identified. Among them, a novel element homologous to *Tan1* from *A. niger* was identified and tentatively designated *AoTan1* that shows multiple characteristics of class II transposon [2]. Changes of the transcripts from several transposable elements were analyzed under extreme stress conditions such as $CuSO_4$ or heat shock by the method of RT-PCR and 3'-RACE. The mRNA analyses revealed that cryptic splicing occurred in the mRNA from *gag*-like elements in a retrotransposn *AoLTR1* and from a deduced DNA transposon(AO090023000251) homologous to *implala* under the normal culture condition. In the case of *AoTan1*, cryptic splicing could not be observed, whereas premature polyadenylations were detected within coding region of the transposase. By the stress treatments, the increasing in mature mRNA molecules from those elements was caused, allowing the full-length to be produced. These results suggested that *A. oryzae* might possess a common defense system against the potential transposable elements by post-transcriptional regulation such as cryptic splicing or premature polyadenylation as observed in the active transposon *Crawler*. 1)H. Ogasawara *et al. Fungal Genet. Biol.*, **46**, 441-449 (2009) 2)H. Ogasawara *et al.* 26th*FGC Abstract Book*, p148 (2011)

PR10.16

***Aspergillus fumigatus* mycovirus infection is not dependent on the genetic up-make of the host**

Jeannine M. Refos[1] Kimberly Eadie[1] Alieke G. Vonk[1] Jerome R. Lo Ten Foe[2]Wendy W.J. van de Sande[1]

1. Department of Medical Microbiology and Infectious diseases, Erasmus MC, Rotterdam, The Netherlands

2. Department of Medical Microbiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Introduction: Mycoviruses are viruses that selectively infect fungi and are ubiquitous in all major groups of filamentous fungi. Most mycoviruses have dsRNA genomes and replicate cytoplasmatically. Although most of the mycoviruses cause cryptic infections, mycoviruses are of scientific interest since some of these viruses can cause fungal hypovirulence such as reduced growth rate, altered pigmentation and sporulation. Viruses are considered to be host-specific, but within each host some individuals are more prone to develop a viral infection than others. Therefore, we determined if the genetic make-up of *Aspergillus fumigatus* was correlated to the presence of a mycovirus.

Materials & Methods: A collection of 112 clinical *A. fumigatus* isolates from the Erasmus MC, Rotterdam, The Netherlands was screened for mycovirus presence by isolating dsRNA from fresh lyophilized mycelial cultures using a Trizol/chloroform method. To determine genetic relatedness of *A.fumigatus* the cell surface protein (CSP) gene was typed by sequencing.

Results: Of the 112 clinical *A.fumigatus* isolates 16 (14.3%) contained dsRNAs. The *A.fumigatus* collection could be divided into 12 different CSP types, indicating that the collection used was of heterogenous origin. *A.fumigatus* isolates which contained dsRNA mycoviruses had similar CSP types as non-infected isolates. In both cases, the CSP types 1, 2, 3 and 4 were the most prevalent which was comparable to the CSP types observed in other Dutch collections.

Conclusion: Mycovirus infection is not related to a specific genetic *A. fumigatus* lineage

PR10.17

The genetic basis of conidial pigmentation in *Aspergillus niger*

M.A. van Welzen[1] T.R. Jorgenson[1,2] J. Park[1] M. Arentshorst[1] G. Lamers[1] P.A. vanKuyk[1] R.A. Damveld[1] A.F.J. Ram[1,2]

1. Leiden University, Molecular Microbiology and Biotechnology, 2. Kluyver Centre for Genomics of Industrial Fermentation

A characteristic hallmark of *Aspergillus niger* is the formation of black conidiospores. We have identified four loci involved in spore pigmentation of *A. niger* by using a complementation approach. First, we characterized a newly isolated color mutant, *colA*, which lacked pigmentation resulting in white conidia. Pigmentation of the *colA* mutant was restored by a gene (An12g03950) which encodes the *A. niger* ortholog of the 4'-phosphopantetheinyl transferase protein (PptA). The loci giving rise to fawn, olive, and brown color phenotypes were identified by complementation. The fawn mutant was complemented by the polyketide synthase A protein (PksA, An09g05730), the *olvA* mutant by An14g05350 (OlvA) and the *brnA* mutant by An14g05370 (BrnA), the respective homologs of *PksP/alb1, ayg1* and *abr1* in *A. fumigatus.* Targeted disruption of the *pptA, pksA, olvA* and *brnA* genes confirmed the complementation results. The different color genes are expected to function in a linear pathway producing the black melanin. To determine the epistasis for the fawn, olive and brown mutants, double mutants were constructed in all possible combinations. As expected, *pksA* is epistatic over both *olvA* and *brnA,* and *olvA* is epistatic over *brnA.*

PR10.18

Genetic diversity of *Rhizoctonia solani* isolates from UK potato crops

James Woodhall, Jeff Peters

Food and Environment Research Agency

Rhizoctonia solani is an important pathogen of potatoes, causing infection of stems and roots and tuber blemish diseases. The fungus is a species complex consisting of 13 related but genetically distinct anastomosis groups (AGs). Isolates are assigned to AGs on the basis of hyphal interaction between isolates. In the UK, AG3 predominates accounting for over 90% of findings but isolates of AG2-1 and AG5 have also been found in potatoes. Although knowledge of the diversity of AGs present is known at a national level in the UK, little is known about the diversity of isolates at the field scale and no studies have been undertaken to investigate the genetic diversity of UK AG3 isolates. To address this, mulitple isolates were taken from three different fields in the UK and compared with other UK isolates. All isolates were determined to be AG3 by using an AG3 specific TaqMan assay. The genotypes present within the AG3 isolates were determined using a range of previously published polymorphic co-dominant single locus PCR-RFLP markers or by sequencing the ITS region and part of the translation elongation factor and cytochrome oxidase genes. In addition, hyphal fusion tests were done between pairs of isolates to determine vegetative compatability group (VCG) for each. Analysis revealed that the population of AG3 isolates within a field is diverse with multiple genotypes and VCGs recovered from the same $30m^2$sampling area. This suggests that AG3 isolates from UK potatoes are not a clonal population.

PR10.19

The role of conidial anastomosis tubes in *Colletotrichum lindemuthianum*

Francine Hiromi Ishikawa [1] Elaine Aparecida de Souza [2] Nick D. Read [3] Maria Gabriela Roca [4]
[1]Universidade Federal do Vale do Rio Doce, Brazil,[2]Universidade Federal de Lavras, Brazil, [3]University of Edinburgh,UK, [4] Universidad de Cordoba, Spain

Conidial anastomosis tubes (CATs) is a common phenomenon in bean pathogen *Colletotrichum lindemuthianum.* We used different strains to perform a microscopical analyse of the fusion between germlings and within mature colonies. In mature colonies, vegetative hyphal fusion between two incompatible strains caused rapid disappearance of nuclei and cell death. In marked contrast, CAT fusion between incompatible strains suppressed the heterokaryon incompatibility response. Heterokaryotic mycelium within the same colonies were produced, and dissected, resulting in different phenotypes. Vegetative incompatibility occurs after fusion and is triggered very fast in mature colonies, but is repressed and delayed during colony initiation. The heterokaryon mycelium originated from fused germlings survives long enough, to allow cytological and nuclear mixing, with important consequence for the cells involved. Through CAT fusion is possible to generate fungal genetic diversity.

PR10.20

Morphological and Molecular Identification of *Trichoderma* Species from West of Iran

Mozhgan Roughanian[1] Jahanshir Amini[1] Dostmorad Zafari[2] Jafar Abdollahzadeh[1]
[1.] College of Agriculture, Kurdistan University [2.] College of Agriculture, Bu-Ali sina University

Some 500 *Trichoderma* isolates were obtained from soil samples and bark of trees collected from west parts of Kurdistan province. Cultures were grown on PDA and purified on 2% water agar by hyphal tip method prior to identification. The isolates were identified using morphological features, including colony characters (pigmentation and growth rate on PDA) and microscopic characters, including shape of conidiophores, shape and size of conidia and phialides. The microscopic features were studied and recorded 3-5 days after inoculation on cultures grown on CMD at 25°C under ambient laboratory condition. Nine species identified, including *T. citrinoviride, T. longibrachiatum, T. saturnisporum, T. asperellum, T. atroviride, T. harzianum, T.arundinaceum, T. brevicompactum* and *T.virens*, in addition six isolates possess conidiophor similar to species in section *Longibrachiatum* were morphologicaly different from others and introduced *Trichoderma* species until now, therefore these isolates can be a new species of *Trichoderma*. In order to accurate identification and support morphological studies internal transcribed spacers of the rDNA, translation elongation factor 1-alpha (*tef-1alpha*) and a fragment of the gene coding for endochitinase 42 (*ech42*) these isolated were nucleotide sequenced and compared by using blast search with introduce *Trichoderma* species. Result of molecular study supported morphological studies and these isolates could be a new species of *Trichoderma*, belonging to *Longibrachiatum* section.

PR10.21

The use of whole genome microarrays to study viral interactions with *Agaricus bisporus*

Gregory L. Deakin, Edward Dobbs, Julie M. Bennett, Kerry S. Burton
East Malling Research, Kent, UK ME19 6BJ

Fungal viruses were first identified in the cultivated mushroom *Agaricus bisporus* in1962. Viral diseases can cause widespread devastation and are difficult to eradicate in mushroom crops which are vegetatively propagated. The latest viral disease known as Mushroom Virus X (MVX) has become a serious economic problem and is associated with changes in agronomic practices, in particular bulk substrate colonisation. Recent research has provided strong evidence that this disease is caused by a number of double-stranded RNA (ds-RNA) viruses, for example transcripts which are not of *A. bisporus* origin but hybridise to ds-RNAs, and are found at very high levels in tissues exhibiting strong disease symptoms.

Statistical analysis of microarray data has identified host transcripts differentially expressed (at the 0.05% level) between MVX infected and non-infected samples. *Agaricus* mycelium in casing had 755 genes up-regulated upon infection while infected mycelium in compost displayed an up-regulation of 2,173 genes. Surprisingly small numbers of genes were identified as up-regulated in fruitbodies where the symptoms are visible, or up-regulated in mycelium growing on defined (agar) culture. This suggests an interaction between viral action and tissue type/differentiation.
Oxidoreductases identified as potentially involved in the degradation of nutrients related to the mushrooms' ecological niche (e.g. laccases and aromatic peroxidises) were found to be routinely down regulated in MVX infected mycelium grown in compost.

Although the infected fruitbodies exhibited symptoms of brown colouration the transcriptomic data reveals that two of the tyrosinase genes (often associated with tissue browning) were down-regulated in infected fruitbodies by more than 6-fold.