



Symposium

Biotransformation by Fungal Cells or Fungal Enzymes

September 30th – October 2nd, 2010

at

Hochschule Lausitz (FH), Senftenberg, Germany

Invited speaker presentations:

Cellulases for second generation biofuels
David B. Wilson, Cornell University, USA

Selective oxidation of steroids by fungal cells
Jens Pilling, Bayer Schering Pharma AG, Germany

**Penicillin production: Improvements after genome sequencing of
*Penicillium chrysogenum***
Marco van den Berg, DSM-Anti-infectives, The Netherlands

Structure-Function Relationships and Applications of CotA-laccase
Lígia O. Martins, Universidade Nova de Lisboa, Portugal

Efficient gene expression in fungi
Helmut Schwab, Graz University of Technology, Austria

The organizing committee wishes to thank



VAAM Special Group "Fungal Biology and Biotechnology"



VAAM / DECHEMA Special Group "Biotransformationen"



Deutsche Forschungsgemeinschaft



Hochschule Lausitz (FH)

for their support.

Organizing committee:

Klaus-Peter Stahmann
Karl-Heinz van Pée
Beatrix Krautz
Grit Hagenberger
Birgit Jahn

Senftenberg, September 2010

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I. Time Schedule

Thursday, 30 September 2010

11:00 Registration; Mounting of posters

14:45 Opening remarks

I. Session, Chair: Günter H. Schulz

15:00 Cellulases for second generation biofuels
David B. Wilson; Cornell University Ithaca, New York, USA

15:40 Enzymatic hydrolysis in a capillary segmented flow reactor – impact of liquid properties on flow pattern and stability
Mahesh Huddar; Brandenburgische Technische Universität, Cottbus, Germany

16:00 Lipase production by fungi: robust monoseptic cultivation system under non-sterile conditions
Susann Barig; Hochschule Lausitz, Senftenberg, Germany

16:20 Coffee Break

16:40 Poster Session I

19:00 Mixer with bar music (piano) at building 15
Possibility to have a look into the laboratories
Organisation of Saturday excursions

Friday, 1 October 2010

II. Session, Chair: Ulrich Kück

09:00 Penicillin production: Improvements after genome sequencing of *Penicillium chrysogenum*
Marco van den Berg; DSM-Anti-infectives, Delft, The Netherlands

09:40 A multi-protein complex regulates secondary metabolism and morphogenesis in the industrial fungus *Penicillium chrysogenum*
Ulrich Kück; Ruhr-Universität Bochum, Germany

10:00 The role of GliT in the *Aspergillus fumigatus* biosynthesis of gliotoxin
Daniel Scharf; Hans-Knöll-Institut, Jena, Germany

10:20 Coffee Break

10:40 Poster Session II

12:30 Lunch

III. Session, Chair: Karl-Heinz van Pée

13:30 Structure-function relationships and applications of CotA-laccase
Lígia O. Martins; Universidade Nova de Lisboa, Oeiras, Portugal

14:10 Two novel fungal laccases for dye decolourisation
Anke Matura; Technische Universität Dresden, Germany

14:30 Biotransformation of disinfectants by yeasts
Rabea Sietmann; Ernst-Moritz-Arndt-Universität Greifswald, Germany

14:50 Production, purification and characterization of an extracellular peroxygenase from the agaricomycete *Marasmius rotula* with promising potential in regio- and stereoselective biocatalysis
Glenn Gröbe; Hochschule Lausitz, Senftenberg, Germany

15:10 Coffee Break

15:20 Poster Session III

IV. Session, Chair: Ursel Kües

16:00 Selective oxidation of steroids by fungal cells
Jens Pilling; Bayer Schering Pharma AG, Bergkamen, Germany

16:40 Selective oxidations catalysed by fungal aromatic peroxygenases
Matthias Kinne; International Graduate School of Zittau, Germany

17:00 Oxidation by laccases – Industrial applications
Bernd Nebel; Universität Stuttgart, Germany

17:20 Production of technical enzymes by solid state fermentation of white-rot fungi in a bioreactor with modular design
Ulrike Böhmer; Technische Universität Dresden, Germany

18:00 Bustransfer to Schloss Sallgast

19:00 Dinner at Schloss Sallgast

23:00 Bus transfer back to Senftenberg

Saturday, 2 October 2010

V. Session, Chair: K.-Peter Stahmann

- 09:00 Efficient gene expression in fungi
Helmut Schwab; Technische Universität Graz, Austria
- 09:40 Overexpression of chloroperoxidase in *Caldariomyces fumago*
Markus Buchaupt; Karl-Winnacker-Institut, Frankfurt, Germany
- 10:00 The secretome of *Candida utilis*
Denis Tielker; Heinrich-Heine-Universität Düsseldorf, Germany
- 10:20 Coffee Break
- 11:00 Functional expression of the cholesterol side-chain cleavage cytochrome
P450_{scc} system and the P450_{c17} in the yeast *Yarrowia lipolytica*
Stephan Mauersberger; Technische Universität Dresden, Germany
- 11:20 Molecular mechanism for undergoing environmental stress – a multidrug
transporter gene of the ectomycorrhizal fungus *Tricholoma vaccinum*
Ines Schlunk; Friedrich Schiller Universität Jena, Germany
- optional:
- 12:00 Lunch
- 13:30 Excursion A
INTEGRA GmbH, Hoyerswerda; Production of Shii-take fruiting bodies;
organised tour; end of tour back in Senftenberg around 17:00
- 15:00 Excursion B
NATURPARADIES GRÜNHAUS, Lichterfeld; wild life coming back to a
closed brown coal mining area;
organised tour; end of tour back in Senftenberg not later than 20:00

II. List of Posters and Abstracts

listed alphabetically by presenting or first author

- P 01 Christian Bodinus, Gerold Barth; Dresden
Enhancing the itaconic acid production in *Pseudozyma tsukubaensis* by overexpression of the *cis*-aconitic acid decarboxylase
- P 02 Julia Böhm, Birgit Hoff, Simon Wolfers, Stefanie Pöggeler, Ulrich Kück; Bochum
MAT1-1- a regulator of asexual development and hyphal morphogenesis in the penicillin producer *Penicillium chrysogenum*
- P 03 B. Cherdchim, A. Majcherczyk, U. Kües; Göttingen
Effects of wood extractives against fungal decay and induction of fungal laccase decaying *Abies grandis* wood
- P 04 Markus Hille, Jennifer Wirth, Benjamin Gol, Reinhard Pätz; Köthen
Production of enzymes in different solid-state fermentation systems on agricultural residues
- P 05 Alexandra Katschorowski, Katarina Kopke, Ulrich Kück; Bochum
FLP/FRT recombination system: A new molecular tool for marker recycling in filamentous fungi
- P 06 Katja Koschorreck, Vlada B. Urlacher; Düsseldorf
Phenolic C-C coupling using fungal and bacterial laccases
- P 07 Erika Kothe, Theodore Asiimwe, Katrin Krause, Doreen Schachtschabel, Wilhelm Boland; Jena
Conversion of tryptophane to indole-3-acetic acid and its role in ectomycorrhiza formation
- P 08 A. Majcherczyk, U. Kües; Göttingen
Analysis of the *Heterobasidion annosum* secretome
- P 09 Elena A. Martinova; Moscow
Divers effects of fumonisins group B on eukaryotic cell signaling pathways
- P 10 Susanne Nieland, K.-Peter Stahmann; Senftenberg
Riboflavin accumulation and *RIB3*_{promoter}*lacZ* induction correlated in one of four cell types of *Ashbya gossypii*
- P 11 Christina Otto, Venelina Yovkova, Stephan Mauersberger, Gerold Barth; Dresden
Variation of the by-product spectrum under KGA production conditions by overexpression of tricarboxylic acid cycle genes in *Yarrowia lipolytica*

- P 12 M. Rühl, S. Kilaru, A. Pomowski, O. Einsle, U. Kües; Göttingen
Characterisation of five different laccases of the basidiomycete *Coprinopsis cinerea*
- P 13 Petra Weißhaupt, Wolfgang Pritzkow, Matthias Noll; Berlin
Exploring the nitrogen uptake of *Trametes vesicolor* and *Oligoporus placenta* by EA-IRMS and stable isotope labelling

P 01

Enhancing the itaconic acid production in *Pseudozyma tsukubaensis* by overexpression of the *cis*-aconitic acid decarboxylase

Christian Bodinus and Gerold Barth

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Itaconic acid (IA) is an unsaturated dicarbonic organic acid which is used as an additive in polymers. Therefore, IA is a component of applications like polyesters, plastics, resins, adhesives or coatings. IA is exclusively manufactured microbially by fermentation of the fungus *Aspergillus terreus*. The main route of the IA biosynthesis in *A. terreus* is most likely via the tricarboxylic acid cycle and the decarboxylation of aconitic acid by the *cis*-aconitic acid decarboxylase (CAD). This enzyme has been sequenced and characterized recently. There are several yeasts known to secrete itaconic acid, including yeasts of the genera *Ustilago*, *Candida* and *Pseudozyma*. However, no industrial process has ever been established to produce IA with yeasts due to low productivities and yields.

We identified the non-conventional yeast *Pseudozyma (P.) tsukubaensis* to produce IA in rather low amounts of 6-8 g/l (Specht *et al.*, 2009). *P. tsukubaensis* is a basidiomycetal yeast which is closely related to the phytopathogenic fungus *Ustilago maydis*. By UV mutagenesis, we generated a mutant, *P. tsukubaensis* H488-M15, which is capable to produce about 14 g/l IA within 16 days of cultivation. Therefore, we cloned the *CAD1* gene from *A. terreus* and transformed it into the *P. tsukubaensis* mutant. For this purpose we established a new yeast-gene-transfer system. One of the obtained transformants produced $54,4 \pm 2,1$ g/l IA within 9 days in flask experiments. Showing a yield of 40 % based on the metabolized glucose the highest productivity was found to be 0,5 gIA/(l*h).

Specht, R., Aurich, A., Kreyß, E., Bodinus, C. and Barth, G. (2009). Method for biotechnologically manufacturing itaconic acid. Patent WO 2009106627

MAT1-1 – a regulator of asexual development and hyphal morphogenesis in the penicillin producer *Penicillium chrysogenum*

Julia Böhm¹, Birgit Hoff¹, Simon Wolfers¹, Stefanie Pöggeler², and Ulrich Kück¹

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Penicillium chrysogenum is best-known as the major industrial producer of penicillin. Although no sexual propagation has been reported so far, we recently were able to detect mating type loci in different strains of *P. chrysogenum*, indicating a sexual lifecycle in this biotechnically relevant fungus. Isolates of opposite mating type were found in near-equal proportion in nature and we observed transcriptional expression of both mating type loci as well as the pheromone and pheromone receptor genes [1]. As a first step for functional characterization of the mating-type genes, a *MAT1-1* knockout strain was generated by gene replacement with a phleomycin resistance cassette in a $\Delta Pcku70$ [2] background. In this mutant asexual conidiophore development was affected; it produces significantly higher quantities of conidia relative to the recipient strain $\Delta Pcku70$, although the ratio of light-to-dark conidial production levels remains the same. Using $\Delta MAT1-1$ and *MAT1-1* overexpression strains we found a significant role of *MAT1-1* in hyphal morphogenesis. This is of significant relevance for pellet formation in industrial fermentation processes. Our results highlight the diverse regulatory roles of the *MAT1-1* gene in developmental processes other than sexual reproduction.

[1] Hoff B, Pöggeler S, Kück U (2008) Eighty years after its discovery, Fleming's *Penicillium* strain discloses the secret of its sex. *Eukaryot Cell* 7: 465-470

[2] Hoff B, Kamerewerd J, Sigl C, Zadra I, Kück U (2010) Homologous recombination in the antibiotic producer *Penicillium chrysogenum*: strain $\Delta Pcku70$ shows up-regulation of genes from the HOG pathway. *Applied Microbiology and Biotechnology* 85: 1081-94

Effects of wood extractives against fungal decay and induction of fungal laccase decaying *Abies grandis* wood

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Abies grandis is a fast growing coniferous tree with a high potential for sustainable wood production and applications in the wood product industry. In wood block decay tests,

A. grandis wood was easily degraded by brown-rot fungi but showed a comparably good resistance against white-rot species. Wood extracted with water and acetone however lost resistance. Wood extractives were shown to inhibit growth of the white-rots

Trametes versicolor, *Pleurotus ostreatus* and *Coprinopsis cinerea*. Moreover, wood extractives in concentration-dependent manner induced production of extracellular laccases but no other oxidative enzymes. We identified extractable wood compounds by gas chromatography and mass spectrometry (GC-MS) and tested the importance of these compounds in wood degradation processes as mediators in laccase oxidation of wood lignin. Some of the natural phenolic compounds have a potential as natural inducers in laccase production. Wood particles were treated with laccases to elucidate their potential in biofuel production. Raw wood extractives, respectively specific phenolic compounds within the raw wood extractives act as inhibitors in hydrolysis of cellulose by cellulase. Phenolic compounds in wood extractives can be degraded by laccase activity, allowing better performance of cellulases in the production of glucose.

Production of enzymes in different solid-state fermentation systems on agricultural residues

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Professor Dr. rer. nat.

Saprophytic mushrooms represent an outstanding expression system for a multiplicity of biomolecules due to their organizational structure and the associated enzyme diversity. Among them there are interesting compounds as the β -Glucane, in addition, the enzymes and enzyme mixtures themselves. The meaning of enzymes produced by fungi for industrial processes will further constantly rise due to the catalyzed reactions. In order to use the metabolic potential of fungi it isn't almost necessary to vary the organisms on a genetic level of regulation. However the fermentation straight of filamentous growing fungi represents a procedural challenge, which is examined not enough by now. In the context of different projects in the working group Bioprocess engineering of the department Applied Life Sciences and Process Engineering of the Anhalt University of Applied Science Koethen were examined different mushrooms. Apart from technical problems, caused by the growth of hyphae, in continuously agitated systems above all the parameters oxygen supply, substrate composition, activity of water, shear load and temperature could be recognized as parameters affecting growth, enzyme- and product formation. It turned out that a high shear stress of the cells in the initial stage of the growth of hyphae led to smaller pellets with smaller diameters. Thus the technical problems, which are known from the CSTR, could be minimized. The same behavior could be observed in the airlift loop reactor with internal loop. As particularly interesting the variant of Slurry fermentation has to be regarded. As Slurry fermentation solid-substrate fermentation is to be understood a liquid continuous phase apart from a disperse phase of a firm substrate. Here it could be shown that hardly growth of hyphae could be observed macroscopically because all of the hyphae had been grown on the substrate surface. One has to assume after today's knowledge level the mushroom settles the rigid surfaces solid particles. As substrates barley pellets with different particle diameters, straw, poplar, corn silage, digestate from anaerobic fermentation of corn silage and garden and landscape conservation material were used. Likewise as solid-substrate chitin containing exoskeletons of crustaceans were used. For the attempts a particularly arranged airlift reactor with eccentric air entry, which is characterized by its zoning into a turbulent and air-satisfied range, a degassing zone and a non-turbulent range with microaerophilic conditions as well as a sedimentation zone, was used. In relation to classical solid-state fermentations it could be shown here that the advantages of submerged-liquid fermentation and solid-state fermentation can be used here in a system. It could be shown for lignolytic enzymes and Cellulases and Hemicellulases that in both systems similarly good results could be received. From the view of Process engineering however the Slurry fermentation could be recognized as favorable. The possibility of the regulation of temperature and the dosage of dietary supplements and other materials used for controlling different parameters were regarded particularly favorably. A further advantage of the Slurry fermentation with the production of enzymes and enzyme mixtures is apart from the fact mentioned the fact that by process engineering strategies it can be achieved that enzyme mixtures independently of the fermentation duration with continuously high activities can be produced.

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P 05

FLP/*FRT* recombination system: A new molecular tool for marker recycling in filamentous fungi

Alexandra Katschorowski, Katarina Kopke, Ulrich Kück
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The FLP/*FRT* recombination system from the yeast *Saccharomyces cerevisiae* has successfully applied to other eukaryotes. So far however any attempts have failed to use this system for marker recycling in filamentous fungi. We recently have constructed a codon-optimized recombinase gene for successful expression in filamentous fungi. This system is fully functional in filamentous fungi and can be used for site-specific recombination (Kopke et al. 2010). Furthermore it could be extended by the construction of a *nat1*-flipper-cassette for one-step marker recycling. This consists of the codon adapted flippase gene and the bacterial *nat1* resistance marker flanked by two *FRT* sites. The application of the FLP/*FRT* recombination system was demonstrated by the generation of marker free knockout strains from the two filamentous fungi *Penicillium chrysogenum* and *Sordaria macrospora*. In addition we will provide data where mutated *FRT* sites were used *in vivo* for flippase mediated recombination. In summary we have developed an optimized FLP/*FRT* recombination system as a molecular tool for the genetic manipulation of filamentous fungi.

Kopke K, Hoff B and Kück U (2010) Application of the yeast FLP/*FRT* recombinase system in filamentous fungi: Marker recycling and construction of knockout strains devoid of heterologous genes. Appl. Environ. Microbiol. doi:10.1128/AEM.00670-10

Phenolic C-C coupling using fungal and bacterial laccases

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Laccases (E.C. 1.10.3.2.) belong to the group of multicopper “blue” oxidases capable of oxidizing several phenolic and non-phenolic compounds through the concomitant reduction of molecular oxygen to water. Due to the broad substrate spectrum of laccases and wide range of catalyzed reactions, like cross-linking of monomers, degradation of polymers and oxyfunctionalization of aromatic compounds, they are considered to be industrially relevant enzymes. In nature laccases are widely distributed among fungi, plants, insects and bacteria.

The white-rot fungus *Trametes versicolor* produces at least four different isoforms of laccase, so called Lcc α , Lcc β , Lcc γ and Lcc δ . These four laccases were cloned and actively expressed in *Pichia pastoris* under control of the methanol inducible *AOX1* promoter.¹ High cell density fermentations of *P. pastoris* yielded volumetric activities of laccases of up to 3400 U l⁻¹. This provides the opportunity to use these enzymes for biotechnological applications.

Biochemical characterization of the laccases showed, that Lcc α and Lcc β were much more stable than Lcc γ and Lcc δ at both room temperature and elevated temperatures. Furthermore, the catalytic activities of the laccases were quite different. Although all four isoenzymes catalyzed the oxidative phenol coupling of sinapic acid to dehydrodisinapic acid dilactone, Lcc δ showed the highest activity with 98% conversion after 20 min. Only 34%, 50% and 48% conversion was reached with Lcc α , Lcc β and Lcc γ , respectively, after 20 min of reaction. The highest activity towards polycyclic aromatic hydrocarbons was obtained with Lcc β . In the presence of the redox mediator 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) over 80% conversion of anthracene, acenaphthene and acenaphthylene was reached with Lcc β after 72 h. Thus higher conversions have been achieved using the Lcc β -ABTS system compared to results described in the literature with laccase mixtures from *T. versicolor* and ABTS.² From these results, Lcc β was identified as the most stable laccase of *T. versicolor* with a high activity towards various substrates and is potentially applicable in biocatalysis. Structural analysis of these laccases followed by mutagenesis demonstrated the impact of positions 164 and 265 located at the substrate binding sites on activity of enzymes towards polycyclic aromatic hydrocarbons.

¹ Koschorreck K, Richter SM, Swierczek A, Beifuss U, Schmid RD, Urlacher VB. 2008 Comparative characterization of four laccases from *Trametes versicolor* concerning phenolic C–C coupling and oxidation of PAHs. *Arch Biochem Biophys* 474: 213-219.

² Johannes C, Majcherczyk A, Huttermann A. 1998 Oxidation of acenaphthene and acenaphthylene by laccase of *Trametes versicolor* in a laccase-mediator system. *J Biotechnol* 61: 151-156.

P 07

Conversion of tryptophane to indole-3-acetic acid and its role in ectomycorrhiza formation

Theodore Asiiimwe, Katrin Krause, Doreen Schachtschabel, Wilhelm Boland and Erika Kothe

The phytohormone indole-3-acetic acid (IAA) has been controversially suggested to play a role in ectomycorrhizal development, albeit with unknown mechanisms. We investigated the possible involvement of IAA in ectomycorrhiza development using studies on gene expression, fungal IAA biosynthesis, effect of IAA on fungal morphogenesis and on the subsequent differentiation in *Tricholoma vaccinum*-spruce ectomycorrhiza. *T. vaccinum* produces IAA presumably through a tryptophan-indole pyruvate-(indole-3-acetaldehyde)-IAA biosynthetic pathway, with the last step most catalyzed by an aldehyde dehydrogenase. The encoding gene, *ald1*, was highly expressed in ectomycorrhiza, and IAA increases fungal growth and hyphal ramification. Acting as a diffusible signal, IAA increased Hartig' net formation and the phytohormone is a prerequisite for effective colonization of roots by the fungus.

Analysis of the *Heterobasidion annosum* secretome

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Heterobasidion annosum is a common devastating root rot in conifer plantations and natural forests in Europe and the USA (northern hemisphere). It is one of the most important tree pathogens and the most harmful fungus to the modern forest industry in causing root and butt rot disease. Infecting trees *H. annosum* causes typical white rot, degrading simultaneously or selectively lignin. Sequencing of the *H. annosum* genome by JGI in 2009 provided the first comprehensive genetic information on a pathogenic white rot and allowed efficient studies on the fungal proteome.

To study the largely unknown secretome of this basidiomycete the fungus was grown in liquid medium with *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 from 2D-gels or by application of a shot-gun method on complex protein mixtures. Using the annotated *H. annosum* genome, 118 unique 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 induced secretion of specialized glycanases, lipases and proteases.

This work was supported in frame of a Common Lower-Saxony-Israel Project (ZN 2043) by the Ministry of Science and Culture in Hannover, Germany. The work conducted by the U.S. Department of Energy Joint Genome Institute is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

Divers effects of fumonisins group B on eukaryotic cell signaling pathways.

Martinova Elena A.

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Fumonisins are mycotoxins produced by fungi *Fusarium moniliforme*, *proliferatum*, *anthophilum*, *damini*, *napiforme*, etc. Consumption of corn contaminated by fumonisins resulted in acute and chronic diseases. Fumonisins B2 (FB2, C₃₄H₅₉NO₁₄, M705) and B3 (FB3, C₃₄H₅₉NO₁₄, M705) differ from fumonisin B1 (FB1, C₃₄H₅₉NO₁₅, M721) by OH-radicals in C5 and C10 position of carbohydrate chain that determines the divergence in its biological effects. Sphingolipid origin of fumonisins allowed to discover the ceramide synthase as a target for FB1 [Merrill et al, 1993]. We found the plasma membrane sphingomyelinase as a target for FB1 [Martinova et al, 1995].

Our current results elucidated the different regulation of cell signaling pathways by FB1 and FB2. At first, the reciprocal response of immune cells to FB1 and FB2 has been shown. Secondly, FB2 induces the stronger apoptotic effect in immune cells compared with FB1. Thirdly, FB1 and FB2 differently regulate the cell cycle progression and expression of inhibitors p21, p27 as well as Rb proteins and cyclines. Because both, cell proliferation and arrest of cell cycle are dependent on signaling from mTOR, we study the influence of fumonisins on proteins connected with these signaling pathways.

The serine / threonine kinase mTOR (mammalian Target of Rapamycin) regulates a cell growth by coordinating the nutrient- and energy-sensitive signals. mTOR forms two multiprotein complexes. The mTORC1 complex, provides signals to the mRNA translation and protein synthesis (a major target is the p70 ribosomal S6 kinase-1 (p70S6K1)). The mTORC2 complex works rapamycin-independently and switches the insulin and PI-3-kinase/Akt signals. Both mTORC1 and mTORC2 complexes are regulated by inhibitors TSC1 and TSC2 (hamartin and tuberlin). In vivo and in vitro using primary lymphocyte culture we found that FB1 and FB2 reciprocal activate and inhibit the TSC1 and TSC2 protein expression followed by signaling transition through mTORC1 or mTORC2 complexes. Expression of other proteins connected with mTOR (Raptor, Rictor, and others) have been found to be regulated differently by fumonisins B1 and B2. Graded influence on mTORC1 and mTORC2 complexes resulted in the diverse effects of FB1 and FB2 on the cell proliferation and apoptosis.

References:

MARTINOVA, E.A. & IVANCHENKO, O.B. Biological effects of fumonisins and contamination of corn products, 2 Edition, revised and augmented, **Kazan', State educational publish house, 2006, – 200 P., ill. – ISBN 5–94688–022–5**

MARTINOVA, E.A. & IVANCHENKO, O.B. Mycotoxin fumonisin B1 and regulation of microbiota. – Moscow, Publish House of the Federal State Institution "All-Russian Scientific Research Institute of Civil-Defense and Emergencies", EMERCOM of Russia, - 2006. – 350 P., ill. – ISBN 5–93970–021–7

**Riboflavin accumulation and *RIB3*_{promoter}*lacZ* induction correlated
in one of four cell types of *Ashbya gossypii***

Susanne Nieland and K.-Peter Stahmann

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The hemiascomycete *Ashbya gossypii* can overproduce 100mg riboflavin (Vit B₂) per g biomass. Less than 1% is produced in chemostatic culture at constant dilution rate [1]. An explanation for that difference in productivity is a regulation of three *RIB* genes. They encode enzymes of the biosynthesis pathway starting from ribulose 5-phosphate. Induction under nutritional stress was concluded from increasing RT-PCR signals compared with *TEF*. For *RIB3*, encoding the first enzyme, increased initiation of transcription was shown by fusion of its promoter with a reporter gene. Furthermore an increase of specific activity of dihydroxy butanone phosphate synthase (*rib3p*) was determined [2].

Interestingly, these overall changes were only partly consistent with the observed cell morphologies. When growth rate declined a significant part of the population formed spores, another accumulated riboflavin, resulting in a green fluorescence, and a third part appeared to be “lazy”. Quantification of cell types was impossible in the mycelium. But conversion to protoplasts by digestion of the cell walls allowed FACS analysis. Up to 10⁹ protoplasts were liberated per millilitre. Flow cytometry distinguished 50% needle-shaped spores from spherical protoplasts. Up to 80% of the latter emitted green light when excited at 488nm indicating riboflavin accumulation.

By FACS sorting 3,000,000 riboflavin accumulating protoplasts were isolated. In their crude extract more than 90 mU reporter activity was determined per mg protein. Less than 5 mU were detectable in hyaline protoplast showing no riboflavin accumulation.

[1] Three Biotechnical Processes using *Ashbya gossypii*, *Candida famata*, or *Bacillus subtilis* Compete with Chemical Riboflavin Production

K.-Peter Stahmann, José Luis Revuelta, Harald Seulberger (2000) Applied Microbiology and Biotechnology 53: 509-516

[2] Growth stress triggers riboflavin overproduction in *Ashbya gossypii*

Thomas Schlösser, Andreas Wiesenburg, Cornelia Gätgens, Andreas Funke, Ulrike Viets, Swaminathan Vijayalakshmi, Susanne Nieland und K.-Peter Stahmann (2007) Applied Microbiology and Biotechnology 76(3): 569-578

Variation of the by-product spectrum under KGA production conditions by overexpression of tricarboxylic acid cycle genes in *Yarrowia lipolytica*

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The non-conventional yeast *Yarrowia (Y.) lipolytica* is of increasing importance as organism for biotechnological applications as well as eukaryotic model system for studies on several biological processes. The genome of *Y. lipolytica* is completely sequenced and genetical tools, e.g for expression of heterologous genes, are well developed. One of the most prominent features of *Y. lipolytica* is the secretion of high amounts of organic acids from the tricarboxylic acid cycle like α -ketoglutaric (KGA), citric (CA) or isocitric acid under special culture conditions. Typically, an excess of carbon source and simultaneous growth limitation by different factors, e.g. thiamine [KGA] or nitrogen exhaustion [CA], result in overproduction of organic acids up to about 200 g/L. The amount and kind of produced organic acid can be effected by changes of activities of involved enzymes. In this study we show that a gene-dose dependent overexpression of the genes encoding fumarase, malate dehydrogenase or pyruvate carboxylase result in a different product ratio of the secreted organic acids under KGA production conditions.

Characterisation of five different laccases of the basidiomycete *Coprinopsis cinerea*

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Laccases are oxidoreductases containing 4 copper atoms involved in the transport of electrons from a phenolic substrate to an oxygen molecule, which is reduced to water. Laccases are widespread in nature and have distinct functions. In fungi, it is thought that laccases are involved in substrate degradation, fruiting and stress reactions. Thus, different biochemical characteristics can be expected. In this study, five different laccases (Lcc1, Lcc5, Lcc6, Lcc7 and Lcc11) from *Coprinopsis cinerea* were recombinantly overexpressed, purified and biochemically characterised.

The molecular weight for the laccases was within the range of 61-63 kDa and N-glycosylation accounts for 2-6% of the total weight. Different isoform bands of the purified laccases were separated by isoelectric focusing (IEF). The optimal pH for the standard substrate ABTS varied from 3.0 to 5.0. For all enzymes, the optimal temperature was 50 °C. Michaelis-Menten parameters were determined for the purified laccases in reaction with the four mostly used laccase substrates ABTS, DMP, guaiacol and syringaldazine (SGZ). Stabilities were tested against inhibitory chemicals and in different solvents. Stable crystals were obtained for Lcc5 and its structure is presented in this work.

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Exploring the nitrogen uptake of *Trametes versicolor* and *Oligoporus placenta* by EA-IRMS and stable isotope labelling

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Fungi are major decomposers in terrestrial ecosystems. In particular, basidiomycetes and ascomycetes degrade plant litter and dead wood to CO₂ and humic substances. Fungal biomass has a much higher nitrogen content compared to their limiting substrates and sustains nitrogen against washout. Fungi may have additional nitrogen sources like an interaction with atmospheric N₂-assimilating, diazotrophic bacteria. A mutualistic interaction is feasible as wood decomposing basidiomycetes may retrieve bacterial fixed nitrogen and bacteria achieve carbon based substrates released by the fungi. Such an interaction will cover the fungal nitrogen-limitation and will lead to high overall respiration rates and accelerated wood decay.

Therefore, the nitrogen sources of the reference organisms *Trametes versicolor* and *Oligoporus placenta* were investigated with a focus on the interaction to diazotrophic bacteria such as *Azotobacter croococcum*, *Novosphingobium nitrogenifigens* and *Beijerinckia acida*.

By means of EA-IRMS (Elemental analysis isotopic ratio mass spectrometry) the nitrogen turnover of defined sources was investigated. The EA of the biomass and their potential substrates revealed in *in vitro* experiments that the supplied nitrogen was assimilated and the isotopic ratio of fungal biomass was similar to those of the substrates. In addition, labelling experiments with ¹⁵N₂ as gaseous substrate elucidated that diazotrophic bacteria assimilated 10 to 12 % (m/m) of their nitrogen from N₂. Two strategies of the organisms growing on nitrogen limited substrates were observed: i) enlarged spatial adaptation by highly branched fungal mycelium or by polysaccharide slimes in order to absorb even traces of nitrogen; ii) increased bacterial N₂ assimilation rates of up to 10 % (m/m) that bridges nitrogen limitation.

In addition, ¹⁵N enriched bacterial biomass was fed to both fungi. By means of EA-IRMS we were able to elucidate fungal-bacterial interactions, which will enable upcoming approaches in innovative ways of wood protection.

III. List of Oral Presentations and Abstracts

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- V 16 Antje Kröber, Anja Kruggel, Nadine Czekalski, Daniel Gliesche, Annett Mikolasch, Rabea Sietmann, Frieder Schauer;
Biotransformation of disinfectants by yeasts
- V 17 Buerth, C., Heilmann, C. J., Klis, F. M., Ernst, J. F., Tielker, D.;;
The secretome of *Candida utilis*
- V 18 Marco van den Berg;
Penicillin production: improvements after genome sequencing of *Penicillium chrysogenum*
- V 19 Maxim Kostylev, Thu Van Vuong, Yongcho Li, Diana Irwin, David B. Wilson;
Cellulases for Second Generation Biofuels

V 01

Lipase Production by Fungi: Robust monoseptic Cultivation System under non-sterile Conditions

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Applied Microbiology is on the way to replace chemical processes. An example is the microbial production of riboflavin which is economically and ecologically better than chemical synthesis [1]. But that and many other processes are run with cost-intensive sterile technique. Cheap and robust processes with a minimum of sterile technique are needed. Since lipases are already successful in several industrial applications a new production system was developed. A combination of five conditions was found to generate selectivity for stable monoseptic one-week-cultivations: mineral salts medium, nitrate as sole nitrogen source, plant triglycerides as sole carbon and energy source, aeration, and pH 3-4 [2].

Botrytis cinerea and five fungal isolates were found to grow under these conditions. The system worked on Petri dishes, in 100 ml cultures cultivated in 500 ml shake flasks, and in 350 l culture broth filled in 500 litre plastic barrels. Cultures stayed monoseptic over a time span of seven days even when run with beginners lacking any experience in microbial systems. Best results concerning lipase production were obtained with AW02 identified as *Phialemonium curvatum*. In a 500 litre barrel filled with 350 litre culture medium and aerated by 100 litres air per minute 20 k Units were obtained.

Lipase activity was found to be attached strongly on the fungal cell wall. Only shear stress applied by grinding in liquid nitrogen liberated the activity into an extraction buffer. Isoelectric focussing followed by activity staining showed two bands at pH 4.7 and 5.6.

- [1] HP Hohmann, KP Stahmann (2010) Vitamins and vitamin-like compounds *In: L Mander, HW Liu (eds) Comprehensive Natural Products II Chemistry and Biology Elsevier, Oxford, Vol 7 p115-139*
- [2] KP Stahmann, S Nieland, A Wuttke, Patent WO / 2008 / 067882

Production of technical enzyme by solid state fermentation of white-rot fungi in a bioreactor with modular design

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The ability to synthesize a set of extracellular enzymes (e.g. laccases, peroxidases, cellulases) enables white-rot fungi to degrade all components of lignocellulosic material. They are also capable to decompose different aromatic xenobiotics like textile dyes or polycyclic aromatic hydrocarbon (PAH). These characteristics make white-rot fungi prospective for various biotechnological applications, for example in pulp and paper industry and textile industry [1].

The solid state fermentation (SSF) includes all fermentations of microorganisms on a solid substrate without free liquid and it is especially suitable for the cultivation of white-rot fungi. It simulates the natural habitat of the fungi and protects the mycelium against mechanical stress. The presented SSF-reactor was developed by fzmb GmbH Forschungszentrum für Medizintechnik und Biotechnologie. It allows the spraying of various liquids, e.g. water for moisturizing the fungi or rather washing the extracellular enzymes from the mycelium. Furthermore nutrients or liquid containing special additives can be used. The colonized substrate can be mixed as the reactor drum can rotate. These features allow the adjustment of various conditions for the fungal mycelium in the solid state fermenter to achieve optimal process conditions for the production of extracellular enzymes. Moreover the modular assembly of the reactor can be changed to perform other fermentations, e.g. the cultivation of hairy roots.

The white-rot fungi *T. hirsuta* and *B. adusta* were cultivated on pine wood chips and orange peels [2] in the SSF-reactor. A mix of pinewood chips and orange peels proved as the best cultivation material. Also the addition of copper sulphate and xylydine was tested. Expectedly the addition of copper sulphate was able to raise the production of laccase whereas xylydine could not improve the enzyme production. Overall high yields of extracellular enzymes were obtained. Both fungi were also cultivated in submerged conditions and the results of both fermentations types were compared. Solid state fermentation proved as a suitable technique for the production of large amounts of enzymes with the use of a low cost substrate and at a low operating expense.

Literature:

[1] S. Mazumder, S. K. Basu, M. Mukherjee, Laccase production in solid-state and submerged fermentation by *Pleurotus ostreatus*, *Eng. Life Sci.*, Vol. 9, Nr. 1, 2009

[2] V. Elisashvili, E. Kachlishvili, M. Penninckx, Effect of growth substrate, methode of fermentation, and nitrogen source on lignocellulose-degrading enzymes production by white-rot basidiomycetes, *J. Ind. Microbiol. Biotechnol.*, Vol. 35, 1531-1538, 2008

Overexpression of chloroperoxidase in *Caldariomyces fumago*

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The filamentous fungus *C. fumago* secretes the versatile enzyme chloroperoxidase (CPO). This heme containing enzyme exhibits peroxidase, catalase and cytochrome P450-like activities in addition to catalyzing halogenation reactions with chloride-, bromide- and iodide ions. Up to now heterologous overexpression in different expression hosts resulted in inactive enzyme or very low yields. As the high enzyme production costs are the major hurdle for the use of CPO in large scale processes we established tools to overexpress the enzyme in its natural production host. Therefore we integrated a CPO expression cassette into the nontranscribed spacer regions of the rDNA in *C. fumago*. One strain obtained by this procedure exhibited about two-fold higher CPO levels in shake flask and bioreactor cultivations compared to wildtype. The highest CPO concentration observed in the bioreactor cultivation was 1.95 g L^{-1} , which is the highest value ever reported and demonstrates the possibility of turning this natural CPO secreting organism into an optimized production strain by genetic engineering.

Production, purification and characterization of an extracellular peroxygenase from the agaricomycete *Marasmius rotula* with promising potential in regio- und stereoselective biocatalysis.

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Aromatic peroxygenases (APOs) are functional enzyme hybrids sharing catalytic properties with classic peroxidases, chloroperoxidase (CPO) and cytochrome P450 monooxygenases. These heme-thiolate proteins are produced by agaric basidiomycetes (agaricomycetes) colonizing soil, dung or wood, and the peroxygenases from *Agrocybe aegerita* and *Coprinellus radians* are meanwhile well characterized. They are able to [per]oxygenate, brominate and [per]oxidize a wide range of aromatic as well as aliphatic substrates including diverse ethers. Unlike intracellular P450 monooxygenases, the secreted APOs are easy to handle (due to their high stability), show a higher catalytic versatility and need only hydrogen peroxide for function instead of complex/expensive cofactors (NAD[P]H) and auxiliary electron transfer proteins.

In a screening of fungi from special habitats (rotting sub-surface wood, leaf and grass litter) for peroxygenase activities, we isolated a strain of the agaricomycete *Marasmius rotula* (pin-wheel mushroom). In liquid culture, this strain was found to secrete exceptionally high amounts of an APO that can reach activity titers of 40,000 U L⁻¹ roughly corresponding to 200 mg peroxygenase protein per Liter. This is the highest amount of a heme-thiolate protein ever obtained and exceeds the titers reported for APOs of *A. aegerita* and *C. radians* by the factor 20 and more. The enzyme (*Marasmius rotula* aromatic peroxygenase = *Mro*APO) was concentrated and purified using tangential ultrafiltration and anion exchange as well as size exclusion chromatography, respectively. Kinetic data of the purified enzyme for the substrate toluene revealed a preference of side chain vs. aromatic ring hydroxylation. *Mro*APO has the highest molecular mass (~ 53 kDa) of all known peroxygenases and tends to form dimers. In contrast to the “neutral” APOs of *A. aegerita* (*Aae*APO) and *C. radians*, its pH optimum has been found to vary between 5 and 5.5 for most reactions tested. *Mro*APO is highly stable in a pH range from 5 to 10 and in the presence of organic solvents (50 % vol/vol) such as methanol, acetonitrile and demethylformamide.

Using *Mro*APO and *Aae*APO, we have developed simple and reasonable syntheses for the oxy-functionalization of drugs and fine chemicals. For example, the multifunction *beta*-adrenergic blocker propranolol can be regioselectively hydroxylated to form 5-hydroxypropranolol in yields up to 37%, and the achiral ethylbenzene molecule can be stereoselectively converted into *R*-(1)-phenylethanol with an enantiomeric excess (ee) of 99%.

Enzymatic Hydrolysis in a Capillary Segmented Flow Reactor – Impact of Liquid Properties on Flow Pattern and Stability

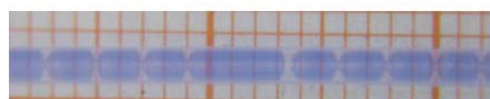
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Enzymatic oil hydrolysis involving the chemical reaction between native oils and an aqueous phase in the presence of a lipase yields free fatty acids and glycerine. Conventional enzymatic processes based on emulsion or membrane reactors possess certain disadvantages which can be overcome by use of a segmented flow reactor. The most important characteristic feature of this reactor type is the high mass transfer rate between the two immiscible phases.



[a]



[b]

Fig. 1 Drop flow formation using segmented flow capillary micro-reactor for enzyme-buffer [blue] and oil [pale yellow] system

[a] at the beginning and end for stable flow

[b] at the end for unstable flow

In the past, segmented flow reactors were used for two phase gas-liquid reactions and liquid-liquid extraction processes which are simple in nature. In contrast the enzymatic hydrolysis is known for its reaction triggered complex interactions between the two phases. e.g. the physical properties are found to vary with conversion to a large extent. Thus, as shown below, large variations in flow patterns are observed along the capillary length leading in some cases to unstable flow conditions. For a reliable design of segmented flow reactors for the enzymatic oil hydrolysis the parameters which control the stability of segmented flow as well as their impact on flow pattern must be known in detail.

Therefore an extensive experimental study of the flow pattern was carried out by varying the physical properties of the two-phase system within their reasonable limits and constructive details of the reactor as well. The results will be shown and discussed in detail.

Oil ml/hr	EB ml/hr	Bubble	Slug	deformed interface	0,2 m	0,4 m	0,6 m	0,8 m	1 m
1	1								
3	3								
5	5								
10	10								
15	15								
20	20								
25	25								
1	3								
1	5								
3	1								
3	5								
3	7								
3	10								
5	1								
5	3								
5	7								
5	10								
7	1								
7	3								
7	5								
7	10								
7	12								

Fig. 2 Checking the flow pattern obtained for different flow ratio and if it is stable (green) or unstable (red) over each 0.2 m length of the reactor.

Selective oxidations catalyzed by fungal aromatic peroxygenases

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Several litter- and wood-decomposing fungi have recently been shown to secrete heme-thiolate peroxygenases, which are involved in a number of selective H₂O₂-dependent monooxygenation reactions. For example, the best-characterized fungal peroxygenase, from *Agrocybe aegerita*, catalyzed the hydroxylation of 2-phenoxypropionic acid to give the herbicide precursor 2-(4-hydroxyphenoxy)propionic acid. Moreover, peroxygenases can be utilized for the synthesis of diverse valuable human drug metabolites such as 5-hydroxypropranolol, 4'-hydroxydiclofenac and O-desmethylnaproxen. The reactions proceeded regioselectively with isomeric purities up to 98%, and reached enantiomeric excesses higher than 60%. ¹⁸O-labeling experiments showed the incorporation of H₂O₂-derived oxygen into the oxidized product, which establishes that the reaction is mechanistically a peroxygenation. Studies conducted with deuterium labeled substrates revealed large intramolecular deuterium isotope effects, which points to a hydrogen abstraction and oxygen rebound mechanism similar to that envisaged for the peroxygenase activity of P450. Our results raise the possibility that fungal peroxygenases may be useful for versatile, cost-effective, and scalable syntheses of diverse chemicals.

V 07

**A multi-protein complex regulates secondary metabolism and morphogenesis
in the industrial fungus *Penicillium chrysogenum***

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The recent discovery of a *velvet* complex containing several regulators of secondary metabolism in the model fungus *Aspergillus nidulans* raises the question whether similar type complexes direct fungal development in genera other than *Aspergillus*. Here we provide a functional analysis of major homologues of the *velvet* complex in *P. chrysogenum* the industrial producer of the beta-lactam antibiotic penicillin. Data from northern hybridizations, HPLC quantifications of penicillin titres as well as detailed microscopic investigations clearly show that all regulators play not only a major role in penicillin biosynthesis but are also involved in different and distinct developmental processes. While for example deletion of the *velvet* homologue PcvclA leads to light-independent conidial formation, dichotomous branching of hyphae and pellet formation in shaking cultures, a Δ PclaeA strain shows a severe impairment in conidiophore formation in both the light and dark. Furthermore, detailed bimolecular fluorescence complementation assays together with yeast two-hybrid screens led not only to the identification for a *velvet*-like complex in *Penicillium chrysogenum* but also to the detection of new components of this complex. Our results extend 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.

Structure-Function Relationships and Applications of CotA-laccase

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Laccases are versatile enzymes capable of coupling the oxidation of a variety of substrates namely phenols, diamines and even some inorganic compounds, to the reduction of oxygen. Fungal laccases are the main object of current fundamental studies and biotechnological applications but, it is now known, that laccases are widespread in bacteria. In the last years, we become interested in exploring the biochemistry and structure of laccases from prokaryotes for which, genetic tools and biotechnological processes are well established. Our research has focused on the CotA-laccase of *Bacillus subtilis* as a model bacterial laccase. The CotA is a thermoactive and thermostable protein with a $T_{op} = 75^{\circ}\text{C}$ and T_m values around 80°C (1). Several X-ray crystal structure studies were undertaken revealing interesting features of the enzyme, namely insights on the nature of binding sites and role of oxidative mediators (2). The characterization of variants obtained by site-directed mutagenesis has been performed contributing for the clarification of the catalytic mechanism of these enzymes (3). In addition, CotA-laccase was tested on its ability to degrade azo and antraquinonic dyes (4). Combining enzymology, electrochemistry, mass spectrometry and nuclear magnetic resonance, a detailed characterization of the laccase decolourisation system was achieved and mechanistic pathways for azo and antraquinone dyes conversion were proposed.

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- (1) Martins *et al.* 2002, JBC, 277,18849; Durão *et al.* 2008, JBIC, 13, 183
- (2) Enguita *et al.* 2003, JBC, 278, 19416; Enguita *et al.* 2004, JBC, 279, 23472; Bento *et al.* 2005, Dalton Trans., 3507
- (3) Durão *et al.* 2006, JBIC, 11, 514-26; Durão *et al.* 2008, Biochem. J., 412, 339; Chen *et al.* 2010, Dalton Trans., 39, 2875
- (4) Pereira *et al.* 2009, J. Biotechnol., 139, 68; Pereira *et al.* 2009, Adv. Synth. Catal., 351, 1857

Two novel fungal laccases for dye decolourisation

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Different white rot fungi from Indonesia were screened for their ability to decolourise industrially used textile dyes. Two of them, a *Marasmius* species and an unidentified white rot fungus were investigated in detail because of their interesting bleaching properties. For both species we identified laccases as the main part of the fungal bleaching system and purified and characterised these enzymes in detail.

The enzyme from the unidentified basidiomycete was purified to homogeneity by ion exchange and hydrophobic interaction chromatography. Characterisation of the native enzyme revealed that the enzyme exists in solution as a monomer with a molecular mass of 54.5 kDa. The laccase is a glycoprotein with an N-terminal amino acid sequence and spectral properties typical for blue laccases. Atomic absorption spectroscopy revealed that it contains 4 copper atoms per enzyme molecule. The new laccase shows K_M values for the substrates ABTS and guaiacol of 4.7 μM and 711 μM , respectively. The pH optimum is at pH 3.0 for the conversion of ABTS and at pH 5.0 for guaiacol with a temperature optimum for both substrates of 50 °C. The enzyme is stable at pH 10 and with high salt concentrations over a long period of time. Highest bleaching activity was found for anthraquinone and formazan-copper dyes followed by monoazo-copper dyes which is consistent with the decolourisation activity observed with the whole fungus.

In the culture filtrate of the *Marasmius* sp., two laccases (laccase I and laccase II) were detected. Laccase I was purified to homogeneity by ion exchange and hydrophobic interaction chromatography. The native enzyme was shown to have a molecular mass of 53 kDa, an N-terminal amino acid sequence with very high identity to that of known laccases and an isoelectric point of pH 3.8. Laccase I accepts typical laccase substrates including ABTS, syringaldazine and guaiacol, but has no tyrosinase activity. The pH optimum is 3.0 for ABTS and 6.0 for syringaldazine and the enzyme is stable up to pH 10. The UV-VIS spectrum of laccase I is non-typical for laccases and metal content analysis showed that the enzyme contains only one copper atom per molecule. This suggests that laccase I could belong to the group of white or yellow laccases, however, no zinc or any other metal ion could be detected in this enzyme, making laccase I a unique laccase. Comparison of the bleaching activity of the whole fungus with that of isolated laccase I showed that laccase I is the major bleaching enzyme produced by this *Marasmius* sp. and the isolated enzyme can bleach a broader range of textile dyes than can be bleached by the whole fungus.

Functional expression of the cholesterol side-chain cleavage cytochrome P450_{scc} system and of P450_{c17} in the yeast *Yarrowia lipolytica*

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Application of recombinant microorganisms, which combine the advantages of microbiological transformation and the high stereo- and regio-specificity of steroidogenic enzymes of mammals, is a highly potential approach for the synthesis of steroids.

The alkane-utilizing yeast *Y. lipolytica* was tested as an alternative host for P450-catalyzed biotransformation of steroids or sterols. Previously, inducible functional expression of the ER-resident P450_{c17} (bovine CYP17A1 cDNA, 17 α -hydroxylase/C17,20-lyase) in *Y. lipolytica* was investigated in comparison with the yeast *Saccharomyces cerevisiae*. The P450_{c17} expressed found to be highly active especially in alkane-growing *Y. lipolytica* cells, catalyzing the biotransformation of progesterone into 17 α -hydroxyprogesterone, indicating significant advantages of this hydrophobic compounds-utilizing yeast as a host to perform P450-catalyzed bioconversion reactions with hydrophobic substrates [1-2]. The construction of new recombinant *Y. lipolytica* strains for functional expression of the cholesterol side-chain cleavage P450_{scc} system (CYP11A1, human and bovine) together with P450_{c17} will be presented in this study.

Two series of integrative multicopy vectors containing cDNAs for the mature forms of the three P450_{scc}-system components (adrenodoxin reductase AdR, adrenodoxin Adx, P450_{scc}, or fused adrenodoxin-P450_{scc} Adx-P450_{scc}) under control of the strong and regulated isocitrate lyase promoter pICL1 were constructed using the basic plasmids p64PT or p67PT (rDNA or LTR *zeta* of Ylt1 as integration targeting sequences, *ura3 Δ 4* as multicopy selection marker, pICL1-*SphI*-ICL1t).

Integration of up to three expression vectors (containing P450_{scc}-system components and P450_{c17} by their simultaneous transformation into haploid recipient strains was demonstrated by Southern blotting. Additionally, further combinations of the different expressions cassettes in one strain were obtained by subsequent diploidisation using haploid multicopy transformants. Thus, new recombinant strains were obtained containing three to five different expression cassettes [2].

For selected strains heterologous protein expression was shown by Western blotting. The functional expression of these mammalian P450 systems was proved by whole cell biotransformation of cholesterol to pregnenolone and further to 17 α -hydroxypregnenolone, or of progesterone into 17 α -hydroxyprogesterone, detected by GLC, HPLC or radio-TLC. The product structures were also confirmed by ¹H-NMR and MS.

The presented method for recombinant strain construction is obviously a useful tool for the heterologous expression of multi-component P450 enzyme systems in *Y. lipolytica*.

[1] Shkumatov VM, Frolova NS, Rudaya EV, Faletrov YV, Mauersberger S, Barth G (2006) Applied Biochemistry Microbiology 42: 472-478

[2] Novikova LA, Faletrov YV, Kovaleva IE, Mauersberger S, Luzikov VN, Shkumatov VM (2009) From structure and functions of steroidogenic enzymes to new technologies of gene engineering. Review. Biochemistry (Moscow) 74:1482-1504

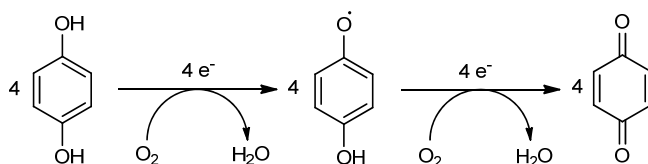
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Enzyme Treated Lignin – Liquid Wood an Alternative to Synthetic Material?

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Laccases [1-3] are regarded as industrially relevant enzymes due to their broad substrate spectrum and wide range of reactions that they catalyze, including cross-linking of phenolic compounds, degradation of polymers, ring cleavage and oxyfunctionalization of aromatic compounds. These enzymes are particularly interesting as biocatalysts because they do not require costly cofactors like NADH or NADPH as many other oxidoreductases. Biotechnological applications of laccases include pulp-pretreatment in the paper production process, dye bleaching in the textile industry, detoxification of xenobiotics, organic synthesis, and bioremediation.

Laccases (benzenediol:oxygen oxidoreductases; EC 1.10.3.2.) belong to the family of multicopper oxidases. Laccases catalyze the four-electron reduction of molecular oxygen to water by the one-electron oxidation of four substrate molecules.



The aim of the “ARBOCAR” project is to replace mineral oil based plastic materials by renewable lignin material. Lignin is a complex polymer mainly present in the secondary walls of tracheary elements and fibers in wood. It is one of the most abundant organic polymers on earth, exceeded only by cellulose. It is necessary to deodorize the crude lignin due to its high concentration of volatile off odour components, because otherwise an industrial processing of lignin at higher temperature is not practical. In the present study, we have investigated the laccase catalysed oxidation of guaiacol, which is the main compound in crude lignin and the role of laccases in reducing the odour of crude lignin.

A process involving the oxidation of the substrate guaiacol by different laccases was developed. Additionally several laccase-mediator systems were tested to increase the oxidation reaction. The different volatile lignin compounds were identified by GC/MS coupled with an automatic headspace auto sampler. Various commercial and isolated laccases were tested. The crude lignin was also pre-treated by supercritical CO₂ and water steam extraction. The outlook of the project is to use pre-treated, deodorized lignin in the automotive industry as a renewable pressure-die casting material.

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1. Koschorreck, K., et al., *Comparative characterization of four laccases from *Trametes versicolor* concerning phenolic C-C coupling and oxidation of PAHs*. Arch. Biochem. Biophys., 2008. **474**(1): p. 213-219.
2. Koschorreck, K., R.D. Schmid, and V.B. Urlacher, *Improving the functional expression of a *Bacillus licheniformis* laccase by random and site-directed mutagenesis*. BMC Biotechnol, 2009. **9**: p. 12.
3. Udayasoorian, C. and P.C. Prabu, *Biodegradation of phenols by ligninolytic fungus *Trametes versicolor**. J. Biol. Sci. (Faisalabad, Pak.), 2005. **5**(6): p. 824-827.

Selective Oxidation of Steroids by Fungal Cells

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The API1) Supply Centre in Bergkamen is the leading production site for steroids and contrast agents of Bayer Schering Pharma AG. For nearly 50 years the Supply Centre has successfully combined chemical and microbiological processes for synthesis of APIs for gynaecology/andrology, dermatology and diagnostic.imaging.

Biotransformations are conducted in agreement with international guidelines in the Microbiological Production facility to generate diverse steroids by use of bacteria, fungi and yeasts. The routine reaction types comprise side chain cleavages (partial and complete), stereo- and regioselective hydroxylations, regioselective dehydrogenations as well as stereospecific oxidations/reductions and fermentation of secondary metabolites from lab up to 200 m³ scale. Subsequent to downstream processing, the products are either used as intermediates for Bayer Schering Pharma API production or sold to customers world wide.

This presentation will focus on selective oxidation of steroidal compounds. It will highlight differences of two different types of oxidations and briefly review the current biological knowledge about this type of conversions. Moreover, some of the challenges during large scale production using fungal oxidations will be illustrated.

1) Active Pharmaceutical Ingredient

The role of GliT in the *Aspergillus fumigatus* biosynthesis of gliotoxin

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Aspergillus fumigatus and other pathogenic fungi have developed various chemical strategies to distress, weaken or even kill their plant or animal hosts. In invasive aspergillosis, the leading cause for death in immunocompromised patients, the fungal secondary metabolite gliotoxin plays a critical role for virulence. Gliotoxin is the prototype of a small family of epipolythiodioxopiperazines (ETPs), which features unique transannular di- or polysulfide bridges. Extensive molecular studies have revealed that this rare structural motif is indispensable for bioactivity and is the key to the deleterious effects of gliotoxin.

Here we describe the function of GliT, an enzyme of the gliotoxin biosynthesis pathway. We could reveal the activity of GliT *in vivo* by means of feeding experiments and *in vitro* by heterologous overproduction and further biochemical characterisation of GliT. We proved that GliT is essential for biosynthesis of gliotoxin and therefore may play a critical role in virulence of *A. fumigatus*.

References: Scharf D. H., *et al.* JACS 2010 Jul 28;132(29):10136-4
Schrettl M., *et al.* PLoS Pathog. 2010 Jun 10;6(6):e1000952

Molecular mechanism for undergoing environmental stress – a multidrug transporter gene of the ectomycorrhizal fungus *Tricholoma vaccinum*

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Fungi are present all over the world in different habitats and in contact with various organisms. Therefore, the fungal cell is often exposed to toxic and harmful substances of biotic and abiotic nature. Several mechanisms are known to prevent cell damages. One possibility is the excretion via multidrug transporters, which can transport a wide variety of compounds. Different classes of multidrug transporters are known, like ABC and MFS transporters, and some of these are well investigated. MATE (multidrug and toxic compound extrusion) proteins also belong to the multidrug transporters, but their role in fungi is marginally understood. Here we report the first MATE family member, *mte1*, of the fungus *T. vaccinum*, which is specifically expressed in ectomycorrhiza with spruce (*Picea abies*). The transporter possesses 12 transmembrane domains, typically for MATEs and shows sequence similarities to other fungal MATE proteins. The gene *mte1* was successfully overexpressed in *S. cerevisiae* and different chemicals were identified as substrates for the transporter including xenobiotics, secondary plant metabolites and metals. For copper sulfate, it was shown that the heavy metal was biotransformed in the cell through linking at glutathione and then excreted via *mte1*. The wide spectrum of transported substrates will be presented facilitating the understanding of the capability of fungi growing on soil with high heavy metal content and as a symbiont circumvent plant defense mechanisms.

Efficient Gene Expression in Fungi

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Fungi play since long time a strong role in biotechnological production. As fungi exhibit many interesting technological and biological features, many fungal species have been developed as host systems for the production of recombinant proteins. However, in most cases more or less basic vectors usually based on strong and regulated promoters and secretion signals for secretory expression are available. Especially in view of production of bio-pharmaceuticals, yeast systems have found most attention. Besides *Saccharomyces cerevisiae*, the methylotrophic yeasts, and among these especially *Pichia pastoris*, have been well developed.

This contribution will on the one hand provide an overview on the recent status of *Pichia pastoris* as expression system. On the other hand, specific examples of addressing problems at various levels of the expression pipeline will be presented. For example, data on molecular analysis of a high-level expression clone (Hasslacher et al., 1997) revealing specific configuration at the site of the integrated expression cassette, dependence of expression on promoter activity and on strategies for improving secretion will be presented.

Finally, the establishment of a new *Pichia pastoris* expression platform based on a genome sequenced host and not depending on existing licence burdens is presented.

References:

Hasslacher, M., Schall, M., Hayn, M., Bona, R., Rumbold, K., Lückl, J., Griengl, H., Kohlwein, S.D., Schwab, H.: High level intracellular expression of hydroxynitrile lyase from the tropical rubber tree *Hevea brasiliensis* in microbial hosts. *Protein Expression and Purification* **11**, 61-71 (1997).

Biotransformation of disinfectants by yeasts

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These days disinfectants are not used for medical purposes only but become more and more important for example for housekeeping, as component of cosmetics, and for industrial purposes. These broad applications result in an increasing input into the environment.

Thus the question of the microbial degradability of disinfectants becomes ever more important. Since the function of disinfectants is to kill microorganisms, their biodegradation is a formidable challenge. Nevertheless, two phenol-degrading yeasts of the genus *Trichosporon* have a high capacity to oxidize, to cleave and to inactivate disinfectants with monoaromatic structure. Initial oxidation of monoaromatic halogenated disinfectants by these yeast strains starts with the introduction of one hydroxyl group into the molecule resulting in dihydroxylated products and continues on up to ring cleavage. The products formed are unchlorinated muconic acid derivatives as well as the corresponding lactones. This implies that a dechlorination step occurred in the course of the ring cleavage. The oxidation of non-halogenated disinfectants with aromatic structure proceeds in an analogous fashion. The disinfectants themselves are toxic to the yeast strains but the ring cleavage products are not. Thus, as the case with other aromatic environmental pollutants (Hammer et al., 1998; Sietmann et al., 2006), the oxidation of compounds with aromatic structure up to ring cleavage represents a detoxification mechanism by yeasts of the genus *Trichosporon*. The results show, that soil fungi may substantially contribute to the aerobic degradation of disinfectants introduced into the environment.

References

Hammer, E., Krowas, D., Schäfer, A., Specht, M., Francke, W. & Schauer, F. (1998) Isolation and characterization of a dibenzofuran-degrading yeast: Identification of oxidation and ring cleavage products. *Appl. Environ. Microbiol.* 64: 2215 - 2219

Sietmann, R., Gesell, M., Hammer, E. & Schauer (2006) Oxidative ring cleavage of low chlorinated biphenyl derivatives by fungi leads to the formation of chlorinated lactone derivatives. *Chemosphere* 64: 6782 - 685

The secretome of *Candida utilis*

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For more than six decades *Candida utilis* is an industrially important yeast, particularly because of its GRAS status. Initially, *C. utilis* was used as a food yeast to produce high quality single cell protein. However, more recent studies on the heterologous expression of several exogenous proteins in *C. utilis* suggest, that the yeast might be an excellent host for the production of recombinant proteins (1, 2).

In order to identify host proteins effectively secreted by *C. utilis*, the secretome of the yeast was investigated. Therefore, cells were cultivated to the logarithmic or the stationary growth phase under different environmental conditions. The genome sequence of *C. utilis* was determined by 454 sequencing (G2L, Goettingen, Germany) in order to facilitate the identification of extracellular proteins found in the respective culture supernatants by both gel electrophoretic and shotgun mass spectrometric approaches. The protein profiles obtained were differentially analyzed and compared to the secretomes of several other yeasts of biotechnological or medical relevance, such as *S. cerevisiae*, *C. albicans* and *C. glabrata*.

References:

1. Ikushima, S., Fujii, T., Kobayashi, O., Yoshida, S., Yoshida, A. (2009). Genetic engineering of *Candida utilis* yeast for efficient production of L-lactic acid. Biosci Biotechnol Biochem. 73: 1818-1824
2. Wei W., Hong-Lan Y., Huifang B., Daoyuan Z., Qi-Mu-Ge S., Wood A.J. (2009). The effective expression of xylanase gene in *Candida utilis* by 18S rDNA targeted homologous recombination in pGLR9K. Mol Biol Rep. in print

**Penicillin production: improvements after genome sequencing
of *Penicillium chrysogenum***

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In the recent years the genome sequences of several industrially important filamentous fungi have been deciphered (i.e. *Aspergillus niger*, *A. oryzae*, *A. terreus*, *Hypocrea jecorina* and *Penicillium chrysogenum*). These sequences started the quest for two answers: (1) why are these specific fungi the champions of their specific applications and (2) how can we further improve them? Generally, these investigations are started by detailed annotation and genome wide omics studies, using platforms like microarrays, proteomics and *in silico* modelling. However, the published genome sequences are obtained from laboratory strains which are quite different from the industrial production strains, which were derived from these laboratory strains after extensive classical strain improvement rounds wherein mutagens induced various chromosomal rearrangements, increasing the complexity level of industrial production strains as compared to the laboratory strains.

Historically, DSM has a long experience in the penicillin G process and has optimized it during the second half of 20th century towards its current efficiency. This is the result of a continuous effort in classical strain improvement and on process optimisation. Recently, we have determined the genome sequence of the international laboratory strain Wisconsin 54-1255 and reported the detailed annotation and initial transcriptome studies*. In parallel, we significantly improved the available toolbox for engineering filamentous fungi and applied that to *P. chrysogenum*. This enabled us to perform a functional exploration of relevant *P. chrysogenum* genes in relation to antibiotic production. The presentation will report on the progress obtained for existing and new antibiotics produced by *Penicillium* and yeast, including 5-membered ring containing penicillins and 6-membered ring containing cephalosporins.

*Van den Berg *et al.* (2008) Genome sequencing and analysis of the filamentous fungus *Penicillium chrysogenum*. Nat Biotechnol. 26:1161-1168.

Cellulases for Second Generation Biofuels

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Most cellulolytic white rot fungi and aerobic bacteria use the free cellulase mechanism to degrade cellulose. In this mechanism a set of cellulases and other proteins are secreted extracellularly. Most of the cellulases contain a carbohydrate binding module, which is joined to their catalytic domain by a flexible linker. The cellulase mixture usually contains both endocellulases, exocellulases and, in bacteria, processive endoglucanases. These enzymes act synergistically on crystalline cellulose with the specific activity of the mixture being up to ten times higher than the activity of any individual cellulase. Most commercial cellulases are produced from white rot fungi, such as *Trichoderma reesei*. The best production strains produce more than 100g/L protein and have the highest activity on crystalline cellulose of known free cellulase mixtures. Extensive research to improve the activity of free cellulase mixtures on pretreated biomass substrates has been carried out over the past five years with some success. The current level of protein production is more than double that of previous production strains. In addition, extensive protein engineering has been carried out to increase the temperature stability of some of the cellulases and to try to increase their activity, although this has not been too successful. However, screening other organisms for proteins that stimulate the activity of *T. reesei* crude cellulase did identify certain family 61 proteins that more than doubled the activity of *T. reesei* crude cellulase on some pretreated substrates. New *T. reesei* strains containing a family 61 gene have been produced and their crude cellulase is significantly more active in hydrolyzing pretreated corn stover. Iogen, a Canadian company, has been producing ethanol from pretreated wheat straw, using *T. reesei* cellulase, for several years in a pilot plant that can produce 5-6 thousand L of ethanol a day from 30-40 tons of wheat straw and is about to build a full scale plant, once they have tested their new technology which should significantly reduce both the capital and operating costs. Other companies also are working to develop similar processes with a variety of biomass feed stocks. One of the most promising feedstocks is bigasse, the residue from sugar cane, as it is at sugar ethanol plants and has been partially processed.

My research has focused on *Thermobifida fusca*, an aerobic cellulolytic bacterium, that has the simplest set of free cellulases with only six. Its genome sequence was determined by the DOE Joint Genome Institute and we have been able to express all six cellulases at a high level in *E. coli*. Three dimensional x-ray structures have been determined for four of these cellulases and reliable model structures are available for the other two. Currently we are using site directed mutagenesis to identify the residues that function to place a cellulose chain into the active site of several of these cellulases, as that appears to be the rate limiting step for crystalline cellulose hydrolysis. This information will then be used to try to engineer new cellulases with higher activity on crystalline cellulose.

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Symposium - Biotransformation by Fungal Cells or Fungal Enzymes
Hochschule Lausitz (FH), September 30th – October 2nd, 2010, Senftenberg

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Symposium - Biotransformation by Fungal Cells or Fungal Enzymes
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