POSTER

Vp-1

THE ARABINOLYTIC PATHWAY IN TRICHODERMA REESEI

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One of the most widespread pentose sugars found in plant biomass is L-arabinose, a constituent of plant cell-wall polysaccharides L-arabinan, arabinogalactans and arabinoxylans. The pentose catabolic pathways are relevant for microorganisms living on decaying plant material but also in biotechnology when cheap raw materials such as plant hydrolysates are used as a carbon source. Genes coding for both extracellular and intracellular enzymes of the arabinolytic pathway have been cloned from the industrially important fungusTrichoderma reesei. The fungal intracellular L-arabinose pathway consists of five enzymes, aldose reductase, L-arabinitol 4-dehydrogenase, L-xylulose reductase, xylitol dehydrogenase and xylulokinase. All the genes coding for the enzymes of this pathway have been cloned in our laboratory. These genes have been utilized to construct an arabinose-utilizing S. cerevisiae strain. We have investigated the expression of the extracellular arabinofuranosidase that cleaves arabinose form arabinoxylans and intracellular arabinolytic genes in T. reesei cells grown on various carbon sources and the regulation of these genes with respect to the carbon catabolite repressor CREI.

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FUSARIUM OXYSPORUM FERMENTATION: SWEET & SOUR ETHANOL

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Fungi are the best known sources of ethanol and many organic acids. While a number of filamentous fungi are known to produce ethanol, Fusarium oxysporum has been the subject of the most intensive research. The interest in filamentous fungi as ethanol producers stems from their expression of the suite of hydrolytic enzymes necessary to degrade complex biomass to monosaccharides, in addition to possessing the primary metabolic pathway for ethanol production. Ethanol and acetate have been cited as products of F. oxysporum fermentations but citric acid cycle intermediates have not. We report that F. oxysporum produces both ethanol and a mixture of C-4 dicarboxylic acids indicative of the functioning of the citric acid cycle in the reverse, or reductive, direction terminating at succinic acid.

MOLECULAR CHARACTERISATION OF A SULPHATE TRANSPORTER IN THE ECTOMYCORRHIZAL FUNGUS TUBER BORCHII VITTAD.

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The filamentous fungi posses a global regulatory circuit that controls the expression of permeases and enzymes that function both in the acquisition of sulphur from the environment and in its assimilation. Sulphate uptake is generally an important point of regulation of sulphur metabolism and appears to occur in fungi, plants, and mammals via a family of related transport proteins.
A Tuber borchii cDNA fragment, showing homology with fungal and plant sulphate transporter has been identified by mRNA Differential Display.
The corresponding full-length cDNA (Sultb1) has been obtained by the screening of a 30-day-old mycelium cDNA library.
This clone contains an insert of 2497 bp in length and encodes a putative 715 amino acid polypeptide. Sequence motifs within the deduced amino acid sequence of this cDNA show homology with conserved areas of sulphate transport proteins from other organisms.
Sequence analysis predicts the position of 8 putative membrane spanning domains.
Southern blot analysis has shown that Sultb1 is a single copy gene in the Tuber genome. Real-time PCR experiments have shown that the sulphate transporter gene is more expressed in T. borchii ripe fruit body respect to the unripe one. Moreover no sulphur containing volatile compounds have been identified by solid-phase microextraction gas chromatography-ion trap mass spectrometry (SPME-GC/ITMS) in completely immature fruit bodies, while several sulphur compounds has been detected in mature one. These data suggest that the increase of the sulphur uptake is probably necessary to produce this class of compounds that represent the major flavouring element primarily involved in truffle aroma.

SORBIC ACID INHIBITS CONIDIAL GERMINATION AND MYCELIAL GROWTH OF ASPERGILLUS NIGER THROUGH INTRACELLULAR ACIDIFICATION

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Growth of the filamentous fungus, Aspergillus niger, a common food spoilage organism, is inhibited by the weak acid preservative, sorbic acid. Here we report that A. niger can degrade sorbic acid and this has consequences for food preservation strategies. Conidia of A. niger inoculated at 10^5/ml medium (pH 4.0) had a minimum inhibitory concentration (MIC) of sorbic acid of 4.5 mM, whereas mycelia from the same spore inocula at 24 h had a three-fold lower MIC. The MIC for conidia and, to a lesser extent, mycelia correlated directly with inoculum size. The mechanism of action of sorbic acid was investigated using ^{31}P NMR. A rapid decline in cytosolic pH (pH_{cyt}) by more than 1 pH unit and a decrease of vacuolar pH (pH_{vac}) in A. niger occurred with sorbic acid. The pH-gradient over the vacuole completely collapsed as a result of this decline in pH_{cyt}. NMR spectra revealed that sorbic acid (3.0 mM at pH 4.0) caused intracellular ATP pools and sugar-phosphomonoesters and -phosphodiesters of A. niger mycelia to decrease dramatically and irreversibly. The disruption of pH homeostasis by sorbic acid at concentrations below the MIC could account for the delay in spore germination and retardation in the onset of subsequent mycelial growth. These studies form a baseline for understanding the action of sorbic acid on A. niger and future work will focus on the underlying molecular mechanisms involved.

Vp-5

FUNCTIONAL ANALYSIS OF MUTATIONS IN THE HUMAN CARNITINE/ACYLCARNITINE CARRIER IN *Aspergillus nidulans*

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Deficiency of the carnitine/acylcarnitine translocase (CACT), the most severe disorder of fatty acid Ǝ-oxidation, is usually lethal in both humans and animals, precluding the development of animal models of the disease. By contrast, CACT deficiency in *Aspergillus nidulans* is conditionally lethal, since loss-of-function mutations in the *acuH* gene, encoding the fungal CACT, allow growth on alternative carbon sources to fatty acids, such as carbohydrates or amino acids.

In this work we have developed a fungal model for the human CACT deficiency based on the ability of the human CACT to fully complement, when expressed at physiological levels, the growth defect of an *A. nidulans*)*acuH* strain on acetate and long-chain fatty acids.

By using in vitro assays and growth analyses by microcalorimetry we performed the functional characterisation of the following human CACT mutations: P30L, P30F, G81R and D32E. Data obtained indicate that P30 is essential for CACT function. The G81R substitution leads to complete loss of CACT function matching its associated lethal phenotype. Finally, we prognosticate for the conserved substitution D32E a mild presentation of the disease.

Results obtained demonstrate the usefulness of this fungal model for evaluating the biochemical phenotype of human CACT mutations and prognosticating their clinical phenotype, being able to distinguish potentially pathogenic human CACT missense mutations from neutral, single residue substitutions causing polymorphism (Perez et al. 2003).

Reference:
- Pérez P, Martinez O, Romero B, Olivas I, Pedregosa AM, Palmieri F, Laborda F, De Lucas JR (2003). Fungal Genetics & Biology 39: 211-220.

Vp-6

ISOLATION OF ACONITASE ACOA INVOLVED IN PROPIONATE METABOLISM IN ASPERGILLUS NIDULANS

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Session 5. Primary metabolism and transporters

Propionate is used as an antifungal agent to prevent spoilage of grain, corn and bread. However, many fungi can grow on propionate as sole carbon and energy source by use of the methylcitrate cycle. This cycle is known from bacteria (1) and fungi (2,3).
We isolated an enzyme, which catalyzes the reaction from (Z)-2-methylaconitate to (2R,3S)-2-methylisocitrate. The specific activity of the purified enzyme was 33.6 U/mg with Z-(2)-methylaconitate as substrate in a coupled assay with methylisocitrate lyase and lactate dehydrogenase. Analysis of the petide masses of this enzyme via MALDI-TOF revealed the pattern of aconitase (AcoA), which is supposed to be active in the citric acid and glyoxylate cycle. Interestingly, the isolated enzyme displayed a comparable lower activity with citrate and cis-aconitic acid compared to (Z)-2-methylaconitate (ratio: 1: 2.4: 14, respectively). This is in contrast to mamalian aconitases, e.g. from bovine heart where the ratio was 1: 2.4: 0.3 (4)
We further investigated enzymatic activities in crude extracts under different growth conditions in order to compare the activities to mRNA levels by northern blot analysis. On propionate as sole carbon source we observed the highest activities with the substrates cis-aconitate and (Z)-2-methylaconitate in a ratio of 3:1. However, samples of the acetate grown cells showed almost the same ratios, but the overall activities were around 30 % decreased.
Northern Blot experiments revealed mRNA levels of acoA, which were consistent to the enzymatic activities. The signals were around 25 % less intense on acetate compared to propionate. That implies that the aconitase Acoa is induced stronger during growth on propionate. Further experiments will have to rule out, whether this is due to a specific induction mediated by propionate.

1) Textor, S., Wendisch, V.F., De Graaf, A.A., Muller, U., Linder, M.I., Linder, D., Buckel, W. 1997. Arch. Microbiol. 168: 428-436
2) Tabuchi, T. Aoki, H., Uchiyama, H., Nakahara T. 1981. Agric. Biol. Chem., 45, 2823-2829
3) Brock, M., Fischer, R., Linder, D., Buckel, W. 2000. Mol. Microbiol. 35, 961-973
4) Beach, R. L., Aogaichi, T., Plaut, G. W. E. 1976. J. Biol. Chem. 252, 2702-2709

IDENTIFICATION AND TRANSCRIPTIONAL REGULATION OF NEW ENZYMES IN THE ASPERGULLUS NIGER GENOME ACTING ON PLANT STORAGE POLYSACCHARIDES STARCH AND INULIN

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Aspergillus niger is well known for its ability to secrete a wide variety of plant Carbohydrate Modifying Enzymes (CMEs). The secreted CMEs are able to degrade the plant cell wall materials as well as its storage carbohydrates, like starch and inulin. Based on its recently finished genomic sequence by Dutch life sciences company DSM, it is estimated that only a fraction of the potential of enzymes produced by A. niger is currently exploited.

Database mining of the A.niger genome resulted in the identification of ten new enzymes, which contain motifs indicating that they might act on starch. In addition to the known starch degrading enzymes of A. niger, glucoamylase (glaA), two alpha-amylase (amyA and amyB), an acid amylase (aamA) and an alpha-glucosidase (aglU), six putative amylases, three putative alpha-glucosidases and one alpha1,4-glucan branching enzyme were identified. It is well established that the expression of starch modifying enzymes is coordinately regulated at the transcriptional level by a pathway specific transcription factor AmyR. AmyR binds to sequences ($CGGN_8CGG$ or CGGAAATTAA) in the promoter region of amylolytic genes. Inspection of the promoter regions of the newly identified amylolytic genes revealed that only three of them contain AmyR consensus binding sites. Whether the expression of the newly identified genes is regulated in response to the presence of starch and whether their regulation is mediated by amyR is currently examined.

The genome of A. niger was also searched for enzymes acting on inulin or inulin derivatives. In addition to the three known inulin acting enzymes, endo-inulinase (inuA) and exo-inulinase (inuX) or sucrose degrading enzymes, invertase (sucA), three new enzymes were discovered. Two genes show sequence homology to the A. niger SucA protein. The third newly identified enzyme showed the highest homology to fungal exo-inulinases. The transcriptional regulation of inulin degrading enzymes is currently investigated.

THE ROLE OF THE CrmA EXPORTIN IN NUCLEAR EXPORT OF THE TRANSCRIPTIONAL ACTIVATOR AreA IN ASPERGILLUS NIDULANS.

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The areA gene encodes a GATA DNA-binding zinc finger transcriptional activator of many genes required for nitrogen catabolism. AreA levels and activity are controlled by autogenous regulation, by differential mRNA turnover and by interaction with the NmrA and TamA proteins. We have shown that an epitope-tagged AreA protein hyperaccumulates in the nucleus under nitrogen starvation conditions. This correlates with a significant elevation of nitrogen catabolic gene expression. Transfer from nitrogen starvation to media containing a nitrogen source triggers rapid exit of AreA from the nucleus, consistent with the lower transcriptional activity of AreA observed during nitrogen sufficiency and limitation compared with nitrogen starvation. We have shown that AreA nuclear hyperaccumulation and nuclear exit are nmrA-independent and do not require residues 60-423 or 844-876 of AreA.
The AreA protein contains a putative binding motif (residues 703-712) for CrmA, the homologue of the *Schizosaccharomyces pombe* CRM1 exportin. CRM1 can be inhibited by treatment with the drug Leptomycin B (LMB), which acts via interaction with a specific cysteine residue. A. nidulans CrmA cannot be inhibited by LMB as the corresponding residue is a threonine. We isolated the crmA gene and used in vitro mutagenesis to introduce the T525C mutation. This mutation was introduced into the A. nidulans genome by gene replacement and shown to confer LMB sensitivity. We are using the $crmA^{T525C}$ mutant and LMB to determine whether nuclear export of AreA is crmA-dependent.

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A GENE SuX(pro) INVOLVED IN REGULATION OF ARGININE AND PROLINE METABOLISM IN ASPERGILLUS NIDULANS, CONTAINS RNA RECOGNITION MOTIFS.

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Regulation of gene expression in Eukaryotes often occurs at the posttranscriptional level. Little is known about the regulation at this level in filamentous fungi. Using transposon mutagenesis to search for new genes involved in regulation of arginine and proline metabolism in Aspergillus nidulans, we identified a suX(pro) gene. Mutation in this gene results in a suppression of proline auxotrophy in proA$^-$ and proB$^-$ mutants. The putative product of the suX(pro) gene is a protein of 259 amino acids residues. This protein comprises two conservative RRM domains (RNA recognition motif), each of them contains two RNP-SC regions (ribonucleoprotein consensus sequence). Proteins comprising RRM domains play a crucial role in posttranscriptional regulation of gene expression at the level of poliadenylation, splicing, transport of mRNAs from nucleus to cytoplasm and in mRNAs stabilization. It is possible that suX(pro) gene participates in regulation of arginine catabolism genes (agaA and otaA). Some data indicate that these genes are regulated at the posttranscriptional level. Enzymatic characterization of suX(pro) mutant was performed. We found no difference in activity levels of arginine catabolism enzymes. We cloned cDNA of suX(pro) gene. Transcriptional and functional analysis of the suX(pro) gene is in progress.

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INTRACELLULAR LOCATION OF ENZYMES INVOLVED IN ALIPHATIC HYDROCARBON BIODEGRADATION PATHWAY

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The principal aim of our investigation is the study of oxidoreductases involved in the first and second steps of hydrocarbon biodegradation pathway using as a model to work a native strain of a filamentous fungus isolated from petroleum contaminated soils named YR-1 strain.
In literature had been reported that in the oxidation pathway of aliphatic hydrocarbons, the first enzymatic step is catalyzed by a monooxygenase enzyme that incorporates one oxygen atom into the aliphatic molecule to form the corresponding alcohol. The second step is catalyzed by an alcohol oxidase to form the fat-alcohol derivate. In the present work we study the intracellular localization of one of this interesting enzymes the alcohol oxidase. In previous work we were capable to make the purification and characterization of this enzyme and it looks like a very different alcohol oxidase from the bulk of methanol-oxidases described and all of them had been localized in peroxisomes. This is very interesting for us because in the case of the filamentous fungi *Penicillium simplicissimum*, has been founded an enzymatic activity called vanyllil alcohol oxidase who's participating in the biodegradation of phenolic compounds and is quit different from methanol oxidases, and interestingly it is localized both peroxisomes and cytosolic fraction.
Strain YR-1 is capable to grow in different hydrocarbons used as sole carbon source and in its free cell extracts is possible to detect alcohol oxidase activity but we don't know intracellular location of the enzyme. There are a few reports about hydrocarbon biodegradation pathways in filamentous fungi, and we consider that is very important to know the intracellular location of these enzymes and the possible existence of a particular organelle that contain the enzymatic activities for the hydrocarbon biodegradation.
YR-1 strain was grown in two different hydrocarbons used each as sole carbon source: Decane 0.5%, hexadecane 0.5% and in glucose 1%. After cells grow, cell free extracts of mycelium from each carbon source were submitted to high speed centrifugation using 10-60% continuous sucrose isopicnic gradients in a VTi50 rotor. In the extract from decane we detect the enzymes with alcohol oxidase activity in vesicles of 1.26 g/ml density. Meanwhile in the extract of mycelium grown in hexadecane, the enzymes with AO activity were detected in vesicles of 1.15g/ml density, we use the peroxidase activity as a peroxisome marker, and it was detected in vesicles of 1.17g/ml density. In the extract of mycelium grown in glucose, neither AO activity could be detected. In all cases alcohol Dehydrogenase activity was used as cytosolic marker showing a density of 1.06-1.07g/ml.

A REDUCTIVE PATHWAY OF D-GALACTOSE CATABOLISM IN *ASPERGILLUS NIDULANS*

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Galactose catabolism in yeast proceeds via the Leloir-pathway, that involves phosphorylation by galactokinase (encoded by *galE*). While yeast *galE* mutants are unable to use D-galactose as a carbon source, *galE* mutants of the filamentous fungus *Aspergillus nidulans* could grow on D-galactose in the presence of ammonium – but not nitrate ions – as nitrogen source. Mycelia of the wild-type *A. nidulans* accumulated intracellular galactitol (50 mM), whereas the *galE* mutant accumulated a 10-fold higher concentration. Unlike an *A. nidulans* mutant in L-arabitol-dehydrogenase, the accumulated galactitol was catabolized lateron in both the wild-type and *galE* strains. Further, an *A. nidulans* mutant in hexokinase (*frA2*) was unable to grow on galactitol, and a *galE / frA2* double mutant was unable to grow on either galactose or galactitol. Mycelia of *A. nidulans frA2* accumulated intracellular L-sorbose on galactitol, indicating it as an end-product of galactitol oxidation. Both the *frA2* and the *galE / frA2* mutants were unable to grow on L-sorbose, indicating that its catabolism involves phosphorylation by the hexokinase. The results therefore provide evidence for a second pathway of D-galactose catabolism in fungi, which involves reduction of the galactose into galactitol, NAD^+-dependent oxidation by an arabitol dehydrogenase to L-sorbose and phosphorylation by hexokinase.

GENETIC MODIFICATION OF CARBON CATABOLITE REPRESSION IN THE FILAMENTOUS FUNGUS TRICHODERMA REESEI FOR IMPROVED PROTEIN PRODUCTION

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The aim of this work was to genetically modify the carbon catabolite repression of Trichoderma reesei to obtain mutant strains derepressed in cellulase production. Several genes coding for regulators of cellulase expression have been isolated and characterized from T. reesei including the cre1 gene mediating the carbon catabolite repression in the presence of glucose. Glucose repression has been shown to occur upon binding of CREI protein to specific sequences in the promoter of the major cellulase gene cbh1. CREI target sequences have also been identified in the promoter regions of other cellulase and hemicellulase genes such as e.g. cbh2 and xyn1.
The CREI protein of T. reesei is similar to many other fungal proteins mediating glucose repression. It was recently shown, however, that a truncated form of CREI (cre1-1) present in the hypercellulolytic T. reesei strain Rut-C30 is responsible for derepression of (hemi)cellulase gene expression on glucose-containing media.
Therefore, to study the effect of cre1 on cellulase and hemicellulase expression, the wild type cre1 gene present in the T. reesei strain QM6a was either replaced by the “Rut-C30 -type” truncated cre1-1 gene or completely removed. Bioreactor cultivations on lactose and glucose media were carried out with these cre1-1 and Δcre1 mutant strains and analysed for cellulase and hemicellulase production. Northern analysis indicated remarkably higher expression levels of several (hemi)cellulase genes in both mutant strains on lactose when compared to the non-modified parent strain. In accordance, 20-fold higher cellobiohydrolase and endoglucanase activities and almost 10-fold higher xylanase activities were detected in the lactose culture medium of mutant strains.

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L-ARABINITOL DEHYDROGENASES OF *HYPOCREA JECORINA* AND *ASPERGILLUS NIDULANS*

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L-arabinitol dehydrogenase has been postulated to be involved in the L-arabinose catabolic pathway in fungi, but genetic evidence was so far lacking. We show here that deletion of this gene (*lad1*) *Hypocrea jecorina* (anamorph *Trichoderma reesei*) in fact impairs growth on L-arabinose or arabinitol. Phylogenetic analysis of putative eukaryotic arabinitol dehydrogenases showed that Lad1 is a member of a terminal clade of putative fungal Lad orthologues which separated during evolution of sorbitol dehydrogenases. The *H. jecorina* genome database contains only a single member of this clade, Lad1. However, the *A. nidulans* genome contains three genes putatively encoding Lad1 orthologues. Since this is in contrast to findings by others that the *A. nidulans araA1* mutation is impaired in L-arabinitol dehydrogenase, and thus unable to grow on L-arabinitol, we cloned and investigated *ladA, ladB* and *ladC*. All three genes are expressed during growth on L-arabinose and L-arabinitol, and accumulate transcripts of the expected length in the wild-type and also in the *araA1* mutant. The three loci have also been sequenced from the *araA1* mutant to reveal possible mutations in any of them. Finally, we have overexpressed the three Lad proteins from *A. nidulans* and compared their substrate specificity with each other and with *H. jecorina*. The data imply that *A. nidulans* has redundant enzyme activities for L-arabinose catabolism and that the interpretation of mutant strain *araA1* thus must be revised.

Vp-14

FUNCTIONAL GENOMICS OF SUGAR UPTAKE BY ASPERGILLUS NIDULANS.

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In recent years considerable advances have been made in the elucidation of the molecular mechanisms of a number of regulatory functions operating at the genetic level which enable A. nidulans to tailor its metabolic versatility to the efficient utilisation of available nutrient sources. In its natural environment A. nidulans lives off decaying matter principally of plant origin, and the expression of genes that encode enzymes required for the utilization of polymeric sources of carbon is regulated depending on the nature of the carbon sources present. Despite the fact that these complex substrates have to be reduced to short polysaccharides and monosaccharides by enzymatic activities in order to serve as useful carbon sources little is known about the means of sensing, signalling and uptake of basic nutrients such sugars in A. nidulans. Given the positive (inducing) and negative (repressing) effects of different sugars on transcriptional regulation, the physiological and genetic characterisation of sugar uptake is of fundamental interest in filamentous fungi. We have recently demonstrated the existence of at least two kinetic components involved in energy-dependent glucose uptake in germinating A. nidulans conidia: a glucose-inducible low-affinity element and a glucose repressible high-affinity element. Repression of the high-affinity component is effected by CreA. The sorA gene, which has previously been suggested to be involved in L-sorbose uptake, appears to play a role in high-affinity glucose transport since the latter is absent in a sorA3 mutant while creA derepressed mutants exhibit enhanced sensitivity to the toxic effects of L-sorbose. In addition to genes previously identified by screening the Oklahoma EST library (mstA, mstB, mstP), recent analysis of the publicly available A. nidulans genome sequence data has yielded a number of genes putatively involved in monosaccharide (mst) transport. A combination of functional genomics, classical genetics and analysis of uptake kinetics is being used to investigate the complexity of sugar transport and the means by which sensing and signalling of sugars operates in A. nidulans.

UPREGULATION OF PDR-TYPE ABC TRANSPORTER GENES CORRELATES WITH THE AZOLE RESISTANCE IN ASPERGILLUS ORYZAE

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Aspergillus oryzae is an industrially important filamentous fungus. It is necessary for strain improvement of A. oryzae to develop the transformation system where multiple dominant selectable markers, such as drug resistant genes, are available. However, A. oryzae has a relatively high resistance to many antifungal drugs that are effective to yeasts and other filamentous fungi. In order to study on the mechanism for drug resistance of A. oryzae, we isolated a spontaneous mutant of A. oryzae that had high resistance to azoles and investigated the involvement of ABC transporter genes in the drug resistance.

The drug-resistant mutant showed the growth on the agar medium containing 10 ppm of clotrimazole or miconazole. Three ABC transporters have been identified in the A. oryzae EST sequencing analysis (http://www.nrib.go.jp/ken/EST/db/index.html). Northern analysis showed that PDR-type ABC transporter genes (atrA and atrF) were overexpressed in the resistant strain, but were expressed at barely detectable level in the wild-type. Further increased expression of the genes was observed in the presence of the azole drugs in the resistant strain. In contrast, an MDR-type ABC transporter gene, atrM, was constitutively expressed irrespective of the drug presence in both strains. In addition, the atrA gene was found to confer the resistance to the azoles, when overexpressed under the control of glaA gene promoter in the wild-type strain. These results indicated that the PDR-type transporters are involved in the azole resistance in A. oryzae.

CONTROL OF GLUCONEOGENESIS IN Aspergillus nidulans

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The role of microbial metabolism in development, secondary metabolism and pathogenesis is assuming a higher priority as studies of the integrated nature of genome wide control mechanisms become possible. Microorganisms growing on two-carbon compounds , fatty acids and amino acids as sole carbon sources require the production of hexose sugars via gluconeogenesis. Regulation of gluconeogenic enzymes is required to avoid futile cycling by the opposing glycolytic pathway. *Saccharomyces cerevisiae* is restricted in carbon source utilisation and transcriptional regulation of gluconeogenic enzymes results from the Cat8 / Sip4 regulatory circuit which also controls acetate utilisation via the glyoxalate cycle. In contrast filamentous fungi are able to use a much wider range of carbon sources metabolised via the TCA cycle and we have found that, in *Aspergillus nidulans,* gluconeogenesis is controlled separately from the glyoxalate cycle.

The *facB* gene, an orthologue of *CAT8* and *SIP4,* is responsible for regulation of the glyoxalate cycle in response to acetate, but does not control gluconeogenesis. Expression of the *acuF* gene encoding phosphoenolpyruvate carboxykinase (PEPCK) is induced by sources of TCA cycle intermediates and analysis of mutants indicates either malate or oxaloacetate as the inducer. The other key gluconeogenic enzyme , fructose-1,6- bisphosphatase (FBP), encoded by *acuG,* is strongly regulated by glucose repression but is also inducible by malate/oxaloacetate. Induction of both of these genes is abolished by mutations in either of the *acuK* or *acuM* genes.

The *acuN* gene is defined by a single allele which results in loss of growth on acetate and other carbon sources that require gluconeogenesis. Isolation of this gene by complementation reveals that it is a unique gene encoding enolase, an essential enzyme for both glycolysis and gluconeogenesis. The mutation turns out to be due to a I-V reciprocal translocation with the breakpoint resulting in truncation of the 5' noncoding region. Expression of *acuN* is not observed in the mutant when grown on gluconeogenic carbon sources but is normal on glycolytic carbon sources. We therefore propose that enolase is subject to separate transcription controls during gluconeogenesis compared with glycolysis.

Vp-17

BIOSYNTHESIS AND UPTAKE OF SIDEROPHORES IS CONTROLLED BY THE PACC-MEDIATED AMBIENT PH REGULATORY SYSTEM IN ASPERGILLUS NIDULANS

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Under iron starvation, most fungi excrete low-molecular-weight, ferric iron-specific chelators - termed siderophores - in order to mobilize environmental iron. Subsequently, the iron from the ferri-siderophore complexes is recovered via specific uptake mechanisms. Synthesis and uptake of siderophores is negatively regulated by iron, and this control has been shown to be mediated by the GATA-transcription factor SreA in Aspergillus nidulans. In this report we show that biosynthesis and uptake of siderophores is additionally subject to regulation by ambient pH: expression of genes involved in this high-affinity iron uptake system is elevated by an increase of ambient pH. Mediation of this regulation by the transcriptional regulator PacC has been confirmed via respective acidity- and alkalinity-mimicking mutants.
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Vp-18

SIDEROPHORE BIOSYNTHESIS IS NEGATIVELY REGULATED BY THE GATA-FACTOR AF-SREA IN ASPERGILLUS FUMIGATUS

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Virtually all organisms require iron for their growth, because this metal is indispensable for many different types of cofactors, e.g. heme moieties and iron-sulfur clusters. Under conditions of low iron availability most fungi excrete siderophores - low molecular-mass ferric iron chelators - in order to mobilize extracellular iron. The filamentous ascomycete A. fumigatus produces, like the closly related A. nidulans, two major siderophores: it excretes triacetylfusarinine C to capture iron and contains ferricrocin as an intracellular iron storage compound. Deficiency in the GATA-type transcription factor Af-SreA caused a partial derepression of extracellular siderophore biosynthesis and accumulation of intracellular siderophores. In Af-sreA-deletion strains, extracellular siderophore production still responded to extracellular iron availability indicating the presence of an additional iron regulatory mechanism. Af-sreA deficiency caused increased sensitivity to the redox cycler menadione, Moreover, an increase of extracellular iron availability caused a decrease of radial growth in this mutant. These data suggest that deregulation of the iron uptake system leads to increased oxidative stress, presumably via Fenton/Haber-Weiss-chemistry.

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THE ACTIVITY OF NIRA, A NITRATE-SPECIFIC TRANSCRIPTION FACTOR IN *ASPERGILLUS*, IS REGULATED ON THE LEVEL OF NUCLEAR LOCALIZATION AND BINDING SITE ACCESSIBILITY.

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Abstract
In the fungus *Aspergillus nidulans*, the assimilatory conversion of nitrate to ammonia is dependent on the activation function of NirA, a transcription factor belonging to the binuclear Zn_2-C_6 cluster family. We have previously shown that sequence-specific binding of the activator to a target sequence of nitrate regulated genes *in vivo* depends on the presence of intracellular nitrate and on the function of a GATA factor (AreA) responsible for chromatin remodelling on the promoter. Nitrate induction promotes NirA binding whereas addition of repressing ammonia leads to gradual dissociation of the activator from its binding site.
Here we show that a functional NirA-GFP protein is excluded from the nucleus under no-nitrate conditions and that addition of the inducer results in rapid and complete translocation of the fusion protein into the nucleus. Using a chromatin enzyme-accessibility assay we further show that the physiologically relevant NirA binding site 2 is uncovered already under no-nitrate conditions which suggests that exclusion of NirA from the nucleus is the main factor determining its DNA binding and transcriptional activity. Interestingly, under conditions of induction plus repression, gradual dissociation of NirA from the binding site coincides with repositioning of the relevant nucleosome –1 but the activator is not transported back to the cytosol. Simultaneous over-expresssion of NirA-GFP and the nitrate transporter *crnA* generates a constitutive nuclear localization signal for NirA and a novel supressor of *areA*$^-$ for nitrate utilization. These data suggest that the nitrate transporter is involved in the generation of the signal and that high levels of the pathway-specific activator can replace the chromatin and activation function of the GATA factor.

FUNCTIONAL GENOMICS IN USTILAGO MAYDIS: IDENTIFICATION OF AN IRON UPTAKE GENE CLUSTER WITH A HIGH AFFINITY IRON PERMEASE AFFECTING PATHOGENIC DEVELOPMENT

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Ustilago maydis is the causative agent of corn smut disease. In recent years, it has been demonstrated that a tightly regulated cyclic AMP signalling cascade is necessary for pathogenic development. In this study we have performed a transcriptome analysis using whole genome microarrays (Affymetrix) to identify target genes of the PKA catalytic subunit Adr1. A set of 400 genes was found to be differentially regulated. In this set were ten genes with a putative function in iron uptake clustered to three chromosomal regions. The cluster contains the known genes sid1 and sid2, involved in siderophore biosynthesis plus 8 new genes, designated fer1-8. We have investigated the expression of these genes in mutants affected in cAMP signalling and show that all genes are repressed by iron. Two genes were analysed in more detail. A new nonribosomal peptide synthetase, fer3, was shown to be required for the biosynthesis of ferrichrome A, while the high affinity ferric permease, fer2, is a critical a virulence factor in Ustilago maydis.

Vp-21

MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF THE NAD-DEPENDENT ADH FROM THE ZYGOMYCETE Mucor circinelloides

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Alcohol dehydrogenase (ADH) catalyses the reversible interconversion of aldehydes and alcohols. This reaction is the final step during alcoholic fermentation in yeast and the initial step in the metabolism of ethanol in a wide variety of organisms. Reductive reactions result in the production of alcohol and oxidized cofactor NAD^+, where the regeneration of NAD^+ is essential for other oxidative and energy-yielding metabolic processes to continue. Many organisms contain multiple ADHs and their physiological roles can sometimes prove difficult to reveal. In previous work we have studied the presence of ADH in the mycelium and yeast phases of M. circinelloides, finding that the same ADH enzyme is produced, though with different specific activity, in both developmental stages. The enzyme from yeast cells was purified to homogeneity by ion-exchange and affinity chromatography; the purified ADH appears to be a tetramer of subunit M_r 37,500. K_m values obtained for acetaldehyde, ethanol, $NADH_2$ and NAD^+ indicated that physiologically the enzyme works mainly in the reduction of acetaldehyde to ethanol. Based on the aminoacid sequence of internal peptides of the enzyme we designed oligonucleotides that allowed the cloning of the adhI cDNA by RT-PCR. This cDNA was used for the cloning of the open reading frame of the genomic copy of the adh1 gene; the analysis of the sequence indicated the presence of two small introns, located towards the 5' end of the gene. The encoded product of adh1 gene has homology with bacterial and fungal ADHs. Southern hybridization analysis indicated that there is a single adh1 gene in the M. circinelloides genome. Northern experiments indicated that the Adh1 gene is expressed in both mycelium and yeast cells, producing a mRNA of about 1.2 kb. In order to test the functionality of the adh1 gene, it's open reading frame was cloned in the M. circinelloides expression vector pEUKA and the construct used to transform the ADH deficient mutant strain M5. The transformant M5/pEUKA-adhI recovered wild type properties, such as the ability to grow in the absence of oxygen and ADH activity.

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Vp-22

OSMOTIC STRESS AND GLYCEROL BIOSYNTHESIS IN THE MYCOPARASITIC FUNGUS *TRICHODERMA ATROVIRIDE*

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Trichoderma atroviride P1 is a mycoparasitic fungus which antagonizes the growth of several plant pathogenic fungi. This antagonization involves, besides formation of cell-wall lytic enzymes and antibiotics, also morphological changes such as coiling and formation of appressoria-like structures. The latter serve to penetrate the host, and usually contain high concentrations of osmotic solutes such as glycerol. Towards a more detailed knowledge of the molecular events in *Trichoderma* leading to appressorium formation, we have cloned and characterized two of the genes involved in glycerol biosynthesis, *gfd1* (glycerol-3-phosphate dehydrogenase encoding) and *gld1* (glycerol dehydrogenase encoding). Epxression patterns of *gfd1* and *gld1* and enzyme activities of the corresponding gene products were determined in the wild-type and a mutant lacking the osmotic stress regulator *seb1* (AGGGG-binding protein-encoding gene) under different kinds of osmotic stress (1 M KCl, 1 M NaCl, 10 % carbon source) and on different carbon sources; additionally polyol pools were measured under the same conditions. *T. atroviride* responds to osmotic stress via increased expression of *gld1*, dependent on the carbon source and the type of osmotic stress, whereas *gfd1* is not upregulated. While the wild-type accumulates glycerol upon osmotic stress, the Δ*seb* mutant shows strongly reduced glycerol levels under osmotic stress conditions and seems to counterbalance this deficiency partially with the enhanced production of other polyols, particularly mannitol. However, intracellular glycerol levels did not strictly correlate with the expression patterns of *gfd1* and *gld1*, indicating that yet additional genes are involved in the accumulation of glycerol in *T. atroviride.*