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COMPARATIVE AND FUNCTIONAL GENOMICS IN ASPERGILLUS

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The completion of several Aspergillus whole genome-sequencing projects marks the beginning of a drive towards identifying and characterising all the genes encoded. Automated annotation programs can quickly identify open reading frames (ORFs) for hypothetical genes, many of which will be conserved across large evolutionary distances, but further information is required to confirm functional assignments. Comparison of the predicted ORFs from Aspergillus niger, nidulans and fumigatus species can evaluate the level of conserved sequence in the genes they represent and allow greater confidence when comparing these genes with those from other organisms. These putative orthologs can then be highly valuable in identifying if pathways or process are also conserved. Transcriptome analysis can provide highly valuable experimental data to elucidate and confirm the function of genes; this approach has been used here to improve the understanding of the Aspergillus secretory pathway. It is hoped that by improving our knowledge of the mechanisms behind protein secretion, we may be able to engineer strains to secrete recombinant proteins more efficiently.

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FUSARIUM OXYSPORUM AS A MULTIHOST MODEL FOR DISSECTION OF FUNGAL VIRULENCE IN PLANTS AND MAMMALS

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Fungi cause disease in plant and animal hosts. The extent to which virulence determinants are conserved between both classes of pathogens is unknown. Large-scale genomic analysis holds the promise to significantly advance our understanding of fungal virulence mechanisms in evolutionary distant hosts. However, this potential is currently limited by the lack of a multihost pathogen system. We present a dual plant-animal infection model based on a single strain of *Fusarium oxysporum*, the causal agent of vascular wilt disease in plants and an emerging opportunistic pathogen of humans. Injection of microconidia of the well-characterized tomato pathogenic isolate 4287 in the lateral tail vein of immunodepressed mice resulted in disseminated infection of multiple organs and death of the animals. Knockout mutants in genes encoding a Pmk1-type mitogen-activated protein kinase, the pH response transcription factor PacC or a class V chitin synthase, all previously shown to be implicated in virulence on tomato plants, were tested in the disseminated mouse model. Our results indicate that some of these virulence factors play functionally distinct roles during infection of tomato and mice. Thus, a single *F. oxysporum* strain can be used to study fungal virulence mechanisms in plant and mammalian pathogenesis.

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EFFICIENT GENE TARGETING IN FILAMENTOUS FUNGI

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In recent years, advancements in high throughput sequencing and bioinformatics have resulted in the accumulation of numerous complete genome sequences. Although this sequence information has already proved useful, a tremendous potential exists to further advance biotechnology, provided that novel molecular techniques are developed, to rapidly and reliably allow precise site-directed genomic modifications. Unfortunately, development of gene targeting techniques in filamentous fungi and many other higher eukaryotes, have been hindered by the fact that foreign DNA is predominantly integrated randomly into the genome and not at the desired sites. Since efficient gene targeting relies on the homologous recombination we have focused our research on developing targeting methods that use substrates that are preferred by the homologous recombination pathway. In one method, using *Aspergillus nidulans* as a model system, we have adapted a yeast-method for precision gene targeting that is based solely on PCR fragments and obviates the need for subcloning to produce the linear targeting substrates. The method reduces the number of false positives and can be used to produce virtually any genome alteration including gene deletion, allele replacement and epitope tagging. The efficiency and perspectives of the method are assessed and discussed.

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MITOCHONDRIAL DNA VARIABILITY IN GIBBERELLA INTERMEDIA (FUSARIUM PROLIFERATUM)

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Strains of Fusarium proliferatum cause diseases on a variety of economically important plants and produce mycotoxins, such as fumonisins. We used restriction fragment length polymorphisms (RFLP) to assess genetic diversity of mitochondrial DNA (mtDNA) among 184 isolates of F. proliferatum recovered from maize, asparagus, palms, and reed. All strains were cross-fertile with standard mating type tester strains of Gibberella intermedia. We identified 16 mitochondrial haplotypes following digestion of DNAs with HaeIII, with seven, seven, five and six different haplotypes from maize, asparagus, palms and reed, respectively. Four haplotypes (I, III, IV, and VII) were found on more than one host. Of these four, haplotype I was the most common and dominated on maize, representing 71% of the isolates. The banding patterns for haplotypes III and IV were >90% similar to the banding pattern of haplotype I. Haplotypes I, III, and IV together accounted for 87% of the isolates from maize, but were less common on the other hosts, accounting for 70%, 52%, and 33% of the isolates from asparagus, palms and reed, respectively. Thirteen of the 16 haplotypes were each recovered from only a single host plant species. When comparing the banding patterns and frequencies of these haplotypes, at least five were recovered at a significantly higher frequency from one host relative to the others. Our results suggest that mtDNA RFLP analysis is a useful indicator of genetic divergence in F. proliferatum.

STUDYING THE POWDERY MILDEW FUNGUS AT GENOME, TRANSCRIPTOME AND PROTEOME LEVEL.

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Powdery mildew caused by *Blumeria graminis* is one of the most severe diseases in cereals, especially wheat and barley. The plant-pathogen interaction is controlled by specific resistance genes in the plant and matching fungal avirulence genes according to the gene-for-gene hypothesis. *B. graminis* is an obligate fungus that develops haustoria inside the epidermal cells and it is important for the fungus to keep the cells alive in order to obtain nutrients from the plant. We are studying the fungus and the interaction at the genome level, the transcriptome level and the proteome level.

A detailed study at the genome level has been carried out by sequencing and analysing 74 kb of genomic DNA. It showed a complex mixture of genes, various kinds of retrotransposable elements and other types of repetitive DNA elements. The study gives a glimpse of what to expect of a whole-genome sequencing project.

At the transcriptome level we are looking at expression profiles of a unigene-set of about 1520 genes from an EST-project using high-density arrays on filters. We have compared 5 isolates at three different stages and are now undertaking a more detailed study of expression during the infection process.

Our proteome analysis is focusing on the intimate interaction. We have developed a method to isolate haustoria from infected leaves and are now doing proteome analysis on this material aiming at identifying proteins involved in uptake and transport of nutrients as well as proteins involved in signalling and communication with the plant.

Results from our studies on the three levels will be presented.

IDENTIFICATION OF GENES PREFERENTIALLY EXPRESSED IN THE PATHOGENIC YEAST PHASE OF *Paracoccidioides brasiliensis* USING SUPPRESSION SUBTRACTION HYBRIDIZATION AND MACROARRAY DIFFERENTIAL ANALYSIS

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Paracoccidioides brasiliensis, a thermodimorphic fungus, is the causative agent of paracoccidioidomycosis (PCM), the prevalent systemic mycosis in Latin America. Pathogenicity appears to be intimately related to the dimorphic transition because strains, which are defective in the transition from mycelium to the yeast phase, are not virulent. Because of the lack of information about *P. brasiliensis* genes expressed in the yeast pathogenic phase, we have undertaken a Suppression Subtraction Hybridization (SSH) and macroarray differential analyses aiming to identify genes preferentially expressed in the yeast phase. Genes identified as being more expressed in the yeast phase, by using both procedures, are involved in basic metabolism, signal transduction, growth and morphogenesis, and sulfur metabolism. Twenty-one of these genes, such as *AGS1* (α-1,3-glucan synthase) and *TSA1* (thiol-specific antioxidant), showed to be more expressed in the yeast phase by using real-time RT-PCR.

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MOLECULAR AND ELECTROPHYSIOLOGICAL CHARACTERISATION OF ION CHANNELS IN FILAMENTOUS FUNGI

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Although it is well established that ion channels play essential roles in many aspects of animal and plant cell biology, very little is known of their roles in filamentous fungi. The development of a laser-assisted patch clamp technique for use on fungi has allowed the unambiguous electrophysiological characterisation of anion and cation channels from the plasma membrane of Aspergillus. Inhibitor studies suggest that they probably have essential roles in filamentous fungal biology.
In an attempt to gain further insights in to the roles of ion channel function in fungal physiology, ion channel genes have been identified in the filamentous fungus, Aspergillus nidulans. This study reports the characterisation of one of these ion channels, AnCLCA. AnCLCA is a 909 amino acid polypeptide which functions as a chloride channel when heterologously expressed in yeast. To define its role in A. nidulans, we created an AnCLCA null mutant. This mutant exhibited acute sensitivity to extracellular copper (Cu), which we show to result from the hyperaccumulation of Cu and the generation of toxic levels of reactive oxygen species. The mutant also exhibited reduced Cu-dependent superoxide dismutase activity (thus exacerbating the oxidative stress) and elevated respiration rates (resulting specifically from enhanced Cu-dependent cytochrome oxidase activity). These results show that the activity of Cu-dependent enzymes is disrupted in the mutant indicating that AnCLCA plays a central role in Cu metabolism in A. nidulans.

Comparison of proposed anion channel function in A. nidulans and Saccharomyces cerevisiae highlights differences in ion channel function between filamentous fungi and yeast. These differences are consistent with the finding that the filamentous fungal genomes possess more anion channel genes than that identified in the model yeast genome. Future work is focussing on determining the roles of anion channels (which are not represented in the yeast systems) in filamentous fungal cell biology.

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GENOMIC VARIATION AND PLASTICITY IN ISOLATES OF THE ECTOMYCORRHIZAL FUNGUS PAXILLUS INVOLUTUS AS ANALYZED BY COMPARATIVE GENOMIC HYBRIDIZATIONS (CGH)

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Like many other ectomycorrhizal fungi, the basidiomycete Paxillus involutus forms mycorrhizal associations with a broad range of boreal and temperate forest trees. To some extent this variation is related to strain specificities, thus different isolates that can have specific host and habitat preferences. To get some insights into the genomic variation between different isolates of P. involutus, the genomes of five strains of P. involutus (ATCC200175, Pi01Se, Pi08Be, Maj and Nau) and P. filamentosus (outgroup) were analyzed using Comparative Genomic Hybridizations (CGH) onto cDNA microarrays. The arrays contained 978 probes from the ATCC200175 strain representing a uniset of 978 EST clones (putatively 978 unique genes). The variation in the ratios of the hybridizations intensities of genomic DNA between the Pi01Se, Pi08Be, Maj and Nau (sample) strains and the ATCC (reference) strain reflected the phylogenetic distance between the isolates. To characterize the genomic mechanisms that could generate the differences in hybridization signals, 15 loci were analyzed in more detail by DNA sequencing. Although there was a correlation between sequence divergence and ratios of hybridization signals in several of these loci, a few cases were encountered where the variation in hybridization signals had to be explained by other mechanisms including gene deletions and amplifications. Analysis of the ratios of hybridization intensities for all 978 genes indicated that approximately 30 % of the genes differed significantly in at least one of the comparisons between the ATCC strain and the sample strains. Except orphans, this “variable” part of the genome contained a high proportion of genes involved in cellular communication and signal transduction, and cell rescue and defense mechanisms.

HYBRIDISATION ARRAY TECHNOLOGY COUPLED WITH CHEMOSTAT CULTURE. GENE EXPRESSION STUDIES IN ASPERGILLUS NIDULANS.

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Although hybridisation array technology is widely used for the analysis of gene expression in a number of different organisms, it is just beginning to be used in the analysis of gene expression in *Aspergillus* species as genome sequence data become available for these organisms. As in many organisms, studies are often carried out in batch culture where the growth rate and environment are continuously changing. Changes in growth rate affect the pattern of gene expression, hence the analysis and interpretation of experimental results obtained in this way are inherently problematic due to the difficulty of discriminating between effects due to the experimental condition and those due to growth rate and other physiological changes. By maintaining cultures at steady state in a chemostat, both the growth rate and the organism's physiology is kept constant. This eliminates these confounding effects and enables experiments to be performed in which a single parameter may be varied while all others remain constant.

In this study, *Aspergillus nidulans* has been grown in chemostat culture under carbon limitation. Samples of biomass have been taken, RNA extracted and cDNA synthesised and labelled with cy3/5 fluorescent dyes and hybridised to *A. nidulans* cDNA microarrays. Expression of genes from the different samples taken from the chemostat culture has been compared to samples taken from a batch culture. Statistical analysis of the expression levels of all the genes on the array illustrates the advantages of chemostat culture in gene expression studies in *Aspergillus*.

TARGETING STRESS-RESPONSE GENES FOR CONTROL OF MYCOTOXIN BIOSYNTHESIS IN ASPERGILLUS

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Certain stress-response genes in aflatoxigenic aspergilli play a significant role for inducing biosynthesis of aflatoxin. For instance, hydrolyzable tannins completely shutdown aflatoxin biosynthesis. The most active constituent of these tannins is gallic acid. By using deletion mutants of Saccharomyces cerevisiae, as a model system, we found the mode of action of gallic acid is as an antioxidant. For example, negative effects on yeasts lacking the antioxidative stress gene cta1Δ exposed to hydrogen peroxide were reversed when the same mutants were treated with gallic acid. Thus, gallic acid counters oxidative stress-response induced biosynthesis of aflatoxin. Examination of a deletion mutant lacking the signal transduction gene sho1Δ, which encodes a transmembrane osmosensor, showed similar results as the cta1Δ mutant. We identified other stress-response genes upstream from the gene cluster of the aflatoxin biosynthetic pathway. Such stress-response genes are responsible for signal transduction, inducing upregulation of transcription factor(s) further downstream to initiate aflatoxin biosynthesis.
To discover genes involved in induction of toxin biosynthesis, we are searching an EST database of A. flavus. Based on results using the model yeast system, described above, we have identified orthologs of yeast MAP kinase-pathway and antioxidative stress-response genes in the A. flavus EST database. In order to study the A. flavus orthologs directly, we are developing a vector system wherein these genes are recombined in yeasts using an artificial chromosome. The system includes a vector having an origin of replication, selectable markers, a yeast centromere, a promoter and cloning sites for insertion of exogenous cDNA. Our approach complements microarray analysis and allows a high throughput assessment of individual, specifically targeted genes.

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DESCRIPTION OF A FUNGAL STEROL ESTERASE ABLE TO HYDROLYZE STEROL ESTERS AND GLYCERIDES: STRATEGY FOR CLONING AND SEQUENCING THIS NEW ENZYME

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A sterol esterase purified from cultures of the sapstain fungus *Ophiostoma piceae* was able to hydrolyze sterol esters and glycerides. The kinetics of sterol esters and triglyceride hydrolysis by this new esterase, estimated using a pH-stat, showed a K_m^{app} in the range of 1 mM. Its ability to hydrolyze both pure sterol esters and natural mixtures of saponifiable lipids from eucalypt wood suggests that this enzyme could be of biotechnological interest for the hydrolysis of sterol esters that form pitch deposits in paper pulp manufacturing.

To obtain an specific probe of *Ophiostoma piceae* sterol esterase, the deglycosylated protein was hydrolyzed with trypsin, the peptides were separated in a C_{18} column and N-terminal sequences were obtained. At present we have obtained a DNA fragment around 1000 pb by PCR using as primers the oligonucleotides corresponding to the N-terminal sequence of the protein and the internal sequences of one of the isolated internal peptides.

On the other hand we have got *O. piceae* esterase antibodies. This could be a second approach for cloning and sequencing the *O. piceae* esterase using the antiserum raised against the purified enzyme to identify esterase-encoding cDNAs in a cDNA expression library.

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DNA METHYLATION IN ASPERGILLUS

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MBD: methyl binding domain; MeC: methylated cytosine; AFLP: amplified fragment length polymorphisms.

We report the presence of methylated DNA in Aspergillus spp. We have used a combination of approaches such as methylation-specific AFLPs, bisulphite DNA sequencing and fractionation (and subsequent sequencing) of DNA using MBD agarose chromatography. Although methylation of DNA has been shown in some fungal species, e.g. Neurospora crassa and Ascobolus immersus, the presence of methylated DNA in Aspergillus spp. has been looked for and either discounted or thought to be at a low level. Recently, Gowher et al. (2001 FEMS Microbiol Lett 205(1), 151-155) demonstrated the presence of low levels of methylated DNA in A.flavus. Even if levels are low the possibility remains that DNA methylation may be functionally significant as an epigenetic mechanism of transcriptional regulation. We sought to examine this by using a variety of methods to detect DNA methylation and had the aim of examining if DNA methylation was restricted to repeat sequences in the Aspergillus genome as is mainly the case (though not exclusively) in N.crassa (Selker et al. 2003 Nature 422, 893-897). We will report our findings with A.oryzae and other members of the Aspergillus Flavi group.

VIEWING ANNOTATED ASPERGILLUS GENOMES VIA THE CADRE WEBSITE

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Three important Aspergillus genomes (A. fumigatus, A. nidulans and A. oryzae) have been sequenced and have undergone first-pass annotation. A worldwide effort is currently underway to manually refine the annotation for genes of interest and to perform preliminary genome-wide analyses. The University of Manchester has been funded to provide a public resource for the long-term annotation and analysis of genomic data from Aspergillus species: to this end, CADRE (Central Aspergillus Data Repository; http://www.cadre.man.ac.uk) was initiated in 2001.

We have already received 922 kb of annotated A. fumigatus sequence, which is centred around the niaD locus, from the Wellcome Trust Sanger Institute: this information was generated as part of the A. fumigatus pilot genome sequencing project. Using Ensembl to implement CADRE, we have established a database for housing the pilot sequence annotation and provided several means of viewing the data. At present, using a Web browser, the sequence contig can be viewed alongside various features (e.g., protein-coding genes and tRNA-coding genes) that have been mapped onto it. For each feature, links are provided to allow the user to retrieve further annotation. For protein-coding genes, such annotation includes: the location, a description of the encoded protein's function; transcripts and their sequence; and links to further information regarding translations. In addition, by virtue of adopting the Ensembl system, we now have a framework in place for housing several other genomes. Therefore, we expect C*A*DRE to greatly expand within the next few months to include three completed Aspergillus genomes: A. nidulans annotation is likely to be made available within C*A*DRE shortly, whereas annotation for both A. fumigatus and A. oryzae will be made available upon the publication of the corresponding genome papers.

EVOLUTIONARY CONSERVATION AND DIVERGENCE OF FUNGAL PROMOTER SEQUENCES

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The recently sequenced and fully annotated genome of the filamentous fungus Ashbya gossypii revealed striking similarity to the baker's yeast Saccharomyces cerevisiae. 90% of A. gossypii genes share homology and also a substantial degree of synteny (gene order conservation) with S. cerevisiae. Although both organisms originate from the same ancestor (carrying about 5000 protein coding genes), the evaluation of synteny was complicated by the fact that their evolutionary paths included not only about 300 translocations and inversions but also a whole genome duplication in the S. cerevisiae lineage followed by loss of 4000 genes. As a consequence the alignment of the A. gossypii genes with homologous S. cerevisiae genes results in many synteny clusters in which one A. gossypii chromosomal region aligns with two chromosomal regions of S. cerevisiae. The clusters themselves are made of gene regions displaying relaxed (incomplete) and stringent (complete) synteny. The latter is found in many small regions of up to eleven genes which, very importantly, are not interrupted by end points of rearrangements. Thus, these regions are particularly suitable for investigations of evolutionary conservation and divergence of syntenic sequences which started diverging over 100 million years ago. In the past, most studies of syntenic regions looked into conservation and divergence of open reading frames (ORFs) and the proteins they encode. We have started an investigation of evolutionary selection regarding size and sequence of inter-ORF regions. A detailed discussion of the subject will be presented taking into account DNA-binding sites of transcription factors, transcription start and terminator sites and inter-ORF lengths discerning between bidirectional or unidirectional promoters and pure terminator-bearing inter-ORFs.

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GLOBAL GENE PROFILING OF FUSARIUM GRAMINEARUM

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We are using a microarray gene profiling approach to identify candidate genes suspected to be involved in Fusarium graminearum pathogenicity. We have constructed nine different F. graminearum cDNA libraries from fungal cultures grown under a variety of conditions to generate a collection of >10,000 expressed sequence tags (ESTs) which group into ~4800 contigs or singletons. Electronic gene profiling has provided a list of genes specifically expressed during actual/simulated plant contact conditions and possibly involved in the fungal infective process. A 4.8K unigene F. graminearum cDNA microarray has been produced and array hybridization experiments are exploring which genes are influenced by plant contact.

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IDENTIFICATION OF MYCOPARASITISM-RELATED GENES OF TRICHODERMA ATROVIRIDE BY RAPID SUBTRACTION HYBRIDIZATION (RaSH)

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Due to its mycoparasitic behaviour the filamentous fungus Trichoderma atroviride is known as a potential agent for the biocontrol of plant pathogenic fungi. Although cell wall lytic enzymes including chitinases, proteases and glucanases have already been studied intensely, until now no approach has been carried out to identify a wider spectrum of genes involved in mycoparasitism.

We used a subtraction hybridisation system to find genes of T. atroviride specifically expressed during the antagonistic interaction with Rhizoctonia solani. cDNA from T. atroviride grown on PDA was subtracted from cDNA derived from different stages (before and after contact with the host) of mycoparasitism. The differential expression of the clones obtained by this method was verified by reversed northern blots. Several clones have been sequenced; in silico analysis of these fragments revealed highest similarities for most of them to a number of lytic enzymes (e.g. proteases, glucanases) known from other filamentous fungi.

We subtracted cDNAs from the same strain on the same media and the only varying factor in this system was the absence or presence of the host fungus R. solani. Due to the high similarity of the compared conditions we obtained only a limited number of different clones. Nevertheless BLAST searches brought out a striking high specificity of the applied method as almost all identified genes could presumably be involved in mycoparasitic interactions.

SEARCHING FOR GENES RELATED TO PRODUCTION OF THE FUSARIUM MYCOTOXIN ZEARALENONE

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Fusarium spp. are a common infection in many cereal grains and as a group the most important food-related fungi in northern temperate regions through their production of mycotoxins. One of these is the polyketide zearalenone which is a potent oestrogenic mycotoxin produced by several Fusarium species, mainly Fusarium graminearum and Fusarium culmorum. Production of zearalenone seems to be induced by low temperature and a high level of moisture. Due to frequent periods with low temperature during the growing season of cereals, a high production of zearalenone should thus be expected in the Nordic countries if zearalenone producing *Fusarium* spp. are present. Correct identification and quantification of mycotoxin-producing Fusarium spp. will give an estimate of the amount of mycotoxin that can be expected to be present in a cereal sample. The goal of this work is to loctate genes related to the production of zearalenone, and further, to develop zearalenone-specific primers for simple detection of zearalenone-producing Fusarium in cereal samples. Two Fusarium graminearum and one Fusarium culmorum strains were tested with ELISA to find the best growth conditions for minimum and maximum production of zearalenone. Differential Display was then used to look for upregulated genes connected to zearalenone production in these 3 isolates. Several good candidates are found, but are not yet sequenced. Alternative strategies are the use of degenerate primers constructed on the basis of known polyketide synthases from other fungi, and screening of the Fusarium graminearum genome database for potential genes. Results from ELISA, Differential Display and genes found with degenerate primers will be presented here.

PHYLOGENETIC ANALYSIS OF PHYTOPHTHORA SPECIES BASED ON MITOCHONDRIAL AND NUCLEAR DNA SEQUENCES

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A molecular phylogenetic analysis of the genus Phytophthora was performed, based on both nuclear and mitochondrial DNA sequence data. Emphasis in our study was on species collected from the Toluca Valley in central Mexico, the presumed center of origin of Phytophthora infestans and other closely related species. A total of 113 isolates from 48 Phytophthora species and 2 Pythium species were used in this analysis. Phylogenetic analyses were performed for combined mitochondrial sequences, for combined nuclear sequences and for all sequences combined, and between-data set congruence was tested. Results indicate that the classical taxonomic grouping as described by Waterhouse (1963) does not reflect true phylogenetic relations. Phytophthora species were redistributed into 8 clades, providing a more accurate representation of phylogenetic relationships within the genus Phytophthora. The evolution and transition of morphological, pathogenic and reproductive traits was inferred from the cladogram generated in this study.

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CHARACTERIZATION OF PHYSIOLOGICAL DIVERSITY IN RECOMBINANT AND CHEMICAL MUTANT STRAINS OF *HYPOCREA JECORINA* BY PHENOTYPE ARRAY ANALYSIS

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Broad-based analyses of an organism's response to genetic alteration, chemical treatment, or changes in environment have become invaluable tools for gaining insight into the effects of these changes in biological systems, mostly at the level of genome-scale DNA, RNA, protein and metabolite analysis. Unlike these, phenotypic analysis reveals the effect of the genetic mutation on the structure and function of the whole organism at the physiological level. Here we describe the development of a phenotype array technique to quantify the physiology of the ascomycete *Hypocrea jecorina* (anamorph *Trichoderma reesei*). To this end, 96-carbon source Biolog FF plates (1) were used, and optimized with respect to inoculum, time and temperature with five wild-type strains, and then used to address the following questions: (a) how stable are phenotypic characters in genealogies of cellulase-producing mutants; (b) how does ectopic DNA-mediated transformation or overexpression under glycolytic promoters influence carbon nutrition patterns; (c) how much does carbon catabolite repression influence carbon utilization in this fungus; and (d) is the Cre1-independent metabolism of the Cre1-mutant strain RUT C-30 comparable to that of wild-type strains? A simple computer program was written to assist the statistic evaluation of the data, and to enable a comparison of variables. The results to be presented demonstrate the utility of phenotype arrays for chemical characterization and gene function determination in *H. jecorina* and likely other filamentous fungi.

(1) Kubicek CP, Bissett J, Druzhinina I, Kullnig-Gradinger C, Szakacs G. (2003) Fungal Genet Biol. 38: 310 - 319.

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THE GEN-AU/MIPS FUSARIUM GRAMINEARUM GENOME DATABASE

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Based on release 2 of the Fusarium graminearum genome sequence from the Whitehead Institute Center for Genome Research, a PEDANT genome database was set up (http://pedant.gsf.de/cgi-bin/wwwfly.pl?Set=Fusarium_graminearum&Page=index) (Frishman et al, The PEDANT genome database. NAR 31: 207-211, 2003). The web interface enables searches across the resulting tables of all applied methods as well as summaries of the method results. Blast searches with a sequence of interest against the contig DNA or the protein set are possible. Applied methods: Blast against a nonredundant Protein database, Blast against individual PEDANT databases (Magnaporthe grisea, N. crassa and S. cerevisiae), extraction of EC numbers, PIR superfamilies, INTERPRO, PFAM, PROSITE, BLOCKS, COGs, Known3D, Scop domains, TmHmm, tRNAscanSE and others.
Protein titles and protein classifications were assigned by applying BLAST searches against manually annotated datasets (N. crassa, S. cerevisiae), an experimental validated subset of the Swissprot database and a nonred protein database with different stringencies. In a second step the Genome Research Environment (GenRE), developed as a general environment for genomic databases at MIPS, was used to maintain and access the F. graminearum annotation and research database. It allows easy data integration for large scale data sets as well as convenient manual annotation access either direct (in house) or for project partners via remote access. Up to now, selected gene models are revised manually and clearly indicated in the entry view. Searches for_syntheny against related fungi, hemiascomycetes as well as selected bacteria are already done. All annotations are stored in a relational ORACLE database. The general user interface (GUI) design achieves a clearly structured entry view and searches across all annotations as well as the main PEDANT results. A graphical view of the contigs with all genetic elements and selected additional information (classification, orthologs etc.) allows intuitive browsing across the genome.

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EFFICIENT TRANSFORMATION OF FOUR STRAINS OF Trichoderma USING PHLEOMYCIN AND HYGROMICIN AS SELECTABLE MARKERS MEDIATED BY PROTOPLAST FORMATION AND BY Agrobacterium tumefaciens AGL1 MEDIATED CONJUGATION.

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The Genus Trichoderma includes many strains that have received an especial attention in last years due to their high potential as biocontrol agents of plant diseases. Some species of this genus are active as mycoparasites and have been tested in field experiments and successfully shown to be effective against to a range of economically important aerial and soil-borne plant pathogens. Different factors act in the growth inhibition of pathogenic fungi and several metabolites involved in this process have been identified such as volatile and non-volatile antibiotics and hydrolytic enzymes, including β(1,3)-glucanases, proteases, chitinases, etc.

The development of a reliable transformation system is a prerequisite for improving the understanding of its genetics and molecular biology leading to enhance the application of these fungi as biocontrol agents.

In this work we describe efficient transformation methods to obtain stable transformants of T. harzianum 2413, T. atroviride B11, T. longibrachiatum T52 and T. asperellum T53 using phleomycin and hygromycin B antibiotics as selectable markers. The transformation protocols were based on protoplast formation by treatment with DTT and lysing enzymes and on the use of Agrobacterium tumefaciens AGL1 strain as vector to transfer the DNA to Trichoderma.

Analysis by Southern blot hybridization of independent phleomycin and hygromycin resistance transformants showed that the phleomycin and hygromycin cassettes were completely integrated in the genome of Trichoderma strains, and that the DNA was randomly integrated in most of the cases.

The stability of each group of pleomycin or hygromycin transformants was evaluated in the different Trichoderma strains. Thus, the best results were obtained in T. longibrachiatum whose transformants obtained both by protoplast formation or by conjugation using Agrobacterium were stable even after four sequential selection rounds of the conidia on both non-selective and selective media.

With this work we evaluate how efficient are the methods used, protoplast transformation or Agrobacterium conjugation, to introduce exogenous DNAs in the four Trichoderma strains belonging to different species used in this study. Thus, this work will allow us to manipulate and improve more easily the different strains of this genus which has an increased interest in microbial and plant biotechnology.

CULTURE INDEPENDENT PCR SCREENING OF ANCIENT SIBERIAN SEDIMENTARY SAMPLES AND CORN WASTE PRODUCTS

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Abstract

Culture independent PCR (ciPCR) is a molecular based discovery technique which can be used to trace the presence of genes from non cultured organisms thus providing a short cut to retrieval of specific genes in a composite multi organic sample. We here report a study on ancient fungal diversity by means of 18s rDNA as long as 510bp from 3-400 kilo years (ky) old permafrost. DNA was amplified from a broad diversity of fungal lineages in ancient samples from Holocene (10 ky) to Pleistocene age (3-400 ky) enabling us to describe ancient cold adapted fungal communities

Recently scientists amplified both mitochondrial- and chloroplast DNA of herbivores and plants from the same Siberian sedimentary samples. However this and other studies have failed in amplification of eukaryotic DNA longer than a few hundred base pair leading most scientists in the field to write off the possibility of amplifying longer fragments due to DNA decaying processes. Data suggested presence of numerous plant pathogenic fungi. DNA traces of several of these parasites host organisms were also present in the samples. In the 3-400 ky sample we were able to associate lichenising fungi of Lecanorales with green algae from Trebouxiaphyceae. Findings of coprofile fungi genera *Sordaria* and *Delitschia* suggest a faecal origin of herbivore DNA in the ancient sediments studied.

Furthermore the technique was used to amplify single copy fungal enzyme genes (glucosyl hydrolase families 7 and 45) from contemporary corn stover and -leaves, both interesting industrial waste products. Enzyme genes originating from the fungi that in nature inhabit the substrate under study could optimally lead to findings of enzymes with higher activity and specificity. This could have great implications in fields such as bioethanol where the efficiency of the enzymes converting cellulose to glucose is a troublesome bottleneck.

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A PHYSICAL CLUSTER OF CALCINEURIN-REGULATED GENES IN *BOTRYTIS CINEREA*

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The necrotrophic Ascomycete Botrytis cinerea is the causal agent of grey mould symptoms on more than 200 plants species including grapes, vegetables and berries. We recently started a genomic approach to identify fungal virulence factors. A library containing 6559 ESTs (Expressed Sequence Tags) allowed the identification of 3032 putative genes (available at http://urgi.infobiogen.fr///Projects/GPiDB/Interface/). The expression level of these genes under different physiological states (starvation, stresses, in planta growth, drug treatments…) or in different genetic backgrounds (WT strains and KO mutants…) is now studied by cDNA arrays technologies. This approach was used to identify the genes that are regulated through the calcineurin signalling pathway which is known to be involved in fungal morphogenesis and pathogenicity. mRNA were extracted from mycelia cultured with 0, 2 or 10 microg/ml of the calcineurin inhibitor Cyclosporin A, labelled with ^{33}P and hybridized to the macroarrays. The resulting data have been analysed with the GENEANOVA software (Didier et al., 2002. bioinformatics 18 : 490-491) to identify genes that are significantly up or down regulated. By using this process, 18 genes with a 2.5 times change in expression have been identified and named CNDs (for calcineurin-dependant) genes (Viaud et al., 2003. Mol. Microbiol. 50: 1451-1456). Genomic sequencing revealed that three of these calcineurin-dependant genes are organised into a cluster. Moreover sequence similarities of these genes with known proteins suggest that they could be involved in sesquiterpenes biosynthesis. A reverse genetics approach is now used to investigate their function in pathogenicity. Preliminary results suggest that they are involved in the production of a small metabolite and in pathogenicity.

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MOLECULAR IDENTIFICATION OF ISOLATES OF *Lentinula edodes* USING MICROSATELLITE MARKERS

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The molecular identification of six isolates of the edible mushroom *L. edodes* was carried out using microsatellite markers. Microsatellite loci have recently become increasingly important as markers in the human, animal and plant genomes. These loci are highly mutable and thus are able to differentiate between related taxa, even at the level of individual isolates in a single species. They are specie-specific and so their isolation must be conducted on each new specimen through the screening of a microsatellites-enriched genomic library. Only a few single-locus microsatellite markers have been isolated in Ascomycetes and Basidiomycetes. This can be due to the structure of Eumycota's genome, which contains only limited amounts of microsatellites. Infact their genome rarely contain more than 5% of repetitive DNA (Wostemeyer J and Kreibich 2002). The screening of the *L. edodes* enriched-genomic library led to the isolation of five loci containing tandem repeats, that allow to discriminate two of the six isolates analysed. The difficulty in the finding of these microsatellites confirms the high reduction in the fungal genome of the non coding sequences.

REPETITIVE DNA: IMPACT ON EVOLUTION OF GENOMIC ARCHITECTURE IN FUNGI

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Looking at the genomes of the supergroup opisthokonts, which puts true fungi (Eumycota) on a common platform with animals (Metazoa), reveals striking observations. Metazoa have a strong tendency towards complex genomes with huge amounts of various repetitive DNA elements, whereas fungi present small, streamlined genomes with little repetitive DNA.
There is one pronounced exception to the general rule of streamlined genomes. The Zygomycota have repeated DNA levels between 30 and 40% and, in this respect, resemble more metazoan than fungal genomes. As zygomycetes reside at the phylogenetic base of the fungi, they are excellent candidates for studying the impact of repetitive DNA on evolution. In addition, the transfer of this DNA across species, genus and even family borders can easily be achieved by parasexuality, either artificially by inter-specific protoplast fusion[6] or naturally by making use of the mycoparasitic fusion biotroph, Parasitella parasitica. P. parasitica infects a huge number of hosts, and transfers nuclei to its hosts, where they disintegrate and their DNA undergoes recombination events frequently.
Introducing foreign repetitive DNA elements into a nucleus may produce dramatic effects on chromosomal architecture. We constructed many independent parasexual recombinants between a prototrophic wild type strain of P. parasitica and a histidine auxotrophic derivative of one of its hosts, Absidia glauca. Probing the genomic DNA of the recombinants with repetitive DNA probes specific for P. parasitica, revealed remarkable genomic arrangements. Typically, the foreign elements are established permanently in the recipient and, in some cases, eventually are amplified to enormous copy numbers, which are much higher than in the donor organism. The data suggest increased activity of repeated DNA in heterologous genetic backgrounds. Thus, inter-specific DNA exchange may play a role beyond introducing potentially coding sequences as a driving force of chromosomal rearrangement and evolutionary velocity.

MINING MICROSATELLITES IN AN EST DATABASE OF *MYCOSPHAERELLA GRAMINICOLA*

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Mycosphaerella graminicola, the cause of septoria tritici blotch of wheat, is developing rapidly as a model organism for fungi in the order Dothideales. Genetic analyses of this fungus are aided by extensive sets of molecular markers, including restriction and amplified fragment length polymorphisms (RFLP and AFLP), and random amplified polymorphic DNA (RAPD). The RFLP markers have been used extensively for population genetics analyses, while AFLP and RAPD markers were used to construct a genetic linkage map. Each of these marker systems has particular limitations, many of which are overcome by microsatellite or simple-sequence repeat (SSR) markers. However, only 12 (check this)nine microsatellite loci have been identified so far in *M. graminicola*, and none has been mapped. To identify additional polymorphic SSR loci, a database of EST data from *M. graminicola* was scanned for di-, - and tri-, tetra- and pentanucleotide units repeated six or more times. Among more than 304,000 EST sequences screened, 104 possible SSR loci were identified and primers flanking these SSR's were developed using an automated software pipeline. To test whether these SSR's are informative for population studies, So far, 38 primer pairs of these putative loci have been tested for polymorphism on on the Dutch parents of the *M. graminicola* mapping population, two isolates from North Dakota, USA, and four isolates of the closely related barley pathogen *Septoria passerinii*. 31Thirty-one of the 38 Most (82%) of the primer pairs (82%) generated a single amplicon, and tested gave good amplification. Among these, 24 18 (5863%) showed polymorphism betweenamong the four field isolates of *M. graminicola* that were tested isolates tested and. Among these 24 microsatellites, 18 were polymorphic between the parents of the mapping population and can be incorporated easily into the existing genetic map. An additional six loci were monomorphic between the Dutch isolates of *M. graminicola* but polymorphic over all four isolates. Only seven (23%) of the loci with good amplification were monomorphic on the extremely limited sample of field isolates tested. Therefore, in total the 38 primer pairs tested identified 24 polymorphic SSR loci. Many of the monomorphic loci may be polymorphic if tested on a larger sample of isolates. Most primer pairs also amplified bands in *S. passerinii*, but they usually had a different size and were less polymorphic less variable (most isolates had similar-sized amplicons) than those amplified with the same primers in *M. graminicola*. The EST database provided a rich source of new microsatellites,SSR which were selected efficiently. These microsatellite markers developed will facilitate integration of the different types of for genetic analyses performed on of this important plant pathogen.

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VISUALIZATION OF OPEN READING FRAMES BY PLOTTING THE CONTENT OF GUANINES AND CYTOSINES IN DNA OF PHYCOMYCES BLAKESLEEANUS AND OTHER ZYGOMYCETE FUNGI

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The Zygomycete fungus Phycomyces blakesleeanus has a 30 Mb genome with a 35% content of guanines and cytosines (G+C). In order to expand our knowledge of DNA composition in Phycomyces we have analyzed the G+C content in Phycomyces genes and fragments of genes available in public databases, the frequency of nucleotides in each codon position, and the codon usage. Phycomyces protein-coding DNA has a G+C content of 48% while non-coding DNA has a G+C content of 30%, a difference of 18%. The difference in G+C content between protein-coding and non-coding DNA is unusually large as compared with the 10% difference in Neurospora crassa or the 8% difference in the human genome. A preference for pyrimidines in the third position of codons has been observed resulting in a biased codon usage were pyrimidine-ending codons are preferred over purine-ending codons for four-codons amino acids, with G as the less preferred nucleotide. The nucleotide at the silent third codon position may change more freely than those on other codon positions due to lower selective constraints. It is then remarkable that the G+C content at silent third codon positions in Phycomyces is 51.3%, similar to the average G+C content of coding DNA but very different of the average G+C content of non-coding DNA (30%).
The 18% difference between the G+C content of protein-coding and non-coding DNA suggested that open reading frames (ORFs) could be visualized by plotting the G+C content along a segment of Phycomyces DNA. We prepared plots of G+C content along the DNA for all the Phycomyces genes present in public databases. As we expected, the position of most exons and introns in Phycomyces genes could be detected by their difference in G+C content using a G+C plot. Additionally, the G+C plot helped to identify a high G+C DNA segment linked to the pyrG genes of the Zygomycete fungi Phycomyces, Mucor, and Blakeslea. Sequence similarities showed that this DNA segment was the 3' end of the gene responsible for the protein kinase C. The G+C plot may be used as a quick and simple method to predict the location of ORFs in Zygomycetes.

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GECCO: A BIOINFORMATICS TOOL for comparative analysis of FUNGAL GENOMES

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Decisive steps in the interactions between hosts and pathogens occur at the onset of infection. During these stages the fungal biomass is still very limited and the majority of mRNAs will originate from the host. To get a better understanding of the genes involved in the infection process we generated cDNA libraries of infected plants. In order to avoid sequencing large numbers of ESTs, before obtaining fungal genes, we have developed a procedure that specifically enriches for fungal sequences during the early (and later stages) of infection. This allowed us to generate large datasets containing pathogen genes involved in the early stages of pathogenesis in various pathosystems. Together with the huge amounts of data available through several genome-sequencing efforts (Whitehead, Sanger, TIGR, JGI/DOE, Genescope) there is a great need for bioinformatics tools to mine and compare these large datasets. A bioinformatics platform has been developed that performs automated analysis of sequence datasets and allows for fast and robust comparison of different databases. Among 1724 *F. graminearum* in *planta* unigenes, we identified three ABC transporters that were not present in the annotated genome of *Magnaporthe grisea*, two of which were also absent in the *Neurospora crassa* genome. Among 4452 *in planta* unigenes from *Mycosphaerella graminicola*, we identified 41 unigenes involved in signal transduction, four of which were not previously identified in *N. crassa.*

Exposure to phleomycin is mutagenic in *Schizophyllum commune* despite acquired resistance

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The most efficient genetic transformation method for *Schizophyllum commune* is PEG mediated transformation of protoplasts followed by regeneration and selection on phleomycin (Schuren and Wessels, 1994). Considering the mode of action of the bleomycins to damage DNA and the fact that resistance is based on a 1:1 binding with the resistance protein, we studied whether phleomycin is still mutagenic in resistant strains. A phleomycin resistant strain of *S. commune* was grown on selective phleomycin medium for one week. Pieces from different parts of the periphery of this colony were grown at non- selective medium. Almost all colonies showed abnormal morphology. The aberrant morphology was stable and hereditary. In 50% of the colonies, the *thn* mutation caused the change in appearance of the colony. This mutation was previously reported to be caused by transposition of the Scooter (Fowler and Mitton, 2000). This and the fact that DNA damage can induce mobilization of transposable elements suggest that the mutagenic effect of phleomycin is (partly) due to transposition. A variety of transposable elements was found in the recently obtained sequence database of *S. commune* (representing 40-50% of the single copy DNA). Some of them are present in high copy numbers. Currently we are assessing the distribution pattern of Scooter and other prominent elements in the genome of phleomycin resistant colonies before and after exposure to the antibiotic.

Fowler T. J. and Mitton M. F. 2000. Genetics; 156(4): 1585 - 1594.
Schuren, F.H.J. and Wessels, J.G.H. (1994). Curr Genet 26 26: 179-183.

In situ hybridization of RNA in filamentous fungi using peptide nucleic acid probes

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DNA probes do not diffuse through the fungal cell wall. Therefore, in situ hybridisations of RNA in yeast are done with protoplasts. However, in case of filamentous fungi the hyphal morphology is lost upon removal of the cell wall. This abolishes the possibility to use in situ hybridisation to localize gene expression at the level of the hypha and the colony. Therefore, we attempted to make the cell wall of Aspergillus niger and Schizophyllum commune permeable by freeze/thawing and by short treatments with lysing enzymes. In contrast to hybridisations with protoplasts, hybridisations with permeabilised hyphae using ALEXA594 labelled 18S rDNA probes were highly irreproducible.

Peptide nucleic acids (PNA) probes are synthetic DNA mimics developed in the 1990's (Egholm et al., 1993; Nielsen et al., 1994). These probes do diffuse through the Saccharomyces cerevisae cell wall and have been used in in situ hybridisations to detect 18S rRNA. We used PNA probes for in situ hybridisations in S. commune and A. niger. Highly reproducible fluorescence was observed after hybridising the fluorescein labelled 18S PNA probe. The signal was absent when the hyphae were pre-treated with RNAse. In situ hybridisation was also successful using a PNA probe hybridising to SC3 mRNA of S. commune. Hybridisation was absent in 2- and 4- day old colonies of a strain in which the SC3 gene has been deleted. In contrast, high fluorescence was observed in a 4-day old colony of a wild-type strain, while signals were lower in 2-day old colonies. The fluorescence intensity correlated with accumulation of SC3 mRNA as determined by Northern analysis. Thus, PNA probes are an excellent tool to study gene expression in filamentous fungi.

Egholm et al. (1993) Nature 365: 556-568.
Nielsen et al. (1994) Bioconjugate Chem 5: 3-7.

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DEVELOPMENT OF GENOMIC RESOURCES FOR THE ROOT ROT PATHOGEN APHANOMYCES EUTEICHES

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Aphanomyces euteiches is an oomycete root pathogen that causes seedling blight and root rot of legumes, such as alfafa and pea, resulting in significant yield reduction in Europe and the United States. The Aphanomyces genus is distantly related to other plant pathogenic oomycetes such as Phytophthora, and includes plant as well as animal parasitic species. Up to now the GenBank database currently hosts only few nucleotide sequence entries for this organism, most of them corresponding to ribosomal and mitochondrial markers. To address this deficiency, and based on the unique phylogenetic position of *Aphanomyces* as a plant pathogenic oomycete, the American Phytopathological Society listed *this organism* as a high priority species for genome sequencing (www.apsnet.org/media/ps/top.asp).

To provide the first foray into gene diversity of *A. euteiches,* a pilot-scale cDNA sequencing project is currently being developed. A total of 3,000 ESTs will be generated corresponding to mycelium grown in liquid medium and starved for 1 week to mimic infection conditions. The ESTs will be processed, assembled, annotated and stored in a publicly available database, the Oomycete Genomics Database (OGD, www.oomycete.org). Data about the first sequence analyses will be presented.

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AUTOMATED ANNOTATION AND ANALYSIS OF THE ASPERGILLUS FUMIGATUS GENOME

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The Aspergillus fumigatus genome has been sequenced by an international consortium including the University of Manchester (UK), The Institute for Genomic Research (TIGR - USA) and the Wellcome Trust Sanger Institute (UK). The assembled genomic sequence was processed through the TIGR annotation pipeline, a collection of software known as Eukaryotic Genome Control (EGC) that serves as the central data management system. EGC processes each sequence through a series of homology computes as well as algorithms for predicting genes (GlimmerM, Exonomy, Unveil, and Phat) and splice sites.

Final gene calls were generated by a program called Combiner, which evaluates each type of evidence separately for its ability to predict translation starts and stops, splice acceptor and donor sites and protein coding regions. Gene models are constructed by merging the most likely gene model signals using statistics generated from the training set provided. This pipeline identified 9,744 genes in the 28.6 Mb sequenced genome, at an average density of 1 gene per 3 kb of sequence.

In order to organize the annotation data for further analysis, proteins were organized into putative paralogous family groupings based on conserved domain composition. Gene products were also assigned to Gene Ontology (GO) terms by transferring the GO associations of the best Saccharomyces Genome Database (SGD) protein match.

The A. fumigatus genome annotation data has been analyzed in the context of a larger consortium involving the Aspergillus nidulans sequencing project (Center for Genome Research, Whitehead Institute, USA), the Aspergillus oryzae sequencing project (Agency of Industrial Science and Technology, Japan) and Aspergillus researchers. We have made the annotated genomes of all three species available to consortium members through a web-based gene evaluation and annotation tool called Manatee(manatee.sourceforge.net). The Manatee interface allows biologists to quickly identify genes and make rigorous functional assessments, using pre-computed search data, paralogous family information, and annotation suggestions derived from automated analyses. Analyzing these genomes simultaneously has highlighted some limitations of automated annotation data, such as merged, split and missing genes, as well as incorrect gene structures.

PROTEIN FAMILIES BASED ON SHARED DOMAIN COMPOSITION IN ASPERGILLUS FUMIGATUS

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The predicted proteome from the completely sequenced genome of Aspergillus fumigatus was classified into putative protein families based on domain composition. These families take into account both previously identified domain signatures (PFAM and TIGRFAM) and potential novel domains identified in the A. fumigatus proteome.

To identify domains from A. fumigatus peptides, we first searched the proteome against PFAM and TIGRFAM HMM profiles. The sequences containing these domains were masked out. The remaining peptide sequences were searched against each other for subsequent clustering and alignment. After resolving clusters with an appropriate link score, resulting alignments were searched back against the A. fumigatus proteome to find additional members. Any A. fumigatus-specific domain alignments containing three or more members were considered true domains for the purpose of building families.

Proteins containing exactly the same set of domains were then classified into families. Families sharing one or more domains between them are considered to be related families. Over fifty percent of the A. fumigatus proteins had domain hits, and slightly less than half were incorporated into families of two members or more. The largest family has 95 members containing the WD domain, G-beta repeat.

We also had the opportunity to run this algorithm on the A. nidulans and A. oryzae genomes and to compare family profiles between genomes.

The identification of putative protein families enables visualization of relationships between proteins/families and allows annotators to evaluate better the function of predicted gene products. We are currently characterizing and curating some of the novel domains identified to further support annotation and to enhance the future functional characterization of fungal protein families.

Transcription profiling of trap cells in the nematode-trapping fungus *Monacrosporium haptotylum*

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The nematode-trapping fungi comprise a large groups of soil-living fungi that can infect and kill nematodes. These fungi enter the parasitic stage by developing specific morphological structures called traps. To obtain some knowledge on the molecular background to the differentiation of trapping cells, we have analysed the global pattern of gene expression in traps and mycelium of the fungus *Monacrosporium haptotylum* (Syn. *Dactylaria candida*). In this fungus the trap is a unicellular spherical cell that develops on the apex of a three celled hyphal branch called stalk. The connections between the knobs and the mycelium can be easily broken and isolated knobs retain their function as infection structures, i.e. they can “capture” and infect nematodes including *Caenorhabditis elegans*. Based on EST sequences, a microarray was constructed containing PCR products of 2.822 cDNA clones of *M. haptotylum.* RNA was isolated from knobs and mycelium, labelled with Cy3 and Cy5 and hybridised onto the cDNA array. Despite the fact that the knob and mycelium were growing in the same medium, there was large differences in the patterns of genes expressed in the two tissues. The number of down-regulated genes were 299 (10.6 % of the total number of fungal clones) and the number of up-regulated genes was 193 (6.8 %) (at a significance level (*P*) of 0.001). A significant part of the EST clones that were annotated in the “transcription”, “cellular transport and transport mechanisms”, “cellular communication/signal transduction”, ”cell rescue, defence, cell death and ageing” and “cell growth, cell division and DNA synthesis” were down-regulated in the knobs as compared to the mycelium. Several of the differentially expressed genes including a Rho1-type GTPase homolog and a Rac homolog are known to play an important role in regulating the organization of the actin component of the cytoskeleton. Among other characterized regulated genes were a Ras homolog, and a homolog to the GAS2 (MAS3) gene that is specifically expressed in appressoria of the rice blast fungus.

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GENOME MINING FOR NOVEL ENZYMES.

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Schizophyllum commune is a basidiomycete that grows predominantly on felled wood. To be able to consume the carbon sources present in this substrate (mainly cell wall polysaccharides, such as cellulose, xylan and galactomannan) this fungus will need to produce a wide range of enzymes degrading these polysaccharides to mono- and small oligosaccharides that can be taken up by the fungal cell.
Plant cell wall degradation has so far been mainly studied in ascomycetes, where many genes have been identified encoding genes involved in this process. These enzymes are widely used in industrial applications.
We have recently obtained a partial genome sequence (13 Mb unique sequence) of *Schizophyllum commune* and have analysed the sequence for genes encoding extracellular enzymes. Amongst the sequences we detected a significant number that had only low homology to ascomycete genes, but instead were highly homologous to genes from mammals, plants or bacteria. These genes are likely to encode enzymes with different properties than those from ascomycete fungi currently used in industrial applications. They are therefore interesting candidates to be used in novel or optimised applications.

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CHARACTERISATION OF REGULATORY ELEMENTS OF CELL WALL HIDROLITIC ENZYMES PRODUCED BY FUNGI OF THE GENUS Trichoderma

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The genus Trichoderma is well-known for its biotechnological potential. Currently there are several commercial products which is based on their abilities to function as biopesicides and bioferilizers. The huge hydrolytic potential of the genus Trichoderma is considered to be a key element in such activities. Nevertheless the knowledge of how many different hydrolytic enzymes there are in the genus; why there are so many isoenzymes for the same activities; how is regulated the expression of the different hydrolytic enzymes, etc. are still poorly understood. In order to get insight into these questions, we have started a functional genomic approach to characterized the hycrolytic machinery of the genus Trichoderma. To do so, a cDNA library have been constructed from the strain T. atroviride B11. The library was built by mixing mycelia coming from different grown condition, including biotic and abiotic stress conditions, different carbon and nitrogen sources and simulated mycoparasitims conditions. To date, we have generated and analyzed approximately 3.000 expressed sequence tags (EST) from that library. Over 5% of the contigs identified code for hydrolytic enzymes or regulatory elements (transcription factors, signalling pathway, etc.). Such EST have been used to construct nylon membrane arrays. At the moment, we are carring out hybridisation experiments in order to identify genes involved in the regulation of hydrolytic enzyme expression in T. atroviride B11.

PCR DIAGNOSTIC METHOD BASED ON MULTI-COPY AND LOW-COPY SEQUENCES FOR THE MAIN OCHRATOXIN A PRODUCING SPECIES IN GRAPES

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Mycotoxin-producing fungi are usually found as saprophytes or plant pathogens colonising both the root and aerial parts of plants, including seeds and fruits. Under suitable conditions these fungi produce highly toxigenic metabolites among which ochratoxin A (OTA) is one of the most important due to its high toxicity to humans. OTA is found in a variety of agro-food products such as cereals, coffee and wine and it is mainly produced by species of the genus Aspergillus (A. ochraceus, A. niger and A. carbonarius) and Penicillium (P. verrucosum), although the presence of OTA-producing species of Penicillium has not been reported in Spain so far. Detection of those OTA-producing strains is a critical point to prevent OTA entering the food chain.

In order to obtain diagnostic sequences of the main OTA-producing Aspergillus species in grapes (A. ochraceus, A. niger and A. carbonarius) we analysed the internal transcribed spacer of rDNA (ITS) and a partial region of the elongation factor 1_ in a number of Aspergillus spp., both from culture collections and isolated from grapes. The specificity and the level of sensitivity of both types of primers, multycopy and low copy, were compared on PCR assays of a wide sample of Aspergillus strains isolated from grapes and cereals confirming the efficiency of the assay to detect OTA-producing Aspergillus isolates.

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Deletion of *hdaA*, a gene coding for a major histone deacetylase of *Aspergillus nidulans*

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During the past years it has become clear that chromatin represents an important regulatory element that affects nuclear processes such as DNA replication, recombination, DNA repair, and transcription by tuning the accessibility of DNA for various factors. Cells have elaborated a specific machinery to modify nucleosomes for specific processes occuring in chromatin. Thereby, acetylation on the N-terminal tails of the core histones is the most prominent modification. Enzymes responsible for the dynamic equilibrium of histone acetylation are histone acetyltransferases (HATs) and histone deacetylases (HDACs). HDACs form highly conserved protein families in many eukaryotic species. Today, histone deacetylases are categorized according to the yeast proteins RPD3 (class 1), HDA1 (class 2), and the sirtuins (class 3).

Recently, we have identified and characterized several HDAC-genes in the filamentous fungus *Aspergillus nidulans*. Further biochemical investigations with partly purified HDAC activities of the fungus revealed that HdaA, a class 2 enzyme, is the major contributor to total HDAC activity of *A. nidulans*. However, the specific function of the enzyme as a histone deacetylase is still unclear. In order to elucidate the biological role of HdaA we generated a *hdaA* deletion strain by direct one-step gene replacement. The deletion of *hdaA* revealed the loss of major HDAC activitiy. Furthermore we investigate the effect of *hdaA* deletion with regard to phenotypical characteristics, metabolic effects, and effects on the regulation of selected genes.

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EXPRESSION PROFILING OF B-MEDIATED GENE REGULATION IN USTILAGO MAYDIS.

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The phytopathogenic fungus Ustilago maydis has a dimorphic life cycle. For successful infection of its host plant maize two compatible haploid sporidia have to fuse on a leaf surface and to form a filamentous dikaryon that is able to penetrate the plant cuticula. Filament formation and the subsequent steps in pathogenic development are controlled by the multiallelic b-mating type locus encoding the homeodomain proteins bE and bW. bE and bW proteins expressed from different alleles can form a heterodimer, which is thought to regulate the b-dependent processes via its function as a transcriptional regulator.
Several approaches helped to identify direct and indirect b-target genes during the past years, but yet did not reveal a complete view of the biological processes regulated by the b mating type locus. Taking advantage of the Ustilago maydis genome sequence, a gene chip was designed that allows parallel expression analysis of about 6200 Ustilago maydis genes. The gene chip technology was employed to monitor the changes in gene expression after formation of the bE/bW heterodimer in a 12 hours time course, leading to the identification of 246 b-responsive genes. The bioinformatic analysis of these genes allows for the first time to visualize the different processes controlled by the *b*-locus, including the alteration of the cell wall composition and cell cycle control.
A large fraction of *b*-regulated genes, however, does not share similarities to known proteins, suggesting that these genes may be related to the dimorphic/pathogenic life-style of *U. maydis*. Other *b*-regulated genes have similarities to proteins that control developmental processes in other fungi; among these, we have identified a gene with similarity to clp1 of Coprinus cinereus as a novel pathogenicity factor for *U. maydis*. In C. cinereus, Clp1 is thought to have regulatory functions during the formation of clamp cells. In U. maydis, no clamp cells have been described yet; the deletion of umclp1 leads to no obvious phenotype in the saprophytic stage, neither growth nor the mating reaction is altered. However, pathogenic development is blocked at the stage of plant penetration. Interestingly, also the constitutive expression of umclp1 renders U. maydis nonpathogenic. Currently we are using the DNA array technology to address the regulatory functions of the Umclp1 during pathogenic development.

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FUNGAL PROTEOMICS AT PACIFIC NORTHWEST NATIONAL LABORATORY (PNNL)

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We are applying proteomics methods to the study of secreted proteins and morphology control in two fungi recently sequenced by the US Dept. of Energy's Joint Genome Institute (JGI), the basidiomycete, Phanerochaete chrysosporium and the ascomycete, Trichoderma reesei. P. chrysosporium is a well studied model white rot fungus with the ability to degrade lignin while T. reesei is an industrially important producer of cellulases and hemicellulases. Our work is directed at understanding extracellular enzymes, especially as it relates to biomass conversion to valuable products and morphology of fungi as it relates to the hyperproductivity observed in some fungal fermentation processes. We report here progress in the application of both 2D electrophoresis and global proteomics tools.

MULTI-GENE GENEALOGIES RECONSTRUCT THE EVOLUTION OF THE ZYGOMYCETES AND DETECT PUTATIVE HORIZONTAL GENE TRANSFER EVENTS

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In order to study the evolution of the zygomycetes in multi-gene approaches, sequences for the nuclear-encoded genes for the small subunit ribosomal RNA (18S), for actin (1) as well as for beta-tubulin (2), for the translation elongation factor EF1-alpha and for the cell division cycle proteinkinase CDC2/28 were determined and applied in concatenated analyses of tree constructions (approx. 6300 nucleotide positions per species).
Special emphasis has given on the Mucorales, the most prominent and largest order of the zygomycetes. Among this order, facultatively mycoparasitic species parasitise on other mucoralean species during the establishment of plasma bridges followed by the unidirectional transfer of genes to the host (3). This parasexual interaction utilises the pheromone trisporic acid for the identification of compatible hosts, similarily to sexual interactions (4). One of the last steps of trisporic acid biosynthesis is catalysed by the 4-dihydromethyltrisporate dehydrogenase (5). The gene encoding this aldo-keto reductase were found in all families of the Mucorales, Mortierellales (6), Entomophthorales and Kickxellales. The sequences were analysed and used for the detection of putative horizontal gene transfer events.

(1) Voigt K & Wöstemeyer J (2001) Gene 270: 113-120.
(2) Einax E & Voigt K (2003) Organisms, Diversity & Evolution 3: 185-194.
(3) Kellner M., Burmester A., Wöstemeyer A., Wöstemeyer J. (1993) Curr. Genet. 23: 334-337.
(4) Wöstemeyer J, Burmester A, Wöstemeyer A, Schultze K, Voigt K (2002). In: Syvanen M, Kado C.I. (eds.) Horizontal gene transfer, 2nd edition, Academic Press, San Diego, CA, pp. 237-247.
(5) Czempinski K., Kruft V., Wöstemeyer J., Burmester A. (1996) Microbiol 142: 2647-2654.
(6) Schimek, C., Kleppe, K., Saleem, A.-R., Voigt, K., Burmester, A., Wöstemeyer, J. (2003) Sexual reactions in Mortierellales are mediated by the trisporic acid system. Mycol. Res. 107: 736-747.

IN SILICO* IDENTIFICATION OF FRUCTAN-MODIFYING ENZYMES FROM *Aspergillus niger

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Fructans are a class of biopolymers which are made up of a series of fructose monomers, usually ending in a terminal glucose residue. The monomers are primarily linked by either β,2-1 (inulins) or β,2-6 (levans) glycosidic bonds. Inulins and its derivatives have found a wide array of applications in the food and pharmaceutical industry, e.g. production of high-fructose syrups and health-promoting prebiotics. Production, degradation and modification of fructans (poly- and oligosaccharides) are carried out by a group of enzymes primarily belonging to the families 32 and 68 of glycosyl hydrolases (http://afmb.cnrs-mrs.fr). Examples of such enzymes have been identified in a large number of plant, bacterial and fungal species, including the industrially important filamentous fungus, *Aspergillus niger*. Although a substantial amount of published data is available for these enzymes in *A. niger*, little is known regarding their functional diversity in this organism.

Recently, the Dutch life sciences company DSM has finished a whole-genome sequencing program of *A.niger*. Under the colours of the national Senter IOP Genomics program (www.senter.nl/asp) we are involved in the further identification and characterization of fructan acting enzymes. As a first approach we have performed *in silico* genome mining for this group of enzymes. Using this approach, 6 potential targets have been identified, displaying homology to previously described sequences from *A.niger* as well as a number of other fungal species.
In this poster, the genome mining steps and findings, identifying potential targets for overexpression, will be presented and discussed.

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CLONING OF A NOVEL PROMOTER FROM OF *Trichoderma harzianum* CONTROLLING A HIGHLY EXPRESSED GENE

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Trichoderma strains are considered to be among the most useful fungi in industrial enzyme production, agriculture and bioremediation. Some Trichoderma strains are able to antagonize phytopathogenic fungi by using substrate colonization, antibiosis and/or mycoparasitism as the main mechanisms. The strong biodegradation activities performed by Trichoderma strains is are the result of an amazing metabolic versatility and a high secretory potential which leads to the production of a complex set of hydrolytic enzymescell wall degrading enzymes (CWDEs). that areSome of these enzymes able to degrade fungal cell walls (cell wall degrading enzymes, CWDEs) and seem to be also involved in the mycoparasitic process. This set of enzymes includes chitinases, β-1,3-glucanases, β-1,6-glucanases, α-1,3-glucanases and proteases.
We have overexpressed some of these CWDEs from T. harzianum in both homologous and heterologous systems (Pichia pastoris) with variable success. Until now, we have used heterologous regulatory signals belonging to a different Trichoderma speciesrelated taxonomic groups (T. reesei) with variable success. Expression vectors containing but it would be desirable to have T harzianum strong promoters and terminators of the same hostcould improve the yield of protein production.. Recently, we have used a genomic approach gene expression profiling to study the level of expression of precise set of genes being expressed at a specific moment and their levels of expression. To do so, 3000 ESTs of T. harzianum CECT 2413 were sequenced and annotated. This database was analyzed for redundancy and and the genes showing the highest values value of this parameter redundancy were selected and membrane-arrayed to study their expression levels in glucose containing media. Following this strategy, we were able to find identify a gene showing high-constitutive expression. Strikingly, this gene didn't show any remarkable homology with any sequences present in databases. The gene promoter of this gene has been cloned and sequenced by means of a "primer walking" technique. Its regulatory function strong gene expression activity has been demonstrated by using a reporter gene system (uidA of E. coli) to transform the filamentous fungi T. harzianum and A. nidulans, and the methylotrophic yeast P. pastoris. In every case, we obtained higher levels of GUS activity than that those obtained with other promoters commonly used for heterologous expression in fungi and yeast.

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DEVELOPMENT OF TOOLS FOR MOLECULAR GENETICS IN ASPERGILLUS

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With a number of Aspergillus genome sequences becoming available, the need for molecular tools that allow analysis of gene function is greater than ever. Although a number of tools are available for use in A. nidulans, rapid methods for functional analysis on a genome-wide scale are very limited, and there is a lack of tools for other species, notably A. niger.

A number of approaches have been employed to develop a molecular tool kit for the aspergilli, including the use of conditional promoters and construction of cassettes for rapid manipulation of genes. Some success has been achieved with using the alcA promoter of A. nidulans to control gene expression in A. fumigatus (Romero et al., 2003). The sulphate permease promoter of A. niger may provide an alternative regulatory system, and is under investigation.

Another approach is the tet system, based on the bacterial mechanism of resistance to tetracycline. This system has been used in plants, mammals and yeast, but not yet in the filamentous fungi. Although a system based on the yeast GAL-4 activator and HOP-1 minipromoter has not proved to be effective in A. nidulans, work is continuing on a system designed for use in mammalian cells, which uses the CMV minipromoter and VP16 activator.

The laccase enzyme, which is native to many filamentous fungi, has been used successfully as a reporter gene in A. nidulans. Under the control of the alcA promoter it demonstrated a good dynamic range of expression, and a low level of background activity, providing an alternative to betagalactosidase and betaglucuronidase as a reporter of promoter activity.

To allow rapid application of these tools across a range of genes and species, development of a cassette-based transformation system is underway. A cassette carrying a selectable marker plus the alcA promoter has been constructed. Two approaches have been used to add flanking homology to such cassettes. Originally overlapping PCR was used to give up to a kilobase of flanking sequence. However, recent evidence shows that 50 base pair extensions on the PCR primers may provide sufficient homology for targetted integration.

TRICHOEST: FUNCTIONAL GENOMICS AND PROTEOMICS OF *TRICHODERMA* ANTAGONIST STRAINS FOR INDUSTRY AND AGRICULTURE

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The EU-project (QLK3-2002-02032) "TrichoEST" started under the 5th Framework Programme "Quality of Life" December 1. 2002. The aims are to identify genes and gene products from *Trichoderma* spp. with biotechnology value, to assess their industrial potential and, to exploit and commercialize them in concert with EU biotechnology strategy. The rationale for the project is that the enzyme produced by *Trichoderma* makes it a fungal genus of great and demonstrable biotechnological value. The project is not a sequencing programme. Instead, it develops integrated functional genomic and proteomic approaches and innovative use of bioinformatics, leading to rapid exploitation of genes and gene products and their transformation into industrial processes. Technologies achieved will have applications in a range of agro-industrial, environmental and medical activities, including innovations in agricultural pest and pathogen management, novel antibiotics, and enzymes with industrial uses involving processing of plant-derived matter, including animal feed, composting and bioremediation.

At the same time the genome of the *Trichoderma* antagonists is poorly surveyed compared with other model microorganisms, due to the great diversity of its species, the absence of optimised systems for its exploration, and the great variety of genes expressed under a wide range of ambient conditions.

The project is coordinated by Dr. Manuel Rey Barrera, Newbiotechnic S.A., Spain, and the partners, besides us, are Dr. Fabrice Lefevre, Proteus S.A., France, Dr. Antonio Llobell González, University of Seville, Spain, Dr. Enrique Monte Vázquez, University of Salamanca, Spain, Dr. Matteo Lorito, University of Napoli, Italy, Dr. Christian Kubicek, Technical University of Vienna, Austria, Dr. Gabi Krczal, Centrum Grüne Gentechnic, and Dr. Paul Cannon, CAB International, UK.

UPR SPECIFIC TRANSCRIPTIONAL REGULATION IN *ASPERGILLUS NIGER*

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The endoplasmic reticulum (ER) is a highly specialized protein folding compartment responsible for the structural maturation of proteins entering the secretory pathway. The folding capacity of the ER can be enormous. To accomplish this, the ER provides an environment highly optimized for efficient folding.
Proteins that fail to be folded correctly are removed from the ER. This is done by retrograde transport to the cytoplasm where ubiquitination and subsequent degradation by the proteasome occur.
Under normal conditions in the ER, import and folding versus dislocation and degradation is in equilibrium. Disruption of this balance, either by an 'overload' of imported proteins or as a consequence of the expression of a particularly difficult to fold protein, results in stress by accumulation of an excess of misfolded proteins in the ER. The cell tries to alleviate this stress by triggering a response, the Unfolded Protein Response (UPR), which induces expression of genes involved in adjusting the cell to cope with the surplus of protein folding intermediates.
Genes in these pathways could be likely targets to optimise the secretion capacity for (heterologous) proteins in *Aspergillus niger.* Our efforts are directed towards identification and characterization of such genes and their products. Both genomics and genetic approaches are taken.
In initial transcriptomics experiments we have screened cDNA microarrays for differential expression under UPR inducing conditions. This resulted in identification of a limited number of new up- or down-regulated genes. Genome-wide Affymetrix chips are presently screened.
The genetic approach employs a hybrid gene in which a selectable trait (*amd*S) is expressed from a UPR responsive promoter. Mutants with altered *amd*S expression are isolated in an *A. niger* transformant with a single copy of this construct and their UPR characterized.

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GENOME SEQUENCING OF *ASPERGILLUS ORYZAE*

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The genome sequencing project for *Aspergillus oryzae* launched in August 2001 by the whole genome shotgun approach. The contigs were assembled from 6X depth of coverage of total sequence and were connected by end sequencing of 5,000 cosmid clones, yielding approximately 30 scaffolds. Most of the scaffolds have already been mapped on the chromosomes and the relative position of the scaffolds on the chromosomes have been almost completed. There are approximately 20 physical gaps remaining right now, most of which will be closed by BAC libraries. The total genome size of *A. oryzae* was estimated to be 36.8 Mb, which was slightly bigger than that estimated by PFGE. We have already obtained 15 independent telomere sequences, indicating most of the chromosomal ends have been identified. However, we found that the longest band on PFGE might include two chromosomes (I and I'). Instead, the shortest band originally predicted to have two chromosomes (VII and VIII) might have only one. The computational prediction of genes by GeneDecoder (Asai et al., http://www.cbrc.jp/) in combination with alignment with *A. oryzae* ESTs suggested existence of more than 11,000 genes in *A. oryzae* genome, among which approximately 40% of genes were predicted to have introns. As was suggested from the *A. oryzae* genome size bigger than that of *Aspergillus nidulans*, there were many redundant genes including energy supplying enzymes, sugar transporters, hydrolytic enzymes found in the *A. oryzae* genome although the genes encoding RNA polymerase remained single. The analyses of genes for transcription factors, metabolic pathways and so on, which are important especially for industrial application of *A. oryzae*, are in progress.

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THE WHOLE GENOME SEQUECE OF THE WHEAT AND BARLEY PATHOGEN, FUSARIUM

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We have generated a draft sequence assembly of the F. graminearum genome that is available on the web for download and query. The sequence is of high quality with the entire 36 Mb assembly consisting of just 511 contigs (> 2 kb) contained within 28 supercontigs. The second genome release (October 2003) contains automated annotation, preliminary genome analysis and integration with the genetic map. Using organism-specific parameters for gene prediction, 11,640 protein-coding genes have been identified, representing over 1,500 more genes than predicted by the same method for the non-pathogenic filamentous fungi, Neurospora crassa and Aspergillus nidulans. A genetic map, currently consisting of 66 SNPs, 29 RFLPs, and 27 microsatellites, has been constructed that anchors 99.5% of the sequence assembly. Details of the automated annotation, efforts toward manual annotation and coordination of functional analysis of the genome will be discussed. The F. graminearum sequencing project was funded by the National Research Initiative, through the USDA/NSF Microbial Genome Sequencing Program.

MOLECULAR STRUCTURE AND EVOLUTION OF HYDROPHOBINS IN THE ECTOMYCORRHIZAL FUNGUS PAXILLUS INVOLUTUS

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Hydrophobins are small hydrophobic proteins that are involved in a number of morphogenetic activities in fungi, including formation of aerial hyphae, spores, fruit bodies and infection structures. They are also involved in cell- to-cell communication and adhesion. We are interested in the molecular structure and evolution of genes encoding hydrophobins in the ectomycorrhiza forming basidiomycete Paxillus involutus. Based on an EST-sequence project seven putative hydrophobin encoding genes (pihydA-G) were found in the Paxillus involutus strain ATCC200175. These seven putative genes were characterised, both on cDNA and genomic level. Based on the positions of the eight conserved cystein residues in the hydrophobin motif the seven hydrophobins fall into two structural groups - pihydA, pihydB, pihydE and pihydF in one and pihydC, pihydD and pihydG in the other. The lengths of the predicted peptides varied between 107-116 residues, except for pihydA which was 141 residues long (the additional residues are positioned in the 5' end). The hydrophobin genes contained two introns, except for pihydA (three introns) and pihydG (no intron). The amino acid sequences showed strong similarity with each other in the 5' end (the signal peptide) and in the 3' end. Phylogenetic analysis showed that the pihydB and pihydE, as well as the pihydD and pihydG were closely clustered and found in two different clades. pihydA, pihydC and pihydF were found at some distance from these clades among hydrophobins from other basidiomycetes. To further understand the evolution of the hydrophobins, we are also analyzing the molecular variation in pihydA-G in closely related strains of P. involutus.

THE PAXILLUS INVOLUTUS / BETULA PENDULA SYMBIOSIS: MICROARRAY ANALYSES OF GENE EXPRESSION DURING NUTRIENT ACQUISITION.

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Boreal forest trees are highly dependent upon symbiotic associations formed between their roots and ectomycorrhizal (ECM) fungi for nutrient acquisition, a process involving coordination between three spatially, morphologically and physiologically distinct tissues. Initial events in nutrient mobilisation and uptake occur at the advancing ECM mycelial front as hyphae proliferate intensively in nutrient-rich soil patches. A network of rhizomorphs distributes nutrients from such patches around the mycelium and to mycorrhizal root tips within which extensive nutrient exchange occurs between the plant and fungal partners. Our study investigates nutrient-regulated gene expression in the Paxillus involutus / Betula pendula association using microarray analysis. This association was grown in semi-natural peat windows into which nutrient-rich patches of different nitrogen and/or phosphorus inorganic or organic compounds were introduced. Three cDNA libraries were constructed from mycorrhizal root tip, rhizomorph and patch mycelial tissues using pooled total RNA harvested from each nutrient treatment. In total 5,429 ESTs were sequenced from these three libraries. A clustering of these 5,429 ESTs with a previous collection of 13,759 ESTs identified 4,968 contigs, each representing a unique transcript of plant or fungal origin. cDNA microarrays, constructed using this unique gene set, are being used in analyses of gene expression a) within patch mycelium in response to the type and quality of nutrient supplied and b) in mycorrhizal tip, rhizomorph and patch mycelial tissues actively exploiting a single nutrient resource.