Poster Category 8: Biotechnology

PR8.1

Establishing a novel protein expression system in *Ustilago maydis*

Janpeter Stock, Parveen Sarkari, Kerstin Schipper, Michael Feldbrügge
Heinrich-Heine University Düsseldorf, Institute for Microbiology

In industrial biotechnology some heterologous proteins cannot be produce with established expression systems. Thus, there is a great interest to develop novel protein expression platforms. In this project we establish a new expression system using the basidiomycete fungus *Ustilago maydis*. This eukaryotic microorganism is very well suited for genetic, biochemical, genome-wide as well as proteome-wide approaches. During filamentous growth microtubule-dependent mRNA transport plays a crucial role. The RNA-binding protein Rrm4 is responsible for long-distance mRNA transport and essential for the efficient secretion of the endochitinase Cts1. With fusions of β-glucuronidase and Cts1 we demonstrated, that Cts1 can mediate the export of active heterologous proteins. β-glucuronidase is inactivated by N-glycosylation during conventional secretion. Using this enzyme as a reporter our data reveal that Cts1 is secreted via an unconventional secretion process avoiding N-glycosylation. In addition, the lack of a canonical N-terminal secretory signal peptide in Cts1 was confirmed by analysis of truncated Cts1 variants. Circumventing N-glycosylation can be beneficial in various applications, for example to prevent undesirable immune responses in medical applications. As a proof-of-principle we successfully expressed codon optimized lipase CalB in *U. maydis* and confirmed its activity by Tributyrin plate assays. Next, we will express difficult-to produce lipases and characterize them by liquid assays.

PR8.2

Approaches for directed strain improvement targeting enhanced biosynthesis of gibberellic acid in *Fusarium fujikuroi*

Sabine Albermann, Bettina Tudzynski
Westfälische Wilhelms Universität Münster

The filamentous fungus *Fusarium fujikuroi* is famous for producing high amounts of gibberellic acids (GAs). These phytohormones exhibit a great biotechnological impact as application of GAs in higher plants induces early flower bud formation and shoot elongation as well as an increased fruit size. Each year about ten tons of gibberellins are used as plant growth regulators by the agricultural and plant breeding industry.

Therefore, we developed strategies to increase GA yields by directed genetic modifications of genes involved in primary and secondary metabolism. Thus, overexpression of the first GA specific gene *Ggs2* (geranylgeranyl-pyrophosphate synthase 2), was performed. In addition, the negative feedback regulation of the key enzyme of the mevalonate pathway, *HmgR* (Hydroxy-methyl-glutaryl-CoA reductase), has been circumvented by deleting the regulatory domains. Overexpression of the truncated gene resulted in higher GA yields. Furthermore, regulation on transcriptional and protein level should be further investigated. This shall be amongst others elucidated by identification of positively or negatively acting transcription factors.

Another approach for enhancing GA-biosynthesis is to knock down a whole set of secondary metabolite pathways competing for the same precursors by deletion of the 4'-phosphopantetheinyl transferase Ppt1. This led to loss of function of all polyketide synthases (PKSs) and non-ribosomal peptide synthases (NRPSs) that are essential for many secondary metabolite syntheses. This modification altered the GA spectrum and resulted in higher GA amounts.

PR8.3

Analysis of the White-Rot Model Pleurotus *ostreatus* Secretome

Manuel Alfaro[1] Andrzej Majcherczyk[2] Mojtaba Zomorrodi[2] Jose L. Lavín[1] Jose A. Oguiza[1] Lucía Ramírez[1] Ursel Kües[2] Antonio G. Pisabarro[1]

1. Grupo de Investigación de Genética y Microbiología, Universidad Pública de Navarra, Spain 2. Division of Molecular Wood Biotechnology and Technical Mycology, Büsgen Institute, Georg-August-University of Göttingen, Germany

The utilization of low-value substrates such as lignocellulosic wastes offers a great potential to reduce the production costs of bioethanol. The biological process of bioethanol production using lignocellulose as feedstock requires its delignification to liberate the cellulose and hemicellulose from their complex with lignin. *P. ostreatus* is a model white rot basidiomycete that produces various ligninolytic enzymes useful in this process.

With the aim to expand knowledge about proteins involved, and through the availability of the whole genome sequence of the two monokaryotic strains PC9 and PC15 that compose the dikaroytic strain N001 obtained in the DOE JGI, we have carried out a study of the secretome of the fungus cultured on three different liquid media using wood, glucose or both as carbon source.

We have used the 2D electrophoresis technique prior to mass spectrometry analyses of the spots in order to make a first approach to the proteins secreted by the fungus. Afterwards, the use of a shot gun technique to deepen in the analysis of the proteins involved, in addition to the analysis of the computationally predicted secretome, have enabled us to compare the enzymatic profile between the monokaryons PC9 and PC15 and the dikaryon N001 and provide valuable insight into how white rot fungi degrade lignocellulosic biomass.

PR8.4

Investigating the yet-unknown biomass degrading and modifying enzymes of Aspergillus *oryzae*

George Anasontzis[1] Gianni Panagiotou[2] Lisbeth Olsson[1]

1. Department of Chemical and Biological Engineering, Industrial Biotechnology, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden 2. Center for Biological Sequence Analysis, Department of Systems Biology, Technical University of Denmark, Kemitorvet, Building 208, DK-2800 Lyngby, Denmark

The associations of plant cell wall components with *A. oryzae*'s extracellular enzyme machinery was investigated from a chemical perspective using an integrated analysis of the transcriptome profile.

*S*train RIB40 of *A. oryzae* was cultured on various carbon sources, namely cellohexaose, mannohexaose, xylopentaose, arabinoheptaose, glucohexaose, glucosyl maltotriosyl maltotriose, galactosyl mannotriose, turanose and sophorose, and the transcribed genes were determined with DNA microarrays. The statistically significant genes were selected and novel hydrolases were identified, which would be further heterologously expressed for subsequent characterization. This work represents a novel way of integrating computational chemical biology and classical enzyme research for improving lignocellulose bioconversion.

More specifically, we aim at the heterologous expression and characterization of seven different hypothetical and non classified proteins of *A. oryzae*, which could prove to be useful tools in the wood biomass separation and modification process.

PR8.5

Transcriptomics-based genome-scale prediction of secondary metabolite gene cluster members in *Aspergillus niger*

Mikael R. Andersen, Dorte M.K. Holm, Lene M. Petersen, Lars Poulsen, Jette Thykær, Kristian F. Nielsen, Thomas Ostenfeld, Uffe H. Mortensen
Technical University of Denmark, Center for Microbial Biotechnology

The biosynthetic pathways for fungal secondary metabolites (SMs) are currently the focus of a large amount of efforts to elucidate the genetic basis of the biosynthesis. Major drivers for this effort are the large potential of fungal metabolites as bioactive compound, as well as an interest in utilizing the enzymes for synthetic biochemistry.

In an effort to alleviate the large amount of work required to identify the biosynthetic genes associated with a given SM synthase, we have previously developed a method for predicting SM clusters size from transcriptomic data, and showed it to be accurate in *A. nidulans*. In this study, we developed a new DNA microarray for *A. niger*, and employed it to build a microarray compendium of 73 samples from a diverse set of growth conditions.

Using the SMURF algorithm (Khaldi et al. 2010), we identified putative NRPS's, PKS's, hybrids and DMATs from the *Aspergillus niger* ATCC 1015 genome. This analysis yielded 81 putative SM synthases. Of those, 75 synthase genes are active in our gene expression catalog. This allowed the assignment of cluster genes for all 75 active synthases. Cluster sizes range from 15 genes (Gene ID: 118581, apparently absent in *A. niger* CBS 513.88) to one gene.

We have further employed the data set to predict cross-chemistry between physically separated gene clusters.

PR8.6

Characterization of the cyp684 gene involved in fenhexamid resistance in the species *Botrytis pseudocinerea*

Azeddine Saad [1] Billard[2] Solignac[2] Bach[2] Debieu[2] Fillinger[2]
1. INRA (Bioger) 2. INRA

The Botrytis species complex responsible for the grey mold disease on multiple crops is composed of several species. The major one is Botrytis cinerea, the second one was called Botrytis pseudocinera. Despite their genetic polymorphism both species cannot be morphologically distinguished. However they differ for their sensitivity to several fungicides, especially to the sterol biosynthesis inhibitor fenhexamid. While B. Cinerea is sensitive to this hydroxyanilide, but can acquire resistance through target site mutations, B. Pseudocinerea is a naturally resistant species. We found a strong synergism between fenhexamid and sterol 14α-demethylation inhibitors (DMIs), especially on B. Pseudocinerea. Since DMIs inhibit Cyp51, a cytochrome P450 protein, we supposed detoxification of fenhexamid by a cytochrome P450 similar to Cyp51 to be involved in B. Pseudocinerea's resistance. The gene with the highest similarity to cyp51, named cyp684, was deleted in a B. Pseudocinerea strain. Cyp684 knock out mutants exhibit increased fenhexamid sensitivity and decreased fenhexamid metabolisation, showing that the Cyp684 cytochrompe P450 is responsible for B. Pseudocinerea's (HydR1) natural resistance to fenhexamid. Although cyp684 is also present in B. Cinerea sensitive to fenhexamid, we observed several polymorphisms: i/ in B. Pseudocinerea the cyp684 promoter shows a deletion of 25 bp, ii/ the peptide sequence varies by 4 amino acid residues between the species. We are currently establishing the cyp684 expression profiles in both species in order to analyze the impact of the promoter deletion on its expression. We will then study which part of the gene is/are responsible for fenhexamid resistance in B. Speudocinerea prior to establish its physiological and enzymatic functions.

PR8.7

Transcriptional and enzymatic profile of *Pleurotus ostreatus* multigene family in submerged and solid state fermentation

Raúl Castanera, Gumer Pérez, Alejandra Omarini, Gerardo Pisabarro, Lucía Ramírez
Public University of Navarre

The functional differences among the members of multigene families can be analyzed by studying their transcriptional profiles under different environmental conditions and different genotypes. The white rot fungus *Pleurotus ostreatus* can be a suitable model for genomics and transcriptomics studies in basidiomycetes due to the information available after the sequencing of the two haploid genomes composing the N001 strain. In this work, we have studied the differential regulation of the laccase gene family transcription using a RT-qPCR approach. The study has been made using different monokaryotic and dikaryotic (isogenic and non-isogenic for the growth-rate containing QTL chromosome VIII) strains, cultured in submerged cultures in the presence or in the absence of a laccase inducer, and in solid fermentation. Our results revealed (1) the importance of measuring the amplification efficiency and of carefully selecting the internal standards for the relative quantification of gene expression, (2) that the *Lacc2* and *Lacc10* genes are the responsible of laccase induction in submerged cultures, (3) that these two genes displayed opposite transcriptional response in PC9 type and PC15 type full-sibs strains, suggesting that laccase induction in submerged fermentation is linked to vegetative growth rate, (4) that the expression of these two genes increased in solid fermentation with increased water availability in the culture, (5) that the enzymatic activities and intracellular/extracellular isozyme patterns confirmed the differential behaviour of fast growing and slow growing strains, and characterized the intracellular and extracellular laccase fractions in solid and submerged cultures.

PR8.8

Real-Time Viability Assay for Fungal cells

Paulo de Boer, Alie de Kat Angelino-Bart, Peter Punt, Remco Kort
TNO, Microbiology & Systems Biology, P.O. Box 360, 3700 AJ, Zeist, The Netherlands

Detection of live microorganisms, including fungi, is currently performed by enumeration of colony forming units (CFUs) on agar plates. Although reliable and widely accepted, this method has disadvantages. Determination of CFUs is timely, laborious and limited to readily culturable microorganisms. Cultivation-independent methods are available nowadays, but they mostly involve multiple incubation steps for assessment of the number of viable cells and do not discriminate between dead or live microorganisms. Here a novel method is presented, capable of specifically monitoring living fungal and yeast cells (and bacteria too) in a real-time manner.

The assay is based on a novel viability criterium, the ability of cells to maintain a neutral pH in an acidic environment. Therefore, we searched for probes that show a fluorescent signal in a neutral environment, and not in an acidic environment. Since fluorescence is only produced in cells with a neutral pH (*i.e.* living cells), the fluorescent intensity is a measure for the amount of viable cells.

We have identified a number of probes that allow real-time viability (RTV) assays of fungal samples. These probes have succesfully been tested on *Aspergillus niger* and *A. fumigatus*, *Saccharomyces cerevisiae*, and *Candida albicans*. Based on these results it is expected that the RTV assay will work for other fungal species too. The RTV assay for fungi opens ways to assess in a fast, automated manner the viability in fungal samples such as spore batches, anti-fungal treated fungal cells and all other applications where viability of fungal cells are of interest.

PR8.9

Hydrophobin fusions for high level intracellular protein production and purification in *Trichoderma reesei*

Eero Mustalahti, Nina Aro, Marika Vitikainen, Markku Saloheimo, Jussi Joensuu
VTT Technical Research Centre of Finland

Insufficient accumulation levels and the lack of efficient purification methods constitute two major bottlenecks hindering the recombinant protein production. Low yields are often seen in the cases where the host is distantly related to the organism from which the product is derived. Microbial production systems where the product is secreted to the medium are hampered by host proteases, which can destroy the target protein. Secretion in eukaryotic cells is finely tuned, and production is frequently limited by inefficient secretion of exogenous proteins. Microbial intracellular protein production, on the other hand, often results in aggregation of the denatured product in inclusion bodies, from which the active conformation is difficult to recover. Hydrophobins are small amphipathic proteins ubiquitously expressed in filamentous fungi. Hydrophobins are capable of altering the hydrophobicity of their respective fusion partner to enable purification by surfactant-based aqueous two-phase separation (ATPS). We have demonstrated that hydrophobin fusions targeted to endoplasmic reticulum (ER) induces formation of large intracellular protein bodies in *Trichoderma reesei*. The fusion protein remains soluble in the protein bodies surrounded by the ER-membrane and can be easily recovered from the cell lysate by ATPS. It is hypothesized that packing of hydrophobin fusions into these protein bodies may exclude the recombinant protein from the host proteolysis, simultaneously protecting the host cell from toxic effects of massive intracellular accumulation of the target protein. The implications of these results in development of novel strategies for production of recombinant proteins will be discussed.

PR8.10

Application of an optimized FLP/*FRT* recombination system in diverse filamentous fungi

Katarina Kopke[1] Birgit Hoff[2] Ulrich Kück[1]
[1] Christian Doppler Laboratory for "Fungal Biotechnology", Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, Universitätsstraße 150, 44780 Bochum, Germany [2] BASF SE, 67056 Ludwigshafen, Germany

A molecular tool for marker recycling was established to overcome the limited availability of resistance markers in filamentous fungi. For this purpose the FLP/*FRT* recombination system from *Saccharomyces cerevisiae* was optimized for the penicillin producer *Penicillium chrysogenum*. In a first approach, we used a two-step strategy to test the functionality of the system. Therefore we generated a nourseothricin resistance cassette flanked by *FRT* sequences in direct repeat orientation (*FRTnat1* cassette) and ectopically integrated this construct into a *P. chrysogenum* recipient strain. In a second step a codon-optimized *Pcflp* recombinase gene were transferred into the *P. chrysogenum* strain, carrying the *FRTnat1* cassette. We observed in several tested transformants the successful recombination event due to the use of a codon-optimized recombinase. To further extend the application of the FLP/*FRT* recombination system, we generated a marker-free ΔPcku70FRT2 strain which enables the production of multiple deletion strains by highly efficient homologous recombination. Moreover a *nat1* flipper was generated to establish a one-step marker recycling. Therefore the FLP/*FRT* system and the *nat1* marker gene were combined in a single construct. For induction of the recombinase gene expression we used the *xyl* promoter. In further experiments we will use different flipper cassettes together with the ΔPcku70FRT2 strain to construct marker-free double and triple mutants.

Moreover the applicability of the developed tool was demonstrated by marker recycling in the ascomycetes *Sordaria macrospora* and *Acremonium chrysogenum* indicating, that the optimized FLP/*FRT* recombination system is suitable to a broad range of filamentous fungi.

PR8.11

Spatially resolving the secretome within the mycelium of the cell factory *Aspergillus niger*

Pauline Krijgsheld[1] A.F. Maarten Altelaar[2] Harm Post[2] Jeffrey H. Ringrose[2] Wally H. Müller[3] Albert J.R. Heck[2] Han. A.B. Wösten[1]

1. Microbiology and Kluyver Centre for Genomics of Industrial Fermentation, Utrecht Universit 2.Biomolecular Mass Spectrometry and Proteomics, Netherlands Proteomics Centre, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University 3.Biomolecular Imaging, Utrecht University

Fungi secrete enzymes to convert organic matter into small molecules that can serve as nutrients. Hyphae at the periphery of the colony are exposed to unexplored organic material, whereas the center of the colony experiences a utilized substrate. This suggests that the enzymes that are secreted by different zones in the colony are different. *Aspergillus niger* is an important cell factory for the industrial production of enzymes. Here, we determined with stable isotope dimethyl labeling the secretome of 5 concentric zones of 7-day-old xylose-grown colonies of *A. niger* that had either or not been treated with cycloheximide. As expected, cycloheximide blocked secretion of proteins at the periphery of the colony. Unexpectedly, protein release was increased by cycloheximide in the intermediate and central zones of the mycelium when compared to non-treated colonies. Electron microscopy indicated that this is due to partial degradation of the cell wall. A total of 124 and 59 proteins were detected in the medium of xylose grown colonies that had or had not been treated with cycloheximide. Apparently, a major part of the proteins are associated with the cell walls of *A. niger*. Taken together, cycloheximide can be used to obtain a (near) complete secretome of *A. niger*. Moreover, the total amount of protein is increased upon treatment with this antibiotic. The composition of the secretome in each of the 5 concentric zones differed. This study thus describes spatial release of proteins in *A. niger*, which is instrumental in understanding how fungi degrade complex substrates in nature.

This project was financed by the Kluyver Centre for Genomics of Industrial Fermentation and the Netherlands Proteomics Centre, which are part of the NGI

PR8.12

D-xylose Concentration-dependent Hydrolase Expression Profiles and the According Role of CreA and XlnR in *A. niger*

Astrid R. Mach-Aigner[1] Jimmy Omony[2] Birgit Jovanovic[1] Anton J.B. van Boxtel[2] Leo H. de Graaff[3]

1. Research Division Biotechnology and Microbiology, Institute of Chemical Engineering, Vienna University of Technology, Gumpendorfer Str. 1a, A-1060 Vienna, Austria 2. Systems and Control group, Wageningen University, P.O. Box 17, 6700 AA Wageningen, The Netherlands 3. Fungal Systems Biology, Laboratory of Systems and Synthetic Biology, Wageningen University, Dreijenplein 10, 6703 HB, Wageningen, The Netherlands

Aspergillus niger is an industrially important organism for the production of industrial enzymes like hemicellulases and pectinases. The xylan-backbone monomer D-xylose is known as an inducing substance for the coordinate expression of a high number of polysaccharide-degrading enzymes. In this study a total number of 22 genes, which encode enzymes that function as xylan backbone-degrading enzymes, accessory enzymes, cellulose-degrading enzymes, or enzymes involved in the pentose catabolic pathway in *A. niger* have been investigated concerning their response to low (1 mM) and high (50 mM) D-xylose concentrations. Notably, genes encoding enzymes that have similar function (*e.g.* xylan backbone-degradation) respond in a similar way to different amounts of D-xylose. Although low D-xylose concentrations provoke - in particular for hemicellulase-encoding genes - highest transcription response, transcript formation in presence of high amounts of D-xylose was also observed. It even turned out that a high D-xylose concentration is favourable for certain groups of genes. Furthermore, the repressing influence of CreA on the transcription of a selection of these genes was observed on D-xylose, regardless whether low or high amount of D-xylose is used. Interestingly, the decrease in transcription of certain genes on high D-xylose concentrations is not reflected by transcription of their activator XlnR. Regardless of the D-xylose concentration applied and whether CreA was functional or not, *xlnR* was constitutively expressed at a low level.

PR8.13

The *de novo* designed antifungal hexapeptide PAF26 is internalized by endocytosis prior to killing fungal cells

Alberto Muñoz[1] Jose F Marcos[2] Nick D Read[1]

1. Fungal Cell Biology Group, Institute of Cell Biology, University of Edinburgh 2. Food Science Department, Instituto de Agroquímica y Tecnología de Alimentos, CSIC

Natural and synthetic antimicrobial peptides (AMPs) provide promising alternatives for the control of microbial pathogens. PAF26 is a *de novo*-designed hexapeptide, cationic tryptophan-rich that has been shown to cross the plasma membrane and be fungicidal against pathogenic fungi. In the present work, the mechanism of internalization of PAF26 has been characterized in detail using the model fungus *Neurospora crassa*. PAF26 possesses two well-defined motifs: a N-terminal cationic and a C-terminal hydrophobic regions. We have shown how these motifs are independently responsible during the three steps of the PAF26 action involving its: (a) electrostatic interaction with cells, (b) cellular internalization; and (c) intracellular toxicity. Live-cell imaging of fluorescently labelled PAF26 and organelle probes, and mutant analyses indicate that it is endocytically internalized at low fungicidal concentrations. PAF26 initially accumulated in vacuoles that expanded, and then was actively transported into the cytoplasm, which coincided with cell death. Deletion mutants of the endocytic proteins RVS-161, RVS-167 and RAB-5 exhibited reduced rates of PAF26 internalization and fungicidal activity. Pharmacological experiments with live-cell probes showed that PAF26 internalization and antifungal action were energy-dependent, primarily actin-mediated, disrupted intracellular calcium homeostasis, and also induced rapid plasma membrane depolarization. PAF26 antifungal activity at low concentrations was shown to rely on its endocytic internalization. However at high fungicidal concentrations, PAF26 internalization was energy-independent and involved passive translocation. Our results provide new mechanistic insights into the mode-of-action of cell penetrating AMPs and for the rational design of more effective PAF26-based AMPs.

PR8.14

Molecular and chemical characterization of secondary metabolite gene clusters in *Fusarium fujikuroi*: the fusarin gene cluster

Eva-Maria Niehaus[1] Karin Kleigrewe[2] Philipp Wiemann[1] Bettina Tudzynski[3]

1. Institute of Molecular and Biology and Biotechnology of Plants 2. Institute of Food Chemistry 3. Institute for Molecular and Biology and Biotechnology of Plants, WWU Münster

The filamentous fungus *F. fujikuroi* is known to produce a variety of structurally diverse secondary metabolites such as the plant hormones gibberellins which cause enormous economical losses in trade of crops. In order to reduce the health risk of mycotoxins in food, feed and biotechnologically produced gibberellin preparations, identification of mycotoxin biosynthesis genes is of great importance. The recently sequenced genome of *F. fujikuroi* contains 16 polyketide synthases (PKS). So far only four of them can be linked to specific products: bikaverin, fusarin C, fumonisin and fusarubin. The focus of this work is studying the biosynthesis and regulation of the mutagenic mycotoxin fusarin C.

Since now only the hybrid polyketide synthase/nonribosomal peptide synthetase (PKS/NRPS) gene from the fusarin C cluster in *F. venenatum* is known. Here we present the characterization of the entire fusarin C gene cluster in *F. fujikuroi* by generating deletion mutants of each single cluster gene including the PKS/NRPS-encoding gene. By using these mutants, we are identifying the intermediates to finally unravel the entire biosynthetic pathway. In addition, we have created a deletion mutant missing all cluster genes except for the PKS/NRPS key enzyme gene to identify the first intermediate in the fusarin C pathway.

Besides, we study the regulation of gene expression for fusarin pathway genes by external signals, such as nitrogen availability and pH and the involvement of potential transcription factors and global regulators such as AreA, AreB, PacC and Velvet.

PR8.15

The Contribution of Melanin to Spore Surface Characteristics in *Aspergillus niger*

Ewald Priegnitz[1] Andreas Wargenau[2] Ulrike Brandt[1] Manfred Rohde[3] Sylvia Dietrich[1] Rainer Krull[4] André Fleißner[1]

1. Institut für Genetik, Technische Universität Braunschweig, Spielmannstraße 7, 38106 Braunschweig
2. Institut für Partikeltechnik, Technische Universität Braunschweig, Spielmannstraße 7, 38106 Braunschweig 3. Helmholtz-Zentrum für Infektionsforschung GmbH, Inhoffenstraße 7, 38124 Braunschweig 4. Institut für Bioverfahrenstechnik, Technische Universität Braunschweig, Gaußstrasse 17

Fungi grow on a great variety of organic and anorganic materials. Usually spore adhesion to solid surfaces comprises the first step of colony establishment or biofilm formation. In liquid culture, many filamentous fungi grow as hyphal aggregates or pellets, a process depending on cell-to-cell interactions of spores and/or hyphae. Pellet formation has been described as two-step processes, comprised of initial aggregation of ungerminated conidia followed by further attachment of spores, germ tubes and hyphae. To test the contribution of the initial aggregation/adhesion of ungerminated spores to pellet and biofilm formation in *Aspergillus niger*, we altered the physical and chemical surface characteristics of conidia by inactivating melanin biosynthesis. Albino mutants were constructed by the deletion of the *alb1* gene, encoding a polyketide synthase essential for pigment biosynthesis. Δ*alb1* conidia exhibit an altered surface structure and changed physiochemical properties. Spore aggregation in liquid culture differs significantly in a pH dependent manner between wild type and mutant. However, further pellet formation and enzyme productivity is unaffected, suggesting a minor role of initial spore adhesion in pellet formation. In contrast, under biofilm promoting conditions, Δ*alb1* mycelium adhere more stably to polymer surfaces, suggesting that initial conidia adhesion promotes sessile growth. Since enzyme productivity of biofilms was significantly increased compared to pellet cultures, we will further focus on biofilm analysis.

PR8.16

Analysis of a New Secondary Metabolite Gene Cluster in *Fusarium fujikuroi*

Lena Studt[1] Philipp Wiemann[2] Karin Kleigrewe[3] Hans-Ulrich Humpf[3] Bettina Tudzynski[2]

1. Institute of Food Chemistry, Institute of Plant Biology and Biotechnology 2. Institute of Plant Biology and Biotechnology 3. Institute of Food Chemistry, WWU Münster

The filamentous fungus *Fusarium fujikuroi* is best known for its production of gibberellins (GAs), natural phytohormones that lead to hyperelongation and chlorosis in rice plants. Besides the production of GAs, *F. fujikuroi* produces a wide range of other secondary metabolites, such as the polyketide-derived mycotoxin fusarin C or bikaverin.

The recently sequenced genome of *F. fujikuroi* revealed 16 polyketide synthases (PKSs) of which so far only a few can be assigned to their respective product. Here we present the discovery and subsequent investigation of an almost forgotten group of polyketides belonging to the fusarubin (FSR) family in *F. fujikuroi*. The corresponding gene cluster was identified and the regulatory network that governs FSR production has been studied. Our results so far indicate a rather complex regulation, including the importance of the initial pH, the nitrogen availability and the controverse involvement of Velvet and G-Protein mediated signaling. This complex regulatory network leads to an even more complex accumulation of the various FSR derivatives, of which the predominant products were determined using different chemical approaches. In addition, single deletion mutants of all *fsr* cluster genes revealed the biosynthetical pathway leading to the formation of the FSR derivatives. Phylogenetic analyses showed close homologies of the PKS Fsr1 to Pgl1 from *F. graminearum* and *F. verticillioides*, indicating the involvement of these pigments in the coloration of the fruiting bodies. Sexual crosses confirmed that the FSR pigments are the so far unknown perithecial pigments in *F. fujikuroi*.

PR8.17

ChemoGenomics: Discovery of novel fungicides and their targets in the phytopathogen *Fusarium graminearum*

Gopal Subramaniam[1] Christopher Mogg[2]
1. Agriculture Canada 2. Carleton University

Chemical genetics screen is based on the ability of small chemical molecules to bind to biological molecules and alter their function. Screening of pharmaceutical libraries has revealed novel molecules effective against cancer and other diseases. We have adopted similar approach and identify bio-active compounds that will block the growth and development of *F. graminearum*. We have developed a 96-well format to monitor the growth *of F. graminearum* in liquid media. The fungus is tagged with a green fluorescent protein (GFP) and the growth is monitored by the measurement of fluorescence of the GFP. This format facilitates high throughput screening for small molecules that could potentially disrupt the growth of the fungus. As proof of concept, we screened ~560 compounds from theTimTec NDL-3000 natural product collection (TimTec LLC,Newark,DE,USA) and identified several compounds with anti-Fusarium properties.

One compound identified form our screen, “Antofine” was purified from *Vincetoxicum rossicum* and was used in subsequent studies, to identify targets in the fungus. We used the gene deletion library of the budding yeast *Sacchromyces cerevisiae* to identify targets for Antofine. Twenty two potential targets of Antofine were identified and GeneMANIA (http://www.genemania.org), an online multiple association network integration algorithm was used to uncover information pertaining to genetic and physical interactions of these targets. Our efforts to identify targets in Fusarium against Antofine will be discussed

PR8.18

Efficient plant biomass degradation by thermophilic fungus *Myceliophthora heterothallica*

Joost van den Brink[1] Gonny van Muiswinkel[2] Sandra Hinz[2] Rob Samson[1] Ronald de Vries[1]
1. CBS Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD, Utrecht, The Netherlands 2. Dyadic Netherlands, Nieuwe Kanaal 7-S, 6709 PA Wageningen, The Netherlands

Rapid and efficient enzymatic degradation of plant biomass into monomeric sugars is currently a major challenge for sustainable production of biochemicals and biofuels. The best studied and most widely used plant-degrading enzymes are produced by *Trichoderma* and *Aspergillus* species, and they are most effective over a temperature range of 40 – 50°C. As a consequence, these moderate temperatures have long reaction times for complete saccharification of plant biomass. It would therefore be desirable to have elevated hydrolysis temperatures using thermostable enzymes. The collection at the CBS Fungal Biodiversity Centre contains several thermophilic fungi, which produce thermostable enzymes up to 70-80°C.

A screening of 32 thermophilic species resulted in several candidates with interesting plant-degrading enzymes. Particularly the genus *Myceliophthora* contains isolates with rapid growth on complex polysaccharides. We elucidated the phylogeny of *Myceliophthora* isolates and distinguished 10 different species, of which four are thermophilic. The isolates with the fastest growth on crude plant material were divided in two species: *M. thermophila* and *M. heterothallica*. The new phylogenetic classification of *M. heterothallica* isolates was further supported by physiological differences between the two species. Also, in contrast to *M. thermophila* isolates, *M. heterothallica* has a functional sexual cycle. *M. heterothallica* isolates were studied in detail for their ability to release sugars from crude plant biomass. Furthermore, crossing experiments between *M. heterothallica* isolates resulted in offspring with an even higher potential in rapid and efficient enzymatic degradation of plant biomass.

PR8.19

Effective production of Itaconic Acid in A. *niger*

Laura van der Straat[1] Marieke Lammers[1] Marloes Vernooij[1] Willy van den Berg[1] Jan Cordewener[2] Ingrid Vermeer[2] Andries Koops[2] Leo de Graaff[1]

[1.] Fungal Systems Biology, Laboratory of Systems and Synthetic Biology, Wageningen University, Dreijenplein 10, 6703 HB Wageningen, Netherlands [2.] Bioscience, Plant Research International, Droevendaalsesteeg 1, 6708 PB, Wageningen, The Netherlands

Itaconic acid is an important building block for the chemical industry that can be produced from sugars in a fermentative process. Currently, *Aspergillus terreus* is most frequently used for the commercial production of itaconic acid. The itaconic acid production pathway in *A terreus* is similar to the citric acid pathway in *Aspergillus niger*. Citric acid is the precursor for *cis*-aconitic acid which can be converted to itaconic acid by decarboxylation. Itaconic acid in *A. terreus* is produced upto a concentration of 80 g/L while citric acid production in *A. niger* reaches concentrations over 200 g/L which show the enormous potential of *A. niger* as a production host for itaconic acid. However, the key-enzyme *cis*-aconitic acid decarboxylase (CadA) in the itaconic acid production pathway is lacking in *A. niger*. Within the genome of *A. terreus* the *cadA* gene is flanked by two putative transporters, a mitochondrial transporter and a plasmamembrane transporter. The expression of the *cadA* gene in an *A. niger* strain optimized for citrate production resulted in the production of itaconic acid. The amount of itaconic acid produced by *A. niger* is further improved by using a codon-optimized version of the *cadA* gene. Still, significant amounts of citrate were produced suggesting that the conversion to itaconic acid is not very efficient. To improve the efficiency and to increase the itaconic acid production both putative transporters are introduced. Introduction of the mitochondrial transporter strongly increased the itaconic acid production.

PR8.20

Efficient expression system for production of natural products in Aspergillus *oryzae*

Khomaizon Abdul Kadir Pahirulzaman, Zahida Wasil, Andrew M Bailey, Thomas J Simpson, Russell J Cox, Colin M Lazarus

University of Bristol, UK

pTAex3 vector has previously been modified by insertion of a GATEWAY destination module into the *amy*B expression cassette to produce pTAex3GS. This facilitates directional transfer of genes such as fungal polyketide synthases (PKS) and hybrid polyketide synthase-non-ribosomal peptide synthases (PKS-NRPS) into the expression site. To simplify plasmid construction for whole-pathway expression pTAex3GS was first converted to a yeast-*E. coli* shuttle vector, pTAYA.GS. An EST database was used to identify genes expressed at a high level under the culture conditions we use for heterologous gene expression in *A. oryzae*, and the promoters of three of them, P*adh*, P*eno* and P*thia*, were evaluated. *A. oryzae* transformants expressing eGFP from P*adh* and P*eno* exhibited intense green fluorescence. We used homologous recombination in yeast to combine P*adh* and P*eno* together with the strong constitutive *A. nidulans* promoter P*gpd*A in pTAYA.GS-Page, a novel multiple gene expression vector which has *Asc*I sites downstream of each promoter. The system was tested by reconstructing and expressing the *Beauveria bassiana* tenellin and *Aspergillus nidulans* aspyridone synthesis pathways, each of which comprises a hybrid PKS-NRPS together with an enoyl reductase and one or more cytochrome P450s, in *A. oryzae*. Yeast recombination between the *Asc*I-cut vector and three PCR products simultaneously placed the tailoring genes downstream of the promoters, creating pTAYA.GSargTen and pTAYA.GSargAsp. Subsequent introduction of the PKS-NRPS gene by GATEWAY recombination created pTAYAargTenellin and pTAYAargAspyridone. Reconstruction of the tenellin and aspyridone biosynthetic pathways proved the multiple gene assembly concept, and chemical analysis showed that 5 of the 11 pTAYAargTenellin transformants analysed produced tenellin, pretenellin B and prototenellin A. Similarly 13 of 14 pTAYAargAspyridone transformants analysed produced aspyridone A and preaspyridone. The results show that our system allows the rapid and simple reconstruction of whole (small) biosynthetic pathways for heterologous expression from a single plasmid in *A. oryzae*. Further development of the system has included replacement of the arginine selectable marker with basta- and phleomycin-resistance genes to allow expression of biosynthetic pathways of up to 12 genes by co-transformation of *A. oryzae* with just 3 plasmids

PR8.21

Genetic And Molecular Characterization Of The *Penicillium chrysogenum* PcrsmA Gene, Encoding A Homologue Of The *Aspergillus nidulans* bZIP Transcription Factor RsmA

Kordula Becker, Sandra Bloemendal, Ulrich Kück

Christian Doppler Labor für „Biotechnologie der Pilze", Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, D-44780 Bochum

Penicillium chrysogenum is the major industrial producer of the ß-lactam antibiotic pencillin. The regulation of cellular processes like secondary metabolism as well as morphogenesis and development in this filamentous fungus is controlled by components of the so called velvet-like complex which was first described in *Aspergillus nidulans*. Previous studies identified *rsmA* (remediator of secondary metabolism) in *A. nidulans* which after overexpression was shown to remediate secondary metabolism defects in knockout strains of the velvet components LaeA and VeA. Hence, it is believed that the putative bZIP transcription factor RsmA may act as a positive regulator of many secondary metabolite pathways. Bioinformatics confirmed the existence of many known and putative bZIP proteins from several fungi such as *P. chrysogenum* and higher eukaryotes which show high homology to RsmA from *A. nidulans*.

Until now, little is known about the biosynthetic regulation of the ß-lactam antibiotic penicillin. Thus, further investigation of putative regulators of secondary metabolism such as PcRsmA, the *P. chrysogenum* homologue to RsmA is necessary to extend the current knowledge of the regulatory network controlling both penicillin biosynthesis and morphogenesis in *P. chrysogenum*. A Δ*Pcku70* strain as recipient for homologous recombination together with the FLP/*FRT* recombination system were used to generate a marker-free Δ*PcrsmA* strain for functional and morphological characterization of PcRsmA. Our results will support deciphering of regulatory networks related to the velvet-like complex in *P. chrysogenum*.

PR8.22

Contamination of peripheral venous catheter associated fungal biofilms

Ouassila Bekkal Brikci-Benhabib[1] Zahia Boucherit-Otmani[1] Kebir Boucherit[1] Christine Gendarme[2] Samir Mehiaoui[1]

[1.] Laboratoire Antibiotiques Antifongiques, Physico-chimie, Synthèse et Activité Biologique, University of Tlemcen, Algeria [2.] Laboratoire de Science et Génie des Surfaces, Service Commun de Microscopie Electronique Nancy, France

Introduction: In the hospital, the use of venous catheters for the administration of drugs exposes patients to increased risk for fungal contamination. Yeasts can form biofilms on the surface of catheters. These properties give them resistance to antifungal agents. In an attempt to integrate this clinical reality, we undertook a study in the neonatal unit of EHS-Tlemcen, for aim to isolate yeasts from venous catheters directly after excision of newborns hospitalized. The approach is to test the ability of yeasts isolated to form biofilms and to test their resistance against amphotericin B.

Materiels and Methods: These samples were taken from implanted venous catheters for 72 hours or more. They are removed directly from patients and placed in Sabouraud liquid medium.The tubes were then agitated in a vortex for 1 minute. Purified strains were identified by API Candida (Biomerieux,France)

Results: From 281 samples, 15 yeasts were isolated, colonizing venous catheters implanted in newborns. *Candida albicans, Candida parapsilosis, Cryptococcus néoformans , Candida famata, Trichosporon spp.* and *Saccharomyces cereviseae* were isolated. C*andida* and *Cryptococcus* species have the ability to form biofilms

Conclusion: Sessile *Candida* species and *Cryptococcus neoformans* isolates were less susceptible than the planktonic populations to AmB.

These results suggest contamination of venous catheters with strains isolates able to form biofilms may be associated with infection.

PR8.23

A *velvet*-like complex in *Penicillium chrysogenum*: the two faces of PcVelC

Sandra Bloemendal, Birgit Hoff, Katarina Kopke, Alexandra Katschorowski, Sarah Milbredt, Jens Kamerewerd, Ulrich Kück

Christian Doppler Labor für "Biotechnologie der Pilze", Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, 44780 Bochum

The filamentous fungus *Penicillium chrysogenum* is the main industrial producer of the pharmaceutically relevant beta-lactam antibiotic penicillin. All three biosynthesis genes are found in a single cluster and the expression of these genes is known to be controlled by a complex network of global regulators. Recently, the velvet complex containing several global regulators of secondary metabolism was described for the model fungus *Aspergillus nidulans*. Next to the founding member VeA, several other velvet-like proteins were meanwhile identified in *A. nidulans* and many other filamentous fungi.

Here we provide a functional analysis of a *velvet*-like complex in *P. chrysogenum* with structurally conserved components that have distinct developmental roles, illustrating the functional plasticity of these regulators in genera other than *Aspergillus*. Data from penicillin bioassays, quantification of conidiospores as well as detailed microscopic investigations of these knockout mutants clearly show that all velvet-like proteins are involved in secondary metabolism and other distinct developmental processes. Interestingly, the velvet-like protein PcVelC seems to be a major regulator of penicillin biosynthesis and conidiation. By protein-protein interaction studies using bimolecular fluorescence complementation, tandem-affinity purification and yeast two-hybrid, we want to extend the analysis of the velvet-like complex in *P. chrysogenum*. These analyses will focus on the velvet-like protein PcVelC to elucidate its opposing roles in the regulation of penicillin biosynthesis and conidiation. Our results widen the current picture of regulatory networks controlling both fungal secondary metabolism and morphogenesis, which is significant for the genetic manipulation of fungal metabolism as part of industrial strain improvement programs.

PR8.24

New hemicellulolytic enzymes for bioethanol production

Ourdia Bouzid[1,2] Marina Nadal[1] Pedro M. Coutinho[3] Bernard Henrissat[3] Ronald P. de Vries[2]

[1] Microbiology and Kluyver Centre for Genomics of Industrial Fermentation, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands, [2]CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT, Utrecht, The Netherlands, [3] Architecture et Fonction des Macromolécules Biologiques, CNRS - Universités d'Aix-Marseille I & II, 163 Av. de Luminy, 13288 Marseille, France

Hemicellulolytic enzymes such as endoxylanases from glycosyl hydrolysis family 11 (GH11) and 10 (GH10) have a major importance in several industrial application such as bioethanol production. They improve the yield of monosaccharides from plant biomass by depolymerization of hemicellulose. This will expand the set monosaccharides available for conversion to bioethanol by yeast.

Most commercially applied fungal endoxylanases are obtained from *Aspergillus* or *Trichoderma* species. However, comparison of fungal genomes identified several species that are much richer in genes encoding these enzymes. One of them is *Podospora anserina*, which has a saprobic life style, and is only found in dung of herbivores [1]

A growth profile of *P. anserina* on various substrates shows a high ability to growth in substrates that are rich in hemicellulose such as wheat straw and Arundo donax. Both substrates are commonly used in ethanol production. Using a combination of comparative genomics and phylogeny new GH10 and 11 enzymes were selected to increase the efficiency of ethanol production.

Reference:

[1] Espagne E, Lespinet O, Malagnac F, Da Silva C, Jaillon O, Porcel BM, Couloux A et al., The genome sequence of the model ascomycete fungus Podospora anserina. Genome Biol. 2008;9 (5) : R77.

PR8.25

Genetic and biochemical investigations of natural product formation in *Boletales*

Jana Braesel, Dirk Hoffmeister
Friedrich-Schiller-Universität Jena

The release of an increasing number of basidiomycete genomic sequences provides valuable insight into their capacity to biosynthesize small molecule natural products. The *Boletales* are represented by *Serpula lacrymans* (dry rot fungus) and *Paxillus involutus* (roll rim mushroom). Assessed by the number of genes encoding polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPSs) in these representative genomes the *Boletales* include particularly prolific producers of secondary metabolites.

This poster presents our current work on genera within the *Boletales* focusing on NRPSs and quinone synthetases, i.e., NRPS-like enzymes. Methodically, we relied on a combined approach of genetic and biochemical methods, complemented by liquid chromatography.

PR8.26

***Aspergillus clavatus* as a potential enzyme source to use in biomass degradation to produce second-generation ethanol: Cloning and expression of hemicelulases after secretomic analysis using different pre-treatment sugar cane bagasse**

Rosymar Coutinho de Lucas[1] Simone Carvalho Peixoto Nogueira[1] André Ricardo de Lima Damásio[2] Fábio Marcio Squina[2] Rolf Alexander Prade[3] Héctor Francisco Terenzi[1] João Atílio Jorge[1] Maria de Lourdes Teixeira de Moraes Polizeli[1] Fernando Segato[2]
[1.] USP/RP-Sao Paulo University, [2] CTBE-National Laboratory of Science and Technology of Bioethanol, [3.] OSU-Oklahoma State University 3

Aspergillus clavatus is a cosmopolitan fungus that had its genome sequenced by The Institute for Genomic Research. A detailed comparative genomic analysis using a large number of fungi database showed that this microorganism is a potential enzymes producer. In addition, there are few studies about this fungus. The plant cell wall polysaccharides are being considered as source of renewable energy such as biofuels owing to the fact that they are the most abundant reserves of carbon. It can be conveniently divided into three groups namely cellulose, pectin and hemicellulose, with hemicellulose being the second most abundant biopolymer component of plant cell wall and composed by xylan, arabinan, mannan, glucomannan, galactomannan, and glucogalactomannan. Due to its complexity a large set of enzymes are necessary to degrade the plant cell wall. In order to study potential enzymes directly involved in degradation of the most abundant brazilian biomass we report in this work, the comparative analysis of the *A. clavatus* secreted in 5 different pre-treatment sugar cane bagasse using glucose as the control, cloning and expression of hemicellulases. The proteomic analyses have identified 135 different proteins where 2% of those are enzymes related with biomass degradation. The relative difference reflect the necessity to use specific enzymatic pool to degrade different pre-treated sugar cane bagasse, because of diference in the sugar compositions. In addition, to perform the heterologous protein expression of *A. clavatus* hemicellulase we used our *Aspergillus nidulans* expression system and the recombinant enzymes were highly expressed and secreted to the culture medium.

PR8.27

Molecular diversity of *Cercospora zeina* on maize in South Africa

Mischa Muller[1] Burton Bluhm[2] Bridget Crampton[1] Dave Berger[1]
1. University of Pretoria 2. University of Arkansas

Grey Leaf Spot is a prevalent worldwide maize disease of global economic importance, and is specifically caused by *Cercospora zeina* in South Africa. In order to implement control measures, it is important to understand the population diversity of the fungus. As no sexual stage of *C. zeina* has been observed, we hypothesised that there would be greater variability among isolates from different populations rather than within a population in South Africa. In order to address this question, we analysed *C. zeina* isolates from GLS infected maize from three regions within South Africa viz. Machadodorp, Greytown and Cedara. We collected 40 GLS infected maize leaves from each region, and isolated and maintained cultures from 40 single *C. zeina* conidia from each area. DNA was isolated from fungal cultures, and a subset of 30 isolates from different regions was screened with 36 Simple Sequence Repeats (SSRs) designed from the genome sequence of a US *C. zeina* isolate. Twelve SSRs were found to display polymorphisms across isolates, and six were selected to score samples against. The amplified SSRs were analysed on agarose gels and scored for variability to assess the diversity amongst isolates within South Africa. From these results we could determine that there was variability within and between isolates from different regions. Thus, we observed greater diversity within *C. zeina* populations than originally anticipated.

PR8.28

Identification And Biochemical Characterization Of Putative miRNAs In The Penicillin Producer *Penicillium chrysogenum*

Tim Alexander Dahlmann, Minou Nowrousian, Jens Kamerewerd
Christian Doppler Labor für „Biotechnologie der Pilze“, Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, D-44801 Bochum

Since the early 1990s small non-coding regulatory RNAs were identified in a variety of different eukaryotic organisms. In fungi, the transient inactivation of gene expression by homologous sequences was first observed in *Neurospora crassa*. The effect of short interfering RNAs (siRNAs) is mediated by the RNA-induced silencing complex (RISC). Besides the class of exogenous siRNAs a previous study shows that there is another class of regulatory small RNAs, which interact with RISC in filamentous fungi. These endogenous small RNAs are derived from RNA hairpin structures of RNA polymerase II transcripts and show typical characteristics of microRNAs (miRNAs), which play an important role in the regulation of gene expression in plants and metazoan.

The aim of this study is to investigate whether miRNA-like regulatory RNAs can be detected in the penicillin producer *Penicillium chrysogenum*. We performed an *in silico* analysis, based on RNA next-generation sequencing data, to predict putative miRNAs hairpin structures. By this approach, sequences with familiar characteristics of previously identified miRNA-precursors could be identified. To confirm the *in silico* predictions, transcript analysis were done *in vitro*. The statements of the predictions and the results from the transcript analysis confirmed the existence of the small RNAs and their precursors. To provide evidence for a regulatory activity of the putative miRNA-like sequences, mRNA-targets were chosen and inducible overexpression constructs of the miRNA-like sequences were generated. The results of this study suggest the existence of a miRNA based silencing mechanism in *P. chrysogenum*.

PR8.29

Secretion of Client Proteins in Aspergillus

Fernando Segato[1] Andre Damasio[1] Thiago Gonçalves[1] Rosymar de Lucas[2] Fábio Squina[1] Stephen Decker[3] Rolf Prade[4]

1. Brazilian Bioethanol Science and Technology Laboratory – CTBE 2. São Paulo University – USP 3. National Renewable Energy Laboratory – NREL 4. Oklahoma State University

Production of pure and high-yield client proteins is an important technology that attends the needs for industrial applications of enzymes as well as basic experiments such as protein crystallization. Client protein expression platforms are available in *Escherichia coli* and the methylotrophic *Pichia pastoris* that result in proteins released to the intracellular cell extract and extracellular medium, respectively. Fungi are utilized in industrial protein production because of their ability to secrete large quantities. In this study we engineer a high-expression-secretion vector, pEXPYR that directs proteins towards the extracellular medium in two *Aspergillii* host strains, examine the effect of maltose overexpression, production time and pH-dependent protein stability in the medium. We describe five client proteins that accumulated 50-100 mg of protein per liter and only one protein was secreted at low quantities. We also test a recyclable genetic marker that allowed secretion of multiple client proteins, enabling the design of an enzyme activity set.

PR8.30

Functional characterisation of Cytochrome P450 genes from the wheat leaf pathogen *Mycosphaerella graminicola*

Mark Derbyshire[1] Andy Bailey[2] Kim Hammond-Kosak[1] Mikael Courbot[3] Jason Rudd[1]

1. Rothamsted Research 2. University of Bristol 3. Syngenta

The Ascomycete fungus *Mycosaphaerella graminicola* (Anamorph *Septoria tritici*) causes the disease Septoria tritici blotch (STB) in wheat. This pathogen is typical of temperate, high-rainfall environments and is of major international importance owing to its ability to substantially reduce agricultural yields. *M. graminicola* infects both hexaploid bread wheat (*Triticum aestivum*) and tetraploid durum wheat (*T. turgidum*) and is largely controlled by fungicide applications. However *M. graminicola* has evolved resistance to several classes of fungicides highlighting the continued need to identify novel targets for disease intervention.

Little is known about fungal metabolism during plant infection. The cytochrome P450 (CYP450) gene superfamily represents an ideal target for further investigation. Members of this group are (1) known to be present and regulated together with clusters of other genes important for the synthesis of secondary metabolites including mycotoxins, pigments and defence compounds; (2) known to act directly upon potentially harmful xenobiotics such as plant defence compounds and potentially fungicides in order to detoxify them; and (3) known to operate in various cellular locations. As a preliminary to this project, Solexa next generation transcriptome sequence analyses were performed at different time points post inoculation of wheat leaves with the fungus. Analysis of expression of members of the CYP450 gene family has identified many which are specifically expressed early during plant infection and some which may reside within secondary metabolite clusters. These will be further investigated initially through the generation of fungal CYP450 gene deletion strains. It is anticipated that this project will provide new insights into the genetic basis underlying the metabolic changes occurring during *M. graminicola* infection of wheat.

PR8.31

Mutation of Genes *areA*, *wcoA*, *cryA* and *acyA* Affect The Regulation of Fusarin Production in *Fusarium fujikuroi*

Violeta Diaz- Sanchez[1] Marta Castrillo[1] Jorge Garcia-Martinez[1] Alejandro F. Estrada[2] Javier Avalos[1] M Carmen Limon[1]

1. Department of Genetics, Faculty of Biology, University of Seville, Spain 2. Growth & Development, Biozentrum, University of Basel, Switzerland

Fusarium fujikuroi is a rice pathogen that produces a diversity of secondary metabolites, that includes terpenoids with biotechnological applications, as carotenoids and gibberellins, and potentially harmful polyketides, as bikaverin and fusarins. In contrast to other compounds, very limited information is available on the biosynthesis and regulation of fusarins in this species. We formerly reported than fusarin production depends on nitrogen availability and is negatively affected by light. To learn more about the genetic basis of this regulation, we have studied the fusarin-producing pattern of targeted mutants on several regulatory genes affecting the synthesis of other metabolites. The loss of the global regulator of the nitrogen metabolism AreA affects fusarin production, but the effect varies depending on the nitrogen source. Our study was extended to two photoreceptor genes, encoding the White Collar protein WcoA and the DASH-cryptochrome CryA, and the gene for the adenylyl cyclase AcyA, that mediates the synthesis of the regulatory signal cAMP. The four classes of mutants investigated exhibit different alterations in the accumulation of fusarins, indicating that this pathway is under control of the regulatory network involved in nitrogen-regulated secondary metabolism. Despite the participation of two putative photoreceptors, the effect of light is mainly explained by light-induced instability of the secreted fusarins.

PR8.32

CefR* Acts As A Regulator Of β-Lactam Transporters In *Acremonium chrysogenum

Marta Fernández-Aguado[1] Fernando Teijeira[2] Ricardo Vicente Ullán[2] Carlos García-Estrada[2] Rebeca Domínguez-Santos[1] Silvia María Albillos[2] Pedro Iván Hidalgo[1] Juan Francisco Martín[1,2]

1. Universidad de León, España 2. Asociación de Investigación INBIOTEC: Instituto de Biotecnología de Léon

Acremonium chrysogenum is an ascomycete filamentous fungus which has been used for the industrial production of cephalosporin-like antibiotics. Our previous investigations about the cephalosporin C biosynthetic pathway led us to discover three genes, *cefT*, *cefP* and *cefM* involved in the secretion of intermediates and therefore in the compartmentalization of this pathway. The genes *cefP* and *cefM* codify membrane proteins that carry the intermediates isopenicillin N and penicillin N, respectively, through the peroxisomal membrane. *CefT* protein takes part in the secretion of hydrophilic β-lactams through the plasma membrane.

However, the regulation of the secretion of the intermediates and the final product of cephalosporin C biosynthetic pathway is still unknown. For this is a reason a search for regulator genes within the early biosynthetic cluster of cephalosporin C was made. A new ORF was found encoding a protein (CefR) which shows homology with other regulatory proteins and bears a “Fungal_trans” domain, characteristic of many fungal regulators. Targeted inactivation of *cefR* diminishes and delays the cephalosporin production but increases the penicillin N secretion. On other side, the overexpression of *cefR* decreases the secretion of penicillin N, preventing the loss of intermediates and then inducing the cephalosporin C production. Northern blot analysis revealed that CefR protein works as a repressor of *cefT* and *cefM* genes, making possible the use of these intermediates in the synthesis of cephalosporin C. In summary, CefR protein represents the first example of a regulator of β-lactam transporters described in *A. chrysogenum.*

PR8.33

PenV Is A Vacuolar Membrane Protein Related To The Penicillin G Biosynthetic Pathway In Penicillim chrysogneum

Fernández-Aguado, Marta[1] Ullán, Ricardo Vicente[2] Teijeira, Fernando[2] García-Estrada, Carlos[2] Domínguez-Santos, Rebeca[1] Albillos, Silvia María[2] Hidalgo, Pedro Iván[1] Martín, Juan Francisco[1,2]

[1.] Universidad de León, España, [2.] Asociación de Investigación INBIOTEC: Instituto de Biotecnología de Léon,

The penicillin G (PenG) biosynthetic pathway is compartmentalized in Penicillium chrysogenum, taking place in the cytosol, where δ-(L-α-aminoadipil-L-cysteinil-D-valine) (ACV) and isopenicillin N (IPN) are formed, and inside peroxisomes, where PenG is synthesized. Transport processes linked to this compartmentalization are largely unknown, but most of them would be mediated by transporter membrane proteins. During the search for this kind of transporters, was found the protein PenV showing a 42% of identical amino acids with the IPN transporter CefP, described in Acremonium chrysogneum. To elucidate the function of the protein PenV, the encoding gene, penV, was silenced by the mechanism of small interfering RNAs. As a consequence of the silencing process an alteration of the transcription levels of the β-lactam biosynthetic genes occurs, together with a drastic decrease of the yield of PenG and the intermediates ACV and IPN. The silencing process also causes alterations in several developmental aspects. The subcellular location of PenV was determined through the expression of the red fluorescent protein PenV-DsRed in the strain Wisconsin 54-1255. Microscopy analysis revealed the presence of PenV, not in peroxisomes but in the vacuolar membrane. Curiously, non ribosomal peptide synthetases appear to be linked to the cytosolic side of the vacuolar membrane. Given these results is purposed the involvement of PenV in the transport of amino acids from the vacuolar lumen to the cytosol, where they would be used as substrates for the synthesis of structural proteins and as precursors of the β-lactam biosynthesis.

PR8.34

The program of iterative fungal PKS-NRPSs

Katja Maria Fisch[1] Ahmed A. Yakasai[1] Zahida Wasil[1] Colin M. Lazarus[2] Andrew M. Bailey[2] Russell J. Cox[1] Thomas J. Simpson[1]

[1.] School of Chemistry, University of Bristol, UK [2.] School of Biological Sciences, University of Bristol, UK

Fungal hybrid polyketide synthase-non ribosomal peptide synthetases (PKS-NRPSs) are huge enzymes that carry out a large number of chemical reactions in the biosynthesis of the natural product. Examples of natural products are tenellin **1**, desmethylbassianin **2** and bassianin **3** produced by different Beauveria strains. Current knowledge about the programming of these enzymes is very limited. Important questions as how the chain length of a polyketide or the methylation pattern is encoded are as yet unanswered.

Tenellin synthase (TenS) and desmethylbassianin synthase (DMBS) produce together with their tailoring enzymes the similar yet not identical natural products **1** and **2**. These natural products differ in chain length and in methylation pattern. The domain architecture of both enzymes is identical, i.e. KS-AT-DH-CMet- ER^0-KR-ACP-C-A-T and homology is over 85%. Domain swaps between different PKS-NRPSs often fails presumably to incompatibility of protein structures. Here we present the successful swap of domains from two highly similar enzymes.

The poster covers domain swap strategy and outcome. It will also outline the use of the domain swap technology to resurrect the extinct compound **3**

References

1. Eley, K. L.; Halo, L.M.; Song Z. *et al., ChemBioChem*2007,*8*, 289-297.
2. Heneghan M. N., Yakasai A. A., Williams K. *et al*., *Chemical Science* **2011**, *2*, 972-979.
3. Fisch K. M., Bakeer W., Yakasai A. A. *et al*., *J. Am. Chem. Soc*. **2011**, *133*, 16635-16641.

PR8.35

Three acidic residues Glu31, Asp142 and Asp171 of *Aspergillus oryzae* cutinase CutL1 are required for both interaction with hydrophobin RolA and consequent stimulation of polyester-degradation.

Kimihide Muragaki[1] Kenji Uehara[1] Toru Takahashi[2] Fumihiko Hasegawa[3] Keietsu Abe[3]
[1.] Grad. Sch. Agric. Sci., Tohoku Univ. [2] NRIB, Japan [3.] New Industry Creation Hatchery Center, Tohoku University

Hydrophobins are amphipathic proteins, and are ubiquitous among filamentous fungi. When the industrial fungus *Aspergillus oryzae* is grown in a submerged medium containing a biodegradable polyester polybutylene succinate-coadipate (PBSA) as a sole carbon source, cutinase CutL1 and hydrophobin RolA are simultaneously secreted into the medium. RolA attached to the surface of PBSA particles specifically recruits CutL1, resulting in stimulation of PBSA hydrolysis (1). In our previous study, we identified amino acid residues involved in the RolA-CutL1 interaction by means of chemical modification and site-directed mutagenesis of RolA and CutL1. As a result, we found that His32 and Lys34 of RolA and Glu31, Asp142, Asp171 of CutL1 are involved in the RolA-CutL1 interaction. In the present study, to quantitatively elucidate the role of the three acidic amino acid residues of CutL1 in the RolA-CutL1 interaction, we characterized kinetics of the interaction between CutL1 variants of the three residues and wild type RolA by using Quartz crystal microbalance (QCM). The QCM analysis revealed that replacement of the three acidic amino acid residues of CutL1 to serine caused increases in K_D values for interaction with RolA. In conclusion, Glu31, Asp142 and Asp171 of CutL1 are critically required for the RolA-CutL1 interaction by multivalent effect.

(1) Takahashi et al. Mol Microbiol. 57:1780 (2005)

PR8.36

A New Method of Increasing the Hydrolytic Activity of *T.atroviride*

Sebastian Graczyk, Urszula Perlinska-Lenart, Wioletta Gorka-Niec, Patrycja Zembek, Sebastian Pilsyk, Joanna Kruszewska
Institute of Biochemistry and Biophysics PAS

Trichoderma species are widely used as biological control agents due to their strong antagonistic activity against phytopathogenic fungi and effectiveness in simultaneously promoting plant growth and defense mechanisms. Biocontrol efficiency may result from a direct interaction between the pathogen and *Trichoderma* when lytic enzymes secreted by the latter degrade the cell wall of the pathogen, causing damage of its cells.

It was observed that many, if not all, lytic enzymes secreted by *Trichoderma* were glycosylated. It was postulated that O-glycosylation of these enzymes was closely correlated with their secretion. Taking into account this correlation we decided to improve the biocontrol abilities of *T. atroviride* P1 against plant pathogens by activation of the mevalonate pathway; in this pathway dolichyl phosphate, a carrier of carbohydrate residues in the glycosylation processes, is produced together with many other biologically active molecules, such as sterols, terpenoids and quinones. The new strains of *T. atroviride* were characterized in terms of the activity of overexpressed enzymes, protein secretion, activity of secreted hydrolases and antifungal properties. Our results showed that activation of the mevalonate pathway could result in higher antifungal activity of the studied new strains.

PR8.37

Transcriptional analysis of *Trichoderma reesei* cultivated in the presence of different lignocellulose substrates

Mari Häkkinen, Mikko Arvas, Merja Oja, Nina Aro, Merja Penttilä, Markku Saloheimo, Tiina Pakula
VTT Technical Research Centre of Finland

Trichoderma reesei is a soft rot Ascomycete fungus able to secrete enzymes extremely efficiently. Availability of the complete genome sequence of *T. reesei* has made it possible to utilise genome wide methods to study protein production by the fungus and to utilise the information obtained to develop new strains with better enzyme production qualities. In order to study the co-expression of enzyme genes, a complete list of CAZymes of *T. reesei* needed to be obtained. Novel CAZymes were searched from the genome by mapping *T. reesei* proteome with Blast search to the protein sequences of the CAZY database. New annotation was given to several genes in order to gain more information on the possible function of novel candidate genes and to specify the annotation of previously identified genes. A phylogenetic approach was used to reveal the functional diversification of *T. reesei* enzyme genes within CAZY families and between the gene duplicates. Expression of the hydrolytic system of *T. reesei* Rut-C30 was studied by cultivating the fungus in the presence of different lignocellulose substrates. Cultures were subjected to transcriptional profiling using oligonucleotide microarrays. Differentially expressed genes were identified and expression profiles of genes encoding lignocellulose degrading enzymes were compared to identify co-regulated groups of genes and genes needed for the degradation of specific substrates. Transcriptional profiling revealed a group of genes co-regulated on all of the substrates and genes which expression profiles were more diverse. Also some examples were found from co-regulation of enzyme genes according to genomic localization.

PR8.38

Novel manganese peroxidases of the litter-decomposing fungus *Agrocybe praecox*

Kristiina Hildén[1] Miia Mäkelä[1] Taina Lundell[1] Kari Steffen[1] David Archer[2] Annele Hatakka[1]
[1.] University of Helsinki [2.] University of Nottingham

Litter-decomposing fungi (LDF) are the primary decomposers of residual plant materials in forest soil. In spite of the ecological significance of LDF, relatively little is known about the molecular characteristics of their lignocellulose-degrading machinery. *Agrocybe praecox* is a litter-decomposing basidiomycete that is capable of mineralising synthetic lignin as well as decomposing various aromatic compounds. We have characterized the primary structure of two different MnP encoding genes of *A. praecox* which encode extracellular, short-type class II heme-containing peroxidases. *Mnp1* corresponds to previously characterized MnP1 enzyme and interestingly, both MnPs are deficient of one of the three conserved acidic amino acids involved in Mn^{2+} binding. Phylogenetically, the closest homologue to MnP1 is a class II peroxidase of the mycorrhizal, agaric basidiomycete *Laccaria bicolor* that lacks all conserved amino acids that bind Mn^{2+}. MnP2 resembles the hybrid-type of MnPs, as described in the wood-decaying, corticioid white-rot basidiomycete *Phlebia radiata*. When the fungus was grown on forest litter, laccase and MnP activities were detected. In birch leaf litter cultures, the transcript levels of expression of *mnp1* and *mnp2* were similar, whereas in cultures on conifer needle litter, transcription of the *mnp1* gene was up-regulated. Molecular characterization of the new MnP enzymes aims to understand better the physiology of litter and lignocellulose decay by *A. praecox*.

PR8.39

Characterization Of Two Redundant *Aspergillus flavus* Peptide Synthetase-like Enzymes

Daniel Schwenk[1] Saori Amaike[2] Ry Forseth[3] Frank Schroeder[3] Nancy Keller[2] Dirk Hoffmeister[4]
1. Hans-Knöll-Institut Jena 2. University of Wisconsin, Madison, WI 3. Cornell University, Ithaca, NY
4. Friedrich-Schiller-Universität Jena

LaeA, a regulator of secondary metabolism in fungi, controls expression of two genes encoding NRPS-like enzymes in Aspergillus flavus, termed LnaA and LnbA. Although these enzymes resemble α-aminoadipate reductases (i.e., primary metabolism enzymes), gene deletion and knockdown experiments pointed to a role for LnaA and LnbA in secondary metabolism. Specifically, the biosyntheses of heterocyclic L-tyrosine-derived natural products are dependent on these enzymes. Genetic data is also suggestive of functional redundancy of these two enzymes.

Hexahistidine-tagged LnaA and LnbA were heterologously produced, and assayed using the amino acid-dependent ATP-pyrophosphate exchange method. L-tyrosine was identified as clearly preferred substrate of both enzymes. Further biochemical characterization established divergent temperature and pH-optima, and differences regarding stereospecificity.

Our biochemical experiments prove that the LnaA and LnbA substrate spectrum is compatible with their tentative heterocyclic products. Further, participation of α-aminoadipate reductase-like NRPSs in secondary metabolism and functional redundancy of LnaA and LnbA, anticipated by genetic methods, is supported by the substrate spectrum of these enzymes.

PR8.40

Genome-wide transcriptome and proteome analysis of *Aspergillus oryzae* in the hypoxic stress condition

Ryutaro Jo, Minori Kouno, Ryouko Hamada, Masafumi Tokuoka, Kazuhiro Iwashita
National Research Institute of Brewing, Japan

In the process of making the Japanese traditional fermented foods, *Aspergillus oryzae* is exposed to hypoxic condition. In this study, we analyzed the effect of hypoxic condition on the physiology of *A. oryzae* using multi-omics analysis. Solid-state cultivation under hypoxic condition effect on morphology of *A. oryzae*, whereas hydrolytic enzyme activities were not significantly different except for glucoamylase. Transcriptional profiling revealed that expression of genes involved in glycolysis and ethanol fermentation were up-regulated under hypoxic (4% O_2) condition, which is supported at the protein level by proteomic analysis. On the other hand, expression of proteins involved in TCA cycle were decreased under hypoxic condition, which is consistent with the observation in the metabolite analysis where the amounts of organic acids in TCA cycle were increased in hypoxic condition. These results suggested that *A. oryzae* adapts to hypoxic condition by activation of glycolysis at transcriptional level and suppression of aerobic respiration at protein level. In addition, we found that gene expression level of BrlA involved in the conidiation was decreased under hypoxic condition. The BrlA over-expression mutant did not exhibit delayed conidiation, suggesting that atmospheric oxygen concentration effects on conidiation through BrlA gene expression. Our results provide the first report on the global physiological response of *A. oryzae* against hypoxia.

PR8.41

Autonomously replicating plasmids as a transient expression tool in *Aspergillus niger*

Ursula Kiesswetter[1] Marzena Blumhoff[2,3] Matthias Steiger[1] Christine Sanystra[1] Diethard Mattanovich[1,3] Michael Sauer[1,2,3]

[1.] Austrian Centre of Industrial Biotechnology (ACIB GmbH), [2.]School of Bioengineering, University of Applied Sciences FH-Campus Wien, [3.]BOKU University of Natural Resources and Life Sciences

The possibility of having an autonomously replicating plasmid for filamentous fungi broadens the horizon of genetic engineering. Tools like recombinases or reporter genes can easily be inserted to cells and just as well be expelled when taking away the selective pressure. Such a system can be used as a transient expression tool for genetic engineering purposes in *Aspergillus niger*.

AMA1 is a 6.1 kb DNA fragment that allows extrachromosomal replication in filamentous fungi and strongly enhances the transformation efficiency. However, plasmid construction is hindered by the two palindromic, inverted sequences that flank the 0.3 kb central region of the AMA1 fragment. Hints in literature led to the assumption that the sequence can be shortened without loosing the positive influence on transformation efficiency.

In this study we characterized different plasmids carrying shortened fragments of AMA1. The transformation efficiencies as well as the plasmid stabilities were analyzed. The conducted experiments demonstrate that only one of the palindromic sequences together with the central region of AMA1 are necessary for autonomous replication in *Aspergillus niger*. Further shortening led to a drastic decline of transformation efficiency. Plasmids are lost at the latest in the 3rd generation when the spores are cultivated without antibiotic pressure. On the other hand, integration of the plasmids seems to be possible if antibiotic pressure is sustained.

PR8.42

Expression Response Of *Aspergillus oryzae* To Different Nitrogen Sources In Batch Cultivations

Christoph Hubert Knuf, Intawat Nookaew, Jens Nielsen

Systems and Synthetic Biology Group, Department of Chemical and Biological Engineering, Chalmers University of Technology

The filamentous fungus *Aspergillus oryzae* is widely used as a microbial cell factory for large scale heterologous protein production. *A. oryzae* is also known as a natural organic acid producer. However, the metabolism and regulation of organic acid production in *A. oryzae* are poorly characterized. Furthermore, the media composition has a major impact on the performance of this organism and the economic feasibility of an industrial fermentation process. We therefore evaluated the global expression response towards and the utilization of different nitrogen sources by *A. oryzae* in batch fermentations.

In this study, we aim for a deep investigation of the cellular mechanisms of the utilization of different nitrogen sources. Firstly, we performed batch cultivations with two strains (NRRL3488 and DSM1862) on defined and complex nitrogen sources using di-ammonium sulphate and peptone, respectively. In addition, transcriptome analysis was performed on samples from these fermentations to analyze the gene expression under exponential growth conditions (mid-exponential phase; 6h) and in nitrogen starvation (stationary phase; 30h) to further identify key-players in the metabolism and regulation of gene expression.

Cluster analysis revealed a conserved response of both strains and helped to identify regulatory sequences among the co-expressed genes. Furthermore the expression data pointed towards malic acid production as a respond to nitrogen starvation stress.

PR8.43

Proteins secrted by *Heterobasidion irregulare* during growth on spruce wood

Andrzej Majcherczyk, Ursula Kües
Georg-August-University Göttingen

Heterobasidion irregulare is a severe conifer pathogen that causes root and butt rot. The white-rot fungus degrades simultaneously or selectively lignin. The genome of the fungus was established by the JGI (http://genome.jgi-psf.org/Hetan2/Hetan2.info.html) and the annotated genome can be used in proteomic studies. Here, *H. irregulare* was grown in liquid medium with and without *Picea abies* wood. Freely secreted and hyphal sheath associated proteins analyzed by 2D-gel electrophoresis revealed a high diversity between wood supplemented and control cultures. Protein identification by ESI-LC-MS/MS was either performed on single protein spots form 2D-gels or by application of a shot-gun method on complex protein mixtures. Using a MASCOT database with the proteome deduced from the *H. irregulare* genome, in total 118 different secreted proteins have been identified. 64 proteins were present under both culture conditions and only seven proteins were suppressed by wood supplementation. Addition of wood resulted in 47 new proteins secreted into the culture media. Redox-enzymes were represented by 23 proteins and most of them were induced by wood. Expression of laccases (except of one) and alcohol oxidases differed not between the two culture media. However, wood induced secretion of FAD-oxidoreductases and redox-enzymes with unknown function and furthermore secretion of specialized glycanases, lipases and proteases.

This work was supported by the Ministry of Science and Culture in Hannover, Germany (Common Lower-Saxony-Israel Project ZN2043). We acknowledge the genome work conducted by the U.S. Department of Energy Joint Genome Institute (JGI) supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

PR8.44

Optimisation of vectors for transformations in Coprinopsis cinerea

Bastian Dörnte, Ursula Kües
Georg-August-University Göttingen

The genetic transformation of the model fungus Coprinopsis cinerea allows the genomic analysis and manipulation of this organism. Initially, transformations were used to study the structure; functions and regulation of expression of genes; in recent years usage for overexpression of industrially important enzymes are also emerging. For the transfer of genetic material, chromosomal integrative vectors are used. These vectors contain a selectable marker gene and/ or a gene of interest under the control of regulatory sequences such as promoter or terminator. Due to lack of systematic experimental data, little is known about the influence of vectors on transformation frequencies. This work targets at improvement of the transformation vector pCc1001 (1). This pUC9-based vector contains a 6.5 kb PstI genomic fragment of C. cinerea with the tryptophan synthetase gene (trp1) that can be used to complement trp1- defects. The vector however shows a surprising phenomenon. In single transformation it gives only low numbers of transformants whereas efficiencies in co-transformation raise by factors of >100%, yielding several hundreds of transformants per experiment. To investigate this phenomenon further, the vector was modified in length and fragments with the trp1 gene were subcloned into pBluescript KS-. The effects on the transformation efficiency were investigated by using several co-transformation experiments. (1) Binninger DM et al. (1987) DNA-mediated transformation of the basidiomycete Coprinus cinereus. EMBO J 6:835-840

PR8.45

Genomics of *Aspergillus oryzae* and effective utilization of large scale genomic information.

Masayuki Machida[1] Hideaki Koike[2] Yoshinori Koyama[2] Hiroko Hagiwara[2] Tomoko Ishii[2] Itaru Takeda[1] Myco Umemura[2] Koichi Tamano[2] Isao Kojima[2] AIST fungal genome[2]
[1.] AIST/TUAT [2.] AIST

Recently, we have determined the genes responsible to the biosynthesis of kojic acid from *A. oryzae*, which is used as a cosmetic whitening agent. Transcriptome analysis and successive gene disruption approach has been successfully applied to the gene identification. We found three genes encoding oxidereductase, transporter and transcription factor, which are localized on the non-syntenic block. It took, however, more than one year for the identification because the target gene prediction was inaccurate and disruption of roughly 20 genes was required before finding the genes. To accelerate identification of the genes responsible to biosynthesis of novel metabolites, we have prepared our pipelines from genome sequencing to gene detection, which includes DNA sequencer, DNA microarray and LC/MS.

We have improved the performance of the *de novo* assembling pipeline for ABI SOLiD, and have optimized it for sequencing of microorganism genomes. Our pipeline generates scaffolds longer than 1 Mb, covering 99% of approximately 40 Mb genome with roughly 100 or less scaffolds in general. Resulted sequences are subjected to annotation, comparative analysis and the highly accurate gene prediction that we have developed. We have found that our pipeline detected the genes for secondary metabolites that have been already known. We are now evaluating the performance in accuracy by disruption and overexpression of some novel genes that have been predicted by the pipeline.

PR8.46

Oxalate decarboxylases of the white-rot fungus *Dichomitus squalens*: expression on wood and in acid-induced cultures

Miia Mäkelä, Outi-Maaria Sietiö, Annele Hatakka, Kristiina Hildén
Department of Food and Environmental Sciences, University of Helsinki, Finland

Oxalate decarboxylase (ODC) is an oxalic-acid decomposing enzyme produced by certain bacteria and fungi with potential for diverse biotechnological applications. Uses of ODC range from assays of oxalate concentration and removal of oxalate salt deposits in industrial processes to transgenic *odc*-expressing crop plants.

In fungi, ODC has an essential role in regulation of toxic concentrations of intra- and extracellular oxalate. Oxalic acid is an important metabolite of basidiomycetous wood-rotting fungi as it assists in wood and lignin degradation by e.g. enhancing the reactions catalyzed by lignin-modifying oxidative enzymes. Moreover, ODC has been suggested to participate in energy production during the fungal vegetative growth.

We found previously that the selectively lignin-degrading white-rot fungus *Dichomitus squalens* secretes oxalic acid during growth in liquid cultures and on spruce wood[1]. The fungus demonstrated intracellular ODC activity after exposure to excess oxalic acid. Mycelial Ds-ODC protein was partially purified, and for the first time for a white-rot polypore species, we succeeded in complete cloning of ODC-encoding gene of *D. squalens*[2].

The whole genome sequence of D. squalens (www.jgi.doe.gov) reveals that the fungus harbours altogether five odc gene models. In this work, we studied the expression of the five Ds-odc genes by real-time RT-qPCR during the fungal growth on solid-state spruce wood and in acid-induced liquid cultures. These results support involvement of differently regulated, individual ODC isoenzymes in primary and secondary metabolism in wood-decaying fungi.

[1]Mäkelä M, Galkin S, Hatakka A, Lundell T (2002) Enzyme Microb Technol 30:542-549
[2]Mäkelä MR, Hildén K, Hatakka A, Lundell TK (2009) Microbiology 155:2726-2738

PR8.47

Transcriptomic And Genomic Approaches To Understand Cellulase Hyper-production In The Filamentous Fungus *Trichoderma reesei*

Thomas Portnoy[1] Frédérique Bidard-Michelot[2] Bernhard Seiboth[3] Frédéric Monot[1] Scott E. Baker[4] Christian P. Kubicek[3] Stéphane Le Crom[5] Antoine Margeot[1]

1. IFP Energies nouvelles, Rueil-Malmaison, France 2. IBENS, Ecole Normale Supérieure, Paris, France 3. Institute of Chemical Egineering, Research Area Molecular Biotechnology, Vienna Univerty of Technology, Vienna, Austria 4. Chemical and Biological Process Development, Pacific Northwest National Laboratory, Richland, WA, USA 5. Université Pierre et Marie Curie-Paris 6, UMR7622 Paris, France; Centre National de la Recherche Scientifique, UMR7622, Laboratoire de Biologie du Développement, Equipe analyse des données à haut debit en génomique fonct

The filamentous fungus *Trichoderma reesei* is well-known for its impressive capacity to secrete high amounts of cellulase and hemicellulase enzymes. These enzymes are key components of most cellulosic biomass to biofuel biological processes, and despite huge R&D efforts, their cost is still too high. Most industrial strains in use today have been obtained by random mutagenesis of the original QM6a isolate. Highest performances range from 40 to above 100 g/L proteins production, depending on the strain and process configuration used. While it is not known whether the already impressive performances of these strains can be further enhanced through targeted genetic engineering, there is still a high interest in understanding the genetic mechanisms leading to cellulase (hyper)secretion and to set up genomic tools that could be used to adapt strains to various industrial conditions. Toward this goal our group has been investigating the onset of cellulase production both on the single-gene level and on the whole transcriptome level using dedicated DNA oligonucleotide microarrays. We are currently completing our previous high-throughput sequencing of the NG14 and RUT C30 strain lineage with the sequencing of five other strains. These genome data are also being completed by transcriptome analysis with RNAseq technology. Our objective is to provide a whole picture of the mechanisms involved in cellulase production by *T. reesei* as well as potential new targets for genetic engineering of industrial strains.

PR8.48

Transposition of the miniature inverted-repeat transposable element mimp1 in the wheat pathogen Fusarium culmorum

Francesca Spanu[1] Matias Pasquali[2] Barbara Scherm[1] Virgilio Balmas[1] Angela Marcello[1] Giuseppe Ortu[3] Marie Dufresne[3] Lucien Hoffmann[2] Marie-Josée Daboussi[3] Quirico Migheli[1]

1. University of Sassari, Italy 2. CRP - Gabriel Lippmann, Luxembourg 3. Université Paris Sud, France

The genome of *Fusarium culmorum*, incitant of crown and foot rot on wheat and type B trichothecene producer, is now being sequenced. The number of predicted genes is estimated to exceed 10,000 and for many of them the function is still unknown. Consequently, there is a strong need for a high-throughput method for functional genomic analysis. Our aim was to test the efficacy of a double component system based on the ability of the *impala* transposase to transactivate the miniature inverted-repeat transposable element *mimp1* of *Fusarium oxysporum*. In this paper we report for the first time on the application of a tagging system based on an heterologous transposon and on the application of the splinkerette-PCR to identify *mimp1* flanking regions in the filamentous fungus *F. culmorum*. Similarly to what was previously observed in *Fusarium graminearum*, *mimp1* was shown to transpose in *F. culmorum* by a cut-and-paste mechanism into TA dinucleotides, which are duplicated upon insertion. Our results also show that *mimp1* reinserts in open reading frames in 16.4 % (i.e., 10 of 61) of the strains analysed, spanning throughout the entire genome of *F. culmorum*. Therefore the *mimp1/impala* double-component system is an efficient tool for gene tagging in *F. culmorum* as confirmed phenotypically for a putative aurofusarin gene. This system allowed also to identify two genes putatively involved in oxidative stress coping capabilities in *F.culmorum* as well as a sequence specific to this fungus, thus suggesting the valuable exploratory role of this tool.

PR8.49

Bioinformatic prediction of *cis*-acting elements in *FUM* gene promoters putatively involved in transcriptional control of fumonisins biosynthesis

Valeria Montis[1] Matias Pasquali[2] Ivan Visentin[1] Petr Karlovsky[3] Francesca Cardinale[1]
1. Department of Plant Physiology - University of Turin 2. Department of Environnement and Agro-biotechnologies - Centre de Recherche Public Gabriel Lippmann 3. Department of Crop Sciences, Molecular Phytopathology and Mycotoxin Research - University of Göttingen

Fumonisins are secondary metabolites produced by the maize pathogen *Fusarium verticillioides*, agent of pink ear rot; these mycotoxins cause agro-economical losses and detrimental health effects in animals and humans. The FUM genes needed for fumonisins biosynthesis are clustered and co-expressed in the fumonisins producers.

In eukaryotes, coordination of gene transcription is mostly attained through transcription factors shared by co-regulated genes, whose specificity relies on the recognition of *cis*-regulatory elements on the promoters of their targets. A bioinformatic analysis of FUM gene promoters in *F. verticillioides* identified a partially degenerated motif potentially involved in the regulation of FUM genes expression, and therefore in fumonisins biosynthesis. The same oligomer was found in the clustered FUM genes of the other fumonisins producers *Fusarium oxysporum* and *Aspergillus niger*; while it is not significantly over-represented in the scattered FUM homologs of the fumonisins non-producing euascomycetes *F. graminearum*, *A. nidulans*, *Magnaporthe grisea* and *Neurospora crassa*.

Comparison of the transcriptional strength of the intact *FUM1* promoter and of a synthetic version, where the motif discovered had been mutated, was carried out *in vivo* and *in planta* by quantifying GFP transcripts in *F. verticillioides* transformants, carrying either promoter upstream of the GFP reporter. Our results show that mutation of the main motif in *FUM1* promoter is sufficient to significantly impair its efficiency, thus validating our *in silico* approach as a discovery tool. The presence of the degenerated 6-mer in all clustered FUM genes suggests that this set of oligomers includes candidate regulatory sequences.

PR8.50

Biosynthesis of Natural Products Through a Fungal Molecular Genetics

Takehito Nakazawa, Takashi Ookuma, Satoru Sugimoto, Satoshi Ichinoseki, Yasutaka Gotanda, Noriyasu Ishikawa, Yuta Tsunematsu, Kan'ichiro Ishiuchi, Hiroshi Noguchi, Kenji Watanabe,
University of Shizuoka

Analysis of fungal genome sequences to date has revealed that ascomycetes possess far greater numbers of gene clusters for biosynthesis of secondary metabolites, polyketides and peptides, than the numbers of natural products that have been isolated from the organisms. Therefore, such cryptic secondary metabolism gene clusters are anticipated to be a source of chemically diverse compounds. However, conventional methodologies in the field of natural products chemistry cannot be applied in hunting of natural products synthesized by enzymes encoded in the fungal genome as silent gene clusters. First, it is necessary to activate the gene cluster to induce biosynthesis of the corresponding compounds. Here, we expressed a gene encoding a transcriptional regulator associated with a target silent gene cluster to induce its natural product biosynthesis. This approach was used successfully to produce two polyketides from *Aspergillus oryzae* and three from *Chaetomium globosum*.

Second, once mRNA can be transcribed from the target gene cluster, cDNA can be synthesized to allow transfer of the cluster genes into budding yeast and achieve heterologous production of compounds. Here, we have developed an innovative method for biosynthesizing bioactive molecules using an engineered *Saccharomyces cerevisiae* strain as a host. We expressed five polyketide synthases and two nonribosomal peptide synthetases from *Aspergillus fumigatus*, *Chaetomium globosum* and *Coprinopsis cinerea*. Subsequent detailed chemical characterizations of the resulting natural products identified six polyketides and two peptides. The methodologies shown in this study can be applied in acquisition of numerous natural products biosynthesized by silent/unknown fungal secondary metabolism gene clusters.

PR8.51

Unravelling the MDR mechanism in new emergent phenotypes of *Mycosphaerella graminicola*

Selim Omrane[1] Anne-Sophie Walker[1] Johann Confais[1] Catherine Lanen[1] Gert H.J. Kema[2] Sabine Fillinger[1]

1. UR BIOGER-CPP, INRA, Avenue Lucien Brétignières, F78850 Thiverval-Grignon, France 2. Plant Research International, Wageningen, The Netherlands

Multidrug resistance (MDR) is a trait developed by many organisms to counteract the effect of chemicals and/or drugs for the control of these agents. The basic MDR mechanism relies on an overexpressed efflux transport system that actively expulses the compound outside the cell. MDR was observed in field populations of the wheat septoria tritici blotch fungus *Mycosphaerella graminicola* since 2007 in France, Ireland and the UK, which evidently threatens control options. Individual strains with MDR (MDR 6 and 7) were characterized on the basis of their high resistance levels to fungicides belonging to the DMI family and to their cross-resistance with QoIs and SDHIs. Two main strategies were adopted: (a) Investigating the relationship between MDR phenotypes and an efflux transport system: Reversal agents inhibiting ABC/MFS transport systems coupled to C14-radiolabeled Prochloraz shed light on the involvement of at least two different transporters. In addition, the Tolnaftate (thiocarbamate) phenotyping of 140 descendant from a cross of MDR6 x MDR7 confirmed allelism or close linkage of genes involved in the MDR phenotype; (b) Analysis of differentially expressed genes in sensitive and MDR isolates:

RNA sequencing profiling of the MDR6/7 strains *vs.* sensitive strains in untreated and Prochloraz treated conditions are ongoing and the latest data and analyses will be presented.

PR8.52

Correlation of gene expression and protein production rate - a system wide study

Mikko Arvas[1] Tiina Pakula[1] Bart Smit[2] Jari Rautio[3] Heini Koivistoinen[4] Paula Jouhten[1] Erno Lindfors[1] Marilyn Wiebe[1] Merja Penttilä[1] Markku Saloheimo[1]

1. VTT Technical Research Centre of Finland, P.O. Box 1000 (Tietotie 2, Espoo), FI- 02044 VTT, Finland 2. NIZO food research, Kernhemseweg 2, 6718ZB Ede, the Netherlands 3. Plexpress, Viikinkaari 6, 00790 Helsinki, Finland 4. Ypap Oy, Hyrsynkulmantie 68, FI-32100 Ypäjä, Finland

Growth rate is a major determinant of intracellular function. However its effects can only be properly dissected with technically demanding chemostat cultivations in which it can be controlled. Recent work on *Saccharomyces cerevisia*e chemostat cultivations provided the first analysis on genome wide effects of growth rate. In this work we study the filamentous fungus *Trichoderma reesei (Hypocrea jecorina)* that is an industrial protein production host known for its exceptional protein secretion capability. Interestingly, it exhibits a low growth rate protein production phenotype. We have used transcriptomics and proteomics to study the effect of growth rate and cell density on protein production in chemostat cultivations of *T. reesei*. Use of chemostat allowed control of growth rate and exact estimation of the extracellular specific protein production rate (SPPR). We find that major biosynthetic activities are all negatively correlated with SPPR. We also find that expression of many genes of secreted proteins and secondary metabolism, as well as various lineage specific, mostly unknown genes are positively correlated with SPPR. Finally, we enumerate possible regulators and regulatory mechanisms, arising from the data, for this response. Based on these results it appears that in low growth rate protein production energy is very efficiently used primarly for protein production. Also, we propose that flux through early glycolysis or the TCA cycle is a more fundamental determining factor than growth rate for low growth rate protein production and we propose a novel eukaryotic response to this i.e. the lineage specific response (LSR).

PR8.53

Enzyme production by Trichoderma reesei Rut C-30 followed by enzymatic hydrolysis of different lignocellulosic materials

Ausra Peciulyte[1] George E. Anasontzis[1] Tomas Larsson[2] Lisbeth Olsson[1]

1. Industrial Biotechnology, Department of Chemical and Biological Engineering, Chalmers University of Technology, 41296 Gothenburg, Sweden 2. Innventia AB, 11486 Stockholm, Sweden

The filamentous fungus *Trichoderma reesei* is one of the main sources for cellulose degrading enzymes. We studied the enzyme profile produced during the fungal growth on cellulosic and lignocellulosic substrates and their capacity to hydrolyze cellulosic and lignocellulosic substrates with different chemical and physical properties. The results brought insight into the bottlenecks of enzymatic hydrolysis.

During the enzyme production study, we grew *T. reesei* strain Rut C-30 in submerged fermentations on Avicel PH-101, commercial cellulose, and industrial-like lignocellulosic substrates from spruce. These substrates were produced during the process of sodium hydroxide cooking, used in pulp and paper industry. Additionally we altered the chemical and physical properties of those substrates by drying and rewetting, treatment of sodium hydroxide and sodium chlorite in order to decrease or increase the surface area and delignify, respectively. We measured cellulolytic enzyme activity by enzymatic assays. Proteins were examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and two-dimensional gel electrophoresis.

The enzymes produced were subsequently used for enzymatic hydrolysis of lignocellulosic substrates and compared to enzymatic hydrolysis of model cellulosic substrates, namely, Avicel PH-101, nanocrystaline cellulose, phosphoric acid-swollen cellulose and cotton, which have defined characteristics. The structural properties of the substrates during the different times of hydrolysis were analyzed by solid-state nuclear magnetic resonance (NMR) technique. Dynamics of the hydrolysis was analyzed by quartz crystal microbalance with dissipation (QCM-D) technique. Hydrolysis products were verified by high performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD).

PR8.54

Structural and Functional analyses of Dehydrin-like proteins in the necrotrophic fungus *Alternaria brassicicola*

Stéphanie Pochon[1] Sandrine Pigné[1] Nelly Bataillé-Simoneau[1] Marjorie Juchaux[2] Emmanuel Jaspard[3] Philippe Simoneau[1] Thomas Guillemette[1]

1. Research Institute Horticulture and Seed – UMR 1345 -Fungisem, Faculté des Sciences, 2 Bd Lavoisier, F-49045 ANGERS (France). 2. SFR QUASAV – IMAC Platform, 2 Bd Lavoisier, F-49045 ANGERS (France)

3. Research Institute Horticulture and Seed – UMR 1345 -Mitostress, Faculté des Sciences, 2 Bd Lavoisier, F-49045 ANGERS (France).

Dehydrins (DHN) are a protein subclass of Late Embryogenesis Abundant proteins (LEA) which, in plants, are involved in the salt and hydric stress resistance. In fungi, dehydrin-like proteins have been identified in *Tuber borchii* and *Aspergillus fumigatus* and a signature pattern has been described for these proteins (1,2). Using this sequence motif, we have identified three DHN-encoding genes in the genome of the necrotrophic fungus *Alternaria brassicicola*. Sequence analysis confirmed that they all shared the characteristic features of dehydrins: high glycine, threonine and serine content, low cysteine and tryptophan content, high hydrophilicity, absence of secondary structures and a high proportion of disordered amino acids. Measures of the expression levels of the three DHN genes in conditions previously reported to induce fungal DHNs transcription (low temperature, salinity and oxidative stress) revealed that they were all up regulated in these conditions. To study their subcellular localization, dehydrin-like proteins were fused to eGfp at their carboxy-terminal end and expressed in *A. brassicicola* under control of their own promoters. Fluorescent protein fusions showed that at least one dehydrin was associated with peroxisomes. A functional analysis has been performed by the construction of knockout mutants deficient for each DHN. Although none of the dehydrin-like mutants were found more susceptible to NaCl than the WT strain, they were all characterized by a stronger susceptibility towards oxidative stress (menadione or H_2O_2). A double-mutant strain exhibited reduced virulence on host leaves and decreased seed transmission rates compared to the parental strain, indicating a role of DHNs in pathogenicity.

1. Abba, S., Ghignone, S. and Bonfante, P. (2006) A dehydration-inducible gene in the truffle *Tuber borchii* identifies a novel group of dehydrins. *BMC Genomics*, 7, 39-54.

2. Wong Sak Hoi J, Lamarre C, Beau R, Meneau I, Berepiki A, Barre A, Mellado E, Read ND, Latgé JP. (2011) A novel family of dehydrin-like proteins is involved in stress response in the human fungal pathogen Aspergillus fumigatus. Mol Biol Cell., 11:1896-906.

PR8.55

Esterases of basidiomycetes as supporting enzymes in degradation of lignocellulosic material

Martin Rühl[1] Alexandra Rojkova[2] Ivan Zorov[2] Arkady Sinitsyn[2] Holger Zorn[1]

1. Institute of Food Chemistry and Food Biotechnology, Justus Liebig University Giessen, Heinrich-Buff-Ring 52, 35392 Giessen, Germany 2. Department of Chemistry, M.V. Lomonosov Moscow State University, Moscow 119899, Russia

In contrast to fossil resources, lignocellulose as the main part of wooden plant material is available in almost unlimited amounts. Its main components - cellulose, lignin and hemicellulose – represent an important feedstock used in various industries, such as in paper manufacturing or bioethanol production. Unfortunately, lignocelluloses are recalcitrant materials, and harsh chemical conditions are needed to degrade them. In nature, fungi are capable of breaking up the wooden material by using a diverse set of extracellular enzymes, the so-called secretome. For degradation of the lignin, a number of oxidases including lignin peroxidases, manganese peroxidases, versatile peroxidases, and DyP-type peroxidases and laccases, are secreted which are able to oxidise aromatic and phenolic parts of the lignin structure. As a second part of the lignocellulosic fungal secretome, hydrolytic enzymes, such as cellulases and esterases, are needed for the extraction of sugars and are involved in the degradation of the lignocellulosic structures, respectively. The different cellulases are able to fracture the cellulose structure, whereas esterases (EC 3.1.1.x) are involved in the hydrolysis of several ester bonds. E.g., feruloyl-esterases (EC 3.1.1.73) participate in the breakup of linkages connecting hemi-cellulose (arabinoxylans) and lignin. Nevertheless, the knowledge on basidiomycete esterases is fragmentary and, thus, their biotechnological potential unknown.

In this work, 30 different basidiomycetes were screened for the ability to degrade several esterase substrates. Interesting candidates were cultured in minimal and complete liquid media with and without addition of lignocellulose. Esterase activities of up to 340 U/L were obtained when cultivating the fungi in shaken flasks at 24 °C. The supernatant of interesting fungal candidates was used together with already optimised cellulolytic enzyme cocktails to improve the degradation of lignocellulosic residues regarding the amount of reducing sugars gained by this bioconversion. Promising candidates will be purified, characterised and heterologously expressed in an ascomyceteous host.

PR8.56

Characterization of Cellobiohydrolase I (CBHI) of the White-Rot Fungus *Dichomitus squalens*

Johanna Rytioja, Aila Mettälä, Kristiina Hildén, Annele Hatakka, Miia Mäkelä
Department of Food and Environmental Sciences, University of Helsinki, Finland

In nature basidiomycetous white-rot fungi are the most efficient wood-decaying organisms. They express extracellular hydrolytic and oxidative enzymes that are needed to degrade all the wood polymers, i.e. cellulose, hemicellulose and lignin, but while their lignin modifying enzymes are well known their cellulolytic enzymes are much less studied. In a large screening of cellobiohydrolases (CBHs) of basidiomycetous fungi from the Fungal Biotechnology Culture Collection (FBCC, University of Helsinki) the white-rot fungus *Dichomitus squalens* strain FBCC312 appeared to be highly cellulolytic and a promising source of novel cellulases. In this work we purified and characterized native CBHI of *D. squalens*. The fungus produced extracellular CBH, endoglucanase, *β*-glucosidase, xylanase and laccase activities in liquid 1% (w/v) microcrystalline cellulose (Avicel) - peptone medium. Ds-CBHI was purified from this culture liquid after 6 to 10 days of cultivation, with anion exchange and size exclusion chromatography. Molecular mass of the purified Ds-CBHI was 45 kDa and p*I* 3.8-4.1, as determined by SDS-PAGE and IEF, respectively. Three internal peptides sequenced by LC-MS/MS were similar with the translated amino acid sequence of the cloned *D. squalens cbhI* gene. The putative polypeptide of Ds-CBHI lacks cellulose binding module and is similar to glycosyl hydrolase family 7 proteins. Ds-CBHI showed wide pH and temperature working ranges with artificial substrate 4-methylumbelliferyl-β-D-lactoside (MULac). Optimum temperature of Ds-CBHI for MULac reaction was +65°C and optimum pH 4.0. Purified Ds-CBHI resembled the known white-rot fungal CBHs by its acidic p*I* and catalytic optimum pH.

PR8.57

Associated biocontrol of cotton pest *Dysdercus peruvianus* by the fungus *Metarhizium anisopliae* and environmental isolates of *Pseudomonas fluorescens*

Lucelia Santi, Walter O. Beys da Silva, Jorge A. Guimarães, Augusto Schrank, Marilene H. Vainstein
Biotechnology Center, Universidade Federal do Rio Grande do Sul

Dysdercus peruvianus is an insect pest of cotton culture, which causes serious economic damages. The method currently used to control this plague is by the use of chemical pesticides. However, this practice is increasingly questioned by society, due environmental impact, high costs, low specificity and risk in its handling. *Metarhizium anisopliae* is a filamentous fungus used in biological control with attested effect on several arthropod pests, including *D. peruvianus*. The main barrier to apply this fungus, or any other biocontrol agent, is the time of death of target pest; which is generally greater than the corresponding chemical pesticide. In this work, the isolation, identification and evaluation of environmental bacteria in association with *M. anisopliae* were performed to optimize the biocontrol of the *D. peruvianus*. Four bacteria isolated from soil effectively accelerate the biocontrol of *D. peruvianus*, when associated with the fungus, and the two best bacteria were identified as *Pseudomonas fluorescens*. The formulation containing *M. anisopliae* conidia and bacterial culture of *P. fluorescens* showed efficiency up to 96% in reducing the time of the death of *D. peruvianus*. Besides molecular aspects of this interaction, including the expression of enzymatic arsenal of *M. anisopliae* in association of bacterial isolates were also evaluated. This work attests the efficiency of associated biocontrol applying the fungus *M. anisopliae* and bacterial isolates collected from environment using a specific strategy. Also, this alternative represents a significant increase in efficiency of biological control, increasing the interest in application of this environmentally safe way of pest control.

PR8.58

Morphological and molecular characterization of Hyphodermella rosae the causal agent of dry fruit rot on plum and peach in Iran

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

In June 2011, a new symptoms of dry fruit rot on plum (Prunus domestica) and peach (P.persica) was observed in Mazandaran province of Iran. Initial symptoms, appeared as dark brown, circular necrotic spots on fruits. The fungus Hyphodermella rosae isolated and identified on the basis of morphological characteristics on PDA. Basidiomata were effuse, resupinate, 15 × 10 mm, tubercules small with apical bristles, light orange to greyish orange. Subhymenium composed of vertically arranged, short-celled, non-agglutinated hyphae; subhymenial hyphae were 3-4 µm in diam. Basidiospores were ellipsoid, 7.5× 5.5 µm and their cell walls were thin, hyaline and smooth (1). A CTAB DNA extraction protocol was used to acquire DNA from mycelium culture. The primer pair ITS4 (5-TCCTCCGCTTATTGATATGC-3) and ITS5 (5-GGAAGTAAAAGTCGTAACAA-3) was used to amplify the Internal Transcribed Spacer (ITS) regions including 5.8S from ribosomal DNA (4) and the PCR product was sequenced. The 627-bp and 604bp fragments of Plum and peach isolates was amplified, respectively. After multiple sequence alignment with CLUSTALW software the obtained sequences were compared with the other related sequences of Hyphodermella genus deposited in GenBank. Blast analysis of the MA4099 (plum isolate) and VA1345 (peach isolate) sequences confirmed a 99 and 100% similarity with the sequences of H. rosae (GenBank accession no FN600386.1, FN600385.1) respectively. The pathogenicity of the isolates has been proven. The genus Hyphodermella has been reported causing wood rot on apricot (2), sweet and sour cherry (3). To our knowledge, this is the first report of H. rosae on stone fruit species in the world.

PR8.59

Screening the secondary metabolome of an unidentified basidiomycete for antifungal compounds

Daniel Schwenk[1] Blanchette Robert[2] Dirk Hoffmeister[1]
1. Friedrich-Schiller-Universität Jena 2. University of Minnesota

The isolate BY represents an unidentified anamorphic fungus. Based on the analysis of ITS/IGS-sequence data it was tentatively assigned to the russuloid clade of the basidiomycetes. However, its genus remains obscure. As cultures of this isolate exert strong antifungal effects further research into its secondary metabolism was warranted. A series of natural products has been isolated, structurally elucidated, and tested for antifungal activity. Our results suggest that the bioactivity of BY is not related to a single agent but due to several structurally dissimilar compounds.

They possibly originate from different metabolic pathways, as polyene and phenylpropanoid core structures were found, alongside polyketidic scaffolds. To clarify the respective biosynthetic routes, feeding experiments with 1-13C labeled acetate have been initiated, accompanied by genetic screens for natural product biosynthesis genes.

PR8.60

Heterologous expression improvement in filamentous fungi by using RNA interference tool

Fernando Segato[1] Fabio Squina[1] Rolf Prade[2]

1. Brazilian Bioethanol Science and Technology Laboratory 2. Oklahoma State University

Fungi are industrial workhorses of secretion, where commercially useful proteins are targeted for secretion and high-yield accumulation. High-yield protein secretion and accumulation are amino-acid sequence dependent and in many cases, more often than not, high-yield of secretion is not observed. *Aspergillus nidulans* has been recognized and utilized as an excellent host to homologous and heterologous protein production. Researches have studied the protein production, because it shows an excellent extracellular secretion capability for a large amount of proteins compared to described in other microbial secretion systems such as *E. coli* and *S. cerevisiae*. Some post-translational modifications including glycosylation and folding are expected to take place in *A. nidulans*. For these reasons, it is considered one of the most adequate hosts to produce higher eukaryotic proteins. To enhance the protein production ability, it is important to construct the host applicable to multiple rounds of genetic manipulation. Recently, in order to carry out multiple gene silence and expression rapidly and efficiently, we developed *pyrG* marker recycling system. Using this system, successive rounds including concatenated gene silence for proteases respectively in *A. nidulans* were successfully achieved with gene-targeting frequency. Based in proteomics results, the genes encoding proteases, we concatenated five proteases in only one construction to analyze the effect of RNA interference on heterologous protein production by *A. nidulans*. Moreover, based on these data, we also confirmed the improvement of cellobiohydrolase productivity with the protease deficient strain. As a result, we constructed a quintuple protease genes disruptant having enhanced levels of cellobiohydrolase protein productivity.

PR8.61

Heterologous expression of the human peptide hormone obestatin in *Trichoderma reesei*

Angela Sun[1] Helena Nevalainen[1] Junior Teo[1] Michal Godlewski[2]

1. Department of CBMS, Macquarie University 2. Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences

The filamentous fungus *Trichoderma reesei* is an efficient expression host widely exploited as an industrial workhorse. However despite being regularly used for proteins and enzyme of larger size its capability of expressing small peptides of (T. reesei as an expression host for peptide production by engineering a strain for the expression of obestatin, a small peptide hormone of 23 amino acids in size that suppresses the appetite and regulates body weight gain in human and other mammals. Thus providing an alternative source for this otherwise naturally scarce and expensive to chemically synthesize peptide hormone. Preliminary expression of obestatin, with HIS tag fused at either N- or C- termini of the peptide, was carried out in *Escherichia coli* and the expression level was evaluated by enzyme immunoassay (EIA). The expression of obestatin was subsequently evaluated in *T. reesei* with a C-terminal purification tag, Hydrophobin I tag (HFBI) which is native to the host. Following the successful expression of obestatin in *T. reesei,* modifications of growth conditions were made to optimize the production of the peptide. Results indicated that it was possible to express the small peptide hormone obestatin in *T. reesei* at a higher concentration than it is in the *E. coli* expression system. It was also possible through strain selection and modification of the culture medium to achieve yield of up to 7µg/ml of obestatin in *T. reesei*.

PR8.62

Extracellular enzymes from *Trichoderma harzianum*, *Aspergillus terreus* and AM fungus

Bakhtiyor Umarov, Khurshede Khamidova
Institute of Microbiology, Uzbekistan

Cellulotic enzymes consistitute form the group well described biologically important enzymes,The enzyme cellulase, a multi enzyme complex made up of several proteins, catalyses theconversion of cellulose to glucose in an enzymatic hydrolysis. Which catalyze the transfer of the glycosyl group between oxygen nucleophiles. important role this enzymes in the biology is include degradation celluloses of the biomass fungi and bacteria, degradation of glycolipids in mammalian lysosomes, and the cleavage of glycosylated flavonoids in plants. Fungus Trichoderma and Aspergillus have biotechnologicaly importance, since they are a producer extracellular enzyme. We studies from the new strains selection high cellulotic activities and using them in the biotechnical studies. Studing extracellular enzymes from 3-fungi, Aspergillus terreus, Trichoderma harzianum and Mucorrhiza conducted in minimum ambience, containing wheat bran as single source of the carbon. Enzyme activities were assayed spectrophotometrically by using Samogy-Nelson. Temperature and pH optimum of some purified enzymes were determined also. The isolation and purification cellulotic enzymes we are used the ion exchange chromatography on DEAE TOYOPEARL 650 M gel in the gradient 0,5M NaCl. The test strains of each species ., Aspergillus terreus, Trichoderma harzianum and AM fungi were analyzed on the basis of extent of hydrolyzing ability. Cellulose hydrolysis of all three strains was immensely affected by varying pH and medium. The results indicated statistically significant interaction in all correlating factors of strain, growth medium and pH level

PR8.63

Finding the conserved mushroom developmental pathway

Arend van Peer, Yuichi Sakamoto
Iwate Biotechnology Research Center

Mushrooms are important sources of food, medicinal compounds, for industrial applications, waste recycling and environmental health. However, cultivation of most mushrooms is difficult or yet impossible. A major obstacle for improvement of mushroom cultivation technology is the lack of knowledge on molecular genetic mechanisms that underlie mushroom development. Available information is highly scattered, describing specific, unrelated studies in model mushrooms and more recently including individual studies on major cultivated mushrooms.

Despite the high variations in shape, color, composition, substrate and triggers for fruiting induction, mushroom life cycles follow a general course. Within the developmental stages of the mushroom, especially the formation of hyphal knots and primordia seem highly similar between species. We decided to 'access' the general developmental pathway through interspecies comparison of primordium specific gene expression patterns, including model as well as major cultivated species. Promising universal, primordium specific genes will be studied in detail in the model organisms *Coprinopsis cinerea* and *Schizophyllum commune* by means of gene deletion, fusion to fluorescent markers and quantitative PCR. Once confirmed to play a role in mushroom development, these genes will serve as foundation for compilation of a general mushroom development model in two directions; stages preceding, and stages following primordium formation.

At the moment, superSAGE datasets are being assembled and most mushroom species are still under cultivation. We just started comparison of our first four datasets (dikaryotic mycelium versus primordia) in *C. cinerea* and *Polyporus brumalis*. Considering the effectiveness of other expression-based studies for identification of stage specific developmental genes in mushrooms, we expect to reveal several universal genes to start our model in the near future.

PR8.64

Laccase functions in *trichoderma virens*

Lorenzo Mannella[1] Lorenzo Guglielminetti[2] Giovanni Vannacci[1] Mariarosaria Vergara[3]
1. Dept. of Tree Science, Entomology and Plant Pathology "Giovanni Scaramuzzi", Section of Plant Pathology, University of Pisa, Italy 2. Dept. of Biology, University of Pisa, Italy 3. Scuola Normale Superiore di Pisa, Italy

Fungal laccases are involved in multiple functions, such as lignin degradation, pigments synthesis and degradation, detoxification and pathogenesis. Furthermore, they are useful biocatalysts for several biotechnological applications. Six laccase genes were previously identified in *Trichoderma virens*, an effective biocontrol agent, and one of them was deleted and proved to be involved in the mycoparasitic activity against *Botrytis cinerea* sclerotia. Laccase activity in some *Trichoderma spp.* is also associated with the production of green pigment in conidial spores. Further investigations on the laccase gene family in *T. virens* were performed in order to explore substrate specificity and mechanisms putatively involved in ligninolysis, conidiogenesis and industrial dyes decolorization. Laccase functions in lignocellulosic process and sporulation mechanisms were studied by growing *T. virens* on two different substrates: wheat straw liquid medium, containing lignocellulose as the only carbon source, or solid Hölker medium, formulated to induce spore formation. Laccase expression analysis induced by multiple substrates is in progress to identify the more effective molecules or the pathways involved in *T. virens*. Further biochemical analyses are going on to search laccase isoforms when fungal cultures are grown on specific substrates. Possible variations of intra/extra-cellular enzymatic levels is also under study. In addition liquid cultures containing twelve commercial textile dyes were set up and *T. virens* efficiently decolorized three of them. In conclusion information is gained about the *T. virens* laccase gene family, involved in physiological processes important for fitness or antagonistic attitude and exploitable in biotechnological applications related to textile dyes decolorization or ligninolysis.

PR8.65

Development of a homologous protein carrier system for heterologous protein production in *Myceliophthora thermophila.*

Hans Visser, Sanaz Mokhtari, Jan Wery
Dyadic Nederland BV

Filamentous fungi have proven to produce and secrete large quantities of extracellular enzymes. Species such as *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei* and *Myceliophthora thermophila* C1 are used in industry as work horses for enzyme production. High yields of homologous enzymes can be readily obtained. On the contrary, heterologous proteins are often produced at low levels. One of the main reasons for this is the presence of host proteases that partially or fully degrade the heterologous protein. In addition at the level of transcription, translation and transport and processing through the secretion pathway problems may be encountered. In order to increase the chances of success, protein carriers have been used for improved heterologous protein production in filamentous fungi[1]. It is believed that the carrier protein will "drag/guide" the heterologous protein through the (initial stages) of the secretion pathway protecting it from mis-folding and proteolysis. Previously, we have used this technology successfully in the expression of human antibodies in C1[2]. The carrier protein used in that study was the catalytic domain of *Aspergillus niger* glucoamylase A, which by itselfis a heterologous protein to C1. In the present study we investigated whether the homologous C1 glucoamylase yields higher levels of a heterologous xylanase sensitive to proteolysis. [1] Gouka *et al*. (1997) Efficient production of secreted proteins by *Aspergillus*: progress, limitations and prospects. Appl. Microbiol. Biotechnol. 47: 1-11. [2] Visser *et al*. (2011) Development of a mature fungal technology and production platform for industrial enzymes based on a *Myceliophthora thermophila* isolate, previously known as *Chrysosporium lucknowense* C1. Ind. Biotechnol. 7(3): 214-223.

PR8.66

Closely related fungi employ diverse enzymatic strategies to degrade plant biomass

Isabelle Benoit[1] Helena Culleton[2] Ad Wiebenga[2] Pedro M. Coutinho[3] Carlo P.J.M. Brouwer[1] Vincent McKie[4] Barry McCleary[4] Bernard Henrissat[5] Ronald P. de Vries[2]

[1.] Microbiology, Utrecht University, The Netherlands [2.] CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands [3.] Architecture et Fonction des Macromolécules Biologiques, CNRS - Universités d'Aix-Marseille I & II, Marseille, France. [4.] Megazyme International, Bray, Ireland [5.] Architecture et Fonction des Macromolécules Biologiques, CNRS - Universités d'Aix-Marseille I & II, Marseille, France

Fungi can grow in many biotopes and on many different carbon sources. In natural biotopes, plant biomass is the predominant carbon source for most fungi. Plant biomass consists largely of polymeric compounds of which polysaccharides are the main components. Fungi cannot take up the intact polysaccharides, but need to degrade them extracellularly to monomeric and small oligomeric compounds. To achieve this, fungi produces diverse enzymatic mixtures that are tailored specifically to the available polysaccharides.

A recent study demonstrated significant differences in the polysaccharide degrading ability of three Aspergilli, while only small differences were detected in their growth on various plant polysaccharides (Coutinho et al, 2009). This suggests that related fungal species may have developed different approaches to plant biomass degradation, employing different enzyme sets. A better understanding of these strategies will not only increase our insight in fungal biodiversity, but will also help in designing more efficient industrial processes for plant biomass degradation.

In this study we have compared the plant biomass degrading potential and strategy of 8 Aspergilli and demonstrate that they have developed a highly diverse approach to using these complex carbon sources. Although all eight species contain the main transcriptional activators involved in plant polysaccharide degradation (AmyR, XlnR, AraR, InuR) the enzymatic sets produced by them differs hugely, suggesting a species specific fine-tuning of plant biomass degradation.

PR8.67

Induction of lignocellulose degrading enzymes in Neurospora crassa by cellodextrins

Elizabeth Znameroski, Samuel Coradetti, Christine Roche, Jordan Tsai, Anthony Iavarone, Jamie H. D. Cate, N. Louise Glass

University of California, Berkeley, United States

Neurospora crassa colonizes burnt grasslands in the wild and metabolizes both cellulose and hemicellulose from plant cell walls. When switched from a favored carbon source such as sucrose to cellulose, N. crassa dramatically upregulates expression and secretion of a wide variety of genes encoding lignocellulolytic enzymes. However, the means by which N. crassa and other filamentous fungi sense the presence of cellulose in the environment remains unclear. Here, we show that a N. crassa mutant carrying deletions of two genes encoding predicted extracellular β-glucosidase enzymes and one intracellular β-glucosidase enzyme (Δ3βG) lacks β-glucosidase activity, but efficiently induces cellulase gene expression and cellulolytic activity in the presence of cellobiose as the sole carbon source. These data indicate that cellobiose, or a modified version of cellobiose, functions as an inducer of lignocellulolytic gene expression and activity in N. crassa. In addition, we have identified two cellodextrin transporters involved in sensing cellulose. A N. crassa mutant carrying deletions for both transporters is unable to induce cellulase gene expression in response to crystalline cellulose. Furthermore, a mutant lacking β-glucosidase enzymes and transporters (Δ3βGΔT) does not induce cellulase gene expression in response to cellobiose. We are currently in the process of characterizing the transport kinetics of each individual transporter in the Δ3βG background with the goal of understanding how the transport of cellodextrins influences cellulose sensing and induction of cellulase gene expression.

PR8.68

A novel group of class II hydrophobins from *Trichoderma* with biased occurrence

Agnieszka Przylucka [1] Liliana Espino Tenorio de Rammer [1] Christian P. Kubicek [1,2] Irina S. Druzhinina [1,2]
[1] ACIB GmbH, c/o Vienna University of Technology, Institute of Chemical Engineering, Getreidemarkt 9/1665, 1060 Wien, Austria [2] Vienna University of Technology, Institute of Chemical Engineering, Getreidemarkt 9/1665, 1060 Wien, Austria

Hydrophobins are small secreted proteins containing eight positionally conserved cysteine residues, are unique to Pezizomycotina (Ascomycota). These proteins assemble in amphiphilic layer on the outer fungal cell wall, where they mediate interactions between the fungus and its environment. The amphiphilic properties have also raised considerable industrial interest in the application of hydrophobins for the processes requiring surface modification. *Trichoderma* spp. possess an amplified arsenal of class II hydrophobins compared to other fungi. In order to exploit this richness for biotechnology, we performed a genus-wide screening for novel *Trichoderma* hydrophobins. Thereby we discovered a group of class II hydrophobins which is restricted to *T. virens* (teleomorph *Hypocrea virens*) and taxa from closely related Harzianum Clade (both section Pachybasium). The intraspecific nucleotide diversity π is in the range <0.062, which is similar to the interspecific diversity of other *Trichoderma* class II hydrophobins, indicating a high rate of evolution. Fishers exact test showed that the gene is under purifying selection and exhibits a high relative synonymous codon usage (>0.8). The expression of these new hydrophobins under different conditions and the characterization of their amphiphilic properties will be reported.

PR8.69

The HFB4 family: novel class II hydrophobins of *Trichoderma* with universal infrageneric distribution and potential for industrial applications

Liliana E. Tenorio- [1] Monika Komon-Zelazowska [1] Doris Ribitsch [2] Katrin J. Greimel [2] Enrique Herrero-Acero [2] Georg Guebitz [2,3] Christian P. Kubicek [1,4] Irina S. Druzhinina [1,4]
[1] ACIB GmbH, c/o Vienna University of Technology, Institute of Chemical Engineering, Getreidemarkt 9/1665, 1060 Wien, Austria [2] ACIB GmbH, Petersgasse 14, 8010 Graz, Austria [3] Graz University of Technology, Institute of Environmental Microbiology, Petersgasse 14, 8010 Graz, Austria [4] Vienna University of Technology, Institute of Chemical Engineering, Getreidemarkt 9/1665, 1060 Wien, Austria

Hydrophobins are small secreted proteins containing eight positionally conserved cysteine residues, are unique to Ascomycota. They usually assemble in amphiphilic structures on the outer fungal cell wall, thus mediating interactions between the fungus and its environment. These amphiphilic properties have also raised considerable industrial interest in the application of hydrophobins for the modification of surfaces. We have previously shown (Kubicek *et al.,* 2008. BMC Evol Biol) that *Trichoderma* spp. possess an highest diversity of class II hydrophobins compared to other fungi. This variability likely arose by iterating patterns of gene duplications and gene loss processes ("birth and death evolution"). Here we studied the HFB4 family, which comprises the most conserved clade of class II hydrophobins in Trichoderma. HFB4 orthologues occur almost in all infrageneric groups of *Trichoderma*, with the exception of the the *Hypocreanum, Psychrophila* and *Lutea* clades. HFB4 sequences from some species exhibited a significant amino acid sequence variation (e.g. *HFB4s in Trichoderma* section Trichoderma), whereas that of other taxa was identical (e.g. section *Longibrachiatum*). The K_a/K_s ratio of 1.93, as well as Tajima's test (significantly positive) and Fishers exact test (< 0.3; see ref. above) showed that *T. atroviride (*teleomorph *Hypocrea atroviridis*) HFB4 is apparently under positive selection pressure, whereas the other confirmed the birth and death mechanism. The expression of *hfb4* gene accompanied the conidia formation of *T. atroviride*, *T. reesei* (teleomorph *H. jecorina*) and *T. virens* (teleomorph *H. virens*). The amphiphilic properties of HFB4 were confirmed by overexpression of the protein from the three above mentioned species in *E. coli,* purification and analysis by contact angle measurement after dropping water on the surface of hydrophilic and hydrophobic surfaces, respectively. HFB4 may comprise a new group of class II hydrophobins with potential industrial properties.

PR8.70

***In Vitro* Assessment of Chitosan on *Ganoderma boninense*, Pathogen of Basal Stem Rot Disease in Oil Palm**

Menaka Ganeson, Peter G. Alderson, Christina V. Supramaniam
School of Bioscience, Faculty of Science, The University of Nottingham Malaysia Campus, Jalan Broga, 43500, Semenyih, Selangor, Malaysia

Chitosan is a natural by-product polymer derived from chitin component of crustaceans, insects and fungi which exhibits antimicrobial properties against various pathogens. This study aims to evaluate the *in vitro* antifungal poperties of chitosan on *Ganoderma boninense*, the causal agent for basal stem rot disease in oil palm. Five concentrations of chitosan (1.0, 1.5, 2.0, 2.5 and 3.0 % w/v) in Potato Dextrose Agar (PDA) media were tested for their efficacy to control the *in vitro* growth of *G. boninense* mycelium during culture for 21 days. All of the concentrations tested significantly reduced mycelial growth compared with the control treatment. Chitosan exhibited a fungistatic effect on mycelial growth of *G. boninense* and markedly reduced radial growth via dose-dependent manner. The highest inhibition of radial growth (PIRG) of 90.09 % was observed with chitosan at 3.0 % (w/v). Chitosan also caused morphological changes in *G. boninense* mycelium including the occurrence of small vesicles due to coagulation of fungal cytoplasm and formation of excessive abnormal hyphal branching at higher concentrations. Spores of *G. boninense* treated with the same five concentrations of chitosan during the 21 days incubation period were unable to germinate.

PR8.71

Post-genomic analyses of fungal lignocellulosic biomass degradation reveal the unexpected potential of the plant pathogen *Ustilago maydis*

Marie Couturier [1] Marie-Noëlle Rosso [1] David Navarro [1,2] Didier Chevret [3] Mireille Haon [1,2] Anne Favel [1,2,4] Laurence Lesage-Meessen [1,2] Bernard Henrissat [5] Pedro M. Coutinho [4,5] Jean-Guy Berrin [1]
[1]INRA, UMR1163 Biotechnologie des Champignons Filamenteux, 13288 Marseille, France. [2]INRA, UMR1163 CIRM-CF, 13288 Marseille, France. [3]INRA, UMR1319 Micalis, PAPPSO, 78352 Jouy-en-Josas, France. [4]Aix-Marseille Universités, 13288 Marseille, France. [5]CNRS, UMR6098 AFMB, 13288 Marseille, France.

Filamentous fungi are potent biomass degraders due to their ability to thrive in ligno(hemi)cellulose-rich environments. During the last decade, fungal genome sequencing initiatives have yielded abundant information on the genes that are putatively involved in lignocellulose degradation. At present, additional experimental studies are essential to provide insights into the fungal secreted enzymatic pools involved in lignocellulose degradation.

In this study, we performed a wide analysis of 20 filamentous fungi for which genomic data are available to investigate their biomass-hydrolysis potential. A comparison of fungal genomes and secretomes using enzyme activity profiling revealed discrepancies in carbohydrate active enzymes (CAZymes) sets dedicated to plant cell wall. Investigation of the contribution made by each secretome to the saccharification of wheat straw demonstrated that most of them individually supplemented the industrial *Trichoderma reesei* CL847 enzymatic cocktail. Unexpectedly, the most striking effect was obtained with the phytopathogen *Ustilago maydis* that improved the release of total sugars by 57% and of glucose by 22%. Proteomic analyses of the best-performing secretomes indicated a specific enzymatic mechanism of *U. maydis* that is likely to involve oxido-reductases and hemicellulases.

This study provides insight into the lignocellulose-degradation mechanisms by filamentous fungi and allows for the identification of a number of enzymes that are potentially useful to further improve the industrial lignocellulose bioconversion process.

PR8.72

Expression profile of beta-galactosidases in *Penicillium chrysogenum*

Szilvia Jäger, Erzsébet Fekete, Michel Flipphi, Anita Kondás, Norbert Ág, Levente Karaffa
Department of Biochemical Engineering, Faculty of Science and Technology, University of Debrecen, H-4010, Egyetem tér 1, Debrecen, Hungary

Penicillium chrysogenum is used as industrial producer of penicillin. We investigated the catabolism of lactose, an abundant component of whey that has been used extensively in pencillin fermentation, comparing NRRL 1951 as a wild-type reference with the industrial penicillin-producer ASP-78.

Both strains grew similarly on lactose under batch conditions. The time-profile of sugar depletion concurred with the presence of intra- and extracellular beta-1,4-D-galactosidase (bGal) activities. Upon growth on D-glucose, D-fructose, D-xylose, D-galactose and glycerol, neither extra- nor intracellular bGal could be detected. However, L-arabinose induced activity to about half the values measured on lactose. The measured bGal activities were similar for the two investigated strains.

In silico analysis revealed that P. chrysogenum features at least five putative bGal-encoding genes at the annotated loci Pc22g14540, Pc12g11750, Pc16g12750, Pc14g01510 and Pc06g00600. The first two proteins appear to be orthologs of the Aspergillus nidulans intracellular family 2 glycosyl hydrolases AN3201 and AN3200. The latter three P. chrysogenum proteins feature an N-terminal secretion signal and appear distinct paralogs to the extracellular bGal from Aspergillus niger, LacA.

Transcript analysis of Pc22g14540 and Pc12g11750 showed that they were expressed exclusively in response to lactose but completely repressed on the mixed growth substrate glucose/lactose. Pc16g12750 was seemingly co-expressed with the two putative intracellular bGal genes, while its two paralog genes were apparently not transcribed under any condition tested. This expression profile is distinct from those in other ascomycetes, like Trichoderma reesei or A. nidulans, where bGal genes are induced by the monosaccharides D-galactose and/or L-arabinose.

PR8.73

D-galactose uptake of Aspergillus niger

Erzsébet Fekete[1] Christian P. Kubicek[2] Ronald P. de Vries[3,4] Bernhard Seiboth[2] Erzsébet Sándor[5] Patricia A. vanKuyk[6] Levente Karaffa[1]
[1]Department of Biochemical Engineering, Faculty of Science and Technology, University of Debrecen, Hungary [2]Research Area Biotechnology and Microbiology, Institute of Chemical Engineering, TU Wien, Austria [3]Microbiology, Utrecht University, Padualaan 8, The Netherlands [4]CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands [5]Institute of Food Processing, Quality Assurance and Microbiology, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Hungary [6]Molecular Microbiology, Institute of Biology Leiden, Leiden University ,The Netherlands.

The majority of Black Aspergilli (Aspergillus Section Nigri), including *Aspergillus niger*, as well as many other Ascomycetes fail to germinate on D-galactose as a sole carbon source. Here, we provide evidence that the ability of *A. niger* to transport D-galactose is growth stage dependent, being absent in the conidiospores but partially present in the mycelia. Despite earlier claims, we could identify galactokinase activity in growing cells and all genes of the Leloir-pathway (responsible for channeling D-galactose into the EMP-pathway) are well induced on D-galactose (and also on lactose, D-xylose and L-arabinose) in the mycelial stage. Expression of all Leloir pathway genes was also detectable in conidiospores, though *galE* (encoding a galactokinase) and *galD* (encoding a UTP-galactose-1-phosphate uridylyl transferase) were expressed very poorly. These results suggest that the D-galactose-negative phenotype of *A. niger* conidiospores is due to the lack of inducer uptake.

PR8.74

Approaches for evaluating the performance of lignocellulosic biomass hydrolysates obtained by using fungal enzyme cocktails

Ying Zha [1,2] Leon Coulier [1,2] Richard Bas [1] Johan van Groenestijn [1] Peter Punt [1,2]
1. TNO Microbiology & Systems Biology 2. Netherlands Metabolomics Centre

The feedstock for 2nd generation biofuel is lignocellulosic biomass, such as wheat straw, bagasse and corn stover. For microorganisms to utilize the biomass, a pretreatment and a hydrolysis step are needed to release fermentable sugars into the lignocellulosic biomass hydrolysate. Fungal enzyme cocktails are used in the hydrolysis step. The hydrolysates differ in their compositions and effects on growths of yeasts and fungi, due to (i) the biomass type (ii) the pretreatment and hydrolysis method (iii) strain characteristics. To select the best combination of these factors is a key step in conducting 2nd generation biofuel research.

In this study, methods were developed to determine the composition of various hydrolysates, generated from diverse biomass and by different pretreatment and hydrolysis methods. In particular, a HPAEC-MS method was found suitable to identify the limiting and interfering factors to the activities of fungal enzyme cocktails. The results of this analysis allow targeted optimization of these cocktails. The performance of these hydrolysates was screened in Bioscreen C Analyzer using *Saccharomyces cerevisiae* CEN.PK 113-7D as model strain.

The results show that our approaches are effective for evaluating and selecting the most suitable hydrolysate for a specific production purpose. The developed analysis methods have potential to enhance the enzymatic hydrolysis efficiency in the hydrolysates, and enable the systematic study on the hydrolysate inhibitory effects.

PR8.75

Enhance itaconic acid production in Aspergillus via cultivation condition optimization

An Li[1] Nina Pfelzer[2] Robbert Zuijderwijk[3] Anja Brickwedde[2] Peter J. Punt[1]
1. TNO Microbiology & Systems Biology 2. Hochschule Bremen 3. Leiden University

The black filamentous fungi Aspergillus niger has a long tradition of safe use in the production of enzymes and organic acids, and is widely used in biotechnology as host for the production of food ingredients, pharmaceuticals and industrial enzymes. Besides, Aspergillus niger grows on a wide range of substrates under various environmental conditions. In our research we have addressed the production of one of the commercially interesting building-block organic acids, itaconic acid. The transcriptomics analysis from A. terreus identified the most relevant itaconic acid related genes. Subsequently, expressing the specific cis-aconitate decarboxylase (CAD) encoding gene in A niger lead to itaconic acid production in the fermentation medium. To enhance itaconic acid production level via medium improvement, 20 different media were designed based on a reference medium from A. terreus and a 96-well micro-titer plate screening assay was applied for screening . The best medium increased the production level in controlled batch fermentation. This was confirmed showing that various levels of one of the trace elements correlated with itaconic acid production under these conditions. In addition, several other parameters such as pH, temperature and dissolved oxygen tension (D.O.) in controlled batch fermentations were shown to be important for itaconic acid production .

PR8.76

Brazilian mangrove fungi in biological synthesis of silver nanoparticles

Rodrigues, A. G.[1] Liu, Y. P.[1] Marcato, P. D.[2] Alves, O.L.[2] Melo, I. S.[3] Tasic, L.[2] De Souza, A. O.[1]
[1]Butantan Institute, Biochemistry and Biophysics Laboratory, 05503-900, São Paulo, SP, Brazil [2]University of Campinas, Chemistry Institute, 13083-970, Campinas, SP, Brazil [3]EMBRAPA Environmental, Environment Microbiology Laboratory, 13820-000, Jaguariúna, SP, Brazil

Mangrove is an ecosystem in which there is a high competition for nutrients among microorganisms and where lack of oxygen supplies enables many oxido-reduction reactions to occur. In this context our study has been focused on the biological synthesis of silver nanoparticles (Ag NP) by some fungi isolated from Sao Paulo State's (Brazil) mangrove and evaluation of Ag NP antimicrobial activities. Fourteen fungi were cultivated in Potato Dextrose Broth at 25°C and 150 rpm for 72 h. The biomass was filtered and incubated at the same conditions with water (0.1 g mL^{-1}). The biosynthesis of Ag NP was performed adding $AgNO_3$ (1 mM) into fungal filtrate. The Ag NP formation was confirmed by Plasmon resonance band (λ = 440 nm). The nanoparticles were characterized applying TEM, size, zeta potential, and protein portion adhered to Ag NP was analyzed by SDS-PAGE electrophoresis. Antimicrobial activities were tested against some Gram-negative and Gram-positive bacteria and *Candida* species. The results showed that the fungi coded as L-2-2, R-2BI-4, MGE-201, MGE-202 and R-3BI-10 were able to produce Ag NP in satisfactory yields, pronounced antimicrobial activities, spherical morphology, and size in a range of 10-30 nm. TEM and SDS-PAGE revealed the presence of proteins around the Ag NP with molecular weight in the range of 75 to 328 kDa and further investigations are being performed to characterize these proteins. Fungi were taxonomically identified as *Bionectria ochroleuca* (L-2-2), *Cladosporium* spp (R-2BI-4), *Aspergillus tubingensis* (MGE-201), *A. niger* (MGE-202) and *Fusarium proliferatum* (R-3BI-10), respectively.

Financial support: FAPESP

PR8.77

Identification and characterisation of novel antifungal compounds against fungal human pathogens

Petra D. Keller[1] Anke Burger-Kentischer[2] Karl-Heinz Wiesmüller[3] Karin Lemuth[1] Ekkehard Hiller[2] Isabel Engelhardt[2] Christoph Müller[4] Klaus Schröppel[5] Franz Bracher[4] Steffen Rupp[2]
[1.] Institute for Interfacial Engineering, University of Stuttgart, Nobelstr. 12, D-70569 Stuttgart [2]Fraunhofer Institute for Interfacial Engineering and Biotechnology (IGB), Nobelstr. 12, D-70569 Stuttgart [3.] EMC microcollections GmbH, Sindelfinger Str. 3, D-72070 Tübingen [4.] Department of Pharmacy, Ludwig-Maximilians University, Butenandtstr 5-13, D-81377 München [5.] Institute of Medical Microbiology and Hygiene University Hospital Tübingen, Elfriede-Aulhorn-Str. 6, D-72076 Tübingen

Fungal infections represent a serious health problem for immune suppressed patients who can be highly susceptible to life-threatening systemic infections. The increasing number of fungal infections and the development of resistance as well as the significant side effects result in the need for the identification of novel antifungal drugs. To identify, evaluate and optimize new tolerable and potent compounds with antifungal activity we have developed an *in vitro* High-Throughput-Screening Activity-Selectivity Assay (AS-HTS-Assay). This assay mimics the smallest unit of a natural infection by incubating host cells with the pathogen, e.g. *Candida species*, in the presence or absence of antimicrobial compounds. Thereby, it covers all potential targets of pathogen and host simultaneously in one assay and provides the minimal inhibitory concentration of active compounds in a host context and the tolerability of these compounds by the host cells. Using this assay we screened more than 100,000 compounds for antimycotic activity. One hit, a benzimidazole derivative, showed high antifungal activity against Candida spp. and good compatibility with human cells. This compound showed a good tissue penetration, tolerability and efficiency in complex 3D-epithelial tissue models and in multicellular organisms as demonstrated in first nematode models. The results of transcriptional profiling of *Candida albicans* indicated that the compound is a potential inhibitor of the ergosterol pathway. This is in contrast to other benzimidazole-derivatives which target microtubules. To further verify the specific target enzyme in the ergosterol pathway sterol pattern of different Candida spp. were carried out by GLC/MS analysis.

PR8.78

Novel approaches for solving bottlenecks and improving recombinant protein production by *Aspergillus*

N.N.M.E. van Peij, A. Los, J. Bakhuis, L. Wu, J-M. van der Laan,M.M.A. Olsthoorn, J.A Roubos, H.J. Pel
DSM Biotechnology Center, Delft, The Netherlands

The filamentous fungus *Aspergillus niger* is an important micro-organism used for large scale industrial production of enzymes. As a cell factory it combines a large intrinsic protein production capacity with a long history of safe use. Enzyme production in *A. niger* has been optimized in many ways. Classical strain improvement, optimization of expression cassettes and gene copy number increase are relevant approaches to achieve high protein productivity levels. Last decade, functional genomics studies have led to the identification of host genes that can be modified to boost protein expression capacity. To optimize gene designs we have developed algorithms that bring single-codon usage as well as codon-pair usage in line with the usage detected in highly expressed genes. An in-depth comparison of the compositional, physiological and structural features of proteins that are poorly secreted and the corresponding features of proteins that are well-secreted has led to a method to predict if an over-expressed protein will successfully be produced or not. Moreover, the same information has been used to design and produce enzyme variants with adapted amino acid features that have an improved secretion while maintaining their catalytic activity.

PR 8.79

A new method for the production of peptides: insertion and isolation of peptides from ankyrin repeat proteins

Alrik P. Los, Tim G. Homan, Jan-Metske van der Laan, Cees M.J. Sagt, Herman J. Pel
DSM Biotechnology Center, P.O. Box 1, 2600 MA Delft, The Netherlands,

Peptides are widely used in the pharmaceutical industry and are also gaining importance as food ingredients. However, the current state-of-the-art production of oligopeptides is via chemical synthesis, which is expensive especially for long peptides. In addition, peptides can be produced by means of fermentation, wherein multimeric genes encoding tandem repeats of the peptide are fused to a carrier protein. After production of the fusion protein peptides are cleaved from the carrier protein. However the yield of the process is generally low and the fusion protein generally accumulates in inclusion bodies.

We developed a new approach in which peptides are inserted in the variable loops of ankyrin repeats. Ankyrin repeat domains are conserved structures and consists of tandem repeats of a 33 amino acid ankyrin repeat unit. Especially interactions between units are important for stability and folding. Therefore, ankyrin repeat domains usually consist of 4 or 6 units. Interestingly, in between two adjacent ankyrin units inserts up to 47 amino acids has been observed in nature.

We have shown in E.coli and Aspergillus niger that peptides can be inserted in between two adjacent ankyrin repeat units and peptides can be isolated from the protein. In Aspergillus niger, ankyrin repeat proteins comprising peptides were fused to truncated glucoamylase. The chimeric repeat protein comprising peptide was successfully expressed and secreted by Aspergillus niger.

In conclusion, production of peptides by inserting peptides in ankyrin repeat proteins is a successful new approach for fermentative peptide production.

PR8.80

The transcriptional regulator RhaR of *Aspergillus niger* is involved in L-rhamnose catabolism and in degradation of Rhamnogalacturonan-I

BS Gruben [1,2] M Zhou [1] A Wiebenga [1] J Ballering [2] RP de Vries [1,2]

[1.] CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; [2.] Microbiology & Kluyver Centre for Genomics of Industrial Fermentation, Utrecht University, The Netherlands

The *Aspergillus niger* genome contains a broad set of pectinolytic genes, encoding enzymes that act on the different substructures and linkages of pectin. Previous studies demonstrated a complex regulation of these genes, but so far none of the regulators involved in this process have been identified.

We identified the transcriptional activator RhaR is described that is mainly regulates the expression of genes involved in degradation of Rhamnogalacturonan-I. Micro-array analysis revealed down-regulation of genes encoding exorhamnogalacturonases, α-rhamnosidases, rhamnogalacturonan acetylesterases, an unsaturated rhamnogalacturonan hydrolase and a rhamnogalacturonan lyase in the Δ*rhaR* strain compared to the reference strain on L-rhamnose. In addition, a gene encoding a putative pectin acetyl esterase, two genes encoding putative β-1,4-galactosidases and one gene encoding a feruloyl esterase were also down-regulated in the disruptant.

RhaR also appears to regulate L-rhamnose catabolism as growth of *rhaR* disruptant strains on L-rhamnose was abolished and two genes encoding putative L-rhamnose catabolic enzymes were down-regulated in the Δ*rhaR* strain.