Differential expression of genes involved in iron metabolism in *Aspergillus fumigatus*

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In response to low iron availability in the environment filamentous fungi secrete siderophores, which have proved to be related to virulence for some species. The present state of knowledge of the siderophore system in *A. nidulans* has helped facilitate the study of the equivalent system in its close relative *A. fumigatus*. RNA was extracted from *A. fumigatus* grown under different $FeSO_4$ concentrations (0, 1, 10, 100 and 1000µM) and the expression of iron metabolism related genes was assessed using both RT - PCR analysis and Real Time PCR. The siderophore biosynthetic genes *sidA, sidC, sidD,* transporters *mirB, mirC,* permease *ftrA,* oxidase *fetC* and siderophore precursor *amcA* genes were shown to be upregulated by iron limitation. In contrast, *sreA* (siderophore repressor), *catB* (catalase) and *lysF* (homoaconitase) were shown to be downregulated under the same conditions. In addition, expression of *sidD, catB* and *sreA* was further downregulated at iron concentrations of 1000µM. Thus it appears that siderophore biosynthesis and uptake are regulated by iron at the level of gene expression in *A. fumigatus*, with differential expression of genes occurring at various iron concentrations.

VIp-2

Mapping N-dynamics in fungal colonies

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Cord-forming basidiomycete fungi form extensive mycelial networks that efficiently scavenge inorganic and organic nitrogen from the soil environment. They can form a substantial part of the microbial biomass in terrestrial ecosystems and play an essential role in cycling nutrients, particularly from the decay of wood or in soils with low N-input. Nitrogen is assimilated into specific nitrogen-rich amino acids, such as arginine and ornithine that are sequestered into vacuoles. Source and sink regions for N exist in different areas of the fungal network depending on the availability and re-distribution of nitrogen. We are proposing a model for N-assimilation and transport in the cord-forming fungi based on the spatially regulated urea cycle. In this model, nitrogen is acquired from soil, extracellular breakdown of wood resources, or autolysis of redundant mycelium and funneled through glutamate to arginine *via* the anabolic arm of the urea cycle. The arginine is sequestered in vacuoles and moved within the vacuolar system to sink regions. Carrier-mediated efflux is linked to arginine breakdown *via* the catabolic arm of the urea cycle. The NH_4^+ released is re-assimilated into glutamate for biosynthesis at sites of active growth.

The fungal mycelium, depending on its morphology and function can be categorized into five different growth patters: exploitative (growing on a C-, N-rich source); foraging (fuelled exclusively by translocation of nutrients from the source); colonizing (exploiting a C-rich resource but translocating N); corded, (transporting between two connected C-rich resources); resorptive (autolysing parts of the mycelium for reallocation to sites of growth).

To visualize N-transport in complex networks we have developed a novel non-invasive technique to track movement of 14C-labelled N-compounds in foraging mycelium using photon-counting scintillation imaging. Non-metabolised, 14C-labelled amino acid AIB (aminoisobutyric acid) has been imaged through the fungal colony of Phanerochaete velutina over time and its re-distribution has been linked with the five morphological growth patterns showing significant differences.

Our model predicts that key enzymes and metabolites involved in N-assimilation should show an asymmetric distribution between identified source, sink and transport regions.

We have identified several genes of interest in N-assimilation and are mapping the expression of those key markers of nitrogen acquisition and the urea cycle from sub-sampled regions of mycelium corresponding to the five morphological phases established in the microcosm system using reverse transcription PCR (RT-PCR).

Analysis of four putative beta-oxidation genes in *Aspergillus nidulans*

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Filamentous fungi are able to use fatty acids as sole carbon sources via beta-oxidation. The enzymes required are present in both peroxisomes and mitochondria (Maggio-Hall and Keller 2004 Mol. Microbiol. 54:1173–1185). Two putative fatty acyl-CoA dehydrogenases, AcdA and AcdB, and two putative fatty acyl-CoA oxidases, AoxA and AoxB, were identified in the genome of *A. nidulans*. Because of their homology to Fox1p of *S. cerevisiae* and a peroxisomal dehydrogenase of *N. crassa*, they are thought to be involved in the first step of beta-oxidation. Homologues for each were found in other fungal species. Three of the four proteins have a clear PTS1 (peroxisomal targeting sequence), while AcdA has a PTS1-like sequence. Hence, all four proteins are predicted to be peroxisomal. GFP and RFP fusion proteins are currently under construction to prove this hypothesis. In the 5' promoter region (1kb) of each gene, a six basepair sequence (CCGAGG/ CCTCGG) was found at least once. This sequence is predicted to be the core of a fatty acid depending regulation site (Hynes *et al.*, unpublished) suggesting a positive regulation by fatty acids. Northern-blot analyses indicate that the genes are fatty acid inducible. Promoter-*lac*Z fusions are currently being constructed to elucidate the regulation of the genes by fatty acids. Deletion of all four genes does not give a severe fatty acid growth phenotype. Only the *aox*A phenotype is clearly visible. This implies that there is great redundancy amongst peroxisomal proteins involved in the first step of beta-oxidation.

VIp-4

A screening system for carbon sources enhancing β-*N*-acetylglucosaminidase formation in *Hypocrea atroviridis* (*Trichoderma atroviride*)

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To identify carbon sources that trigger β-*N*-acetylglucosaminidase formation in *Hypocrea atroviridis* (anamorph *Trichoderma atroviride*), we have designed a screening system that consists of a combination of Biolog Phenotype MicroArray plates, which contain 95 different carbon sources, and specific enzyme activity measurements using a chromogenic substrate. The results revealed growth dependent kinetics of β-*N*-acetylglucosaminidase formation and we could show that β-*N*-acetylglucosaminidases were enhanced on carbon sources sharing certain structural properties, especially on β-glucans (e.g. glycogen, dextrin and maltotriose) and oligosaccharides containing galactose. β-*N*-acetylglucosaminidase activities were assessed in the wild-type and a *H. atroviridis* Δ*nag1* strain to study the influence of the two β-*N*-acetylglucosaminidases, Nag1 and Nag2, on total β-*N*-acetylglucosaminidase activities. Reduction of β-*N*-acetylglucosaminidase levels was strongly carbon source and growth phase dependent, indicating distinct physiological roles of those genes. Transcript abundance of *nag1* and *nag2* was increased on carbon sources with elevated β-*N*-acetylglucosaminidase activities indicating transcriptional regulation of those genes.
The screening method for the identification of carbon sources that induce enzymes or a gene of interest presented in this paper can be adapted for other purposes if appropriate enzyme- or reporter-assays are available.

Induction of xylulose-5-phosphate phosphoketolase in *Aspergillus nidulans*: the key for efficient production of the acetyl-CoA precursor molecule

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In the industrial exploitation of cell factories for production of chemicals, including many small molecules used as pharmaceuticals, the yield of conversion of substrate to product is of outmost importance. The aim of this study was to use metabolic engineering strategies to modulate the central carbon metabolism such that carbon from the substrate more efficient can be channeled to specific products.

Although there is a great deal of interest in the utilization of xylose as a fermentation substrate, evidence for its metabolism in fungi taking place solely via the pentose phosphate pathway (PPP) rests on doubtful assumptions. The initial metabolic pathway of D-xylose and D-xylulose in all microorganisms involves their conversion to xylulose-5-phosphate which then is channeled into the PPP. In an alternative route phosphoketolase catalyses the cleavage of xylulose 5-phosphate to glyceraldehyde-3-phosphate and acetyl-phosphate. The formed acetyl-phosphate is then converted directly or via acetate to acetyl-CoA, which is the main starter unit for the production of many products including polyketides.

In the present investigation we determined that the inhibition of the oxidation of glyceraldehydes-3-P (G3P) to 1,3-biphosphoglycerate by GEP dehydrogenase, shifts xylulose metabolism in *Aspergillus nidulans* between the PP pathway and the phosphoketolase pathway, resulting in different yield coefficients of the products. Furthermore, overexpression of a putative phosphoketolase gene in *Aspergillus nidulans*, homologous to sequenced phosphoketolase genes from other microorganisms, was used as a strategy to achieve higher fluxes towards acetyl-CoA.

VIp-6

Investigating the regulation of cellulase production by the fungus *Talaromyces emersonii*

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In nature, the bioconversion of cellulose, the most abundant biopolymer on earth, to simple sugars and the recycling of carbon in the biosphere are tasks efficiently conducted by a myriad of fungi and bacteria present in the environment. These microorganisms produce complex enzyme systems required for the degradation of cellulose-rich plant materials, including wastes. Notwithstanding the important ecological role these microorganisms play, the biopolymer degrading enzymes they produce have numerous industrial, environmental and biotechnological applications. Production of these enzymes represents a high energy demand on these lifeforms and is highly regulated at a genetic level. Cellulose hydrolysis by fungi, and the induction of cellulases has been, and continues to be, a topic of much interest, given the potential of cellulose and cellulose-rich wastes as feedstocks for biofuel production. Therefore, understanding the conundrum of how a large insoluble molecule like cellulose can signal the induction of the enzymes required for its complete hydrolysis is very important. Several theories have been put forward by numerous researchers to explain cellulase induction and repression in the cellulolytic fungus *Trichoderma reesei*. However, it is likely that different mechanisms of induction may exist in different cellulolytic fungi. In this study, we investigate factors involved in the induction and repression of key cellulases in the thermophilic fungus *Talaromyces emersonii*, and for the first time, reveal clear differences between the regulation of the cellulase systems of *T. emersonii* and *Tr. reesei* at transcriptional and translational levels.

Activation of alternative carbohydrate metabolic system in the white-rot fungus *Phanerochaete chrysosporium*

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To investigate cellular system of the white-rot fungus *Phanerochaete chrysosporium*, proteomic analysis was performed. Proteins were obtained from the fungus grown on media with 28 mM glucose and either 1.2 mM (low nitrogen; LN) or 12 mM (high nitrogen: HN) ammonium tartrate as the carbon and nitrogen sources, respectively. After a 4-day incubation, extracellular proteins from *P. chrysosporium* were separated by two-dimensional gel electrophoresis (2-DE). Peptide mass fingerprinting (PMF) was obtained with matrix-assisted laser desorption/ionization time of flight mass spectrometry and utilized for protein identification by analyzing the sizes of tryptic fragments via the MASCOT search engines against the *P. chrysosporium in silico* protein library. As a result, the expression patterns of several proteins on 2-DE gels were significantly different between LN and HN conditions. Manganese peroxidases, lignin peroxidases, glyoxal oxidase (GLO) and aryl-alcohol oxidase (AAO) were identified under LN conditions. GLO and AAO are important for the ligninolytic system of *P. chrysosporium* as a physiological H_2O_2 source. These results suggested that the fungal ligninolytic system was activated under LN conditions. On the other hand, a series of enzymes involved in the metabolism of carbohydrates and organic and amino acids were expressed under HN conditions. These enzymes involved several glycoside hydrolases, glutaminase A, lipolytic enzyme, mannose 6-phosphatase, oxalate decarboxylase and peptidases. Glucose starvation was known to be relatively easily induced under HN conditions compared to LN conditions. Most of the identified glycoside hydrolases were supposed to attack the components of plant and fungal cell walls. Therefore, the expressions of these enzymes seem to be induced by the activation of alternative carbohydrate metabolic system. Furthermore, endo-1, 4-beta-xylanase and endo-1, 5-alpha-L-arabinosidase were specifically expressed in the presence of 24 mM sodium glutamate used as nitrogen source. These results at least suggest that this fungus has ability to sense nitrogen concentrations and change the metabolic system in response to nitrogen status. Proteomic analysis on the cytosolic proteins of *P. chrysosporium* is under investigation.

VIp-8

A novel MFS transporter encoding gene by *Fusarium verticillioides* putatively involved in siderophore transport

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Major facilitator superfamily (MFS) is a ubiquitous group of proteins involved in the transport of a wide range of compounds, including toxins produced by fungal species. In this paper, a novel MFS encoding gene (fvmfs), which showed an up-regulation in fumonisin-inducing conditions in a previous work, has been identified and characterized. The deduced protein sequence predicted 14 transmembrane domains, typical of transporter proteins and showed the highest similary to MFS proteins with 14 transmembrane domains. The phylogenetic analysis performed with a representative sample of MFS proteins suggested a possible function of FVMFS as siderophore transporter. A real time RT-PCR protocol was developed to analyze expression of fvmfs gene to investigate the relationship of the FVMFS protein with fumonisin production and the role of extracellular iron in fvmfs expression. The results suggested that fvmfs might encode a siderophore transporter whose expression was repressed by extracellular iron but it did not seem to be functionally related to fumonisin production.

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Analysis of an endopolygalacturonase enzyme and its coding gene (pg1) from the potato pathogen *Rhizoctonia solani* AG-3

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Rhizoctonia canker is caused by the fungus *Rhizoctonia solani.* There are a number of strains of this fungus, called anastomosis groups (AG) that affect a wide range of crops. It has been found that AG-3 of R. solani is the major cause of the Rhizoctonia disease in potato crops. Rhizoctonia canker is present in all potato growing areas of the world. Symptoms and signs of Rhizoctonia canker include cankers on stems, sprouts and stolons, malformation of tubers and sclerotia formed on the surface of mature tubers. Endopolygalacturonases (endoPGs) are plant cell wall-degrading enzymes that have been implicated in the invasion of plant tissue by pathogenic microbes. EndoPGs have been described from bacteria, plants, insects and numerous species of phytopathogenic fungi.

In this work, we report the purification and characterization of an extracellular endopolygalacturonase (PG1) produced by *R. solani* AG3 and its coding gene (*pg1*), which was analyzed and compared with other endopolygalacturonase genes. The expression of this gene was induced by pectin, polygalacturonic acid and glucose in *in vitro* cultures. *Pg1* gene was highly expressed *in planta* assays suggesting that this endopolygalacturonase might be actively involved during infection process.

VIp-10

Characterization and differential regulation of variable manganese peroxidase genes in the white-rot fungus *Physisporinus rivulosus*

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Physisporinus rivulosus strain T241i is a lignin-degrading basidiomycete that is able to selectively remove lignin from wood and is one of the most promising fungi for the use in biopulping. During growth in wood chips, the fungus produces manganese peroxidase (MnP), which is considered as the main ligninolytic enzyme in the lignin degradation. Present study provides the primary structure of two MnP encoding genes *mnpA* and *mnpB* of *Physisporinus rivulosus* T241i. Surprisingly, the *mnp* genes are significantly divergent in sequence, length and intron-exon structure. The *mnpA* gene of P. rivulosus could be classified to the classical MnP –group, whereas *mnpB* shared characteristics with the lignin peroxidase-type MnP –group. Such diversity of *mnp* genes appears to be rare among white-rot fungi, and merits further investigation.

The complex structure of wood makes it difficult to investigate enzyme regulation under natural growth conditions. Thus, to study the expression of two different MnP encoding genes of *P. rivulosus* and their regulation by different chemical compounds, we cultivated the fungus on defined media under nutrient limited or sufficient conditions supplemented with Mn^{2+} or a non-phenolic aromatic compound veratryl alcohol. The expression of the two *mnp* genes in agitated liquid cultures implicated quantitative variation and differential regulation in response to Mn^{2+} and veratryl alcohol. The transcription of *mnpA* was induced by the addition of veratryl alcohol but not by Mn^{2+}. In the cultures with sawdust a clear induction of *mnpA* was observed. On the contrary, the transcription of *mnpB* was induced by addition of either veratryl alcohol or Mn^{2+} and only slightly by sawdust. This study suggests that the regulation of MnP production in *P. rivulosus* is obviously multifactorial. Genes encoding enzyme isoforms are expressed differentially and the inducers act both separately and in conjunction.

Enzyme, intra and extra cellular metabolite analysis in oxygen limited cultivations of *Aspergillus niger*

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Aspergillus niger is known to be a strictly aerobic organism, meaning it is not using the reductive part of the TCA cycle to produce organic acids like fumarate and succinate. In the present study, our aim was to investigate the effect of different oxygen levels on the physiology and especially the organic acid production of *Aspergillus niger*. The hypothesis is that under strict oxygen levels *Aspergillus niger* might need an additional electron acceptor next to oxygen leading to more organic acid production in the form of fumarate or succinate by the reductive part of the TCA cycle. Five batch cultivations were performed under different aeration rates (0.01, 0.02, 0.05, 0.1, and 1 vvm). Intra and extra cellular metabolites were analysed together with the quantification of enzyme activities in the glycolysis, gluconeogenesis and TCA cycle. We measured 10 different enzymes: glucose-6P dehydrogenase, pyruvate dehydrogenase, pyruvate decarboxylase, pyruvate carboxylase, malic enzyme, malic dehydrogenase, fumarase, succinate dehydrogenase, isocitrate lyase and citrate synthase. The results indicate that the different metabolic pathways are drastically influenced by the oxygen availability. Furthermore, by a detailed analysis of the metabolite fingerprint of *Aspergillus niger* we expect to verify the aforementioned conclusion and reveal more information for the regulation of the different pathways by oxygen.

VIp-12

The unproessanle isopenicillin N acetyltransferase variant (IATC103S) of *Penicillium chrysogenum* located into peroxisomes regulates the processing of the wild-type protein and decreases the penicillin production

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The isopenicillin N acyltransferase (IAT) of *Penicillium chrysogenum*, catalyzing the last step of penicillin biosynthesis, is synthesized as an inactive 40-kDa proIAT that is activated by self-processing forming the active α-β heterodimer (11 + 29 kDa). Previous comparative studies in *P. chrysogenum* and *Aspergillus nidulans* suggested that the efficiency of self-processing is an important differential factor in these two fungi. Hence, we obtained a mutant IAT unable to be self-processed (IAT^{C103S}). Expression of this protein was efficiently induced in *P. chrysogenum* from the strong *gdh* promoter. The unprocessable 40-kDa IAT^{C103S}, like the wild-type protein, was located into peroxisomes (microbodies) as shown by immunoelectron microscopy, but lacked the isopenicillin N acyltransferase (benzylpenicillin-forming) activity. *P. chrysogenum* transformants containing both the endogenous (processable) IAT expressed from its native promoter and the unprocessable IAT^{C103S} (expressed from the *gdh* promoter), were also obtained. Co-expression of these two proteins gave rise to a decrease in benzylpenicillin production and to a delay in the accumulation of the β subunit until the unprocessable protein decayed, suggesting that the presence of the unprocessable IAT^{C103S} inhibited the processing of the native IAT. The inhibition of self-processing of the native wild-type IAT by the unprocessable IAT^{C103S} was confirmed in *E. coli*, where both proteins were efficiently co-expressed under IPTG induction. In this bacterium, almost complete inhibition of the processing of the native IAT by the mutant proIAT was observed.

Transcriptional and bioinformatic analysis of the 56.8 kb DNA region amplified in tandem repeats containing the penicillin gene cluster in *Penicillium chrysogenum*

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High penicillin-producing strains of *Penicillium chrysogenum* contain 6 to 14 copies of the three clustered structural biosynthetic genes, *pcbAB*, *pcbC* and *penDE* (Barredo *et al.*, 1989; Smith *et al.*, 1989). The cluster is located in a 56.8 kb DNA region bound by a conserved TGTAAA/T hexanucleotide that undergoes amplification in tandem repeats (Fierro *et al.*, 1995; Newert *et al.*, 1997). Transcriptional analysis of this amplified region (AR) revealed the presence of at least eight transcripts expressed in penicillin producing conditions. Three of them correspond to the known penicillin biosynthetic genes. To locate genes related to penicillin precursor formation or penicillin transport and regulation we have sequenced and analysed the 56.8 kb amplified region of *P. chrysogenum* AS-P-78, finding a total of 16 open reading frames including the tree structural genes *pcbAB*, *pcbC* and *penDE*. Two of these ORFs have functional orthologues in the databases. Other ORFs showed similarities to specific domains occurring in different proteins and superfamilies which allowed to infer their probable function. ORF11 encodes a D-amino acid oxidase that might be responsible of the conversion of D-amino acids in the tripeptide L-α-aminoadipyl-L-cysteinyl-D-valine or other β-lactam intermediates to deaminated by-products. ORF12 encodes a predicted protein with similarity to saccharopine dehydrogenases that seems to be related to biosynthesis of the penicillin precursor α-aminoadipic acid since *P. chrysogenum* npe10 strain (lacking the entire AR including ORF12), shows a partial requirement of L-lysine for growth. ORF13 encodes a putative protein containing a Zn(2)-CY6 fungal-type DNA-binding domain, probably a trascriptional regulator. Although some of the ORFs in the AR may play roles in increasing penicillin production, none of the 13 ORFs other than *pcbAB*, *pcbC* and *penDE* seem to be strictly indispensable for penicillin biosynthesis.

VIp-14

Lack of *sir2* in *Podospora anserina* increases life span under rich glucose medium.

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Dietary restriction (DR) extends the life span of many organisms such as *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Drosophila melanogaster* and mammals. The *sir2* gene is required for this effect at least in yeast and in *Drosophila* and its overexpression extends life span of yeast, worm and fly. It has been recently shown that DR also extends the life span of the ascomycete *Podospora anserina* (Maas *et al.* 2004). We present here the variations of growth rate, mycelium phenotype and longevity of *Podospora anserina* grown under a series of glucose concentrations and the effect of gain or loss of function of the *sir2* gene. While overexpression of *sir2* does not extend life span, the loss of function allele leads to a spectacular increase of life span (about 200%) on a rich glucose medium, specifically. This result shows that the role of *sir2* in the control of life span is conserved through evolution but the ways it takes to do it may be different.

Searching for the molecular basis of QTLs related to yield in *Pleurotus ostreatus* (oyster mushroom).

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Pleurotus ostreatus is a commercially important edible mushroom commonly known as oyster mushroom. This fungus is industrially produced as human food, for the bioconversion of agricultural and industrial lignocellulose wastes, and as a source of enzymes and other chemicals for industrial and medical applications. Previous work in our laboratory resulted in identification of several genomic regions with quantitative trait loci (QTLs) involved in the control of yield. The QTLs have been mapped to the genetic linkage map of this fungus using molecular markers that co-segregate with them. The hypothesis of this project is that this genomic region would have genes responsible for the QTLs. In order to prove this hypothesis, the markers where used as probes to isolate two BAC clones from a genomic library. The clones were sequenced using the "whole BAC shotgun sequencing" method to 9.5X coverage and an accuracy at the nucleotide level of 99,99%. The annotation of the sequence was performed by both manual and automated methods using advanced BLAST tools and GlimmerHMM, a gene prediction software that were trained for *Pleurotus ostreatus*. The analysis of the synteny among *Pleurotus ostreatus*, *Coprinus cinereus* and *Ustilago maydis* was carried out using the FISH (Fast Identification of Segmental Homologies) algorithm, the detection of several sequence repeats and transposons using customized BLAST databases and the prediction of conserved domains and putative gene function using InterproScan and BLASTP. The annotation consists of 82 protein-coding genes, 4 large repeated sequences, and 75 SSRs. A significant block of conserved synteny between *Pleurotus ostreatus*, *Coprinus cinereus* and *Ustilago maydis* was also found. Most of the putative gene functions where related to cell cycle, meiosis, sporulation, genetic recombination, DNA repair, signal transduction to nucleus and metabolism. The probable role of genes in biological networks is under evaluation. 116 PCR primers where designed in order to study the structural organization of the sequence by comparison to homologous chromosomes in strains segregating for the QTLs and to perform an analysis of gene expression levels using the PCR products as probes for macroarray experiments.

VIp-16

Degradation of sorbic and cinnamic acids by spoilage moulds and yeasts

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The ability of fungi (both moulds and yeasts) to colonise food products and cause spoilage results in significant economic losses and imposes potential safety hazards for the food industry. Several weak acids, including sorbic or benzoic acids, are routinely added to foods and beverages as preservatives to prevent spoilage. Other weak acids are added as acidulants (e.g. acetic or citric acids) or as flavourings (e.g. cinnamic or butyric acids). In the present study, conidial spores of *Aspergillus niger*, a common food spoilage mould, rapidly degraded sorbic and cinnamic acids to the volatile compounds 1,3-pentadiene and styrene, respectively. Spores of other mould genera, isolated from food spoilage incidents, including *Aspergillus*, *Trichoderma* and *Penicillium*, also showed similar effects. Some yeasts (e.g. *Saccharomyces cerevisiae*), also isolated from spoilage incidents, similarly degraded sorbic and cinnamic acids, but to a much lesser extent than the moulds. In separate experiments, Pad1p (encoded by *PAD1*, phenylacrylic acid decarboxylase) from *S. cerevisiae* facilitated the degradation of sorbic and cinnamic acids. In contrast, *S. cerevisiae pad1*Δ mutants were unable to cause such degradation. *PAD1* homologues were identified in the genomes of several yeasts, including *Candida albicans* and *Debaryomyces hansenii*, while other species, including *Zygosaccharomyces rouxii* and *Saccharomyces bayanus,* lacked such homologues. This ability of yeasts containing *PAD1* homologues to degrade both sorbic and cinnamic acids supports the hypothesis that Pad1p is the active moiety. Interestingly, *S. cerevisiae pad1*Δ mutants, despite being unable to degrade sorbic and cinnamic acids, showed no hypersensitivity to these compounds, compared to the wild type phenotype. These results therefore suggest that the degradation of weak acids is not an important resistance mechanism in *S. cerevisiae*. A *PAD1* homologue has been identified in *A. niger* (designed *padA*) and gene deletion studies are in progress.

Functional characterization of β-1,6-glucanase from *Trichoderma virens* and enhanced antifungal activity of transformants constitutively co-expressing β-1,6-glucanase and β-1,3-glucanase

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The mycoparasitic activity of *Trichoderma* spp. against phytopathogenic fungi and oomycetes due to lytic activity of cell wall degrading enzymes, such as chitinases, proteases or β-1,3-glucanases, has been widely studied. In contrast, the function of β-1,6-glucanases in these processes is not well defined. The role this enzyme plays in the biocontrol activity of *T. virens* was examined by gene expression analyses, and by constructing a series of transformants in which the *T. virens* β-1,6-glucanase (*TV-BGN3*) gene was disrupted, constitutively over-expressed, or co-expressed with the *T. virens* β-1,3-glucanase (*TV-BGN2*). Our results reveal induction of *TV-BGN3* in the presence of fungal cell walls and demonstrate enhanced ability of single or double over-expression transformants to inhibit growth of the plant pathogens *Pythium ultimum*, *Rhizoctonia solani*, and *Sclerotinia minor*. Strains disrupted in *TV-BGN3* displayed reduced capability to inhibit growth of *P. ultimum* and *S. minor* as compared to the wild-type. Plant bioassays revealed enhanced protection of cotton plants against *P. ultimum* with *T. virens* wild-type, over-expression and co-expression strains, and reduced protection with disruption strains. These results clearly indicate involvement of β-1,6-glucanase in the mycoparasitic activity of *T. virens*. Successful overproduction of two genes in *T. virens* afforded new opportunities to investigate construction of *Trichoderma* strains expressing 'multigene' combinations to achieve even greater protection against a broader range of plant pathogens.

VIp-18

The antifungal protein PAF produced by *Penicillium chrysogenum* induces hyperpolarization of the fungal membranes

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The antifungal protein PAF is a small, cysteine-rich protein secreted by the filamentous fungus *Penicillium chrysogenum*. PAF inhibits the growth of various important plant and zoopathogenic filamentous fungi (1). Among numerous physiological detrimental effects and changes in morphology, PAF evokes K^+-efflux in sensitive test organisms, *e.g.* in *Aspergillus nidulans* (2). To investigate whether the observed K^+-efflux is due to an unspecific membrane damage or to the activation of specific K^+ -channels, plasma membrane potential changes were measured in PAF-treated *A. nidulans* hyphae by using the amino-naphtylethenylpyridinium dye di-8-ANEPPS. An unspecific ion-leakage should lead to the depolarization, whereas a specific selective K^+ -permeability would result in the hyperpolarization of the fungal membrane. We detected changes in fluorescence staining and intensities with a laser scanning microscope LSM 510 by recording emissions at 560 nm (F_{560}) and 620 nm (F_{620}). The microscopic images were first selected manually and then the membrane potential changes were monitored by calculating the fluorescence emission ratios (R, $R= F_{620}/F_{560}$) in the first hyphal segments between the hyphal tip and the area next to the first septum (3). By this method, we proved the hyperpolarization of the plasma membrane in PAF-treated *A. nidulans*. The membrane potential in fungi is maintained by active H^+-efflux and it is likely that PAF directly or indirectly interacts with the plasma membrane H^+ pumps or alternatively influences ion effluxes, such as K^+-efflux by activating specific K^+ pumps. This stands in good agreement with the observed increase of extracellular potassium concentration in PAF-treated *A. nidulans*.

(1) Marx (2004), Appl Microbiol Biotechnol 65:133-142, Review.
(2) Kaiserer *et al.* (2003), Arch Microbiol 180:204-210.
(3) Leiter *et al.* (2005), Antimicrob Agents Chemother 49:2445-2453.

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Mannitol metabolism in the wheat pathogen *Stagonospora nodorum*

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We are investigating the role of mannitol metabolism in the necrotrophic wheat pathogen *Stagonospora nodorum*. This ascomycete is the causative agent of stagonospora nodorum blotch of the leaf and glume and is responsible for up to 31% loss of yield in wheat annually. Strains were created harbouring either a disrupted mannitol 1-phosphate dehydrogenase gene (*Mpd1*) or a disrupted mannitol 2-dehydrogenase gene (*Mdh1*). The mutants lacked any detectable activity for the enzyme encoded by the disrupted gene. NMR spectroscopy revealed the *mpd1* strain contained significantly less mannitol and arabitol, but more trehalose than the wild-type strain, when grown on glucose. The phenotype of the *mpd1* strains *in vitro* was identical to that of the wild-type, with the exception that the mutants grew poorly on mannitol as a sole carbon source. This suggests that Mpd1 has a significant role in the catabolism of mannitol. Pathogenicity assays showed that the *mpd1* strains remained pathogenic but were unable to sporulate. The phenotype of the *mdh1* mutants, both *in vitro* and *in planta*, was identical to the wild type, including growth on mannitol. The ability of this strain to utilise mannitol as a sole carbon source questions the proposed mannitol cycle. This implies that either the dephosphorylation of mannitol 1-phosphate to mannitol is reversible, or there is an alternative pathway for mannitol catabolism.

VIp-20

Aspergillus fumigatus sidI encoding an Acyl CoA-ligase, is essential for biosynthesis of extracellular siderophore desferri-triacetylfusarinine C

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Iron is essential for virtually all organisms. Most fungi utilize siderophores for acquisition and storage of iron. The opportunistic pathogen Aspergillus fumigatus produce two major siderophores: it excretes desferri-triacetylfusarinine C (DC-TAFC) to mobilize extracellular iron and accumulates desferri-ferricrocin for intracellular storage of iron. The necessity of the siderophore system for virulence of A. fumigatus in a murine model of invasive aspergillosis has recently been demonstrated, which underscores the importance of the role of iron during infection. In A. fumigatus expression of genes involved in iron homeostasis is generally controlled by SreA, a repressor of genes involved in iron acquisiton. Genome–wide microarray analysis identified several genes displaying this expression profile. To functionally analyze one of these, termed sidI a deletion mutant was generated. The sidI deficient strain showed normal desferri-ferricrocin production but lacked DF-TAFC synthesis. SidI shows similarity to Acyl CoA-ligases suggesting a role in activation of the fatty acid moiety in the biosynthesis of DF-TAFC, which is a cyclic peptide consisting of three N5-cis-anhydromevalonyl-N5-hydroxy-L-ornithine residues linked by ester bonds. In agreement with a role in iron homeostasis, sidI-deletion strains displayed decreased radial growth and conidiation under iron-limiting conditions.

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Cell lipoperoxidation in *Aspergillus ochraceus* is a fundamental step in ochratoxin A biosynthesis

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Aspergillus ochraceus, a widespread fungus which contaminates different food commodities like cereals, vegetables and fruits, is an important ochratoxin A (OTA) producer in warmer climates (Wheele *et al.*, 1991). OTA interferes with some basic metabolic processes as protein synthesis, by the inhibition of phenylalanyl-tRNA synthase, leading to nephropaty in humans (Petkova-Bocharova and Castegnaro, 1998; Stoev, 1998). Several enzymatic activities are involved in the OTA formation, a polyketide synthase for the synthesis of dihydroisocoumarin, a chlorinating enzyme, a methylase, an esterase and a peptide synthetase (Farber and Geisen, 2004). Recently it has been shown that a polyketide synthase gene pks is fundamental in OTA biosynthesis, in fact pks-disrupted *A. ochraceus* mutants are not able to synthesise the toxin (O'Callaghan *et al.*, 2003). In other Aspergillus species (*A. nidulans, A. flavus*), which colonize seeds, lipoperoxides play an important role in the regulation of mycotoxin biosynthesis, sporulation, conidia and sclerotia formation (Wilson *et al.*, 2001; Calvo *et al.*, 2002). The aim of this study is the investigation of the role, if any, of cell lipoperoxidation in the biosynthesis of OTA in Aspergillus ochraceus. As first step a lox-like sequence (DQ087531) was obtained in *A. ochraceus* by the use of degenerated primers designed on the basis of the conserved consensus sequence of fungal and plant lipoxygenase gene sequences present in the gene bank. This fragment share an high homology (47-78%) with *A. nidulans* and plants lipoxygenase (LOX) like genes. The protoplasts of the wild type strain 1035 of *A. ochraceus* was then transformed with pAN7.1::aolox-like, a plasmid obtained by inserting a 700 bp fragment of the aolox-sequence. The knock-out mutant was tested both in in vitro and *in vivo* conditions, by culturing it in Potato Dextrose Broth, Potato Dextrose Agar and on wheat seeds. In these conditions the mutant showed a different colony morphology (delay in conidia formation and increase of sclerotia production), a reduced lipoxygenase activity with subsequent very low formation of lipoperoxide in the mycelium (quantified by LC-MS analysis) and a strong reduction of OTA biosynthesis in comparison with the wild type strain. In conclusion from this study experimental evidences emerge that support a correlation between lipoxygenase activity and OTA biosynthesis in *A. ochraceus* even if the actors that mediate these events remain still unveiled.

VIp-22

Aflatoxin biosynthesis in *Aspergillus parasiticus*: a role for yap-1 like gene

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It has been established that lipoperoxide formation in *Aspergillus parasiticus* promotes aflatoxin biosynthesis in conducive and low-conducive media (Potato Dextrose Broth and Czapek Broth, respectively). In low-conducive medium oxidative stress was induced by addition of cumene hydroperoxide (1mM), a lipoperoxidation inducer. The two regioisomers of linoleic acid hydroperoxides (9-HODE and 13-HODE) were analysed in *A. parasiticus* mycelia grown in both the used media with and without cumene hydroperoxyde addiction, by liquid chromatography-mass spectrometry (LC-MS). The addition to the conducive and low-conducive medium of phenolic antioxidants inhibits aflatoxin biosynthesis of about 80-90% furtherly supporting the role played by lipoperoxides in aflatoxin formation. The activation of some oxidative stress related transcription factors such as *yap1*-like, *skn7*-like and *hsf2*-like appears to drive the correlation between oxidative stress in the mycelia and aflatoxin production in the cells as well as antioxidant enzyme activities superoxide dismutase and glutathione peroxidase. In *Saccharomyces cerevisiae* Yap1 coordinates the response to oxidative stress probably acting as a sensor of oxidation by a cysteine rich domain that readily undergoes oxidation in the presence of lipoperoxides and other reactive oxygen species in fungal cell. The inhibition of aflatoxin biosynthesis that occurs when antioxidants are added to the media could be related to enhancement of the oxidative stress related transcription factors like *yap1*-like that in turn lead to the enhancement of the activity of superoxide dismutase and glutathione peroxidase. *Yap1*-like gene fragment was inactivated in *A. parasiticus* NRRL 2999 by homologous recombination with the plasmid p3SR2 in which was inserted fragments of *yap1*-like sequence. The mutant ApΔyap1-like inoculated in aflatoxin conducive medium advanced and increased aflatoxin biosynthesis in comparison to wild type. This result suggests that the deletion of *yap1*-like alters the defence response of the fungus against oxidative stress settling a cell environment proner to support an earlier toxin formation. In conclusion reactive species as lipoperoxides create a stressing environment that fungal cell tries to control by activating oxidative stress related transcription factors and antioxidant enzymes. In this context *yap1*-like appears to play a modulating role in the metabolic events that lead to aflatoxin biosynthesis.

Identification of the *mstE* gene encoding a glucose inducible, low-affinity glucose transporter in *Aspergillus nidulans*

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The *mstE* gene encoding a low-affinity glucose transporter active during the germination of *Aspergillus nidulans* conidia on glucose medium has been identified. *mstE* expression also occurs in hyphae, is induced in the presence of other repressing carbon sources besides glucose, and is dependent on the function of the transcriptional repressor CreA. The expression of MstE and its subcellular distribution have been studied using a MstE-sGFP fusion protein. Concordant with data on *mstE* expression, MstE-sGFP is synthesised in the presence of repressing carbon sources and fluorescence at the periphery of conidia and hyphae is consistent with MstE location in the plasma membrane. Deletion of *mstE* has no morphologically obvious phenotype but does result in the absence of low-affinity glucose uptake kinetics, the latter being substituted by a high-affinity system.

VIp-24

Accumulation of deoxynivalenol and its 15-acetylated form is significantly modulated by oxidative stress in liquid cultures of *Fusarium graminearum*

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Fusarium graminearum is a fungal pathogen that infects cereals and corn. It is one of the causal agents of the Fusarium Head Blight (FHB) leading to reduced yields and therefore economic losses. Furthermore, some *F. graminearum* strains can produce mycotoxins belonging to the group of type B trichothecenes (TCTB), especially deoxynivalenol (DON) and its acetylated derivatives (3- and 15ADON).

Acute TCTB toxicity is widely demonstrated upon animals and human beings. TCTB are very stable and resist to biodegradation and food processing. No suitable detoxification procedure is currently available. Maximum DON level in cereals and corn food stuffs has been recently settled in Europe (1). New strategies allowing an efficient management of the mycotoxic risk could be implemented thanks to a better understanding of events leading to TCTB accumulation in kernels.

Previous works focused on the effects of *in vitro* substrates and kernels biochemical composition on TCTB yield. *In planta*, the substrate composition may be greatly modified when *Fusarium* invasion occurs, triggering into the host several defence mechanisms. The occurrence of an oxidative burst of H2O2 inside the plant tissues infected by *Fusarium* has been suggested (2). Since many oxidation steps are involved in TCTB biosynthesis, the very strong oxidant characteristic of H2O2 may interfere with the fungus metabolism and modulate TCTB yields. Then, the effect of oxidative stress on toxins production *in vitro* by *Fusarium graminearum* was determined (3).

Liquid cultures were supplemented with H2O2 or other oxidative compounds and trichothecenes accumulation kinetics were followed. At a non lethal concentration, H2O2 treatments modulate toxins accumulation depending on the way of supplementation. When H2O2 is added at the time of inoculation, higher levels of toxins accumulate 30 days later. Conversely, adding H2O2 two or seven days after inoculation has little effects. When H2O2 is daily added, trichothecenes accumulation is rapidly and strongly enhanced. H2O2 regulation of trichothecenes accumulation may be specific since paraquat, another pro-oxidant compound, inhibits trichothecenes production.

Considering H2O2 is a major component of the oxidative burst occurring in pathogen/host interactions, these data support trichothecenes may act as virulence factors for *Fusarium graminearum*.

(1).Règlement (CE) N 856/2005 de la Commission du 6 juin 2005 Vol. L 143: 3-8. 300
(2) Zhou, *et al.*, 2004; 2nd Symposium on Fusarium Head Blight 1: 233
(3) Ponts *et al.* submitted FEMS Microbiology Letters

Expression profiling indicates that CPC1 is mainly involved in primary metabolism in *Fusarium fujikuroi*

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The rice pathogen *Fusarium fujikuroi* produces gibberellins (GAs), a group of economically important phytohormones. The production of GAs and the polyketide pigment bikaverin is subject to nitrogen metabolite repression: The central regulator of nitrogen repression, AREA, had been shown to directly activate the transcription of 6 out of 7 biosynthetic genes under nitrogen limitation conditions. Beside AREA, we are interested in the identification of other key elements of nitrogen regulation affecting GA production in *Fusarium fujikuroi*.

The Cross Pathway Control protein CPC1 in filamentous fungi (GCN4 in yeast) has been shown not only to affect the availability of amino acids, but also secondary metabolism, stress response and pathogenicity. The deletion of the glutamine synthetase gene *glnA* had led to an increased *cpc1* expression in *F. fujikuroi* and an unexpected loss of nitrogen regulated secondary metabolites. One explanation for this down-regulation of secondary metabolism could be that CPC1 is directly or indirectly responsible for the repression of secondary metabolite genes under glutamine starvation as it was proposed for the repression of penicillin biosynthesis in favour of lysine biosynthesis in *A. nidulans*.

However, the deletion of the *cpc1* gene did not restore expression of the gibberellin and bikaverin genes under glutamine starvation conditions, ruling out the possibiliy that CPC1 is responsible for the repression of secondary metabolism. To better understand the biological function of CPC1 in *F. fujikuroi*, macroarray experiments were performed comparing wild type and *cpc1* mutant. A set of CPC1 target genes were confirmed by northern nalysis. Among them are amino acid biosynthetic genes, stress responsive genes, genes of protein catabolism and glycogen metabolism.

VIp-26

Functional analysis of the fumitremorgin gene cluster of *Aspergillus fumigatus*

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Aspergillus fumigatus strains have been reported to produce a variety of secondary metabolites, including toxic prenylated alkaloids such as fumitremorgin C, and many putative secondary metabolic gene clusters have been identified in the genome reference strain Af293 following the completion of the genome sequence. A gene ftmA encoding a dimodular non-ribosomal peptide syntetase (NRPS) was found within one of the putative secondary metabolic gene clusters. ftmA was overexpressed in strain Af293 by insertion of multiple copies, and in the naïve host *Aspergillus nidulans*, which lacks the equivalent gene cluster, under the control of the alcA promoter. Though neither fumitremorgins nor the dipeptide intermediate brevianamide F, cyclo-L-Trp- L-Pro, could be detected in wild-type strains, brevianamide F accumulated in liquid cultures of both species following increased expression of the NRPS gene. The cyclic dipeptide brevianamide F is the precursor of a variety of prenylated alkaloids, including fumitremorgins A, B, C, tryprostatin B and verruculogen. RT-PCR indicated that some of the genes in this cluster are poorly transcribed in Af293. We are currently investigating a collection of *A. fumigatus* strains in order to identify fumitremorgin producers, and to understand the regulation of expression of this secondary metabolic cluster.

Induction and carbon catabolite regulation of a putative lactose permease of *Aspergillus nidulans*

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Lactose metabolism by *Aspergillus nidulans* is essentially intracellular, indicated also by the fact that no extracellular beta-galactosidases have been described from this species. In silico analysis of the *A. nidulans* genome suggests that lactose is transported into the cell by a specific lactose permease with high similarity to the protein from *Kluyveromyces lactis*, thereby inducing the activity of the intracellular beta-galactosidase(s), which then hydrolyses it into glucose and galactose. In the absence of relevant data on fungal lactose permeases on the molecular level, a study was undertaken to clone the gene and investigate its expression. The lapA (lactose permease encoding) gene is not constitutively expressed, as no transcript was formed on any other carbon sources but lactose and D-galactose in the wild-type strain. While expression of lapA in a wild-type *A. nidulans* strain is completely repressed by glucose, this repression does not occur in a CreA-loss of function mutant delta 4. This finding was essentially confirmed by examining the effect of glucose on 14C-lactose transport by whole mycelia of an *A. nidulans* wild-type and a creA-mutant strain. We conclude that the *A. nidulans* lapA gene encoding a lactose-permease is induced by lactose and D-galactose, and repressed by glucose in a CreA-dependent manner.

VIp-28

alcS, an AlcR-responsive gene, and two other ethanol- and ethylacetate-induced genes in *Aspergillus nidulans* encode members of the novel GPR1/FUN34/YaaH membrane protein family

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Aspergillus nidulans, like other filamentous fungi, is able to grow on a very wide range of compounds as alternative source of nutrients. Especially, it can utilize two-carbon compounds, such as ethanol and acetate, as sole carbon sources. The ethanol utilization pathway (*alc* system) of *A. nidulans* requires two structural genes, *alcA* and *aldA*, which encode the two enzymes (alcohol dehydrogenase and aldehyde dehydrogenase, respectively) allowing conversion of ethanol into acetate via acetyldehyde, and a regulatory gene, *alcR*, encoding the pathway-specific autoregulated transcriptional activator. The *alcR* and *alcA* genes map on chromosome VII and are closely linked to three other genes that are positively regulated by AlcR : *alcO*, *alcM* and *alcS*. These three latter genes are dispensable for growth on ethanol, and their function remains unknown. However, their AlcR-responsiveness and their cluster organization with *alcR* and *alcA* suggest that the pathway(s) they are involved in, and the ethanol catabolism are intimately linked. This is reinforced by the fact we have recently identified a *A. fumigatus* gene that likely encodes a functional homologue of *A. nidulans* AlcS (69% identity) and that is clustered with two other genes encoding a putative alcohol dehydrogenase and a putative aldehyde dehydrogenase. We have shown that *alcS* is strictly co-regulated with *alcA*, and encodes a 262-amino acid protein that is located in the plasma membrane. Sequence comparison with protein databases detected a conserved domain that is characteristic of the novel GPR1/FUN34/YaaH membrane protein family including, among others, *Y. lipolytica* Gpr1p and *S. cerevisiae* Ady2p. Although definite biochemical function has not been established for any protein classed in this family, some interesting phenotypic effects, relative to acetate metabolism, have been described in yeasts species for *gpr1* and *ady2* mutants. However, deletion or overexpression of *alcS* did not result in any obvious phenotype. Blast analysis against the *A. nidulans* genome led to the identification of two novel ethanol- and ethylacetate-induced genes encoding other members of the GPR1/FUN34/YaaH family, AN5226 and AN8390. To elucidate the functionality of AlcS and the other GPR1/FUN34/YaaH membrane proteins in *A. nidulans*, the null mutants *delta 5226* and *delta 8390* as well as the double *delta 5226 delta alcS* and *delta 8390 delta alcS* mutants have been constructed and their phenotypic characterisation is now in progress. Moreover, a functionnal complementation analysis of the *S. cerevisiae* null *delta ady2* mutant with *alcS*, *AN5226* and *AN8390* has been undertaken.

Regulation of autolysis in *Aspergillus nidulans*

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Regulation of autolysis was studied in carbon-starved *Aspergillus nidulans* cultures. Autolysis was described as an energy-consuming, well-regulated process, in which cell death was accompanied with a bulk degradation cell wall biopolymers caused by the induction of extracellular hydrolases (*e.g.* chitinases and proteinases), and which resulted in dry cell mass declination, pellet disorganisation and hyphal fragmentation.

The FluG-BrlA pathway – which induces all the sporulation specific genes in *A. nidulans* – proved to be essential in the initialisation of autolysis after glucose depletion. Inactivation of either FluG or BrlA resulted in a non-autolytic phenotype. Glucose repressed autolysis *via* CreA, the main transcriptional regulator of carbon catabolite repression in *A. nidulans*, as demonstrated by the hyperautolytic phenotype of the *creA* null mutant strain. Since glucose and the glucose antimetabolite 2-deoxy-D-glucose repressed autolysis and sporulation even in the *creA* null mutant, the involvement of a CreA-independent glucose repression pathway in the regulation of autolysis was probable. The FadA/FlbA and GanB/RgsA heterotrimeric G-protein mediated pathways had only minor effects on autolysis itself; however, mutations in these signalling pathways affected proteinase production, glutathione degradation and the induction of certain antioxidant enzymes during carbon starvation. Surprisingly, neither the *creA* null mutant with hyperautolytic phenotype nor the loss-of-function *fluG* and *ΔbrlA* mutants with non-autolytic phenotype affected the age-dependent decrease of viability and the development of apoptotic markers after glucose depletion.

To sum it up, cell death and autolysis appeared to be concomitant but independently regulated processes in carbon-starved cultures. The regulation of autolysis was tightly connected to the regulation of sporulation and highly depended on carbon source dependent signalling pathways. We suggest that the main physiological function of autolysis was the liberation of nutrients from dead cells to support conidiogenesis initiated during carbon starvation.

VIp-30

Isolation and characterization of the gene enconding a 2,3-oxidosqualene-lanosterol cyclase type protein from the clavaric acid producer *Hypholoma sublateritium*.

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The *oscl1* gene encoding a oxidosqualene-lanosterol cyclase type protein was cloned and sequenced from the basidiomycete *H. sublateritium*, that produce a steroidal antitumor molecule, clavaric acid. The DNA sequence of this gene has been determined and found to contain an open reading frame of 2190 nt (including stop codon) that encoded a predicted protein of 729 amino acids.The predicted molecular mass of the *H. sublateritium* oxidosqualene-lanosterol cyclase type protein, 83,3 kDa is similar to the molecular masses of the oxidosqualene-lanosterol cyclase from another fungi. At the level of predicted amino acid sequences, the *H. sublateritium* and *Pneumocystis carinii* cyclases share 58 % identity. Tryptophan and tyrosine residues are abundant in the predicted amino acid sequences of oxidosqualene-lanosterol cyclases, leading to a hypothesis that electron-rich side chains from these residues are essential features of cyclase active sites.

For elucidation of the role of the oxidosqualene-lanosterol cyclase gene in cell growth and secondary metabolism, gene disruption and overexpression experiments were done. The *oscl1* disruptant grew as well as the wild-type strain, but the disruption of this gene bloqued the producction of clavaric acid. One additional copy of this gene increased the level of clavaric acid producction two fold. The cloned gene is therefore, involved in the biosynthetic pathway of the clavaric acid molecule.

Cloning and analysis of the IPP (isopentenyl pyrophosphate isomerase)-coding gene of *Mucor circinelloides*

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Mucor circinelloides is a beta-carotene-accumulating zygomycetous fungus, which has largely been used to study the biosynthesis of carotenoids at the molecular level. Carotenoids are long, hydrophobic, and usually coloured molecules, synthesised by a side-route of the general isoprenoid biosynthetic pathway. All isoprenoids are chemical compounds derived from the repeated condensation of only a few simple building blocks, what means that the amount of precursor molecules synthesised in the early isoprenoid biosynthesis can be considered as a limiting step of the final isoprenoid production. Given this fact, we wondered if the production of carotenoids in this fungus could be increased by modifying the biosynthesis pathway. This could be carried out by over-expression of the genes encoding the rate-limiting enzymes of the isoprenoid pathway. Recently, two genes responsible of these early steps were isolated and characterised in *M. circinelloides*: *isoA*, encoding farnesyl pyrophosphate synthase (1) and *carG*, encoding geranylgeranyl pyrophosphate (2).

In this work, we present the cloning and analysis of a *M. circinelloides* gene that encodes the IPP (isopentenyl pyrophosphate isomerase), and takes part as a key-enzyme in the isoprenoid pathway. A functional cDNA clone was isolated by screening a non-homologous expression system in *E. coli* with the *M. circinelloides* cDNA library. The entire gene with the downstream and upstream flanking regions was determined by inverse PCR, sequenced and analysed. Then, different expression vectors, containing the *McIPP1*, *isoA* and *carG* genes were constructed. These vectors were introduced into a double auxotrophic (*leu*-, *ura*-) strain of *M. circinelloides* by PEG-mediated transformations. Co-transformations with the possible vector-pairs were also performed. Carotene production in the transformants and in the recipient strain was measured by high-performance liquid chromatography. In comparison with the original strain, when the transformants contained one of the plasmids with *McIPP1*, *isoA* or *carG*, they produced about 1.5-, 1.4- or 1.7-fold more carotene, respectively. In the co-transformant strains carotene production increased about 2 fold. The applicability of these genes to improve the carotene production in *Mucor* will be discussed.

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VIp-32

Non-ribosomal peptide synthetase genes in the *Omphalotus olearius* genome

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The homobasidiomycete *Omphalotus olearius* produces omphalotins, highly methylated, cyclic dodecapeptides with nematicidal activity which are predicted to be synthesized by non-ribosomal peptide synthases (NRPS). Basidiomycetous fungi are the source of many peptides with broad structural diversity and bioactivity. Contrary to bacteria and ascomycetes, NRPS are hardly studied in basidiomycetes. The known enzymes from ascomycetes have a modular organization, and act to connect amino acids in an assembly-line resembling mechanism (Finking and Marahiel, 2004). In order to identify the putative omphalotin synthetase gene and other peptide synthetase genes in *O. olearius*, random sequencing projects were undertaken. GSTs (genome sequence tags) were obtained from two different genomic libraries. Sequences were also obtained using a fosmid gene bank. Genes identified by BlastX searches were functionally categorized using COGS (Clusters of Orthologous Groups) as defined by NCBI. The use of three different genomic DNA libraries allowed the discovery of many genes putatively involved in the secondary metabolism in *O. olearius*. Analysis of about 2500 GST clones and about 3000 fosmid clones led to the identification of two putative polyketide synthase encoding genes, three putative NRPS genes and one stand-alone adenylation domain. The NRPS genes and their regulation were further analysed. The NRPS gene *fso1* was found to encode a ferrichrome A synthetase which is clustered with two genes essential for siderophore synthesis (Welzel *et al.*, 2005). The Fso1 protein product has been detected in crude extracts from cultures grown under iron depletion and is currently being investigated.

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Biosynthesis and metabolism of zearalenone (ZON)

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The resorcylic acid lacton zearalenone (ZON) is an estrogenically active polyketide mycotoxin produced by some species of the genus *Gibberella*/*Fusarium*. Recently, a gene cluster comprising two polyketide synthase genes (*PKS13*, *PKS4*) and two other genes (*ZEB1*, encoding an oxidase; *ZEB2*, encodig a transcriptional regulator) required for ZON biosynthesis in *Gibberella zeae* has been identified [Kim *et al.*, 2005]. We have additionally identified a 4'-phosphopantetheinyl transferase (*PPT1*) which carries out post-translational modification of the acyl carrier protein domains on the polyketide synthases therefore being essential for their functionality [see poster presented by Peruci *et al.*]. Currently, we are trying to simultanously express all *F. graminearum* cDNAs beeing necessary for ZON-biosynthesis in *Saccharomyces cerevisiae* to test whether they are also sufficient. As a genetic background we use yeast strains which harbour estrogen inducible reporter genes (e.g. *3xERE-URA3*), expressing in *trans* the human estrogen receptor (hER).

Interestingly, ZON which is exogenously added to ZON biosynthesis deficient mutants of *F. graminearum* is rapidly converted to zearalenone-4-O-sulfate which can also be found in unaffected cultures of *F. graminearum* grown on rice. Using the above mentioned yeast bioassay and an *in vitro* assay we could show that zearalenone-4-O-sulfate is no longer estrogenically active. Attempts to clone the according sulfotransferase gene have been initiated, first results will be presented.

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VIp-34

A chitinase gene, *chiB*, plays an important role in the autolytic process in *Aspergillus nidulans*

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Chitin, a homopolymer of beta-1,4-linked *N*-acetyl-D-glucosamine (GlcNAc), is one of the major cell wall components of filamentous fungi. Chitinases (EC 3.2.1.1.14) are enzymes that hydrolyze chitin at beta-1,4-bonds between GlcNAc residues. Chitinases are considered to play roles in the processes that require cell-wall degradation and modification, such as conidia germination, tip growth and branching of hyphae, differentiation of conidia, and autolysis. However, the detailed functions of chitinases in these processes remain unclear. We cloned a gene (*chiB*) encoding a chitinase from *Aspergillus nidulans*. The amino acid sequence of its gene product (ChiB) deduced from 5'- and 3'-RACE analyses suggests that ChiB is a protein of 398 amino acids and does not have a secretory signal sequence at its N-terminus. ChiB expressed in *Escherichia coli* had chitin-hydrolyzing activity, indicating that *chiB* encoded a chitinase. Deletion of *chiB* affected neither germination efficiency nor hyphal growth rate, but considerably reduced the intracellular and extracellular chitinase activities. The decrease in hyphal dry weight during autolytic phase was slower in the mutant than in the wild-type strain. The amount of ChiB significantly increased when the wild-type mycelia were starved for carbon sources, a condition that induced hyphal autolysis. These results suggest that *chiB* plays an important role in the autolytic process in *A. nidulans*.

"PolyPomics": A comprehensive analysis of polyphosphate accumulation in *Saccharomyces cerevisiae*

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Inorganic polyphosphate (poly P) is a linear polymer that consists of chains of ortho-phosphate residues linked by high-energy phosphoanhydride bonds, which has been found ubiquitously in all living organisms and every cell that was studied so far. Poly P serves as a phosphate storage, but is also implicated in gene regulation, the activation of proteins, as an energy storage and in other cellular and biological processes. Despite these important functions and its omnipresence, surprisingly little is known about the synthesis, degradation and functions of poly P. Even in yeast, where poly P can comprise as much as 20% of the dry weight, it is neither known how poly P is synthesized, nor how it is mobilized from the vacuole. In order to identify genes required for the synthesis and accumulation of poly P in yeast, we screened the complete knockout and mutant collections of all non-essential and essential yeast genes for poly P content. Overall, about 8% of all yeast genes were required for normal poly P accumulation. Many of the identified genes necessary for poly P accumulation function in the secretory pathway, in nucleotide, RNA or carbohydrate metabolism, or in signaling and protein synthesis. The data from this poly P screen were compared with published gene expression data and results from protein interaction studies to further characterize poly P synthesis. This analysis suggested a link between ATP and poly P metabolism, which was subsequently confirmed by experiments. From this work it was concluded that poly P accumulation is a highly interconnected process that strongly depends on many pathways from primary metabolism and is a sensitive measure for the metabolic or physiological state of a cell.

VIp-36

Evidence for a phytopathogenic gene in the thermophilic, saprophytic fungus *Talaromyces emersonii*

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Several phytopathogenic fungi produce saponin-detoxifying enzymes that enable them to detoxify anti-fungal saponins present in many plant species, and facilitate successful colonization of such plants by these fungi. To-date saponin-detoxifying enzymes have only been reported in phytopathogens. Studies conducted in our group revealed that *Talaromyces emersonii,* a saprophytic fungal species,could detoxify the saponin avenacin as demonstrated by its ability to grow on agar plates containing avenacin. Furthermore, when the fungus was grown in the presence of oat plants, which produce avenacin to protect against fungal attack, and the culture filtrate was assayed for avenacinase, deglycosylated avenacin was observed on thin layer chromatography. This finding suggested the production of an 'avenacinase' enzymeby*T. emersonii*. The full-length gene encoding a putative avenacinase was cloned from *T. emersonii.* This gene and the deduced protein product exhibit greatest identity with other fungal saponin-hydrolysing enzymes and with GH family 3 b-glucosidases. Expression of this putative avenacinase gene in a heterologous host, such as the yeast *Pichia poastoris*, is underway to elucidate the exact role of this protein. This work presents the first report of an avenacinase gene encoding a protein capable of hydrolyzing avenacin A-1 from a thermophilic, saprophytic fungal source, and suggests that this gene is not exclusive to plant pathogens. We propose that production of saponin-detoxifying enzymes, such as avenacinase, by *T. emersonii,* a fungus whose natural habitats include soil and composting biomass, would allow this organism to utilize saponin-containing plant material very efficiently in its environment as a nutrient source and would enhance this organism's potential as a potent mediator of plant biomass degradation.

Characterization of the sulfur metabolism network in the phytopathogenic fungus *Magnaporthe grisea*

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Knowledge on the molecular mechanisms involved in the fungal-plant interactions is still very limited. In this study, enzymes involved in the sulfur metabolism of the pathogenic fungus M. grisea are studied with regards to their role during infection of host plants. In filamentous fungi, the methionine (Met) / cysteine (Cys) biosynthetic pathways are complex. A preliminary model for the sulfur network in M. grisea, genes and proteins involved in the pathway, arises from the characterization of auxotrophic mutants in Neurospora crassa, Aspergillus nidulans and a systematic analysis of recently sequenced genomes. From sulfate to sulfur amino acids, the pathways consist of features reported in plants and bacteria together with those described in the yeast Saccharomyces cerevisiae.

Our goal, using the rice blast fungus M. grisea as a model, is to identify the genes and proteins involved in the sulfur network and to study their role during development at molecular, biochemical and physiological levels. Studies are conducted on (a), the biosynthesis of cysteine (reverse transsulfuration and the cysteine pathway); (b), the biosynthesis of homocysteine (direct transsulfuration pathway) and finally (c), the synthesis of methionine. Genes of interest are identified and their physiological role analyzed using auxotrophic mutants obtained by gene replacement. For the characterization and analysis of the generated mutants, we develop classical trophic complementation on minimal medium in presence of various sources of sulfur compounds. Secondly, quantitative PCR analysis is performed to follow the expression of the identified sulfur genes in the mutants in comparison to the wild type strain. In parallel, the major sulfur metabolites are analyzed through reversed chromatography using HPLC. All these complementary approaches allow understanding of the complex fungal sulfur pathway.

VIp-38

A glutamate decarboxylase gene from Japanese koji mold, *Aspergillus oryzae*

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Gamma-aminobutylic acid (GABA) is an amino acid that is widely distributed in nature, in animals and plants, and it is particularly known as a major inhibitory neurotransmitter in mammalian brain tissues. GABA-abundant diet components such as tea, germinated brown rice, and beni-koji (solid culture of *Monascus pilosus* grown on steamed rice) are marketed as supplements in Japan. GABA is generated by the decarboxylation of L-glutamate by glutamate decarboxylase (GAD), and GAD-encoding gene from various species, such as *E. coli*, *A. thaliana*, mouse, and human has been already cloned. The Japanese fermented foods produced with rice-koji contain GABA. The GAD of *Aspergilus oryzae* was purified and characterized, and the *A. oryzae* GAD-encoding gene has been cloned (GADA). We cloned a genomic DNA encoding a new glutamate decarboxylase (GAD2) from *Aspergillus oryzae* using information of sequence data from *A. oryzae* genome data base. Nucleotide sequence analysis showed that the cloned gene encoded 508 amino acid residues. The deduced amino acid sequence of *A. oryzae* GAD2 showed a highly degree of similarity with those of *A.oryzae* GADA, *Oryza sativa* GAD, and *Lactococcus* GAD. The expression of GAD genes of *A. oryzae* in solid state and submerged culture was studied.

Functional characterization of genes of the carotenoid oxygenase family in *Fusarium* and *Neurospora*

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Carotenoids are terpenoid pigments synthesized by photosynthetic organisms and many non-photosynthetic microorganisms, including bacteria and fungi. Apocarotenoids are cleavage products generated by enzymes of the carotenoid oxygenase family. They have diverse biological functions, such as vitamins, visual pigments and signaling molecules in animals (retinoids), or hormones, pigments and aroma compounds in plants. The fungi *Fusarium fujikuroi* (*Gibberella fujikuroi*, mating group C) and *Neurospora crassa* accumulate neurosporaxanthin, an apocarotenoid produced through the oxidative break of the precursor torulene. In addition, both fungi contain proteins from the opsin family, which use an apocarotenoid (retinal) as prostetic group. We have identified in the genome of these fungi two genes coding for putative carotenoid oxygenases. The mutation of one of them, *carX* of *Fusarium*, results in a partial derepression of the carotenoid pathway (Thewes *et al.* 2005; MGG 274: 217-228). Here we report on the biochemical characterization of the two genes from *F. fujikuroi*, *carX* and *carT*, and the genetic analysis of one of them from *Neurospora*, homologous to *carT*.

A cDNA version of *carX* was expressed in carotenoid-accumulating *E. coli* strains, and their carotenoid content was analyzed by HPLC and GC-MS. These assays showed retinal formation in *E. coli* cells producing beta-carotene. The retinal product was converted by *E. coli* to retinol and a retinyl ester, most probably retinyl acetate. Similar experiments were done with *carT*, but no apocarotenoid formation from beta-carotene was detected. Both proteins were expressed and purified for further biochemical analysis. In vitro assays showed that CarX cleaved only the all-*trans* isomer of beta-apo-8´-apocarotenal and beta-carotene, indicating stereospecificity for CarX substrate recognition. CarX was found to be also active with other intermediates of the pathway, as gamma-carotene and torulene. These results indicate a role for CarX as the enzyme responsible for retinal synthesis in *Fusarium*.

In vitro, CarT was unable to cut beta-carotene, showed a low activity on lycopene and cut efficiently torulene to produce beta-apo-4´-apocarotenal, the aldehyde version of the final product neurosporaxanthin. Targeted mutants of the *carT Neurospora* counterpart, *cao-2*, obtained by gene replacement with a hygR cassette, accumulate torulene instead of neurosporaxanthin. In addition, three torulene-accumulating mutants, two of *Neurospora* and one of *Fusarium*, contain mutations in their corresponding *carT*/*cao-2* alleles. These results, together with the biochemical analysis of the CarT protein, confirm that this gene codes for the enzyme responsible of the oxidative break of torulene in the arotenoid pathway of these fungi.

VIp-40

Analysis of genes involved in the regulation of carotenoid biosynthesis in *Fusarium*

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The ascomycete *Fusarium fujikuroi* (*Gibberella fujikuroi*, mating group C) accumulates the xanthophyll neurosporaxanthin and other carotenoids by means of the enzymes encoded by the genes *carRA*, *carB*, and *carT*. The first two genes are clustered and coregulated with *carO*, coding for an opsin-like protein, and *carX*, coding for a carotenoid oxygenase. A similar carotenoid pathway exists in *Neurospora*, mediated by the products of the genes *al-1*, *al-2* and *cao-2* (see communication by Prado-Cabrero *et al.*). Transcription of these genes is stimulated by light in both fungi, a response mediated in *Neurospora* by the products of the *wc-1* and *wc-2* genes. To check if the same mechanism is operating in *Fusarium*, the homologous *wc-1* gene from this fungus, called *blrA*, has been cloned. In contrast to *wc-1*, *blrA* mRNA levels are not induced by light. Albino mutants blocked in this transcriptional light induction have been identified. Unexpectedly, they show a semidominant phenotype in forced heterokaryons with the wild type. Experiments to determine the eventual relation of these mutants with *blrA* and the role of this gene in photocarotenogenesis are under way. Mutants of the *carS* gene of *F. fujikuroi* exhibit carotenoid overproduction in the dark. Such regulatory mutants are obtained in different *Fusarium* species but are not found in *Neurospora*, indicating differences in the molecular mechanisms that control their respective carotenoid pathways. The CarS protein is not needed for the light regulation, as judged by the significant *carB* and *carRA* photoinduction still found in *carS* mutants. The *car* genes are derepressed in the *carS* strains in the dark. We postulate that *carS* codes for a transcriptional repressor, able to bind the car promoters. To check this hypothesis, the bidirectional *carRA*/*carX* promoter was chosen for protein binding analysis through electrophoretic mobility shift assays. The wild type extracts contain at least a protein able to bind the upstream DNA sequences for both genes. The shift is not apparent when the same DNA is exposed to extracts from a *carS* mutant, indicating the lack of a functional binding protein. Comparison of the *car* promoters reveals putative conserved regulatory elements whose ability to support protein binding will be analyzed. Complementation analysis between *carS* mutants and albino strains holding deletions of the structural car genes indicate the linkage of *carS* to the *car* cluster. Two candidate linked genes, containing zinc-finger and ring finger motifs, respectively, are under investigation.

The intra- and extracellular proteome of *Aspergillus niger* during growth on different carbon sources

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For a comprehensive understanding of cell physiology, metabolism and growth processes of *Aspergillus niger*, the intracellular and extracellular proteome was analysed during batch cultivation on different carbon sources.

The glucoamylase producing strain *A. niger* AB 1.13 was grown on different carbon sources, namely, the glucoamylase inducing carbon source maltose and the non-inducing carbon source xylose. Changes in cell physiology during cultivation with different carbon sources were followed by monitoring time-dependent changes of the intra- and extracellular proteome using two-dimensional gel electrophoresis. The protein patterns were compared using ProteomWeaver™ software. About 200 proteins were analysed using mass spectrometry and identified by *Aspergillus niger* gene database search. Identified proteins include proteins in central metabolic functions, secretion, and stress management.

This work was supported by the SFB 578 and DSM provides access to the Aspergillus niger *database.*

VIp-42

The D-xylose reductase of *Hypocrea jecorina* is a general aldose reductase involved in cellulase induction on lactose

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The *Hypocrea jecorina* (anamorph: *Trichoderma reesei*) D-xylose reductase encoding gene (*xyl1*) was cloned and characterized. The corresponding protein Xyl1 is a member of the aldo/ketoreductases superfamily and shows high similarity (up to 60%) to its orthologues in *Neurospora crassa* and *Aspergillus niger*. The *xyl1* transcript was most abundant during growth on D-xylose, xylan, L-arabinose and L-arabinitol but low levels were also found on lactose and D-galactose. The recombinantly expressed enzyme catalyzes the reduction of D-xylose, L-arabinose, D-ribose, D-galactose and D-glucose with NADPH as cofactor. Deletion of the *xyl1* gene strongly reduced growth on D-xylose and L-arabinose but the remaining growth and residual aldose reductase activity indicate the presence of at least one other enzyme with similar substrate specificities. In addition, delta-*xyl1* strains also show slower growth on D-galactose and reduced D-galactose reductase activity. Strains deleted in *xyl1* and in the *gal1* gene – which encodes galactokinase, the first enzyme in the Leloir pathway of D-galactose catabolism – are severely impaired in growth on D-galactose. Deletion of *xyl1* also resulted in a strong reduction of cellulase transcript levels during growth on the disaccharide lactose (1,4-O-ß-D-galactopyranosyl-D-glucose). These findings indicate that the *H. jecorina* D-xylose reductase is involved in the reductive catabolism of a number of aldoses and that the reductive and the Leloir pathway operate in parallel during normal growth on D-galactose. The operation of both pathways is necessary for high level induction of cellulases on lactose.

Pathway crosstalk of heme biosynthetic pathway with glycolytic pathway and TCA cycle in the lignin-degrading basidiomycete *Phanerochaete chrysosporium*

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A functional proteomic analysis for surveying heme-binding proteins in the white-rot basidiomycete *Phanerochaete chrysosporium* was performed utilizing biotinylated heme-streptavidin beads system. Citrate synthase (PcCS) in the TCA cycle was identified as a heme-binding protein. Kinetic and spectroscopic studies revealed that PcCS was effectively inhibited by the heme with physiological concentrations. In our previous proteomic studies, the metabolic flux shift was found to occur in the TCA cycle at isocitrate against exogenous addition of aromatic compounds. Under nutrient sufficient conditions, *P. chrysosporium* utilizes a so-called short-cut TCA cycle where isocitrate is directly converted to succinate; thus, 2-oxoglutarate and succinyl-CoA are skipped. However, the addition of aromatic compounds caused an immediate metabolic shift into the TCA cycle, which in turn resulted in the activation of the heme biosynthetic pathway branched at succinyl-CoA. *P. chrysosporium* is known to produce a large amount of heme-peroxidases and a large number of cytochrome P450s for aromatic degradation. On the other hand, a free heme is highly toxic to the cell. Thus, overproduction of heme molecules should be avoided. From this point of view, the inhibition of PcCS by the heme may be a physiological event to regulate heme biosynthesis. Furthermore, glyceraldehyde 3-phosphate dehydrogenase (PcGAPDH) in the glycolysis pathway was also identified as a heme-binding protein. Inhibition of this enzyme by the heme may cause the change in the carbon flux into the pentose-phosphate cycle for NADPH production, which seems to be important for a cellular redox balance. Finally, 5-aminolevulinate synthase, an initial and rate-determining enzyme of the heme biosynthetic pathway, was found to be inhibited by the heme. These data indicated the presence of a novel pathway crosstalk of the heme biosynthetic pathway with the glycolysis pathway and TCA cycle in *P. chrysosporium*. This unique crosstalk seems to play an important role in activating and optimizing aromatic degradation system.

VIp-44

Isocitrate lyase is required for growth in acetate in *Aspergillus fumigatus*

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Isocitrate lyase (icl) and malate synthase (mas) are the principal enzymes of the glyoxylate cycle, which allows some microorganisms to grow using two-carbon compounds like acetate or ethanol as sole carbon sources. This mechanism is important for survival in nutritionally poor environments, and has been linked to pathogenesis in a number of different microorganisms that survive phagocytosis by macrophages during the process of infection. For example, it has been reported that Candida albicans induces the expression of genes related to the glyoxylate cycle when internalised by macrophages. In a disseminated murine infection model, a strain lacking the icl1 gene was less virulent than the wild type strain (Lorenz, 2002). Aspergillus fumigatus conidia are also internalised by macrophages and lung epithelial cells, and survive to produce life-threatening infections in immunocompromised patients (Latge, 2001). Therefore the glyoxylate cycle may be involved in facilitating the fungus in surviving phagocytosis by macrophages.

In this study we report on the expression of the icl and mas genes in A. fumigatus, which occur only under conditions of glucose starvation. In addition we created an Δicl mutant in A. fumigatus AF293, and monitored growth on AMM containing glucose, ethanol or acetate. Growth in these carbon sources was evaluated in liquid cultures and plates. The Δicl mutant was unable to grow on acetate or ethanol, but retained its ability to grow on glucose.

These results suggest that the glyoxylate cycle and its two main enzymes are the only mechanism by which A. fumigatus can obtain energy from two-carbon sources. This is the first report of the molecular characterisation of genes involved in the glyoxylate cycle in A. fumigatus.

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Schizophyllum commune in wood colonization and degradation

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Schizophyllum commune is frequently found in nature on decaying dead wood and occasionally also on living trees. This basidiomycete is believed to be a white-rot fungus. In laboratory tests in pure cultures however, the fungus shows only poor ability of wood degradation. Wildtype strains and mutants of the hydrophobin *Sc3* are able to colonize wood. Mass losses in beech wood of up to 10 % were observed after six month of incubation compared to 85-90 % mass loss caused by *Pleurotus ostreatus*. Fourier transform infrared (FTIR) microcopy was used to localize *S. commune* wildtype strains and the *Sc3* mutants inside the wood. Fungal mycelium growing on wood distinguishes from mycelium within wood vessels. On wood, dikaryons distinguish from monokaryons and *Sc3* mutants from wildtype strains. Within wood, the mycelium of the different fungal strains were not discriminated.

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VIp-46

Saccharomyces cerevisiae genes reacting on volatile organic compounds from wood related sources

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Saccharomyces cerevisiae reacts on unfavorable stress conditions such as presence of toxic compounds by altering expression patterns of genes. Stress responding and detoxifying genes are overexpressed and other genes are down-regulated. In this study, yeast cells were exposed to volatile organic compounds (VOCs) from fresh pine wood, to α-pinene as a VOC emitted from wood and to formaldehyde used in wood composite production. RNA was isolated, cDNA produced and hybridized to micro-slides carrying 6240 double-spotted genes of yeast. The responses of the yeast genome differed in the three treatments. For example, treatment with formaldehyde increased transcription of genes for ribosomal proteins whereas genes for energy metabolism were down-regulated. Promoters of induced genes were fused to the *lacZ* reporter gene in vector YEp356R and tested for reactions upon exposure of volatiles of transformed yeast cells.

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Localization and quantification of inorganic polyphosphate in fungal cell walls

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Inorganic polyphosphate (poly P) consists of linear chains of phosphate residues linked by high energy phosphoanhydride bounds. Poly P is an ubiquitous polymer that appears to be involved in many different cellular processes. It is for example involved in phosphate storage, energy metabolism and stress response or the regulation of enzyme activities.

Poly P has long been described as a counter-anion to the positively charged chitosan in the cell wall of Zygomycota. However, until now it has not been possible to stain or quantify poly P in fungal cell walls specifically. Here we describe specific biochemical and microscopical methods to localize and quantify poly P in fungal cell walls. Poly P was released from the cell wall by a gentle, high salt extraction, without disrupting cells (which was confirmed by measuring the ATP content of the extracts). Following, the poly P was purified and quantified. For the microscopical localization of poly P we used specific poly P binding proteins, for example the ATPase domain of the Lon protease and the C-terminus of an exopolyphosphatase, which were detected with a binary fluorescence labelled antibody system.

The biochemical experiments showed high contents of poly P in the cell wall of Mucorales (the major group within the Zygomycota), which were typically around 0.1% of the dry weight. In contrast, only low amounts or no poly P could be measured in cell walls of various Asco- and Basidiomycota, although some species contained high levels of intracellular poly P. The high poly P contents of zygomycotan cell walls suggest a function as a phosphate storage. But it remains to be shown, if the cell wall poly P can be remobilized under conditions of phosphate deprivation, for example by the secretion of polyphosphatases.

Staining of Mucorales with poly P binding proteins resulted in a strong fluorescence of the cell wall. Competition experiments with an excess of poly P or other poly P binding proteins indicated specificity of this staining method. The high sensitivity of this method was demonstrated by staining of Mucorales grown under low phosphate conditions, which contained only minute amounts of poly P. The specificity of poly P binding proteins and the high sensitivity of immunofluorescence allowed for the first time the direct visualization of poly P in fungal cell walls and will serve as a useful tool for the study of this poorly understood polymer in fungi.

VIp-48

Sequential deletion of the *Hypocrea jecorina* glucokinase and hexokinase genes

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In *Hypocrea jecorina* (anamorph: *Trichoderma reesei*) multiple gene deletions are limited by the number of readily available selection markers. We have therefore constructed a blaster cassette which enables successive gene knock-outs in *H. jecorina*. This 3.5 kb *pyr4* blaster cassette contains the *H. jecorina pyr4* marker gene encoding orotidine-5'-monophosphate (OMP) decarboxylase flanked by two direct repeats of the *Streptoalloteichus hindustanus* bleomycin gene (Sh ble), which facilitate the excision of the blaster cassette by homologous recombination after each round of deletion. Functionality of this *pyr4* blaster cassette was demonstrated by deletion of the *glk1* encoding glucokinase and *hxk1* encoding hexokinase from a *pyr4* negative *H. jecorina* strain. For excision of the *pyr4* blaster cassettes, Δ*glk1* strains were selected for growth in the presence of 5-fluoroorotic acid. Recombination between the two *Sh ble* elements resulted in uridine auxotrophic strains which retained their respective glucokinase negative phenotype. Subsequent transformation of one of these auxotrophic Δ*glk1* strains with the hexokinase blaster cassette resulted in *pyr4* prototrophic strains deleted in both *glk1* and *hxk1*. We have now started to analyze the role of these two genes in carbon metabolism and signalling of carbon catbolite repression. Initial experiments showed that Δ*glk1* strains showed reduced growth on d-glucose and d-fructose whereas Δ*hxkl* strains showed reduced compact growth on d-glucose but were unable to grow on d-fructose as carbon source. The Δ*glk1*Δ*hxk1* strain was completely unable to grow on either d-glucose or d-fructose. Furthermore Hxk1 is also essential for the catabolism of different other sugars and polyols including the degradation of d-mannitol, an important carbon reserve compound of the fungal spore, d-sorbose, d-sorbitol and d-galacitol, which supports the role of Hxk1 in a second pathway for d-galactose metabolism. The role of both kinases in carbon catabolite signalling is currently under investigation.

Functional and structural studies of the nitrate transporter

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The result of various genome projects indicated that up to 30% of proteins encoded by eukaryotic cells including fungi are membrane proteins. Although several major facilitator superfamily (MFS) protein structure has been determined the structure and function of the subfamily high-affinity nitrate permeases such as NrtA and the functional role of the amino acids in this protein responsible for nitrate transport are not fully understood. The lower eukaryote *Aspergillus nidulans* NrtA protein has a secondary structure consisting of 12 hydrophobic transmembrane domains (TM). This transport membrane protein has 507 residues and belongs to the NRT2 family, one of the 17 members of the MFS. DNA sequences for *nrtA* homologues from other organisms facilitate the comparison between proteins consequently assisting the identification of certain conserved amino acids for mutational studies. Previous work has shown that nitrate provides a major nitrogen source for the growth and yield of microorganisms and plants. The main objective of this study is to reach a better understanding of the function and the structure of NrtA by changing potentially interesting amino acids which may play a critical role in nitrate transport using oligonucleotide mediated site-directed mutagenesis. Amino acids were selected and prioritized for mutational studies on the bases of their properties and conservation across 118 homologues. In this present study 52 NrtA amino acids, were altered, these mutants were phenotypically analysed. Certain mutants did not grow well in nitrate indicating that such amino acids replacements were not tolerated and replacement resulted in loss of nitrate activity. In contrast, certain mutants did grow well in nitrate, clearly tolerating the replacements indicating that such replacement did not markedly affect the nitrate transport through the membrane. The characteristic, enzymology and significance of such residues changes will be presented.